Bioactives Compendium

Meat & Livestock Australia

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contents

The Australian bioactives industry

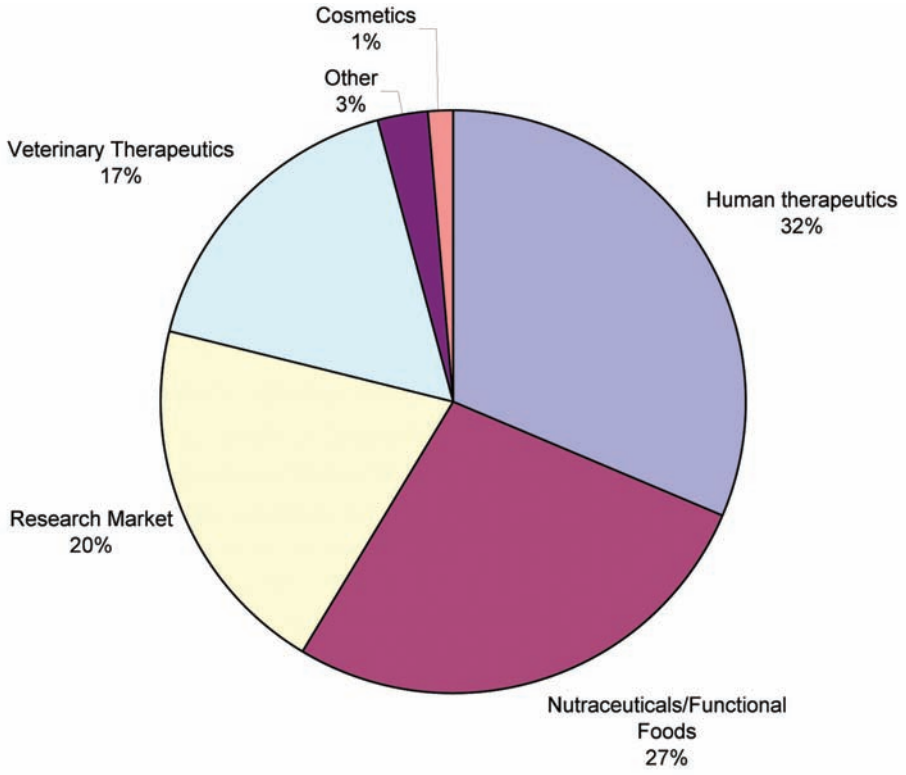
In accordance with common industry practice, bioactives are defined as animal and/or plant derived molecules and compounds which can interact with living tissues and form the active ingredients in functional foods, nutraceuticals, functional cosmetics and pharmaceutical products. Functional foods, cosmetics and nutraceuticals all contain active ingredients that have a biological effect on living organisms independent of, and in addition to, the actual nutritional or cosmetic benefit that they provide. Such bioactives can also be key active ingredients in human and veterinary pharmaceuticals.

The markets for products containing bioactives are large and varied. Examples of products where bioactives ingredients may have, but are not limited to, market applications include:

- cosmetics for lessening the effects of ageing;
- ingredients in perfumes, fragrances and scents;
- ingredients in oral and physical hygiene treatments;
- supplements for prevention or management of specific health conditions;
- enhancing lifestyle, energy and/or mood;
- enhancing sports endurance;
- specific health and nutrition requirements of children, women etc;
- ingredients in organic or natural foods;
- ingredients in research products such as cell culture media; and
- animal health treatments and dietary supplements.

In Australia, many bioactives ingredients are derived from a range of plant and animal sources and are often taken from the waste stream. These are then processed specifically for the market in which the final products are sold. In 2005, an MLA survey of the key players in Australia found that they were 73 companies that manufactured a total of 147 ingredients and final products targeted for different markets. Fifteen companies made 46 products aimed at the human therapeutics market, while 30 companies made 40 products aimed at the nutraceuticals/functional foods market. Together, these made up 59% of the products (Figure 2.1).

Figure 2.1: Market applications for animal and plant derived bioactives ingredients

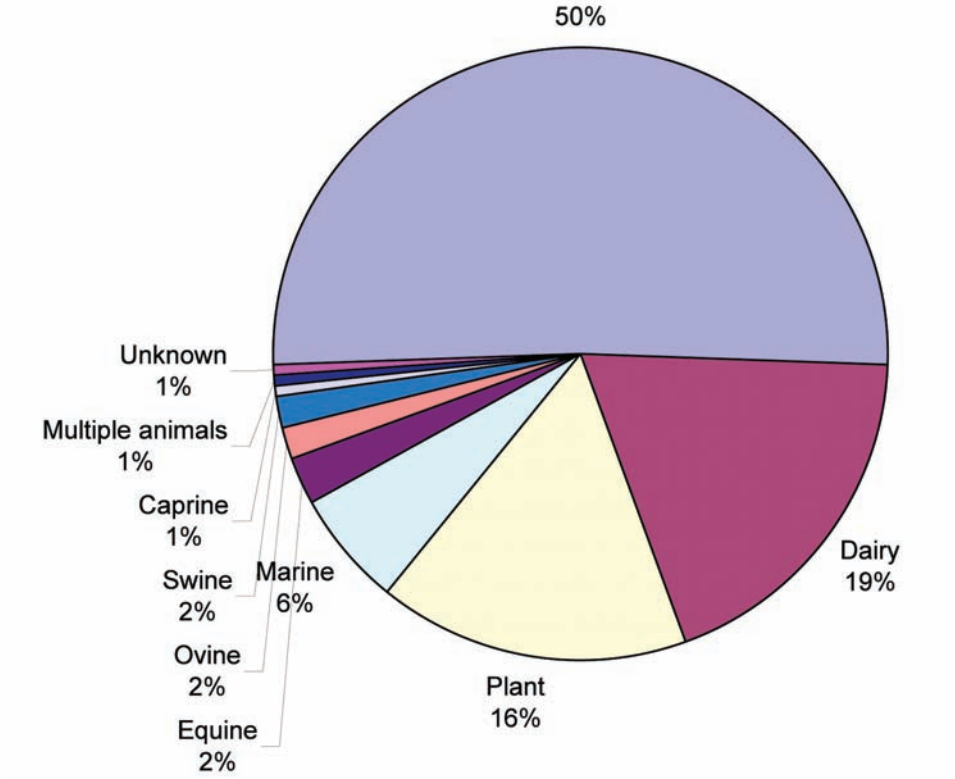


Bioactives may not only need to be extracted but purified, formulated and included in final products. Of the proprietary 147 products identified in the MLA survey, 76% were being sold in bulk as ingredients with the remainder as final products. In general, ingredients manufacturers dominate in all markets except for veterinary products and cosmetics, where manufacturers of final products predominate.

In general, these markets are easier to access than either human foods or therapeutics due to lower regulatory requirements.

The 2005 MLA survey also found that the main source for the molecules identified was beef cattle, followed by dairy herds and plants (Figure 2.2). Other land animal sources were used by 7% of producers/ manufacturers.

Figure 2.2: Sources of bioactives ingredients produced in Australia



Bioactives Value Chains

Bioactive agents from carcases and dairy products fetch high prices at retail; however, this is rarely translated into high returns for primary producers and processors. This is partially due to the amount of processing required further along the chain from the co-products exiting the meatworks, and partially due to the current value chain.

In 2006, MLA undertook to investigate the structure of various bioactives supply chains. The value chains all begin with the producer and the processor, who raise animals and process them, and end with the retailer who sells a finished product to the ultimate consumer. Depending on the particular product, there are two or three processing steps in between.

The "value adder," the next step after the processor, receives raw material from the processor, often derived from a waste stream, and produces a bulk ingredient. For clarity, the value chains treat the value adder as a separate organisation, although it would certainly be possible for a processor to take on a value-adding role as well. Raw material from a waste stream generally has a very low value. In some cases, the processor may actually be paying to dispose of it. The value adder therefore usually pays very little for raw material.

The next step in the value chain is the manufacturer, who buys the bulk ingredient from the value adder and converts it into a finished product. The steps involved may include further purification, formulation, encapsulation, and so on. Again for clarity, this step in the value chain is shown separately, although it would be possible for a manufacturer to do the value-adding as well. Some manufacturers sell to distributors, who in turn sell to retailers, but including that step in the analysis would add little to our understanding, so it has been omitted.

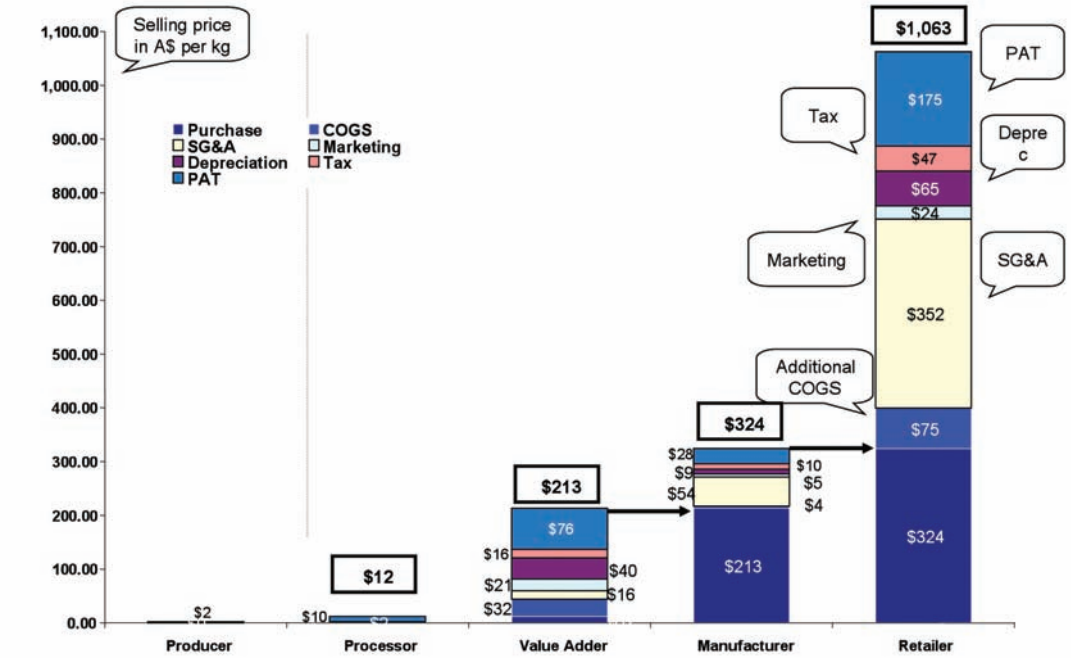
In order to illustrate the relative contribution of raw material – the item of interest to producers and processors – to overall costs and profits, the economics of each step in the chain have been broken down into the major cost components as follows: cost of raw material, additional costs of good sold (COGS), sales, general, and administrative (SG&A) costs, marketing, depreciation, tax, and profit after tax. Obviously, the selling price at each step in the chain becomes the cost of raw material at the next step. Because the material is changed and enhanced as it moves along the value chain, it is natural that its cost increases.

The value chain was created using known data on selling prices at various steps in the chain and information about company-wide economics from CSIRO and published financial reports. The resulting value chain can be assumed to be sufficiently accurate to be used as the basis for developing insight about the opportunities for competing at various steps in the chain.

The value chain analysis is designed to assist players at all points in the value chain to make strategic decisions about where to focus and how best to leverage their capabilities. Four theoretical value chains were built based on the relevant bioactives market applications.

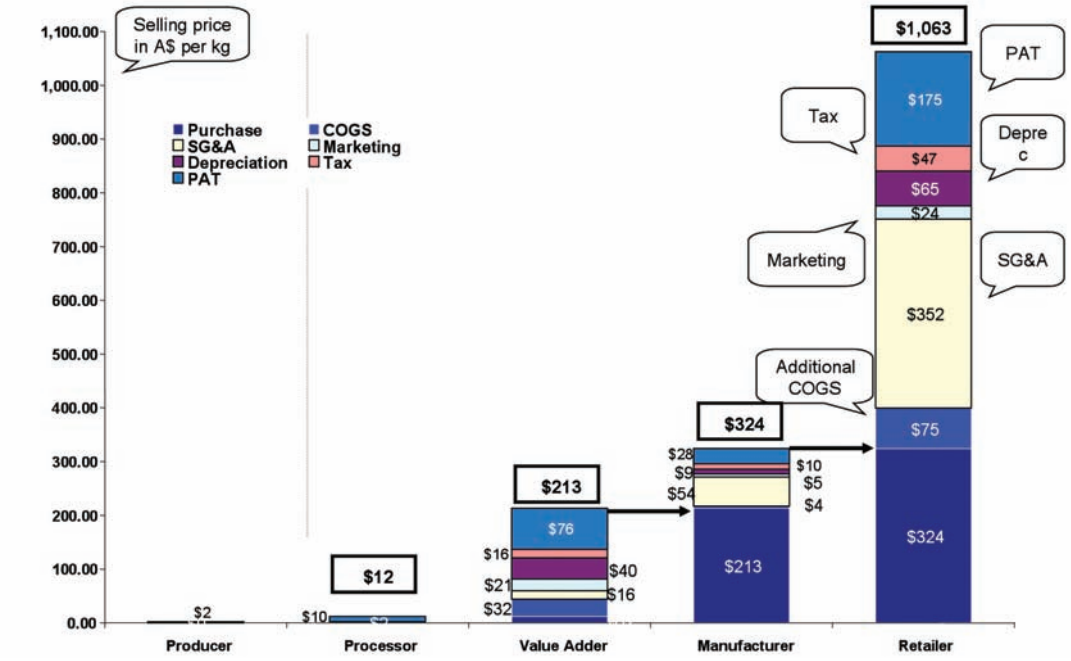
The generic nutraceutical value chain was developed based on chondroitin sulphate food grade, which is a popular supplement used by people and animals suffering from joint pain. Chondroitin sulphate can be sourced from animal tracheas and nasal septum.

Figure 2.3: Generic nutraceutical value chain



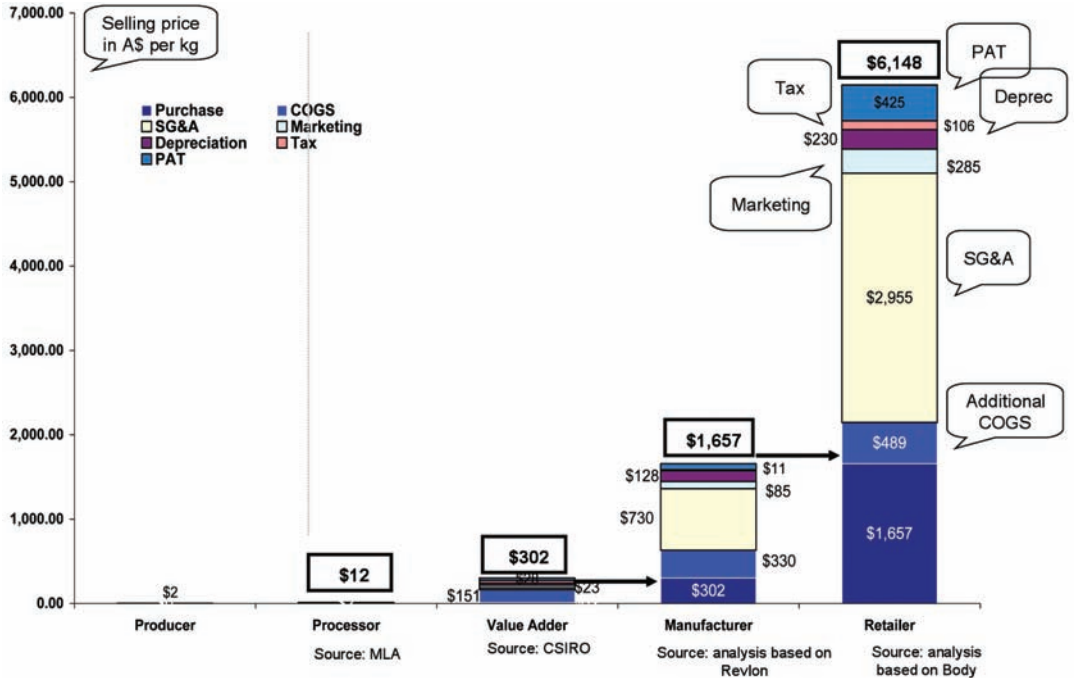
The generic pharmaceutical value chain was developed based on chondroitin sulphate pharmaceutical grade, which is much purer than its food counterpart and claims higher prices.

Figure 2.4: Generic pharmaceutical value chain



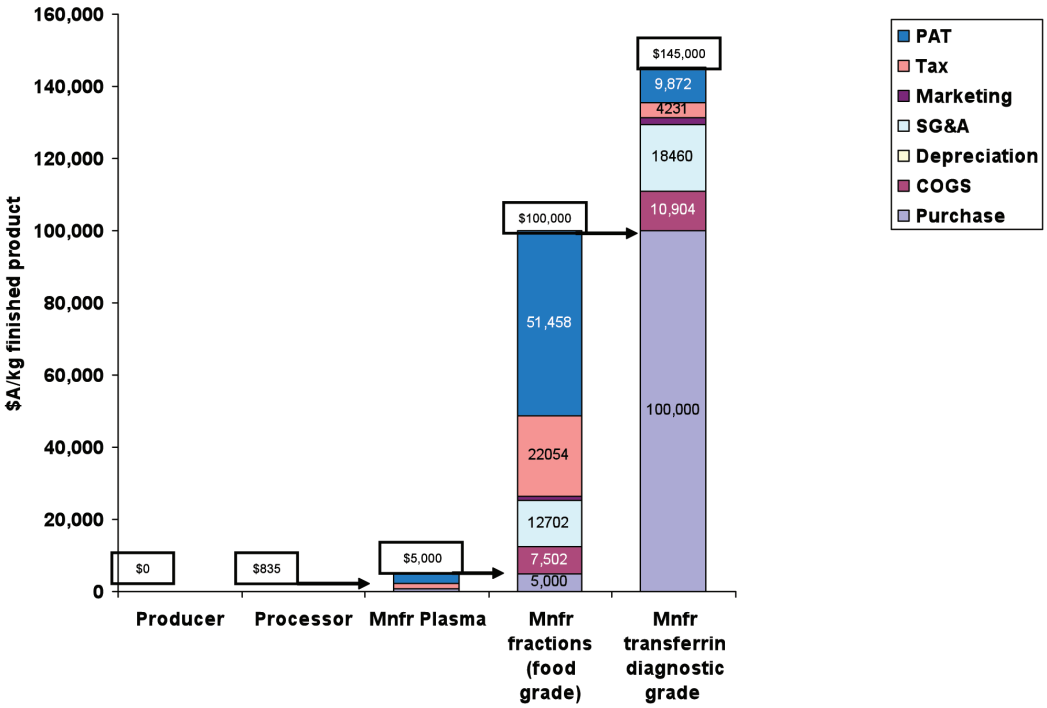
The generic cosmetic value chain was developed based on collagen type II used in lip balm. Type II collagen is used in many cosmetic and nutraceutical products, generally in a mixture with other ingredients. It can be sourced from animal skin, tendon and bone.

Figure 2.5: Generic cosmetic value chain



The generic pharmaceutical value chain was developed based on chondroitin sulphate pharmaceutical grade, which is much purer than its food counterpart and claims higher prices.

Figure 2.6: Generic diagnostic value chain



Whilst these value chains are based on approximations, industry benchmarks and assumptions, they do show that value is concentrated at the downstream. This reflects the elaborate transformation, investment and risk that these participants incur. There are, however, potentially opportunities for producers and processors at the value-adding stage.

Overview of regulatory framework for bioactives

The regulatory framework for bioactives, functional foods, therapeutics and the like is complex and plays a major role in determining the commercial feasibility. Regulations cover the use of certain ingredients and claims that may be made about them in relation to health and nutrition. In general, it is easier to obtain approval for ingredients/components which are derived from existing approved sources, provided the extraction process does not introduce toxic trace compounds.

Regulation of functional foods and nutraceuticals

Food regulation in Australia is administered initially by Food Standards Australia and New Zealand (FSANZ), which is responsible for developing and reviewing standards and recommending adoption of standards to the Australia New Zealand Food Standards Council. Their scope includes the composition, production, packaging, storing or handling of food; information about food including labelling, promotion and advertising; the interpretation of other standards; and other public health matters.[1] FSANZ also oversees matters of food surveillance, carries out research relevant to the standard-making function, develops food safety education initiatives, co-ordinates food recalls, develops policies on importing food, advises the Commonwealth Minister on food matters and develops codes of practices for industry on matters relevant to standards. Many of these functions are carried out in consultation with the States and Territories.

The latest edition of the Food Standards Code became operational in 2002 and covers general food standards, food product standards, food safety standards, primary production standards, transitional standards for health claims (see below), labeling, food additives, contaminants and residues, novel foods, microbiological and processing requirements.[2] There are specific requirements for meat products including implementation of a food safety management system that identifies, evaluates and controls hazards, and meets specified requirements.

Many food manufacturers and retailers also choose to be accredited under HACCP – Hazard Analysis and Critical Control Point quality system. HACCP is recognised internationally as a system to prevent food contamination and to promote safe handling practices. HACCP is not compulsory for food manufacturers, but many choose to seek accreditation because it provides independent benchmarking of their handling systems and provides them with access to larger retailing chains (who require HACCP accreditation of their suppliers). HACCP approved manufacturers must have a food safety plan which covers purchasing, receiving, handling, preparing, packaging, transport, hygiene, temperature controls, equipment testing and calibration, record keeping and auditing. HACCP is administered by State and Territory governments.

HACCP is not a requirement for development of functional foods or nutraceuticals. However, a number of other national agencies may be involved in managing, monitoring or approving use of bioactives and their incorporation in functional foods. In addition to FSANZ, these are:

- Australian Quarantine Inspection Service [AQIS] – export certification of processors (e.g. abattoirs) and import approvals (e.g. for functional ingredients or source organisms);
- Australian Taxation Office [ATO] – GST applied to functional foods (exemption issues, dependent on final product or raw ingredient); and
- Therapeutic Goods Administration (TGA) – certification of GMP premises (for pharmaceutical products).

Regulation of human therapeutics

The process of developing a new therapeutic is complex and involves a number of steps which include testing in animals, health of human subjects, and human subjects with the disease that is the subject of the drug being developed. The process is similar for all classes of compounds and can be prolonged, taking many years.

[1] www.foodstandards.gov.au

[2] http://www.foodstandards.gov.au/foodstandardscode/index.cfm#_FSCchapter1

While it is not necessary to use compounds manufactured under GMP conditions for preclinical development or for use in Phase I clinical trials, GMP grade material will be required for later clinical development. In addition, consistency of batches used during preclinical development and during clinical phases of development is essential. For this reason, and because GMP is the manufacturing standard for drugs, many companies prefer to use GMP-grade material for the generation of all data that will be used for product registration.

Regulatory authorities, including the Australian TGA, set standards of manufacture required for each therapeutic product. The Code of Good Manufacturing Practice (cGMP) describes the principles and practices that are necessary to provide assurance that each batch of a therapeutic product[3] contains the same active components and is safe and reliable. Each Code sets out requirements relating to premises, equipment, personnel, documentation and quality control. The aim is to ensure high quality production and to avoid contamination, deterioration, omissions and errors. Compliance with cGMP is ascertained by carrying out regular on-site audits, involving detailed examination of the operations and procedures of the factory and review of batch documentation, quality control testing and independent testing of product.

Regulation of veterinary products

The National Registration Scheme for Agricultural and Veterinary Chemicals was established under Commonwealth and State legislation and ensures that these products are effective on target species; safe when exposed to humans and non-target species either through direct exposure or residues in treated food stuffs; environmental friendly; and are labelled and packaged correctly. The Department of Agriculture, Fisheries and Forestry manages the legislation under which the National Registration Scheme operates.[4]

The Australian Pesticides & Veterinary Medicines Authority (APVMA) sits within the Department's portfolio and is the statutory body that administers the National Registration Scheme. APVMA evaluates, registers and regulates agricultural and veterinary chemicals up to the point of sale. The States are responsible for control of use.

Companies wishing to register a product are required to provide extensive data supporting the safe and environmentally friendly status of the product. As part of the assessment process, the APVMA receives input from other Commonwealth agencies, including:

- The Therapeutic Goods Administration (regarding, e.g. use of antibiotics in animal feed and potential impact on management of human diseases);
- The Department of Environment and Heritage (regarding, e.g. the environmental impact of agricultural and veterinary chemicals);
- Office of the Australian Safety and Compensation Council;
- Food Standards Australia New Zealand (FSANZ);
- Office of the Gene Technology Regulator (regarding, release of genetically modified organisms);
- The Expert Advisory Group on Antimicrobial Resistance (EAGAR); and
- The Australian Quarantine and Inspection Service (AQIS).

The APVMA requires manufacturers to follow the Australian Code of Good Manufacturing Practice for Veterinary Chemical Products.[5] The basic requirements of this are that all manufacturing processes are clearly defined and are systematically reviewed; critical steps and any changes to manufacturing are validated; all necessary facilities for Good Manufacturing Practice are provided;

instructions and procedures are documented, operators are trained correctly; records of procedures and manufacturing are maintained; a recall system is in place; and complaints about marketed products can be examined. The APVMA also has a system in place to ensure that overseas manufacturers of such products meet equivalent standards.

[3] http://www.health.gov.au/tga/pubs/pubs.htm

[4] www.daff.gov.au

[5] www.apvma.gov.au /qa/mls.shtml. The system has been reviewed and a revised system came into operation on 1 May 2006.

[6] Department of Health and Ageing (2005): *Regulation of Cosmetic Chemicals – Final Report and Recommendations*, Canberra, 2005

[7] Robert Forbes regulatory consultants

Regulation of cosmetic products

In Australia, cosmetic ingredients are regulated under the National Industry Chemicals Notification and Assessment Scheme (NICNAS) and, where cosmetics make therapeutic claims, by the TGA.[6] There is no requirement to follow cGMP in the manufacture of cosmetics.[7] There are also differences between Australia and other countries in the definitions and subsequent regulation of cosmetic products.

Other regulatory and accreditation schemes

Another two regulatory and accreditation schemes are relevant to this report: ISO 9001 accreditation and the expert certification of meat processors by the Australian Quarantine and Inspection Service (AQIS).

ISO 9001 is an internationally recognised quality standard used to demonstrate the ability of a business to meet customer's need and relevant regulatory requirements. It is administered by the International Standards Organisation (ISO). In the case of ISO systems, the term "quality" relates not the quality of the end product but the management and review of the process of production – basically the policies, processes, procedures, people, tools and equipment and other resources. ISO 9001 is a generic standard that is based on five key components: system documentation; management responsibility; resource management; product/ service realisation and checking. Compliance with ISO 9001 is voluntary and is used by some customers as an indicator of the strength or reliability of a company.

AQIS is responsible for administering the Export Control Act, with the latest arrangements being based on the 2005 Australian Standard for the Hygienic Production and Transport of Meat and Meat Products for Human Consumption.[8] These arrangements are based on HACCP (see above) and cover slaughter, boning, processing, cold storage, holding, air transport, dry storage, casings and the container depot.[9] Where receiving countries have specific regulations, AQIS also ensures that these are followed: for example, those abattoirs which export to Europe must comply with the requirements of the European Union Cattle Accreditation Scheme (EUCAS).[10] Similarly, AQIS sets standards for E. coli testing,[11] anti-bacterial residue minimisation[12] and the like.

[8] www.affa.gov.au

[9] Department of Agriculture, Fisheries and Forestry: *Introduction of New Regulations: Export Control (Meat and Meat Products) Orders 2005*

[10] AQIS Notice Number Meat 2004/02 and Meat 2005/07; assure traceability of all hormonal growth promotant-free cattle slaughtered.

[11] AQIS Notice Meat 98/10

[12] AQIS Notice Meat 99/26

Useful Links

Food Standards Australia New Zealand (www.foodstandards.gov.au)

FSANZ is the body responsible for developing, varying and reviewing standards for food available in Australia and New Zealand. FSANZ develops food standards, and joint codes of practice with industry, covering the content and labelling of food sold in Australia and New Zealand. Food standards provide the minimum regulatory burden necessary to maintain a safe food supply and informed consumers. In addition, the agency develops Australia-only food standards that address food safety issues – including requirements for primary production – and maximum residue limits for agricultural and veterinary drug residues.

Therapeutic Goods Administration (TGA) (www.tga.gov.au)

The Therapeutic Goods Administration (TGA) is a unit of the Australian Government Department of Health and Ageing and is responsible for administering the provisions of the legislation. The TGA carries out a range of assessment and monitoring activities to ensure therapeutic goods available in Australia are of an acceptable standard. At the same time the TGA aims to ensure that the Australian community has access, within a reasonable time, to therapeutic advances.

The Australian community expects that medicines and medical devices in the marketplace are safe and of high quality, and of a standard at least equal to that of comparable countries. The objective of the Therapeutic Goods Act 1989, which came into effect on 15 February 1991, is to provide a national framework for the regulation of therapeutic goods in Australia to ensure the quality, safety and efficacy of medicines and ensure the quality, safety and performance of medical devices.

The regulatory framework is based on a risk management approach designed to ensure public health and safety, while at the same time freeing industry from any unnecessary regulatory burden.

Essentially therapeutic goods must be entered on the Australian Register of Therapeutic Goods (ARTG) before they can be supplied in Australia. The ARTG is a computer database of information about therapeutic goods for human use approved for supply in, or exported from, Australia.

The Therapeutic Goods Act 1989, Regulations and Orders set out the requirements for inclusion of therapeutic goods in the ARTG, including advertising, labelling, product appearance and appeal guidelines. Some provisions such as the scheduling of substances and the safe storage of therapeutic goods are covered by the relevant State or Territory legislation.

National Industry Chemicals Notification and Assessment Scheme (www.nicnas.gov.au)

NICNAS is the Australian Government regulator of industrial chemicals. NICNAS regulates chemicals for the protection of human health and the environment. Manufacturers of cosmetic ingredients must have their ingredients approved by the NICNAS. While cosmetics themselves do not need separate approval prior to sale, those that make therapeutic claims must be approved by the TGA. There is no code governing cosmetics manufacture.

US Food and Drug Administration Agency (www.fda.gov)

FDA is an agency within the Department of Health and Human Services in the US. FDA is the federal agency responsible for ensuring that foods are safe, wholesome and sanitary; human and veterinary drugs, biological products, and medical devices are safe and effective; cosmetics are safe; and electronic products that emit radiation are safe. FDA also ensures that these products are honestly, accurately and informatively represented to the public.

The FDA is responsible for protecting the public health by assuring the safety, efficacy, and security of human and veterinary drugs, biological products, medical devices, our nation's food supply, cosmetics, and products that emit radiation. The FDA is also responsible for advancing the public health by helping to speed innovations that make medicines and foods more effective, safer, and more affordable; and helping the public get the accurate, science-based information they need to use medicines and foods to improve their health.

European Union European Medicines Agency (http://www.emea.europa.eu/htms/aboutus/emeaoverview.htm)

The European Medicines Agency (EMEA) is a decentralised body of the European Union with headquarters in London. Its main responsibility is the protection and promotion of public and animal health, through the evaluation and supervision of medicines for human and veterinary use.

- The EMEA is responsible for the scientific evaluation of applications for European marketing authorisation for medicinal products (centralised procedure). Under the centralised procedure, companies submit a single marketing authorisation application to the EMEA. Once granted by the European Commission, a centralised (or 'Community') marketing authorisation is valid in all European Union (EU) and EEAEFTA states (Iceland, Liechtenstein and Norway).
- All medicinal products for human and animal use derived from biotechnology and other high technology processes must be approved via the centralised procedure. The same applies to all human medicines intended for the treatment of HIV/AIDS, cancer, diabetes, neurodegenerative diseases, autoimmune and other immune dysfunctions, and viral diseases, as well as to all designated orphan medicines intended for the treatment of rare diseases. Similarly, all veterinary medicines intended for use as performance enhancers in order to promote the growth of treated animals or to increase yields from treated animals have to go through the centralised procedure.
- For medicinal products that do not fall under any of the abovementioned categories, companies can submit an application for a centralised marketing authorisation to the EMEA, provided the medicinal product constitutes a significant therapeutic, scientific or technical innovation, or the product is in any other respect in the interest of patient or animal health.
- The safety of medicines is monitored constantly by the Agency through a pharmacovigilance network. The EMEA takes appropriate actions if adverse drug reaction reports suggest changes to the benefit:risk balance of a medicinal product. For veterinary medicinal products, the Agency has the responsibility to establish safe limits for medicinal residues in food of animal origin.
- The Agency is also involved in referral procedures relating to medicinal products that are approved or under consideration by Member States in non-centralised authorisation procedures.

BioRegs Online (www.bioregs.gov.au)

BioRegs Online is an Australian government website that offers comprehensive information about the regulation and development of biotechnology and related products in Australia.

You can find out which Commonwealth Government agencies regulate your product, what compliance steps are involved and where to go for more information. You can also find out about

- technology licensing,
- business planning,
- good manufacturing practice,
- exporting, government assistance and
- many other useful business topics of relevance to biotechnology

This website has a biotech web tool wherein by working through a decision tree, researcher, companies and other users can develop a customised package of the key regulatory and business development requirements to take a specific biotechnology product.

Good Manufacturing Practice (GMP)

In most cases manufacturers of therapeutic goods distributed within Australia must hold a licence issued by the Therapeutic Goods Administration. To obtain a licence to manufacture therapeutic goods, a manufacturer must demonstrate, during a factory audit, compliance with manufacturing principles which include codes of good manufacturing practice.

Guidance can be found at the following links: http://www.tga.gov.au/manuf/index.htm

- For all manufacturers
- Medicines manufacturers
- Medical device manufacturers
- Blood & tissues manufacturers
- Overseas manufacturers

Key Steps

- Become familiar with Australian GMP codes;
- Ensure manufacturing facility complies with Australian codes for GMP;
- Apply for licence to manufacture therapeutic goods
  - o Application form
  - o Application fee;
- Be prepared for GMP facility audit by Therapeutic Goods Administration (TGA);

- For products manufactured overseas:
  - o Ensure overseas facility complies with Australian codes of GMP;
  - o Obtain evidence stating compliance with Australian codes of GMP;
  - o Where no evidence exists, arrange for TGA audit of overseas manufacturing facility.

What is Good Laboratory Practice (GLP)?

GLP is a quality system concerned with the organisational process and the conditions under which non-clinical studies are planned, performed, recorded, archived and reported. The system and processes are not concerned with the technical validity or otherwise of the studies themselves. Non-clinical studies for therapeutic products and devices, veterinary medicines and pesticides, and industrial chemicals may cover:

- physical-chemical testing;
- toxicity, mutagenicity, environmental toxicity, bioaccumulation and residues studies;
- studies of effects on ecosystems; and
- analytical chemistry associated with such studies.

Why do you need to follow GLP?

The basis for the development of GLP is to provide assurance regarding test data from non-clinical studies. This is particularly related to the hazard assessment of chemicals (pharmaceuticals, veterinary and agricultural chemicals, industrial chemicals) when manufacturers are seeking to register products for use.

Please note that MLA strongly advises to seek professional advice when assessing the regulatory framework for any candidate bioactives.

Bioactive Extraction and Purification Technologies

A variety of technologies are available for obtaining bioactive agents from the source tissue. Once extracted or separated, the substance must be purified to the standard required—nutraceutical, cosmetic, diagnostic, pharmaceutical—and then treated in some way to preserve the bioactive molecular activity.

Extraction

Extraction process

Extraction is the process by which the bioactives that are in a tissue are freed from the tissue and dissolved in a liquid. The extracting liquid may be water (in which case the process is called an aqueous extraction) or in an organic solvent such as alcohol (in which case the process is called a solvent extraction). The resulting liquid is referred to as the extract and is typically a very dilute solution.

Aqueous 2 phase extraction

Aqueous two-phase systems allow recovery of proteins and enzymes from crude mixtures. The system is based on the formation of two distinct phases, both of which are aqueous (water based), one of which "floats" on the other. The two phases are created by dissolving a mixture of 2 polymers e.g. Polyethylene Glycol and Dextran, in water, one of which is slightly hydrophobic (has an affinity for oil based compounds). The two polymers separate into two distinct layers (phases) in the solution. The "upper phase" is formed by the more hydrophobic polyethylene glycol (PEG), which is of lower density than the "lower phase," consisting of the more hydrophilic and denser dextran solution. When the tissue from which a bioactive is to be extracted is mixed in such a system, the various tissue components which are extracted tend to accumulate in one or other of the phases. The characteristics of phases are determined by concentrations, pH, temperature and ionic strength of the two components. Aqueous 2 phase extraction is a valuable process because it can result in both extraction and a degree of concentration and purification in the one step. The two phases can be separated by decanting or by centrifugation.

Organic Solvents

Organic solvents (such as alcohol) are used to dissolve bioactives which are oil soluble rather than water soluble. For example, vitamin E is oil soluble and is found in cell membranes and can only be extracted in an organic solvent in which the vitamin E can be dissolved.

Supercritical fluids

Supercritical fluids are highly compressed gases than can be used as extraction solvents. The most common is carbon dioxide. When compressed to very high pressures it becomes a liquid with solvent extraction properties. Carbon dioxide is a relatively small molecule and can be quite effective in extracting organic compounds from cell mass.

Separation

Centrifugation

Centrifugation is a process used to separate or concentrate materials suspended in a liquid medium. Centrifugal forces amplify the effect of gravity on the mixture resulting in phases separating according to their relative densities. Centrifugation is commonly used to separate plasma from blood or to separate waste tissue from the liquid following an extraction process. Centrifuges can be batch or continuous. In a batch centrifuge all the material remains in the centrifuge until it stops spinning whereupon the liquid or lighter phase can be decanted. In a continuous centrifuge, the separation of phases occurs as the mixture to be separated flows through the device. The heavier material can be discharged either continuously through nozzles or intermittently when the material accumulates to a target level.

Microfiltration, Ultrafiltration

Microfiltration is a filtration process in which the pores in the filter range from 0.1 to 10 micrometres (microns). The particles are retained by the appropriate sized membrane while the solvents and accompanying solutes are allowed to pass through.

Ultrafiltration is fundamentally similar to microfiltration, the only difference being the smaller pore size, ranging from 0.1 to 0.001 micrometres. Microfiltration and ultrafiltration filters are generally polymer membranes and can be found in a number of configurations: Hollow fibre, tubular, plate and frame, spiral wound. The simplest mode of use of a filter is as a dead end filter, in which a batch of material to be filtered is pumped through the filter, creating a cake of material (retentate) on the "dirty" side while the filtered material (filtrate) passes through. This mode of use is mainly reserved for final filtration or polishing, because the accumulated filter cake can create a secondary membrane and blind the filter. To overcome the blinding phenomenon, most microfiltration and ultrafiltration operations are run in "cross-flow" or "tangential" mode, in

which the solution to be filtered is pumped across the face of the membrane, in order to sweep any accumulated cake away and prolong the life of the membrane before cleaning is required to recover filtration rates.

Diafiltration

Diafiltration is a process in which residual compounds retained incidentally in the retentate along with the target compound are successively diluted and filtered out. This can be done batch-wise e.g. passing a fresh solution of say water through a filter cake whilst it is still on the filter, or continuously e.g. adding fresh solution of say water continuously as the filtrate is captured downstream of the filter. Continuous diafiltration is more efficient in terms of volumes of diafiltration liquid used.

Rotary vacuum filters

Rotary vacuum filters are slow moving rotating cylinders covered in filter or filter medium. Solution to be separated is sprayed over the surface as the cylinder revolves and the solids are retained while the filtrate passes through. It can be used as a primary separation process to remove tissue from a bioactives extraction stream.

Flotation

Flotation is a process in which fine bubbles of air are pumped into a liquid solution in order to separate some components from the main body of the liquid. Surface active compounds such as proteins are preferentially attracted to the surface of the bubbles and carried upwards into a foam phase. The foam can be skimmed from the solution and collected or discarded as required. A version of flotation known as foam fractionation can be used to separate target proteins from a mixture of proteins if the target has a different surface activity from the rest.

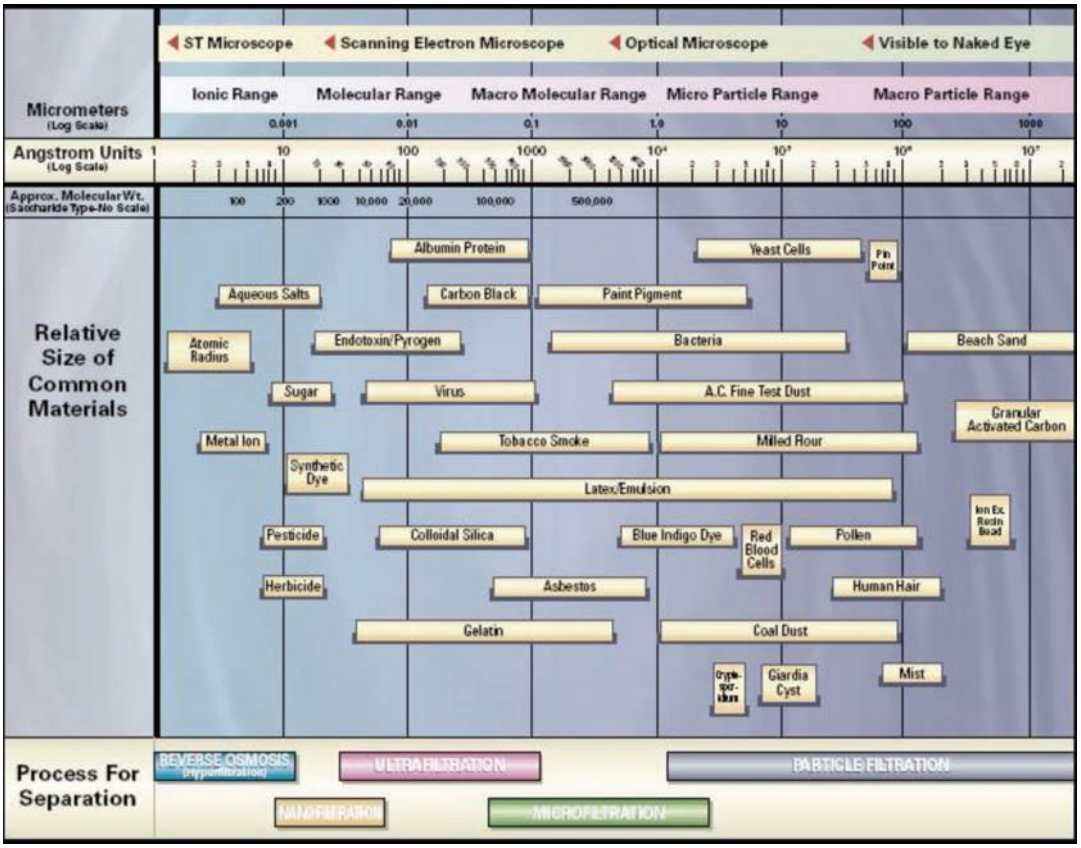
Purification

Reverse Osmosis

Reverse osmosis (RO) is a separation process that uses pressure to force a solution through a membrane that retains the solute on one side and allows the pure solvent to pass to the other side. More formally, it is the process of forcing a solvent from a region of high solute concentration through a membrane to a region of low solute concentration by applying a pressure in excess of the osmotic pressure. This is the reverse of the normal osmosis process, which is the natural movement of solvent from an area of low solute concentration, through a membrane, to an area of high solute concentration when no external pressure is applied. The membrane here is semipermeable, meaning it allows the passage of solvent but not of solute. In the water treatment industry there is a chart of types of contaminants, their sizes and which ones pass through the various types of membranes.[1] Membrane pore sizes can vary from 1 to 50,000 angstroms depending on filter type. “Particle filtration” removes particles of 10,000 angstroms or larger. Microfiltration removes particles of 500 angstroms or larger. “Ultrafiltration” removes particles of roughly 30 angstroms or larger. “Nanofiltration” removes particles of 10 angstroms or larger. Reverse osmosis is in the final category of membrane filtration, “Hyperfiltration”, and removes particles larger than 1 angstrom.

Adsorption

Adsorption is the process in which components of a fluid stream (which could be either liquid or gas) bind to the surface of a solid. For a given solid (adsorbent) the components of the fluid stream tend to each bind with different strengths, and it is this property which allows adsorption to be used to selectively “fish out” target compounds. The most common types of adsorption operations encountered in bioprocessing are ion exchange and chromatography. Adsorption should not be confused with absorption. In absorption the relative levels of all the components in the original solution stay the same right down to the surface of the solid, whereas in adsorption the composition on the surface is different from that in the bulk liquid, meaning that some form of purification has taken place.



Source: http://www.hfpurewater.com/index.htm

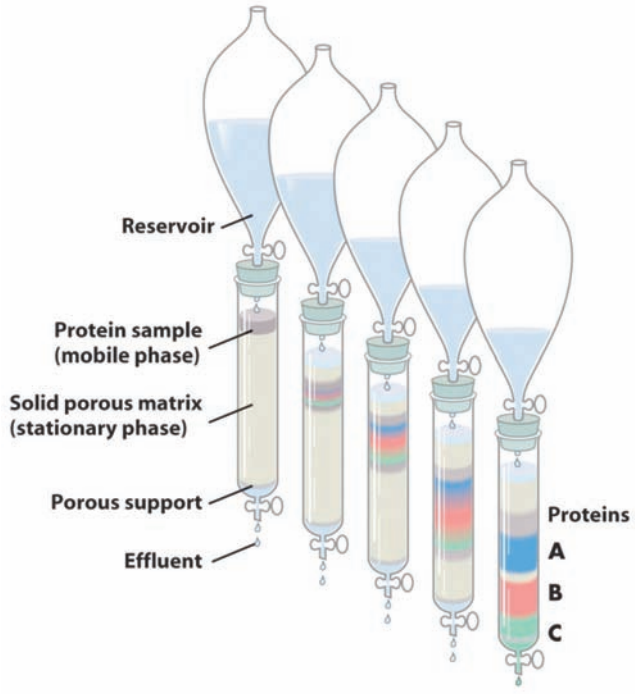
Chromatography

Chromatography is a physical method in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances interact with the stationary phase to be retained and separated by it.

Chromatography materials are held in a column, and flow through the column usually results in zones of different materials. Purification is achieved by collecting each zone separately.

The target molecule is one of the key factors when designing the most appropriate. Below are examples of techniques used.

"Size exclusion" or "gel filtration" chromatography separates molecules according to their size. Larger molecules pass more freely, appearing in the earlier fractions leaving the column.



Diagrammatic representation of chromatographic separation

Ion exchange chromatography uses a charged stationery phase to separate compounds according to their charges, which will interact with oppositely charged groups. Proteins will move through the column at the rate determined by the strength of their net charge, which varies with pH.

Affinity Chromatography uses a highly target-specific matrix to capture the bioactive. The target compound e.g. a protein is eluted by adding reagent which competes with it for the binding sites on the adsorbent.

Preservation

Bioactive compounds tend to lose their bioactivity over time due to processes such as oxidation, microbial spoilage and thermal degradation. Bioactives are seldom sold as pure substances and are usually found in a stabilising solution if liquid or bound on inert fillers if solid. Stability in liquid solutions depends on the pH and mineral compositions of the liquid and is very dependent on temperature. Freezing is commonly used to prolong shelf life of liquid suspensions of bioactives. Drying can be very effective in stabilising bioactive compounds because most chemical reactions (including those involved in loss of bioactivity) require a liquid phase. Dried products are still prone to oxidation if exposed to air and even under vacuum or nitrogen flushing gradually lose activity. Activity loss does occur during drying and choice of the most appropriate drying process is critical. The major processes are spray drying, freeze drying and ball drying.

Spray drying

Spray drying is involves rapid heating of a liquid suspension of the target compound, usually containing a “carrier” compound, pumping it under pressure into a large container where there is a flash evaporation of the liquid. It is one of the cheapest drying processes, but the high temperatures can damage bioactivity.

Freeze drying

In Freeze Drying a solution of the target compound (often with the addition of a protective agent (cryoprotectant) is snap frozen and then placed in a vacuum. The ice in the frozen material evaporates off into the vacuum from where it is condensed on an ice bank, leaving the rest of the compounds in the dried material. Freeze dried products have the best stability but require special packaging and gas flushing, because they tend to by hygroscopic (water hungry).

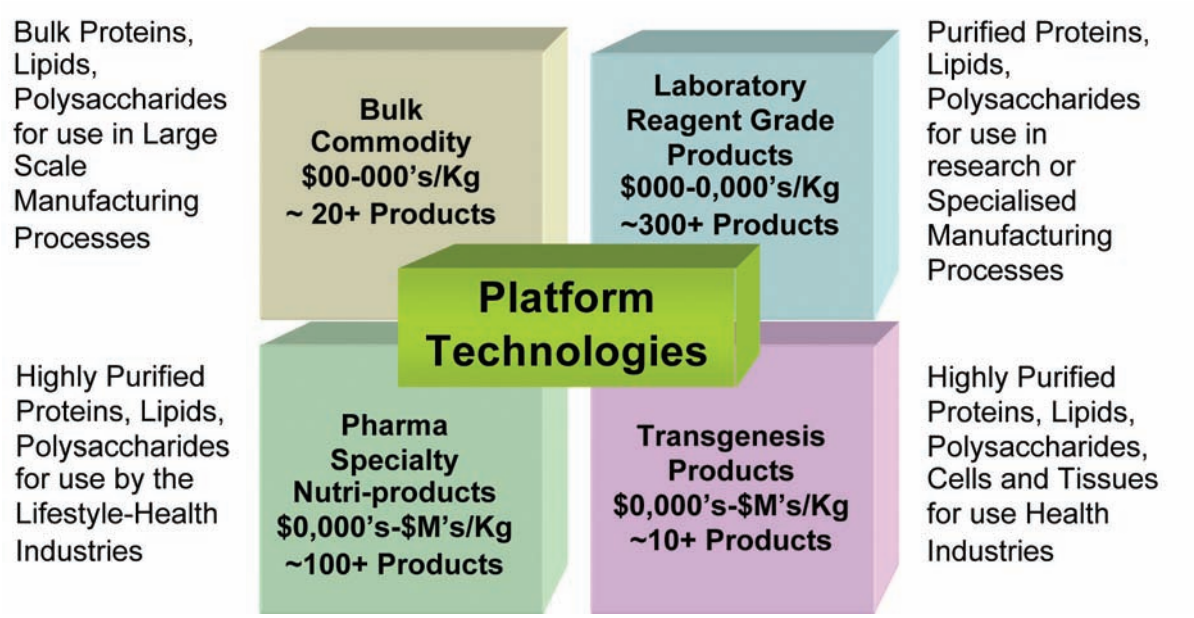
Fluid Bed drying

A paste of bioactives with a solids content of around 30% or more may be extruded into “noodles” and then dried. When warm air is pumped through a bed of noodles at a high enough rate the noodles are dislodged from the bed and bounce around in the air stream. The noodles flow over each other and the bed behaves like a fluid and is therefore referred to as a fluid bed. One advantage of fluid bed dried products is that they tend to generate less powder and therefore are easier and safer to handle and show better yields.

Platform technologies

Investments decisions in bioactives processing technologies should consider multiple products on different time horizons. As revenue is generated though first generation products , such as bulk or crude bioactives extracts, further technology investments can be made to sustain other more refined and higher value bioactives.

What product horizon can be realised?



Anticoagulants

Apoprotin

Antithrombin III

Herapin

Plasminogen

Protein C

Antithrombin III

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Use	Supplier
Antithrombin III	non-activated citrated bovine plasma: 11.34kg	200 mg/mL plasma	Anticoagulant	Aniara Corp.; Innovative Research, Inc; Stratech Scientific

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Antithrombin III is an inhibitor of thrombin and other serine proteases of the coagulation cascade (a complex process by which blood forms clots).

Commercial Applications

- According to data from 2002, bovine Antithrombin III was modified and showed potential to be an anti viral agent in the treatment of HIV-1 patients [4]
- Antithrombin was recently shown to inhibit angiogenesis (the growth of new blood vessels) and tumour growth. It was concluded that antithrombin is widely expressed in prostate cancer but is gradually lost in tumours and that antithrombin acts as a local anti-angiogenic factor, the effect of which is partially lost in poorly differentiated prostatic tumours. [7]
- Antithrombin was also shown to be able to reverse endotoxin-induced adhesion of leukocytes to endothelium by ligation of syndecan-4 with antithrombin's heparin-binding site. This however only applies to human ATIII. It is unknown if similar function is seen with bovine ATIII[10]

Price

Company	Unit	Price	Source	Reference	Source Date
Sigma	1mg	$2419	Bovine plasma	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;A9141	2008
Sigma	1mg	$3520	Human plasma	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;A9141	2008
Sigma	1mg	$8000	Rat plasma	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;A9141	2008

Market size

AT III has a small market with the main users being research institutes and universities.

It is unknown what the size is of the global market for commercial Bovine Antithrombin. From the research, the market appears very small.

- Human Antithrombin represents major market potential [12]
  - o Worldwide potential exceeds $500 million (Human Antithrombin)
  - o The potential market value of the Bovine Antithrombin can not be found
- The market for bovine will be substantially smaller

Conclusion

No discernable need can be identified or use outside of research purposes for the bovine form of antithrombin, this bioactive compound has no economic viability for the red meat industry.

Aprotinin

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Use	Supplier
Aprotinin	Lung: 2.85kg	10-15 mg/Kg	Anticoagulant	Biochain Institute; Boehringer Mannheim; Sigma

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Aprotinin, also known as bovine pancreatic trypsin inhibitor, is a protein that is used as medication administered by injection to reduce bleeding during complex surgery, such as heart and liver surgery. Its main effect is the slowing down of fibrinolysis, the process that leads to the breakdown of blood clots.

Aprotinin inhibits several protein degrading enzymes, specifically trypsin, chymotrypsin and plasmin.

Its action on kallikrein leads to the inhibition of the formation of factor XIIa. As a result, both the intrinsic pathway of coagulation and fibrinolysis are inhibited. Its action on plasmin independently slows fibrinolysis.

Commercial Applications

The aim in its use is to decrease the need for blood transfusions during surgery, as well as end-organ damage due to hypotension (low blood pressure) as a result of marked blood loss.

In cardiac surgery with a high risk of significant blood loss, aprotinin significantly reduced bleeding, mortality and hospital stay. Beneficial effects were also reported in high-risk orthopedic surgery.

Withdrawn

On 6 November 2007, Bayer Australia Ltd announced a worldwide suspension of the supply of Trasylol (aprotinin) injection. This follows the release of preliminary results from a clinical trial that suggested an increased risk of death for patients receiving Trasylol (aprotinin) compared to those receiving the alternative medications of aminocaproic acid or tranexamic acid for control of bleeding during heart surgery.

http://www.tga.gov.au/alerts/trasylol.htm

Research

Research-grade aprotinin, for laboratory use, plays a part in preventing proteolysis of reagents [9] and in cell culture [13].

Price

Company	Unit	Price	Source	Reference	Source Date
Sigma	250mg	$2719	Bovine lung	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage	2008

Market

Sales of aprotinin for medical procedures have fallen significantly since its withdrawal and links to deaths and complications.

The main supplier of medical aprotinin (Trasylol) Bayer no longer produces the product. However before its ban in 2007 the prescription-grade product Trasylol had annual sales of around U.S $120 million (2004).

Aprotinin can still be used for research purposes and the market for the research-grade version used in bioproduction was perhaps a tenth medical grade aprotinin (2004). The impact of aprotinins' medical use ban may have affected the research market but no information could be found relating to this.

http://www.in-pharmatechnologist.com/Materials-Formulation/Aprotinin-from-plants-could-end-supply-shortage

Commercial Aprotinin was produced primarily from bovine lung and the market growth of aprotinin-based products was limited by the availability of uncontaminated cow's lungs [7].

Production of the protein in transgenic crops had been developed and was to be released around the same time that aprotinin was taken off the market.

Conclusion

Aprotinin could have been a highly profitable bioactive for the Australian red meat industry. It does highlight the fact that the medical/health industry can change very rapidly and that even the market leading product from a major multinational company can vanish in two years.

Heparin

Bioactive compound	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Heparin	Intestine mucosa: N/A Lung: 2.85kg	40 –120 mg/Kg	A naturally occurring substance in the blood that works by interfering with the body's natural blood clotting mechanism.	Widely used for past 50 years in therapy, separation, cell culture and other applications	Merck, Sigma

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Heparin is an anticoagulant glucosaminoglycan (GAG) from the same family of chemicals as chondroitin sulphate. It is used to decrease the clotting ability of the blood and help prevent harmful clots from forming in the blood vessels.

Heparin binds to the enzyme inhibitor antithrombin causing a conformational change which results in its active site being exposed. The activated antithrombin then deactivates thrombin and other enzymes involved in blood clotting, most notably factor Xa.

Heparin increases the deactivation rate of antithrombin by a 1000 fold.

Although used principally in medicine for anticoagulation, the true physiological role in the body remains unclear. Heparin is usually stored within the secretory granules of mast cells (immune cells) and released only into the blood stream at sites of tissue injury.

It has been proposed that, rather than anticoagulation, the main purpose of heparin is in a defensive mechanism at sites of tissue injury against invading bacteria and other foreign materials.

Commercial Applications

Medical use

Heparin acts as an anticoagulant, preventing the formation of clots and extension of existing clots within the blood. While heparin does not break down clots that have already formed (unlike plasmin activator), it allows the body's natural clot degrading mechanisms to work normally to break down clots that have already formed.

Heparin is often used as a treatment for certain blood vessel, heart, and lung conditions. Heparin is also used to prevent blood clotting during open-heart surgery, bypass surgery, and dialysis. It is also used in low doses to prevent the formation of blood clots in certain patients.

Heparin gel (topical) may sometimes be used to treat sports injuries to help reduce inflammation.

It can also be used to form an inner anticoagulant surface on various experimental and medical devices such as test tubes and renal dialysis machines.

Current sources

Pharmaceutical and medical grade heparin is derived from mucosal tissues of slaughtered meat animals such as porcine intestine or bovine lung/ intestine. Heparin is found in almost all animals but the majority of the worlds' heparin is produced from porcine and bovine sources.

There is no significant difference in the anticoagulant effectiveness of beef lung-derived heparin as compared with heparin obtained from pork intestinal mucosa.

Heparin is not produced from microbial sources and it would be difficult to develop as it is a very large molecule with a very negative charge.

Heparin from bovine and porcine sources can be tainted and has led to several deaths since 2007. The majority of tainted heparin in this period was produced in China and then exported worldwide.

The development of synthetic heparin is difficult as it is a very large complex molecule however the potential market is very large at over $4 billion a year. There are many companies and research laboratories attempting to develop synthetic heparin and every year there is a news headline stating that someone has done it and that production will commence in 2 years.

Price

Company	Unit	Price	Source	Reference	Source Date
Merck	715mg	$150	Bovine lung	http://www.merckbiosciences.com/product/375093	2008
Sigma	1000mg	$235	Bovine intestine	http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/51536	2008

World Market

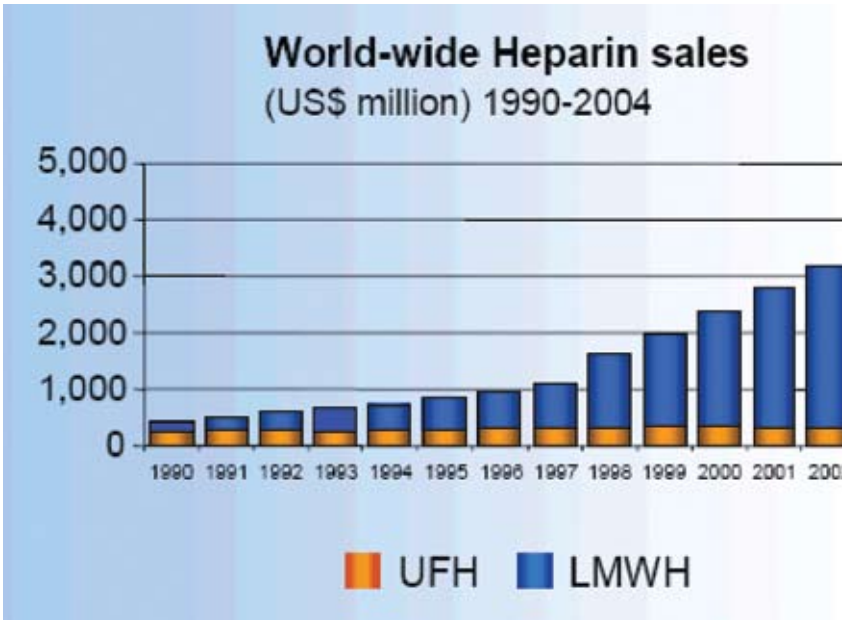
Currently the annual world heparin sales are estimated to be worth US$3-4 billion. (pharmaceutical grade).

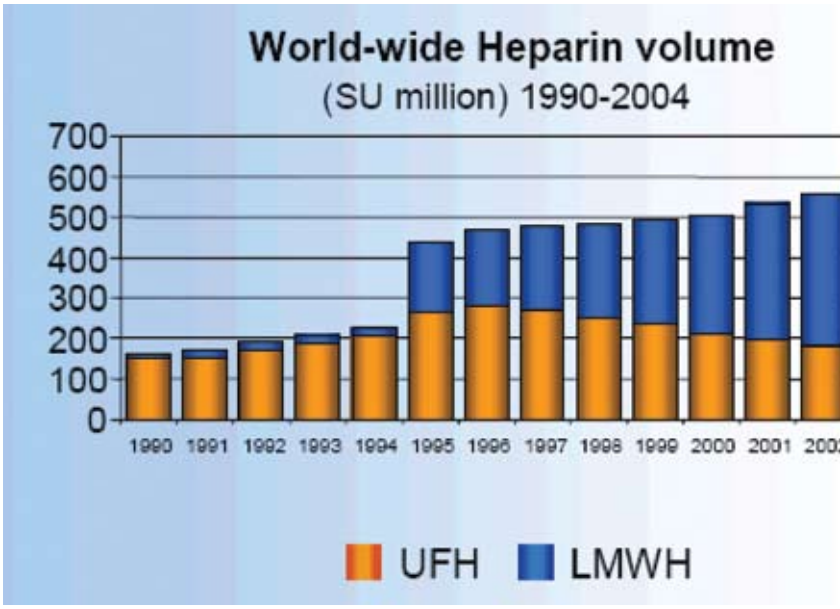
(http://www.bio-medicine.org/biology-news-1/American-Chemical-Societys-Weekly-PressPac----Aug--13--2008-4523-2/)

Most of the world's crude heparin comes from China, which has become a major supplier of raw ingredients to pharmaceutical companies. Though no information was found on the animal source for Chinese heparin it is assumed that it is primarily porcine intestinal mucosa. The Chinese have had to recall sales of medical grade heparin due to contamination resulting in several deaths around the world.

The huge effort into the production of synthetic heparin is primarily driven by market demand for a safe alternative to current sources. If a safe, trouble free natural heparin were to be produced there could be a strong market for it and the push for the development of a synthetic heparin may subside.

The Anticoagulant Market has shown to be receptive to new and improved treatment regimens





Source IMS Health, PADDs, MAT Q4 2004
UFH unfractionated heparin (injectable) LMWH low molecular weight heparin (injectable)
www.bayer.de/de/20051208rdinvestordaymisselwitz.pdfx

What product processing is involved?

The isolation process involves hydrolysis of the mucosa followed by extraction of the heparin.

U.S. Pat. No. 5,607,840, describes a process for hydrolysis of mucosa tissue.

The method involves hydrolysis of an aqueous mixture containing mammalian mucosa with a proteolytic enzyme at a temperature of about 55°C, adsorption of polyanions to an anion exchange resin and subsequent recovery of the anions from the resin and the protein hydrolysate from the digested aqueous solution. In order to stabilise the raw mucosa material and to prevent bacterial growth, salts in the form of an oxygen scavenger or bacteriocides are introduced into the solution.

The heparin content in the mucosa-containing aqueous medium is very low and consequently large amounts of mucosa tissue have to be processed.

http://www.patentstorm.us/patents/6232093/description.html

Strengths:

- Large market
- Heparin from bovine sources is well excepted and widely used. There is a cloud over the quality of product from the major manufacturer of crude heparin, presenting a potential opportunity for new market entrants.

Weaknesses:

- Primarily used in the medical industry in low doses
- No functional food or cosmetic use

Threats:

- Market is well established and competitive

Plasminogen

Bioactive compound	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Plasminogen	non-activated citrated bovine plasma: 11.34kg	80 mg/L	Zymogen of plasmin, which degrades many blood plasma proteins, most notably fibrin clots	Diagnostics, veterinary uses	Aniara Innovative Research, Pel-Freez Laboratories,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Plasminogen is an inactive precursor for the active protease, plasmin. Plasmin is an important enzyme present in blood that degrades many blood plasma proteins, most notable, fibrin clots. The degradation of fibrin is termed fibrinolysis.

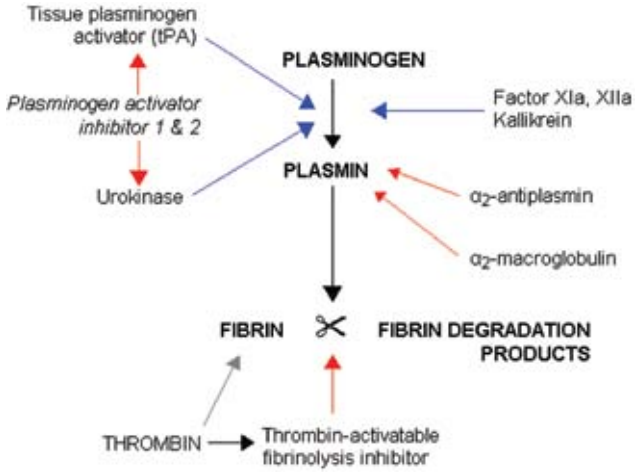
Plasminogen is produced in the liver and released into the circulatory system, it remains inactive until activated by plasmin activator.

Plasmin activator is produced in blood vessel walls, from which it is released following vascular injury. Further, the process of blood coagulation may foster the activation of plasminogen. Moreover, the presence of a fibrin clot enhances activation of plasminogen by many agents, perhaps because plasminogen is adsorbed to the fibrin and thus separated from its inhibitors in the plasma.

Plasmin can act on many protein substrates. It liquefies coagulated blood by digesting fibrin (fibrinolysis), the insoluble meshwork of the clot; it also digests fibrinogen, the precursor of fibrin, rendering it incoagulable. The digestion products of fibrinogen and fibrin are anticoagulant substances which further interfere with the clotting process.

Apart from fibrinolysis, plasmin proteolyses proteins in various other systems: It activates collagenases, some mediators of the complement system (a biochemical cascade that forms part of the immune system) and weakens the wall of the Graafian follicle (leading to ovulation).

Bovine and human plasminogens possess many common features but may react differently due to differences in amino acid sequence.[2]



Source http://en.wikipedia.org/wiki/Plasmin

Commercial Applications

Anti-coagulants are used extensively in medicine to remove clots that have developed in the body. Although clots are essential for the healing of external and internal injuries they can sometimes develop when they are not needed. Blood clots can block veins and capillaries stopping the flow of blood and oxygen to organs and tissues causing stroke, deep vein thrombosis and many other diseases. When these diseases are detected or patients are deemed at risk of developing negative blood clots they are given anti-coagulants to treat the symptoms.

Plasmin is just one of many anti-coagulants produced by the body, and human plasmin is being investigated in blood clot disease studies to determine the use of the enzyme as a potential anti-coagulant to counter clotting disorders such as deep vein thrombosis. Human plasmin can be directly infused at a local site where a clot is present. 3

Although plasmin is the enzyme responsible for breaking down clots it is seldom used that often in the treatment of stroke and other blood clot diseases. Instead the enzyme that activates plasminogen into plasmin, plasmin activator is used. Plasmin activator is used extensively in medicine as it can be produced by recombinant bacteria and it is very effective. Plasmin activator world sales were approximately $U.S 600 million in 2004. http://pharmalicensing.com/public/articles/view/1130421281_4360dc215fedf

The commercial applications for bovine plasminogen appear to be very limited. As plasminogen is rarely used in medicine and there are no other commercial applications for the protein it would be primarily used in research. As bovine plasminogen and human plasminogen are different this would also reduce its economic importance.

Current Commercial Sources: 4

- Bovine Plasma
- Rat Recombinant
- Mouse
- Human Plasma

Current Suppliers & Unit Cost

Company	Source	Amount	Price	Unit Cost	Reference	Source Date
Innovative Research (USA)	Bovine Plasma	1.0mg	US$195	$195/mg	http://www.innov-research.com/plasminogenproductsbovine.htm	2008
Biopur (Switzerland)	Bovine Plasma	1.0mg	US$160	$160/mg	http://www.biopur.com/product_info.php?products_id=564	2008
Pel-Freez Biologicals (USA)	Bovine Plasma	1.0mg	US$260	$260/mg	http://www.pelfreez-bio.com/programs/productdescription.asp?prodid=27004-1	2008
Sigma-Aldrich	Bovine Plasma	250mg	AU$1700	$14.85/mg	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/P9156	2008
Aniara Corporation	Bovine Plasma	5.0mg	US$750	$150/mg	http://www.aniara.com/store/products/AN42.html	2008

Market Size

Although there are many suppliers of bovine plasminogen the price per milligram remains high (average $150/mg), which indicates that production costs may be high. The limited applications of bovine plasminogen mean that the world wide demand will remain low.

Opportunity potential: negligible

Summary of Commercial Potential for Australian Beef Industry:

Strengths – bovine sourced plasminogen is sold commercially by companies such as Sigma Aldrich

Weaknesses – differences between human and bovine based plasminogen

Opportunities – negligable

Threats – interest in human plasmin and therapeutics

Market Applications:

- Precursor for plasmin activation
- Research reagent

Emerging Technologies:

Research in thrombolytic pharmaceuticals and therapies is growing but limited to anticoagulants and plasminogen activators which trigger the human clot dissolving mechanisms.

Concentration in Bovine Tissue:

Bovine Plasma

Bovine Plasminogen	
Product	Yield
Plasminogen	~8mg/100ml citrated plasma

Johann SCHALLER, Peter W. MOSER, Gabrielle A. K. DANNEGGER-MÜLLER, Susanne J. RÖSSELET, Urs KÄMPFER, Egon E. RICKLI , 'Complete amino acid sequence of bovine plasminogen Comparison with human plasminogen', Eur. J. Biochem. 149, 267-278 (1985)

References

1 http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/P5661

2 http://www.blackwell-synergy.com/links/doi/10.1111/j.1432-1033.1985.tb08921.x/abs/

3 http://www.genengnews.com/news/bnitem.aspx?name=20137259

4 http://biocompare.com/search.asp?option=all&groupID=1&maxrecords=115&search=plasminogen&s=plasminogen

Protein C

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Protein C	Purified from fresh citrated bovine plasma: 11.34kg	5-10 mg/L	Catalyses proteolytic inactivation of factors Va and VIIIa. Activated protein C also contributes to the fibrinolytic response	Anticoagulation, cell biology	Haematological Technologies, Inc.; Enzyme Research Laboratories

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Human protein C (HPC) is an antithrombotic factor dependent on vitamin K and is activated in the presence of thrombin[1]. It inactivates factors Va and VIIIa in the coagulation pathways and modulates the blood's capacity to form clots (prolongs clotting time).

Human protein C appears to be structurally and functionally similar to its bovine counterpart although anticoagulant activity of both proteins is species-specific[2].

In humans, protein C deficiency is a genetic disorder that leads to higher than normal blood clotting in blood vessels and organs, which in turn causes acute thrombolytic episodes[3].

Commercial Applications

Drotrecogin alfa (activated) (Xigris, marketed by Eli Lilly and Company) is a recombinant form of human activated protein C that has anti-thrombotic, anti-inflammatory, and profibrinolytic properties. Drotrecogin alpha (activated) belongs to the class of serine proteases. It is used mainly in intensive care medicine as a treatment for severe sepsis.

Xigris was designed to fight sepsis, a condition that kills more than 200,000 Americans annually. It is the only approved drug for sepsis, and it costs $8,000 to treat a single patient. Lilly hoped it would be a blockbuster, with sales of at least a billion dollars a year. But after five years on the market, sales are only $200 million.

Current Suppliers & Unit Cost

Company	Source	Amount	Price	Unit Cost	Reference	Source Date
DiaPharma (USA)	Bovine/ human	1mg	N/A	N/A	http://www.apcresistance.com/apc/diapharma-pp-apc.htm	2008
Haematologic Technologies Inc. (USA)	Bovine/ human/ mouse	0.1mg	US$67	$670/mg	http://www.haemtech.com/Pricing/Pricing_Zymogens.htm	2008
Innovative Research (USA)	Bovine plasma	1.0mg	US$350	$350/mg	http://www.innov-research.com/Bovineproteins.htm	2008

Competitive Products

Xigris (Drotecogin alpha) 8 – available since 2001 and has been the first and only FDA-approved therapy for severe sepsis in adults. The drug aims to combat the high risks of death from sepsis by increasing the levels of human activated protein C.

Heparin and Coumarin Derivatives9,10 – both are anticoagulants used to treat excessive clotting in the blood circulation. Coumarin is a phytochemical developed from plants.

Emerging Technologies

Protein C-Like Molecules 6,7– used in the treatment of coagulation disorders. They are novel conjugates of polypeptide variants of protein C. The modified protein C molecules have desirable characteristics such as decreased sensitivity to inhibitors.

Global Market

Xigris' nearest competitors failed clinical trials at the end of 2001 leaving the company open as the sole distributor of protein C based therapy eutics.

The company appears to have failed to meet expectations in terms of sales.

"Consequently, many investment analysts expected Xigris' sales to break through the $1bn threshold. However, following its US launch in November 2001, sales of Xigris have been flat with total sales for the first half of 2002 at $44.6m, rising to $66m by the end of the third quarter and only $100m for the full year. Industry analysts had initially predicted global sales of $300-500m in 2002."

Opportunity potential: possible "research reagent"

Summary of Commercial Potential for Australian Beef Industry:

Strengths – bovine Protein C commercially available

Weaknesses – bovine anticoagulant properties are specific for bovine

Opportunities – niche but open market with very few competitors for sepsis treatment

Threats – recombinant and modified forms of protein C are available

Concentration in Bovine Tissue:

Bovine Plasma – Assumption: similar order of magnitude concentration in bovines

Human Plasma	
Product	Yield
Protein C	5mg / 15L plasma

W. KISIEL, 'Human Plasma Protein C: ISOLATION, CHARACTERIZATION, AND MECHANISM OF ACTIVATION BY a-THROMBIN', J. Clin. Invest., Volume 64 September 1979 761 -769

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5 http://www.haemtech.com/Mouse_Proteins.htm

6 http://www.freepatentsonline.com/7226999.html

7 http://www.patentstorm.us/patents/5837843.html

8 http://www.xigris.com/index.jsp

9 http://www.phytochemicals.info/phytochemicals/coumarin.php

10 http://www.rxlist.com/cgi/generic/heparin.htm

11 The Therapeutic Proteins Outlook to 2007 (2003). An analysis of leading products and late-stage pipeline developments. *Business Insights*.

Antioxidants

Coenzyme Q10

Cytochrome C

Glutathione Peroxidase

Superoxide Dismutase

Coenzyme Q10

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Coenzyme Q10	Heart: 1.5kg Cheek: 1.86kg Brain: 0.34kg	N/A		Supplement, neutraceutical	Numerous

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

This oil-soluble vitamin-like substance is present in most animal cells, and is concentrated in the mitochondria - the 'power plants' of the cell.

It plays a vital role in the production of chemical energy by participating in the production of adenosine triphosphate (ATP), the body's so-called 'energy currency'.

Ninety-five percent of the human body's energy is generated this way. Therefore, those organs with the highest energy requirements – such as the heart and the liver – have the highest CoQ_{10} concentrations. CoQ_{10} also works as an antioxidant.

Antioxidants are substances that scavenge free radicals, which are damaging compounds in the body that can alter cell membranes, damage DNA, and even cause cell death. Free radicals occur naturally in the body, but environmental toxins (including ultraviolet light, radiation, cigarette smoking, and air pollution) can also increase the number of these damaging particles. Free radicals are believed to contribute to the aging process as well as the development of a number of health problems including heart disease and cancer. Antioxidants such as CoQ_{10} can neutralize free radicals and may reduce or even help prevent some of the damage they cause.

CoQ_{10} (also known as Ubiquinone) is produced by the body, though production begins to drop after the age of about 20.

CoQ_{10} is a large compound which is intensely lipophilic, which means that it is primarily associated with cell membranes and fat/oil phases.

CoQ_{10} is found in foods, mainly animal tissue, and the average daily intake is about 10 milligrams.

Research

A recent study in Japan by Michihiro Kon recruited 18 elite Japanese kendo student athletes and randomly assigned them to receive daily supplements of CoQ_{10} 300 mg.

After 2 weeks the levels of markers associated with increased wear and tear in the muscle, like creatine kinase and lipid peroxide, were significantly lower in elite Japanese kendo athletes after consuming CoQ_{10} for 20 days, compared to placebo.

Migraine headaches

Supplementation of CoQ_{10} has been found to have a beneficial effect on the condition of some sufferers of migraine headaches.

Brain health and neurodegenerative diseases

Recent studies have shown that the antioxidant properties of coenzyme Q_{10} benefit the body and the brain in animal models. Some of these studies indicate that coenzyme Q_{10} protects the brain from neurodegenerative disease such as Parkinson's, although it does not relieve the symptoms. Dosage was 300 mg per day.

Blood pressure

There are several reports concerning the effect of CoQ_{10} on blood pressure in human studies. The research group concluded that coenzyme Q_{10} has the potential in hypertensive patients to lower systolic blood pressure by up to 17 mm Hg and diastolic blood pressure by up to 10 mm Hg without significant side-effects.

Lifespan

Studies have shown that low dosages of coenzyme Q_{10} reduce oxidation and DNA double-strand breaks, and a combination of a diet rich in polyunsaturated fatty acids and coenzyme Q_{10} supplementation leads to a longer lifespan in rats.

Heart Disease

The ingredient received a tremendous boost, particularly in the US, as its use sky-rocketed in parallel with the rise of statin drugs that have become popular to fight heart disease, high blood pressure and control cholesterol build-up.

Statin drugs deplete the body's natural stores of CoQ_{10} and are particularly popular with over-40s males. They have become the highest selling drugs in the world.

Commercial applications

Coenzyme Q_{10} is available as a supplement in several forms, including softgel capsules, oral spray, hard shell capsules, and tablets.

Coenzyme Q_{10} is also added to functional foods and drinks.

Anti-aging creams are also adding CoQ_{10} to boost their effectiveness.

Global Market

The global CoQ_{10} market is estimated at more than 500m ($850m Aus) by various market analysts. In 2007 approximately 170 tons of CoQ_{10} were sold in the U.S alone. (nutraingredient.com)

Kaneka supplies a naturally fermented form of CoQ_{10} and is the biggest of four Japanese suppliers that dominate the global CoQ_{10} market. The others are Niishin, Mitsubishi Gas Chemical and Asahi.

While demand remains strong for the ingredient, particularly in the US and Japan, the raw material price has dropped out of the market as new supply channels have come on-board in places like China.

Only a few years ago the raw material spot price for CoQ_{10} was $8500 per kilogram or more, but it now rests at about $500-700 per kg, a situation that is encouraging companies like Kaneka to drive into the supply of value-added, premium ingredients such as Ubiquinol, the reduced and bioactive form of CoQ_{10}.

http://www.nutraingredients.com/Industry/Kaneka-targets-CoQ10-at-European-supplements-market

http://www.nutraingredients.com/Research/CoQ10-may-cut-muscle-injuries-for-athletes

Concentration in Animal tissues

According to Meat science International Journal:

CoQ_{10} content of beef cattle (in mg 100^{-1g}) (2003)

Cheek	Heart	Liver	Semitendinosus muscle
6.79	6.05	4.6	2.18

CoQ_{10} concentration means in lamb muscle

Longissimus lumborum	Biceps femoris	Gluteus medius	Psoas major	Quadriceps femoris	Semimembraneous
1.81	3.05	2.48	2.45	2.40	2.63

Strengths – CoQ_{10} is found in high concentrations in bovine tissues like the heart and cheek.

CoQ_{10} market is growing and the enzyme has many commercial applications in foods and cosmetics and is used extensive as a cardiac health supplement.

Weaknesses – The market is reaching maturity and is becoming increasingly competitive.

CoQ_{10} is currently produced most efficiently by microbial fermentation and it is unlikely that bovine CoQ_{10} will be able to compete in the market on price due to higher production costs.

Opportunities – If bovine or animal CoQ_{10} were found to be a more effective for human health compared to current microbial sources the Australian red meat industry would be in a good position to expand into the market. Alternatively, a crude heart extract might have market potential as a "natural source" and would be cheaper to produce than a purified CoQ_{10} product.

Cytochrome C

Bioactive compound	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Cytochrome C	Heart: 1.5kg	~ 50 mg/Kg	A small haeme protein essential for oxidative phosphorylation at the mitochondrion capable of undergoing oxidation and reduction and also an intermediate in apoptosis	Diagnostics;	Invitrogen LLC; Sigma Aldrich

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Cytochrome C is generally regarded as a universal catalyst of respiration; it forms the essential electron-bridge between the respirable substrates and oxygen.

Cytochrome C is found in all cells and it is found where most cellular respiration occurs, the mitochondria (power house) in animal and plant cells.

Cytochrome C is also an intermediate in apoptosis, a controlled form of cell death used by the organism to kill cells in the process of development or in response to infection or DNA damage.

Commercial Applications

Cytochrome C is a highly conserved protein across the spectrum of species, found in plants, animals, and many single celled organisms. This, along with its small size (molecular weight about 12,000 daltons), makes it useful in studies of cladistics. (Cladistics is the hierarchical classification of species based on evolutionary ancestry.)

In medicine Cytochrome C injections are given to treat patients with Ischemia (a restriction in blood supply).

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	5000mg	$3405	Bovine Heart	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C2037	2008

Market Size

- The Cytochrome C has no bulk commercial uses.
- The extent to which Cytochrome C is used in research and for some medical procedures has not been determined.

What product processing is involved?

Minced muscle is defatted with acetone and benzol, dried, pounded, washed three times with water and extracted with decinormal sulphuric acid. The extract is neutralized the precipitate centrifuged off, and the fluid considerably reduced in bulk by evaporation. The solution is then fractionated with ammonium sulphate giving finally cytochrome containing 0.17% of iron which is about 50% pure. Further purification was carried out by adsorption on barium sulphate and elution with decinormal HCl or by adsorption on cellophane, which is sectioned with a freezing microtome, and elution with dilute ammonia (Keilin and Hartree, 1937).

Chromatography on DEAE-cellulose and Ultrogel AcA 44.

The product is mainly the oxidized form of the protein. The reduced form of Cytochrome C can be prepared with either sodium dithionite or sodium ascorbate, followed by gel filtration.

Yield:

- 16.5g/100kg heart muscle (Keilin and Hartree, 1937)
- 165mg/kg

Keilin, D. and E.F. Hartree (1937) “Preparation of Pure Cytochrome c from Heart Muscle and some of its Properties.” Proceedings of the Royal Society of London. Series B, Biological Sciences, Vol. 122, No. 828. (May 1, 1937), pp. 298-308. http://www.jstor.org/view/00804649/ap000377/00a00010/0

Glutathione peroxidase

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Glutathione peroxidase	Red Blood Cells: 4.96kg Mammary gland: 5kg Liver: 5.1kg	~1 mg/Kg	A selenium-dependent enzyme that catalyzes the reduction of H202 or organic hydroperoxides to the corresponding alcohol with a specific requirement for the reductant substrate, GSH.	Bioconversion of various organic hydroperoxides, as well as hydrogen peroxide,	US Biologicals, Calzyme Labs Inc

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Glutathione Peroxidase is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.

An example reaction that glutathione peroxidase catalyses is:

$$2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O$$

Where GSH represents reduced monomeric glutathione and GS–SG represents glutathione disulfide. Reduction of the oxidized form of glutathione (GS-SG) is then catalysed by glutathione reductase.

Commercial Applications

Glutathione Peroxidase is an antioxidant; however it is not commonly used in foods or other products.

Price

Company	Unit	Price	Source	Reference	Source date
Sigma	5mg	$274	Bovine Blood	http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/49753	2008
Sigma	10mg	$501	Bovine Blood	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G6137	2008

Market

Global Market Size

Information relating specifically to Glutathione Peroxidase is not available. However Glutathione Peroxidase is an antioxidant and the U.S. market for antioxidants totals over half a billion dollars, with sales through natural supermarkets reaching a level of $102 million in dollar sales. However Glutathione Peroxidase is not one of the very commonly used antioxidants, according to Sigma-Aldrich.

Current Global Supply

Sigma-Aldrich stated that the global supply by Sigma-Aldrich of Glutathione Peroxidase is approximately less than US$10,000. Some industrial orders may increase this estimate but unlikely.

Sigma is a major player in the scientific supply market. Based upon their annual sales of Glutathione Peroxidase we can estimate that worldwide sales would be below $100,000 a year.

Conclusion

Glutathione Peroxidase is not a complex enzyme, therefore it can be expressed as a recombinant protein in bacteria.

Superoxide Dismutase

Bioactive compound	Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Superoxide Dismutase	Red blood cells: 4.86kg Liver: 5.1kg Heart: 1.5kg Kidney: 1.2kg	~ 120 -200 mg/Kg depending on the procedure	Catalyses the decomposition of superoxide into oxygen and hydrogen peroxide. Presence of SOD has been shown to help protect many types of cells from the free radical damage.	Anti-inflammatory uses; used in cosmetic anti-aging skin and wrinkle rejuvenation products to reduce free radical damage to skin; diagnostics, bioconversions in food and nutraceutical industries	Worthington Biochemical CalbioChem; Sigma-Aldrich; Calzyme Lab

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

The enzyme superoxide dismutase (SOD) converts oxygen free radical into oxygen and hydrogen peroxide. As such, it is an important antioxidant defence in nearly all cells exposed to oxygen.

SOD is one of the most important antioxidants because it neutralises the very reactive oxygen molecule O_2- or superoxide.

Simply stated, SOD out competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. SOD is produced by almost all organisms exposed to oxygen.

Bovine red blood cell SOD has been extensively studied. It is identical to the SOD from human red blood cells (Bannister et al. 1971; Keele *et a*l. 1971 and Nyman 1960).

Commercial Applications

Superoxide dismutase supplements (tablets) are taken to neutralise free radicals (harmful by-products of cellular respiration). SOD supplements are widely available and prices vary from $12 to $50 for approximately 30g of SOD tablets. The majority of SOD supplements are plant based.

Superoxide Dismutase is added to cosmetic creams to repair damaged cells in the dermis and epidermis, used heavily in many anti-wrinkle products.

Superoxide Dismutase has been successfully used to treat scar tissue, but its anti-wrinkle properties have not been analysed.

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	100mg	$538	Bovine RBC	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/S2515	2008
Worthington	10mg	$141	Bovine RBC	http://www.worthington-biochem.com/SODBE/cat.html	2008
Calzyme	1mg	$0.50	Bovine liver and kidney	http://www.calzyme.com/commerce/catalog/product.jsp?product_id=1228&czuid=1197640984051	2008
Sigma	30mg	$500	Microbial	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/S2515	2008
Calzyme	1mg	$1.20	Bakers yeast	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1098	2008
Coregene	1000mg	$25	Porcine Blood	http://www.diytrade.com/china/4/products/4353582/Superoxide_Dismutase.html	2008

Market Size

The market size for SOD was not found, however there are several companies supplying SOD at the retail level. SOD is reported to be a highly effective antioxidant if it is ingested and as a cosmetic.

These two industries are extremely large and are worth several billion dollars a year.

The majority of the world's SOD appears to come from plant sources however there are no clear reasons why this is so.

Bovine red blood cells, liver and kidney appear to be excellent sources of SOD.

SOD represents a high value widely used product that could be an excellent source of revenue for the red meat industry. The Australian and New Zealand red meat industries are free of any potential diseases like BSE that could affect sales of bovine SOD. Bovine and Human SOD are reported to be identical, but whether this makes bovine SOD a more effective antioxidant than plant SOD is not known.

A Chinese company Coregene quoted $25 a gram for Superoxide dismutase that was suitable for use in food and cosmetic products. Coregene stated that their SOD has an activity of 3000 units per milligram, which is approximately 37% less active than Sigma's SOD 4750 U/mg. Though the SOD from Sigma is 200 times more expensive.

What product processing is involved?

Initial anion exchange chromatography, followed by a hydrophobic interaction chromatography and gel filtration chromatography.

Catabolic Enzymes

Creatine Kinase

Enolase

Glucose Oxidase

Glycogen Phosphorylase Isoenzymes

Lactate Dehydrogenase

Malate Dehydrogenase

Malate Hydrolase

Pyruvate Kinase

Creatine kinase

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Creatine kinase	Heart: 1.5kg	Represent ~10-20% (w/v) of muscle cytoplasmic protein depending on process	Primarily concerned with ATP regeneration	Diagnostics	USB Corp, Sigma, Worthington Biochemicals, BioChain, RayBiotech

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Creatine kinase is an important energy regeneration enzyme as it catalyses the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP), consuming phosphocreatine and generating creatine.

CK is expressed in most tissues however it is more abundant in tissues that consume energy rapidly, especially skeletal muscle, but also brain and smooth muscle.

Commercial applications

- Modified physiological levels of creatine kinase are used as indications for various diseases and disorders, including heart disease1 and multiple sclerosis2.
- Creatine kinase is used extensively in the testing and monitoring of heart disease in humans (specifically myocardial infarction), and analysis is generally performed on blood samples by pathology service providers around the world.
- Creatine Kinase is also used to determine if rhabdomyolysis (severe muscle breakdown) and acute renal failure are present in patients.

Current Commercial Sources:

- Bovine heart
- Rabbit brain
- Rabbit muscle
- Human heart
- Human brain
- Recombinant yeast
- Human recombinant

Price

Company	Source	Amount	Price	Reference	Source date
Sigma Aldrich	Bovine heart/ rabbit brain / rabbit muscle / human heart	350 mg	AU$593	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C7886	2008
USB Corp.	Rabbit muscle	1g	US$471	http://www.usbweb.com/category.asp?cat=bio&id=13915	2008
BioChain	Human recombinant	50ug	US$209	http://www.biocompare.com/ProductDetails/872231/ProductDetails.html	2008
RayBiotech	Human recombinant	1mg	US$1,122	http://www.biocompare.com/ProductDetails/821226/ProductDetails.html	2008

Market size

Although creatine kinase is an important energy regeneration enzyme there are no commercial applications utilising this function.

The majority of commercial applications involving CK are based upon its detection in patients with heart problems.

There was no market data on CK and it is assumed that the majority of the worlds' CK is used in research laboratories and universities.

Summary

- Although this enzyme is vital for the regeneration of energy in tissues there appears to be no supplements or products containing CK on the market.
- CK would be found in high concentrations in bovine heart and muscles however the latter is already a high value product in the form of meat.
- It would be assumed that CK could not be extracted without severely degrading the quality of the meat.
- CK can also be produced recombinantly however it is many times more expensive than from animal tissues.
- CK is found in all animals and there would be no specific advantage held by bovine sources over other animals.

Concentration in Bovine Tissue

Bovine Uterus

Bovine Uterus (150-200g)	
Product	Yield
Purified creatine kinase	10.8 g

Bovine Heart – Assumption: similar order of magnitude concentration in bovines

Rabbit heart (363g)	
Product	Yield
Purified creatine kinase	11 mg

Yvonne Landt, Hemant C. Valdya, Sharon E. Porter, David N. Dletzler, and Jack H. Ladenson, Immunoaffinity Purification of Creatine Kinase-MB from Human, Dog and Rabbit Heart with Use of a Monoclonal Antibody Specific for CK-MB, CLINICAL CHEMISTRY, Vol.35, No. 6, 1989, p.985

Reference

1 http://www.patentstorm.us/patents/5382515.html

2 http://www.muscle.ca/content/index.php?id=1303

3 http://en.wikipedia.org/wiki/Creatine_kinase

Enolase

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Enolase	Liver: 5.1kg Muscle: N/A		Catalyses the Mg2+-dependent reversible dehydration/ hydration of 2-phospho-glyceric acid (2-PGA)/phos-pho(enol)pyruvate (PEP).	diagnostics, cell biology, bioconversions	Sigma, Cortex

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Enolase is an enzyme present in muscle tissue that acts in carbohydrate metabolism. Enolase catalyses the conversion of 2-phosphoglycerate (2PG) to phosphoenolpyruvate (PEP), which is the ninth step in the conversion of glucose to pyruvate.

There are 5 distinct isoforms of enolase including neuron specific enolase (NSE), muscle specific enolase (MNE) and non-neuronal enolase (NNE) .

All isoforms of enolase share a close functional relationship, differing mainly in their distribution. NNE is present in many different cell types, while NSE and MNE forms are generally found in muscle, glial and neuronal cells .

Commercial Applications

Tissue injuries induce the release of NSE into the blood and cerebral spinal fluids. The NSE test is used in medicine to determine if trauma has recently occurred. This technique is not widely used as it is still being developed.

Price

Company	Units	Price	Source	Reference	Source date
Sigma	1mg	$15.60	Rabbit muscle	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/E0379	2008

Global market Size

An estimation of the direct global market size for this compound could not be determined. Furthermore, market size could not be accurately inferred, as limited information could be found detailing the compounds potential uses.

One patent suggested the use of enolase as the active ingredient in treating chronic inflammatory airway disease, however this was deemed an inappropriate application for inferring bovine enolase market size.

Distribution

Only one, Californian based, supplier for bovine enolase was located, a company called Cortex Biochem. This company has an established worldwide distribution chain.

Competing Products

Rat, rabbit and human enolase is broadly available from retailers

Extraction/Processing Technologies

Processes for extracting competitor animals are easily found, and although a process must exist for extracting bovine gamma enolase (as evidenced by its availability for purchase), details were unable to be found. Likewise, no details were found on processes for extracting/purifying other bovine enolase isoforms, possibly as no processes currently exist.

Other

Given that enolase from competing animals is widely available, the literature suggests that currently too little research has been conducted into bovine derived enolase to justify any commercial investment into this compound at the present time.

Summary

- Bovine enolase appears to have little commercial potential.
- Of all the isoenzymes that are grouped under the enolase banner, only two are dealt with at any length within the literature, or within the research, and commercial sector.
- Of the enolase isoenzymes, most of the available information deals with gamma, or neuron specific enolase.
- For neuron specific enolase, only one supplier was identified worldwide, of.
- Uses and products incorporating bovine enolase were likewise few and far between. Research data including extraction and purification technologies was not identified for bovine enolase. It is assumed that the sole supplier of bovine enolase has scaled up protocols used to extract rat or rabbit enolase for use in bovine tissue extraction.
- Although this appears to be a compound where first mover advantage remains to be taken, the lack of a product, a proven manufacturing process, or a potential market militate against this for the Australian red meat industry.

Referemce

1 http://www.answers.com/topic/enolase

2 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=9169614&query_hl=11&itool=pubmed_docsum

3 http://www.blackwell-synergy.com/doi/abs/10.1111/j.1471-4159.1981.tb01663.x

Glucose Oxidase

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Glucose oxidase	Various	~1 mg/kg	Catalyses the oxidation of beta-D-glucose to glucono-1,5-lactone and hydrogen peroxide	Biosensors, food industry, biocatalysis	US Biologicals; Calzyme Labs Inc

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

The glucose oxidase enzyme binds to beta-D-glucose and aids in breaking the sugar down into its metabolites. GOx is a protein that catalyzes the oxidation of beta-D-glucose into D-glucono-1,5-lactone, which then hydrolyzes to gluconic acid.

Glucose oxidase

Glucose + O_2 + H_2O <=======> gluconic acid +H_2O_2

The glucose oxidase enzyme is commonly used in biosensors to detect levels of glucose by keeping track of the number of electrons passed through the enzyme by connecting it to an electrode and measuring the resulting charge. When produced commercially for this application, it is often extracted from Aspergillus niger.

Commercial Applications

Glucose oxidase enzyme is used in conjunction with tiny electrodes as glucose sensors for diabetics.

Glucose oxidase is found naturally in honey and acts as a natural preservative. GOx at the surface of the honey reduces atmospheric O_2 to hydrogen peroxide (H_2O_2), which acts as an antimicrobial barrier.

Price

Company	Units	Price	Source	Reference	Source date
Calzyme	1000mg	$5500	*Aspergillus niger*	http://www.calzyme.com/commerce/catalog/product.jsp?product_id=1122&czuid=1195831575996	2008
Sigma	1000mg	$134	*Aspergillus niger*	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G2133	2008

Market Size

- Approximately 100-1000kg of glucose oxidase is produced a year with a retail value of $8-20 per kg (Protein Biotechnology 2002).
- The current production source is *Aspergillus niger*.
- It is produced by yeasts due to lower production and extraction costs
- No potential for the red meat industry due to the great production and extraction costs from animal sources compared to microbial sources.

Glycogen Phosphorylase Isoenzymes

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Glycogen phosphorylase isoenzymes	Heart: 1.5kg Corpus luteum: 0.001kg Spleen: 0.66kg	~ 18 mg/Kg	Catalyses the degradation of glycogen to glucose	Biotransformations, pharmaceuticals	US Biologicals; Calzyme Labs Inc

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

- Glycogen phosphorylase breaks up glycogen into glucose subunits in the liver and muscle.
- Glycogen is left with one less glucose molecule, and the free glucose molecule is in the form of glucose-1-phosphate.
- In order to be used for metabolism, it must be converted to glucose-6-phosphate by the enzyme phosphoglucomutase.
- Glucose-1-phosphate provides a readily useable source of energy for living organisms.
- Glycogen phosphorylases have been isolated from a wide variety of organisms, including bacteria, yeast, plants, amphibians and mammals.
- The mammalian glycogen phosphorylases are regulated by phosphorylation, by phosphorylase kinase and by AMP.

Commercial Applications

The commercial applications for glycogen phosphorylase are limited and it is used solely as a research reagent.

Price

Company	Units	Price	Source	Reference	Source date
Sigma	100(mg)	$641	Rabbit muscle	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/P6635	2008
AbD Serotec	25 (mg)	$122	Rabbit muscle	http://www.ab-direct.com/catalog/datasheet-7370-9902.html	2008

Market size

- Limited usage worldwide
- Recombinant sources have been developed so animal sources will become obsolete in the future.

Opportunity potential: possible "research reagent"

Summary of Commercial Potential for Australian Beef Industry:

Strengths – Currently manufactured from bovine sources, relatively high concentration in brain

Weaknesses – No major applications other than research reagent and antigen for generation of antibodies

Opportunities – Supply to fine chemical companies

Threats – No reason for market growth

Concentration in Bovine Tissue

Bovine corpus luteum

Bovine corpus luteum (200-400g)	
Product	Yield
Purified glycogen phosphorylase	16 mg

Said A. Assaf and Adel A. Yunis, 'Purification and properties of glycogen phosphorylase from bovine corpus luteum. Kinetics of salt activation', Biochemistry; 1970; 9(22) pp 4381 - 4388

Bovine skeletal muscle – Assumption: similar order of magnitude concentration in bovines

Pig skeletal muscle (500g)	
Product	Yield
Purified glycogen phosphorylase	8.9 mg

Pascual Lopez Buesa, Fredi Schwägele and Karl O. Honikel, 'Purification and isoenzymic composition of glycogen phosphorylase b from normal and abnormal (PSE) muscles, Z Lebensm Unters Forsch (1995) 201:30-34

1 Characterization of a hyperthermostable glycogen phosphorylase from Aquifex aeolicus expressed in Escherichia coli, Journal of Molecular Catalysis B: Enzymatic Volume 22, Issues 3-4, 2 June 2003, Pages 173-180

Bovine Heart – Assumption: similar order of magnitude concentration in bovines

Rabbit heart (363g)	
Product	Yield
Purified creatine kinase	11 mg

Norbert Berndt and Peter Rösen, 'Isolation and partial characterization of two forms of rat heart glycogen phosphorylase', Archives of Biochemistry and Biophysics, Volume 228, Issue 1, January 1984, Pages 143-154

Current Commercial Sources:

- Rabbit muscle
- Human recombinant
- Human heart

Emerging Technologies:

Human recombinant sources of glycogen phosphorylase are becoming available.

Competitive Products:

Recombinant sources of glycogen phosphorylase

Lactate Dehydrogenase

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Lactate dehydrogenase	Heart: 1.5kg Brain: 0.34kg & various tissues\	100-250 mg /Kg depending on the process and source	Converts pyruvate to lactate	Diagnostics; Multiple isoenzymes are known, bioconversion	Worthington Bio-chemical, Calzyme Labs Sigma

**Assume 300kg HSCW. See Appendix A for other HSCW*

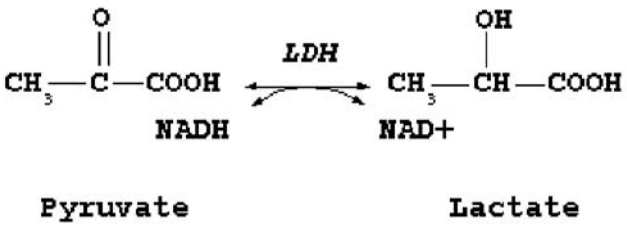
Description

If insufficient oxygen is available, intracellular lactic acid is broken down anaerobically, creating lactate in animals and ethanol in plants. Pyruvate from glycolysis is converted by anaerobic pathways to lactate using the enzyme lactate dehydrogenase and the coenzyme NADH in lactate fermentation

Lactate dehydrogenase (LDH) is an enzyme present in a wide variety of organisms, including plants and animals.

Any of a class of enzymes that catalyse the reversible interconversion of pyruvate and lactate, found predominantly in the liver, kidneys, striated muscle, and heart muscle.

Lactate dehydrogenase catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD+. It converts pyruvate, the final product of glycolysis to lactic acid when oxygen is absent or in short supply, and it performs the reverse reaction during the Cori cycle in the liver.



Commercial Applications

Medical use

Tissue breakdown elevates levels of LDH, and therefore a measure of it indicates e.g. haemolysis. Other disorders indicated by elevated LDH include cancer, meningitis, encephalitis and HIV.

It can also be used as a marker of heart attack. Following a heart attack, levels of LDH peak at 3-4 days and remain elevated for up to 10 days. In this way, elevated levels of LDH can be useful for determining if a patient has had a heart attack.

Lactic Acid

Lactate dehydrogenase is involved in the production of lactic acid by lactic acid bacteria. Lactic acid is commonly used as a food additive for preservation, flavour, acidity and for the manufacture of the biodegradable plastic, polylactic acid (PLA). The global lactic acid market is estimated to be in excess of 100,000 tons per year

Bacterial fermentations with Lactobacilli are common for industrial production of lactic acid

http://www.patentstorm.us/patents/6268189/description.html

The market for bovine LDH would be limited to research as LDH is already produced by microbes at a very low cost.

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	2.5ml	$77	Bovine heart	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/L3916	2008

Market Size:

- The annual production is in the order of 10,000-100,000 litres per year.(Hui, 2006)
- Although the market is large in volume it is not a high value product.
- The market for bovine LDH would be limited to research as LDH is already produced by microbes very cheaply.

Reference

Handbook of Food Science, Technology, and Engineering

By Yiu H. Hui

Published by CRC Press, 2006

Malate Dehydrogenase

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Malate dehydrogenase	Heart: 1.5kg Brain: 0.34kg	~500 mg/ Kg heart	An enzyme in the citric acid cycle that catalyses the conversion of malate to oxaloacetate using nicotinamide adenine dinucleotide (NAD) as a coenzyme and involved in gluconeogenesis, the synthesis of glucose from smaller molecules.	Widely used in coupled systems for determination of biological metabolites; diagnostics; bioconversions.	Worthington Biochemical Corp; Calzyme Labs Inc, Biozyme, Inc., and

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Malate dehydrogenase is an enzyme in the citric acid cycle that catalyzes the conversion of malate into oxaloacetate (using NAD+) and vice versa (this is a reversible reaction).

L-malate + NAD^+ ←→ oxaloacetate + NADH + H^+

Malate dehydrogenase is also involved in gluconeogenesis, the synthesis of glucose from smaller molecules. MDH is found in all eukaryotic cells as two isozymes: mitochondrial and cytoplasmic.

Commercial Applications

Malate dehydrogenase is widely used in coupled systems for determination of biological metabolites as well as bioconversion of cellulose in ethanol production.

MDH is of interest to the clinician in that its activity in serum and cerebral/spinal fluid has been shown to be of diagnostic significance.

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	36mg	$411	Porcine heart	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;M2634	2008
Sigma	5mg	$589	Bovine heart	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/M9004	2008
Sigma	20mg	$43	Thermus flavus	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;M7032	2008

World Market

The market for bovine malate dehydrogenase would be limited as there are several microbial sources of malate dehydrogenase that are produced at a fraction of the cost.

What product processing is involved?

Homogenate supernatant is added to polyethylene glycol to precipitate malate dehydrogenase and purified by DEAE-cellulose column chromatography.

Purity: 95%

Malate Hydrolase

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Malate Hydrolase	Heart: 1.5kg Plasma: 11.34kg	~80 mg/ Kg	Converts L-malate to fumarate	Applications in food industry	US Biologicals; Sigma Aldrich

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Malate Hydrolase, Fumarase (or fumarate hydratase) is an enzyme involved in the Krebs cycle that catalyzes the hydration of Fumarate to L-malate (malic acid)

Fumarate + H_2O ←→ L Malate

Commercial Applications

Malate hydrolase is used to produce malic acid which is used as a food additive

Malic acid is the active ingredient in many sour or tart foods. Malic acid contributes to the sourness of green apples. The worldwide market for malic acid is large with over 7.5 million kilograms produced every year. http://www.freepatentsonline.com/4551432.html

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	13 mg	$914	Porcine heart	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/F1757	2008

The majority of the world's fumarase is produced from bacterial and fungal sources.

The bulk price for fumarase from microbial sources was not found as large quantities can be produced by fermentation in vats on site.

Market

The annual production of fumarase would be 100s of kilos per year. It is difficult to determine the exact amount as fumarase can be produced by those who need it.

The ability to produce fumarase from microbes means that it would be sold cheaply on the market, and any new source from animals would have difficulty entering into the market.

Pyruvate Kinase

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Pyruvate kinase	Muscle: N/A Kidney: 1.2kg Brain: 0.34kg and other tissues	150 mg/Kg	Catalyses the conversion of phosphoenolpyruvate to pyruvate	Pyruvate kinase (PK) is a key enzyme in glycogen metabolism; bioconversion	US Biologicals, Calzyme Lab Inc.,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Current Market Applications:

Research reagent

Description

Pyruvate kinase is an enzyme found in red blood cells that helps change glucose to energy when oxygen is low.

Pyruvate kinase (PK) main role is in the glycolysis pathway where it converts phosphoenolpyruvate to pyruvate, generating ATP from ADP.

ADP + Phosphoenolpyruvate → ATP + Pyruvate

Vertebrates contain four different PK isozymes that are specific to tissue types:

- L Type (Liver)
- R Type (Red Cells)
- M1 (Muscle; heart & brain)

Bovine pyruvate kinase exists in at least three distinct subunit types, K, L and M, which are separable by electrophoresis:

- Type K – predominantly in foetal tissue and persists in many adult tissues.
- Type L – predominant form in adult liver and is also present in the kidney.
- Type M – the main isozyme in the adult bovine brain, skeletal and cardiac muscle.

Pyruvate kinase also exists in plants, bacteria, lower eukaryotes and *E. coli*.[1]

Commercial Applications

Pyruvate kinase has no bulk commercial applications but it is available for research purposes.

In medicine the pyruvate kinase test is done to detect abnormally low levels of pyruvate kinase. If you do not have enough of this substance, red blood cells break down faster than normal. This can lead to haemolytic anemia.

Current Commercial Sources:

- Bovine heart
- Porcine heart[2]
- *Bacillus stearothemophilus* – bacteria found in thermal hot springs.

Current Suppliers & Unit Cost

Company	Source	Quantity	Price	Reference	Source date
USB Corp	Rabbit Muscle	3000mg	US$141	http://www.usbweb.com/category.asp?cat=116&id=20977&special=	2008
Sigma-Aldrich	Bacillus stearothermophilus	200mg	AU$268	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/P1903	2008
Calzyme	Rabbit Muslce/ Human Muscle/ Porcine Heart	4mg	US$2.00	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1092	2008

Market Size

The market Size for pyruvate kinase was unable to be determined, though as the enzyme has no bulk commercial applications the market is likely to be limited.

Pyruvate kinase is also not a viable bioactive product for the red meat industry as the enzyme is produced by all living organisms and as bovine pyruvate kinase does not hold any specific advantage there is no market for the enzyme.

Opportunity potential: negligible

Summary of Commercial Potential for Australian Beef Industry

Strengths – pyruvate kinase is available from multiple organs and tissue of bovine

Weaknesses – pyruvate kinase is found in all living organisms including plants and bacteria

Opportunities – no current use of pyruvate kinase or properties of the enzyme that may be deemed beneficial

Threats – deficiency of pyruvate kinase and excess pyruvate kinase are related to various disease states such as cancer

Current Market Applications:

Research reagent

Concentration in Bovine Tissue

Bovine Neck Muscle (232g)	
Product	Protein (mg)
Purified PK	26.1 mg

J. M. CARDENAS, 'Pyruvate Kinase from Bovine Muscle and Liver', METHODS IN ENZYMOLOGY, VOL 90, 1982

http://www.ebi.ac.uk/interpro/IEntry?ac=IPR001697

Bovine Brain (350g)	
Product	Bioactive compound (mg)
Purified PK	18 mg

GRZEGORZ TERLECKI, 'PURIFICATION AND PROPERTIES OF PYRUVATE KINASE TYPE M, FROM BOVINE BRAIN', Int. J. Biochem. Vol. 21. No. 9. pp. 1053-1060, 1989

Bovine Heart (1000g)	
Product	Protein (mg)
Purified PK	48 mg

J. PARKINSON and J. S. EASTERBY, 'PURIFICATION AND MOLECULAR PROPERTIES OF BOVINE HEART PYRUVATE KINASE', Biochimica et Biophysica Acta, 481 (1977) 471—480

Bovine Liver (1700g)	
Product	Protein (mg)
Purified PK	4 mg

J. M. CARDENAS, 'Pyruvate Kinase from Bovine Muscle and Liver', METHODS 1N ENZYMOLOGY, VOL. 90, 1982

Porcine Kidney (900g) **assumption:** similar order of magnitude concentration in bovines	
Product	Protein (mg)
Purified PK	7.9 mg

L. BERGLUND, O. LJUNGSTROM, and LORENTZ ENGSTROM, 'Purification and Characterization of Pig Kidney Pyruvate Kinase (Type A)', The Journal of Biological Chemistry Vol. 252, No. 17, Issue of September 10, pp. 6108-6111

http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1092

Reference

1 http://www.ebi.ac.uk/interpro/IEntry?ac=IPR001697

2 http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1092

Fibroblast Growth Factors

Lipoprotein Deficient Serum

Serum Albumin

Taurine

Vitronectin

Fibroblast Growth Factors

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
FGF-1, 2,	Brain: 0.34kg Pituitary gland:0.002kg Heart: 1.5kg	Typically, ~0.150 mg/Kg	Over 27 different FGFs, major forms are the acidic (FGF-1) and basic (FGF-2). The basic form present in basement membranes and in the subendothelial extracellular matrix of blood vessel.	Cell Culture, skin care and other growth factor, cytokine activities, angiogenesis; wound healing, tissue engineering	R&D Systems; SigmaAldrich; ProSpec-Tany TechnoGene Ltd., CalbioChem.,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Fibroblast growth factors, or FGFs, are a family of growth factors involved in angiogenesis (blood vessel growth), wound healing, and embryonic development.

FGFs are key-players in the processes of proliferation and differentiation of cells.

Bioactive Function:

In the adult organism, FGFs are homeostatic factors which have function in tissue repair and response to injury. When inappropriately expressed, some FGFs can contribute to the pathogenesis of cancer. A subset of the FGF family, expressed in adult tissue, is important for neuronal signal transduction in the central and peripheral nervous systems.2

FGF is critical during normal development of both vertebrates and invertebrates and any irregularities in their function leads to a range of developmental defects

Commercial applications

FGFs are generally used as cell culture additives (including stem cell cultures) for research purposes.

Fibroblast growth factor-1 or, FGF-1, is a powerful stimulator of new blood vessel growth, a process referred to in the scientific community as “angiogenesis.” This is due to the fact that FGF-1 stimulates the growth and multiplication of the two main cell types of blood vessels, smooth muscle cells and endothelial cells. Extensive work has shown that, when FGF-1 is injected into the hearts of animals with experimentally induced heart disease, new blood vessels grow in the injected areas. Proteins such as Cardio Vascu-Grow™ represent a novel way to circumvent clogged arteries in patients with heart disease.

Current Commercial Sources:

- Bovine brain
- Bovine pituitary
- Human recombinant

Price

Company	Source	Amount	Price	Unit Cost	Reference	Source date
Sigma Aldrich	Bovine pituitary	50µg	AU $1,208.70	$24.17/µg	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/F3133	2008
Bachem	Bovine brain	5mg	US$480	$96/mg	https://www.bachem.com/index.cfm?uuid=538F68571AE411D6B4CA00500465876C&site=detail&action=showchildren&idland=&prodid=4242	2008
R&D Systems	Bovine brain	25µg	US$350	$14/µg	http://www.rndsystems.com/product_results.aspx?k=132-FA	2008

Market

The market for Fibroblast growth factor appears to be limited to research at this point in time. There is only one commercial application for Fibroblast growth factor in the medical industry and this product appears to be in its early stages of development. The use of bovine Fibroblast growth factor in human medicine is unlikely though no information was obtained on this possibility.

Concentration in Bovine Tissue

Bovine brain

Bovine brain	
Product	Yield
Purified FGFs	0.144 mg

K A Thomas, M Rios-Candelore, and S Fitzpatrick, 'Purification and characterization of acidic fibroblast growth factor from bovine brain', Proc Natl Acad Sci USA 1984 January; 81(2): 357–361

http://www.rndsystems.com/mini_review_detail_objectname_MR01_FGFs.aspx

http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed&uid=11276432&cmd=showdetailview&indexed=google

Bovine pituitary

Bovine pituitary (1.8kg)	
Product	Yield
Purified FGFs	0.15 mg

Denis Gospodarowicz, Jannie Cheng, Ge-Ming Lui, Andrew Baird, and Peter Bohlent, 'Isolation of Brain Fibroblast Growth Factor by Heparin-Sepharose Affinity Chromatography: Identity with Pituitary Fibroblast Growth Factor', PNAS, November 15, 1984, vol. 81, no. 22, 6963-6967

Bovine heart

Bovine heart (1kg)	
Product	Yield
Purified FGFs	0.5 mg

H Sasaki, H Hoshi, YM Hong, T Suzuki, T Kato, H Sasaki, M Saito, H Youki, K Karube and S Konno, 'Purification of acidic fibroblast growth factor from bovine heart and its localization in the cardiac myocytes', J. Biol. Chem., Vol. 264, Issue 29, 17606-17612, 10, 1989

Summary

Opportunity potential: possible “research reagent”

Summary of Commercial Potential for Australian Beef Industry

Strengths – Currently manufactured from bovine sources

Weaknesses – human recombinant FGFs commercially available. Low concentration in tissue - pituitary gland low volume stream

Opportunities – Supply to fine chemical companies

Threats – Recombinant proteins have been developed and currently sold by many companies such as Millipore

Reference

1 http://www.rndsystems.com/mini_review_detail_objectname_MR01_FGFs.aspx

2 http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed&uid=11276432&cmd=showdetailview&indexed=google

Lipoprotein Deficient Serum

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Lipoprotein Deficient Serum	Plasma: 11.34kg		Sera that have been depleted in lipoproteins by stripping procedures	Cell Culture	Sigma, Autogen Bioclear.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Lipoprotein Deficient Serum is foetal bovine serum that has had all lipoproteins removed.

Lipoproteins (lipids attached to a protein) are particles that transport dietary lipids from the intestines to other locations in the body via the blood stream.

As fats cannot be dissolved in water a protein has to be attached in order for them to be soluble and able to move within the water based solution of the blood stream.

Commercial applications

- LPDS is used in place of foetal bovine serum for culturing cells to examine Low Density Lipoprotein (LDL) receptor-mediated binding. LPDS is especially useful in medical research as LDLs transport cholesterol to the heart and brain causing heart attacks and stroke.

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	50 ml (1750mg)	$672	Bovine foetal	http://www.sigmaaldrich.com/catalog/search/ProductDetail?ProdNo=S5519&Brand=SIGMA	2008
Autogen Bioclear	50 ml (1750mg)	$580	Bovine foetal	http://www.autogenbioclear.com/PROD_RP-056.html	2008

Market Size

The market for LPDS is not known, though as there are few commercial applications for LPDS the market is likely to be small.

Processes

Lipoprotein Deficient Serum, LPDS, is prepared from bovine serum by isopycnic (density gradient centrifugation) ultracentrifugation at the density limit of the specified density range (Goldstein et al.16), and is extensively dialyzed. The resultant LPDS is membrane filtered and packaged aseptically.

Each lot is evaluated in human skin fibroblast cell culture in conjunction with our LDL and [I125] LDL or DiI-LDL to determine the degree of up regulation of LDL receptors (http://www.btiinc.com/page/cata3.html#Lipoprotein, Havel, R.J., Eder, H.A., and Bradgon, J.H. (1955) J. Clin. Invest. 34:1345.)

Yield: Close to 100%. As only lipoproteins are removed.

Content: 35-120g Protein/L of LPDS

Serum Albumin

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Serum albumin	Plasma: 11.34kg	Typical concentration > 50g/L		Higher value applications include diagnostics, cell culture and various laboratory applications; serology; lower value include wood adhesives, incipients and diluents	Bovogen Biologicals SeraCare ; Invitrogen, Proliant Biologicals Sigma Aldrich; Genataur

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Serum albumin is the most abundant blood plasma protein in humans and other mammals.

Serum Albumin is essential for maintaining the osmotic pressure needed for proper distribution of body fluids throughout the circulatory system and body tissues.

It also acts as a plasma carrier for several hydrophobic steroid hormones and as a transport protein for iron proteins and fatty acids.

Functions of albumin

- Maintains blood pressure
- Transports hormones, particularly fat soluble ones
- Transports fatty acids to the liver
- Transports many drugs
- Competitively binds calcium ions (Ca2+)
- Buffers pH

Serum albumin is only found in the blood of animals and should not be confused with albumin which is a general term which refers to any protein that is soluble in water. Albumin can be found in eggs and in plant seed as it is used as a storage protein for growth.

Foetal bovine serum (FBS) is more valuable than adult bovine serum albumin because FBS is more difficult to extract than BSA and FBS is significantly less abundant than BSA.

FBS has higher concentrations of growth promoting factors and nutrients, making FBS a more potent version of BSA. FBS is used very similarly to BSA in research though as it is more expensive it is used less often in neutraceuticals and cosmetics

Commercial applications

Neutraceutical

Albumin is added to foods to act as a binding agent, though recently bovine serum albumin has been developed as a neutraceutical. BSA is added to foods to boost the protein content and as an immune system enhancer.

Proliant is the market leader with its nutraceutical product NutraGammax. The following information was taken from the Proliant website. www.proliantinc.com

NutraGammax™-WP is a bioactive protein isolate available for use in sports nutrition products and protein supplements. It offers the dual benefit of providing immune support and improving the utilization of amino acids. It also contains beneficial growth factors. NutraGammax™-WP contains very low levels of saturated fat and no lactose. It is free of milk proteins, casein, and gamma-lactoglobulin, which are not well tolerated by all people.

Physical Characteristics

NutraGammax™- WP is an agglomerated, cream colored powder with a neutral flavour and odor. It is designed for enhanced dispersion and dissolution in water when used alone or in combination with other proteins.

Recommended Use

Use to support lean tissue growth, maximize utilization of protein and promote healthy immune function during training. The recommended dosage of NutraGammax™-WP is 10 to 25 grams per day which provide 2.0 to 5.0 grams of IgG.

Labeling

NutraGammax™-WP; serum protein isolate and soy lecithin.

Cosmetic

Limited use of BSA in cosmetics, BSA mainly from milk used in some cosmetics for the treatment of wrinkles, scars and burns.

Research

Bovine serum albumin, or BSA, is commonly used in immunodiagnostic procedures, clinical chemistry reagents, cell culture media, protein chemistry research and molecular biology laboratories.

The rich variety of proteins in bovine serum albumin maintains cultured cells in a medium in which they can survive, grow, and divide.

BSA is used because of its stability, its lack of effect in many biochemical reactions, and its low cost since large quantities of it can be readily purified from bovine blood, a byproduct of the cattle industry.

Summary of research applications

- General additive in growth media for tissue and cell culture
- Protein standard
- General research or diagnostic assay reagent
- Assays where low endotoxin levels are required
- Additive in specific diluents
- Vaccine production
- Blocking agent in immunoassays
- Stabiliser in certain types of protein ligands
- Reagent for protease sensitive immunoassays

Celliance has introduced a new bovine albumin product, manufactured exclusively from animals originating in Australia, that is aimed at applications such as pharmaceuticals where there is a need to ensure the absence of transmissible spongiform encephalopathies (TSEs).

Probumin Plus albumin is a high-quality albumin product manufactured from Australian cattle, which are classified as being BSE (bovine spongiform enchalopathy) free.

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	500g	$1330	Bovine	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIAL/A9056	2008
Invitrogen	150g	$354	Bovine	https://catalog.invitrogen.com/index.cfm?fuseaction=viewCatalog.viewProductDetails&productDescription=200	2008
Genataur	250g	$315	Bovine	http://www.gentaur.com/genprice/acatalog/Online_Catalogue_Biochemicals_330.html	2008
SeraCare	5kg	$2520	Bovine	http://www.seracarecatalog.com/Default.aspx?tabid=396&CategoryID=74&List=1&SortField=ProductName%2CProductName&Level=a&ProductID=438	2008

Market Size

Pharmaceutical grade BSA has a global demand of 200 tonnes annually and at $180 million market value, represents a substantial sector of the biopharmaceutical market.

http://www.cabinet.qld.gov.au/MMS/StatementDisplaySingle.aspx?id=45865

The pharmaceutical industry is the primary market for high grade BSA as it is used extensively in cell culturing and other research procedures.

The demand for Australian BSA has increased over the last few years due to the discovery of BSE in several countries. Australian and New Zealand BSA are sold at a premium and can be imported into almost any country whilst U.S and Canadian BSA is currently restricted.

Several pharmaceutical and research companies offer Australian and New Zealand BSA products. An example of this is Celliance's sales of the Probumin range which was developed in 2004. Celliance sold U.S$4.4 million of Australian BSA in the first quarter of 2005 and sales were predicted to increase while trade in bovine products were restricted.

http://www.in-pharmatechnologist.com/Materials-Formulation/Celliance-extends-bovine-albumin-range

In 1999 the market for albumin was 440 tonnes worldwide though this was from all animals and for various commercial applications.

http://www.springerlink.com/content/v60287h8n6126706/

The market for BSA as a neutraceutical or food additive was not determined though it is estimated that in 2007, 770 tonnes of BSA was sold worldwide. As 200 tonnes of pharmaceutical grade BSA are sold annually it is possible to deduce that approximately 570tonnes of BSA was sold for neutraceutical and other applications.

The value of these sales was not determined though pharmaceutical grade BSA is more expensive than other grades as it requires further purification and processing than lesser grades.

Using this knowledge we can assume that the 570 tonnes of BSA sold for non-research purposes has a retail value equal to or less than the $180 million market size of pharmaceutical grade BSA.

Strengths:

- BSA is widely used in cell culturing
- Readily available tissue source
- Easily extracted
- High value
- Australia is viewed as a reliable and safe source of BSA due to our clean green image.
- The nutraceutical market is growing and BSA represents a high quality protein nutraceutical.

Weaknesses:

- Market is mature and there are several suppliers of BSA

Taurine

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Taurine	Lung: 2.85kg Bile acids: N/A	N/A	Amino acid, intracellular regulation.	Food additive, cell culturing	Sigma, Numerous others

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Taurine is an amino acid that occurs abundantly in the body, and is found in cattle lung and bile, shark blood, mussels and oysters. Taurine is named after the Latin *taurus*, which means bull or ox, as it was first isolated from ox bile in 1827.

Taurine has been proposed to play important roles in such biological functions as regulating intracellular calcium, osmoregulation, brain development (particularly for cerebellum and retina cells), and in the enhancement of bile flow and cholesterol clearance by the liver.

Taurine may be conjugated to bile acids and secreted in the duodenum

Taurine is a derivative of the sulfur-containing (sulfhydryl) amino acid, cysteine. Taurine is one of the few known naturally occurring sulfonic acids.

Taurine levels were found to be significantly lower in vegans than in a control group on a standard American diet. Plasma taurine was 78% of control values, and urinary taurine 29%.[24]

Taurine is essential for cat health, as cats cannot synthesize the compound. The absence of taurine causes a cat's retina to slowly degenerate, causing eye problems and (eventually) irreversible blindness

Synthesis and production

Taurine in the pharmaceutical and lab setting is synthesized through a combination of cysteine, methionine and vitamin E.

It is naturally produced in many mammals.

Taurine is sometimes extracted from the intestines of cattle, many food industry sources, including the popular energy drink Red Bull,[9] make efforts to use synthesized sources that are vegetarian friendly.

Commercial Applications

Taurine is used in many different products. The most important uses are:

- In infant formula to make it similar to human milk: dairy milk is, as known, deficient in taurine.
- In a wide variety of different functional drinks, ranging from "smart drinks" in Europe to "energy tonics" in Asia often in conjunction with caffeine.
- In Asia also for the prevention of the side effects from excess alcohol consumption
- Taurine is added to pet food, especially cat food.

Taurine is used in cell culturing as a source of sulphur for growing tissue cells and organisms.

Price

Company	Unit	Price	Source	Reference	Source date
Sigma	1kg	$420	Non-animal	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/T4571	2008
Sigma	1kg	$632	Synthetic	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/T0625	2008
Sigma	10kg	$464	Non-animal	http://www.sigmaaldrich.com/catalog/search/ProductDetail/ALDRICH/W381306	2008

The retail price for Taurine powder is AUD $245 for 5 kg.
http://www.mfcd.net/store/product.asp?pID=707&cID=23

Market

In 1993, approximately 5,000–6,000 t of taurine was produced; 50% for pet food manufacture 50% in pharmaceutical applications.[39]

The market demand for taurine is likely to have increased substantially since 1993 as the energy drink industry has grown substantially in the last 15years.

In 1993 energy drinks were still being developed and the market was small, since then the market has grown substantially and is now worth over $10 billion worldwide.

http://www.nutraingredients.com/Consumer-Trends/Report-reveals-keys-to-energy-drink-success

The majority of the worlds' taurine is produced synthetically or from plant sources.

The market size for animal sourced taurine was not determined and the use of animal taurine in energy drinks or other human foods was not determined.

Vitronectin

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Vitronectin	Serum: 11.34kg	200-400 mg/L	Major cell adhesion glycoprotein; constituting 0.2 to 0.5% of total plasma proteins	phagocytosis of Streptococcus dysgalactiae in mastitis	Sigma, BioPur, EMD Bioscience, Innovative Research

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Vitronectin is an abundant glycoprotein found in blood plasma and the extracellular matrix of many tissues. Vitronectin serves to regulate proteolysis initiated by plasminogen activation. Additionally vitronectin is a component of platelets and is thus involved in hemostasis.

Vitronectin and fibronectin are the two major adhesive proteins in plasma and serum.

Vitronectin has been speculated to be involved in hemostasis and tumour malignancy.

Commercial Applications

- When used as a thin coating on tissue-culture surfaces, vitronectin is useful to promote cell attachment, spreading, proliferation, and differentiation of many normal and neoplastic cells, and to study cell migration.
- Medium additive for cells with integrin receptors that bind vitronectin; platelets, endothelial cells, melanoma cells, osteosarcoma.
- Vitronectin is also added to eye drop formulations for the treatment of corneal lesions.

Price

Company	Unit	Price	Source	Reference	Source date
Sigma	0.05mg	$336	Bovine plasma	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/V9881	2008
BioPur	0.2mg	$530	Bovine plasma	http://www.biopur.com/product_info.php?products_id=586	2008
EMD Biosciences	0.1mg	$351	Bovine plasma	http://www.merckbiosciences.com/product/681101	2008
Innovative Research	1mg	$2500	Bovine plasma	http://www.innov-research.com/vitronectinproductsanimal.htm	2008

World Market

- The market size for vitronectin was not found, it is likely that the market is too insignificant to have been quantified.
- Market size is likely to be small due to vitronectins' high price and lack of bulk commercial applications.
- Bovine vitronectin is the substitute for human sources when used in research, however it can be produced from almost all mammals.
- Human Vitronectin is used in eye formula, though the market for this application was not determined.
- Bovine Vitronectin would not be a viable economic enterprise on its own; however it could be produced in conjunction with other bovine blood products.

What product processing is involved?

Serum was obtained from plasma by adding calcium and then centrifuging. The heparin-binding activity of vitronectin in human serum was activated with 8 M urea. The activated vitronectin specifically bound to heparin-Sepharose in 8 M urea and was eluted with 0.5 M NaCl containing 8 M urea. This procedure resulted in an approximately 250-fold purification of vitronectin with a 15-30% recovery; 3-6 mg of pure vitronectin were obtained from 100 ml human plasma within 2 days (Yatohgo, T., M. Izumi, H. Kashiwagi, and M. Hayashi. 1988. Novel purification of vitronectin from human plasma by heparin affinity chromatography. Cell Struct. Funct. 13: 281-292 [Medline].) http://www.springerlink.com/content/n377625341041535/

Purity: 95%

Content: 200_g/ml http://www.springerlink.com/content/n377625341041535/

Yield: 60mg/L of plasma

3-6mg/100ml (Yatohgo, T., M. Izumi, H. Kashiwagi, and M. Hayashi. 1988. Novel purification of vitronectin from human plasma by heparin affinity chromatography. Cell Struct. Funct. 13: 281-292 [Medline].)

Coagulants

Factor V

Factor VIII

Factor IX

Factor X

Factor XIII

Fibrinogen

Prothrombin

Thrombin

Factor V

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Factor V	non-activated citrated bovine plasma: 11.34kg	2-5 mg/mL	Procofactor; activated by thrombin to form the active cofactor, factor Va	coagulation; diagnostics, bioconversions	MP Biologicals Inc; Haematological Technologies, Inc.;

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Factor V is involved in the blood coagulation cascade, specifically the formation of Factor Va during coagulation by thrombin via peptide bond cleavages. Factor V differs from many other Factors in the coagulation cascade, as it has no enzymatic activity, but acts as a cofactor.

Factor V can be found in two specific tissues, 80% from plasma and 20% from platelets. Plasma factor V is synthesised in the liver, and platelet Factor V originates from bone marrow.

Commercial applications

Factor V is most often utilised as a research tool, especially in ELISA and Western Blots.

Factor V has been used as a supplement for patients with the rare Factor V deficiency disease (1:1000000), the supplement is administered to control bleeding in surgical procedures such as tooth extraction .

Price

Company	Unit	Price	Source	Reference	Source date
MP Biologicals	0.1mg	$1076	Bovine plasma	http://www.mpbio.com/product_info.php?cPath=491_1_12&products_id=194928&depth=nested&keywords=Factor%20V	2008
MP Biologicals	0.05mg	$1046	Human Plasma	http://www.mpbio.com/product_info.php?cPath=491_1_12&products_id=194928&depth=nested&keywords=Factor%20V	2008

Market

The exact size of this market cannot be defined as specific data is not readily available to the public.

It has been determined in a number of cases that Factor V, extracted from different species, has reacted adversely in humans systems. This has been determined with horse, mouse and rabbit species. Factor V is currently only being utilised for research purposes.

Extraction/Processing Technologies

Factor V is obtained from bovine blood using column chromatographic techniques. Once derived the Factor is converted into a 50% (vol/vol) glycerol mixture, and ideally should be stored at -20°C .

Recommendations

- Factor V is an essential component in the blood coagulation pathway.
- Currently within the market Factor V is extracted from bovine plasma, by a number of research laboratories around the world.
- There appears to be only a limited potential for Factor V to be used as an extracted bioactive commercially, as the majority of the Factor V that is extracted is utilised for research purposes.
- As Factor V is used as a research tool, it proved a difficult to determine the exact amount that was in demand at any one time.
- The current market price for Factor V is quite high at $1076 for 0.1mg. This premium price suggests that the market demand is low.
- Although there is perhaps room in the market for another distributor of Factor V, it would be hard to penetrate a market where there appears to be little demand for the product.

Reference

1 Duga S., R. Asselta, M. L. Tenchini. 2003. Coagulation factor V. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=15147718&dopt=Citation

2 Duga S., R., et al.

3 Entrez PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=9467366&dopt=Abstract

4 Haematologic Technologies http://www.haemtech.com/Cofactors/Factor_V.htm

Factor VIII

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Factor VIII	non-activated citrated bovine plasma: 11.34kg	7 mg/mL plasma	Effective in accelerating the coagulation of hemophilic blood	coagulation diagnostics, bioconversions	Enzyme Research Laboratories; Vital Products, Inc;

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Factor VIII (FVIII) is an essential clotting factor. A deficiency of FVIII causes Hemophilia A, a bleeding disorder that occurs 1 in 5000 births.

Factor VIII participates in blood coagulation; it is a cofactor for factor IXa which, converts factor X to the activated form Xa.

Commercial Applications

FVIII concentrated from donated blood plasma, or alternatively recombinant FVIII can be given to hemophiliacs to restore haemostasis.

Bovine FVIII is never used for medical purposes as it is not compatible with the human immune system.

Price

- No price data for factor VIII found.
- No suppliers of bovine Factor VIII.

Market Size

The world market for human Factor VIII is worth approximately US $4.1 billion.

World demand for human factor VIII is 20 million liters at 200 IU/L. http://www.bbts.org.uk/PDFs/events/1100%20sat%20lomond%20Over.pdf

The market size for bovine factor VIII was not determined; it is likely to be very small as there are no suppliers and no substantial commercial applications for bovine factor VIII.

What product processing is involved?

The purification procedure involves BaSO4, kaolin, and bentonite adsorption to remove contaminants, ethanol, polyethylene glycol, and ß-alanine fractionation, calcium citrate-cellulose chromatography, concanavalin A precipitation, and an Agarose gel filtration step. The final product is homogeneous when examined by gel filtration, density gradient centrifugation, and zone electrophoresis (http://www.jbc.org/cgi/content/abstract/247/8/2512).

Factor IX

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Factor IX	Plasma: 11.34kg	5-10 mg/ mL	The zymogen factor IX is a single chain vitamin K-dependent glycoprotein; catalytic component of the "intrinsic factor Xase complex" (factor VIIIa/ IXa/Ca2+/phospholipid) proteolytically activates factor X to factor Xa.	coagulation; diagnostics, bioconversions	Haematological Technologies, Inc.; Innovative Research Laboratories;

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Factor IX enzyme is a step in the coagulation cascade. Factor IX primary function is to activate factor X to form factor Xa.It does this by hydrolysing one arginine-isoleucine bond in factor X thus forming factor Xa.

Deficiency of Factor IX causes haemophilia B a rare disorder that occurs in 1 in 25000 male births. Factor IX deficiency leads to an increased propensity for haemorrhage. This is in response to mild trauma or even spontaneously, such as in joints or muscles.

Commercial applications

Injections of human factor IX are used to treat haemophilia B.

Bovine factor IX is not used in medicine and it is only used in research.

Price

Company	Amount	Price	Source	Reference	Source date
Innovative Research	2.5mg	$954	Bovine plasma	http://www.innov-research.com/innovative/Literature/Catalog-2008.pdf	2008

Market Size

- The primary use of Factor IX is for treatment of Haemophilia B the size of this market was approximately $ 25 million in the U.S. (1996)
- The current worldwide demand for human Factor IX is approximately 2.5 million litres at 350IU/L.

  http://www.bbts.org.uk/PDFs/events/1100%20sat%20lomond%20Over.pdf
- Factor IX is primarily sourced from human blood donations and bovine factor IX is not used in the treatment of Haemophilia B.
- Bovine factor IX would be solely used for research purposes and there are very few vendors.

What product processing is involved?

Ammonium sulfate fractionation followed by heparin-agarose, DEAE-Sephadex, CM-cellulose, arginine-agarose, and benzamidine-agarose column chromatography.

Purity: 90.00%

Yield: 12.5 mg/L Blood (Plasma)

Content: 108.55 mg/L Bovine Blood Plasma

Factor X

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Factor X	Plasma: 11.34kg	10 mg/L	Serves as a key enzyme component of the prothrombinase complex responsible for the rapid conversion of prothrombin to thrombin.	anticoagulation; diagnostics, bioconversions	Sigma, Biopure, MP Biologicals, Pierce Biotechnology, Haematologic Technologies, Innovative Research.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Factor X enzyme is found in blood, as it forms a crucial role in the coagulation of blood to form clots.

Factor X is inactive until activated by other coagulation cascade enzymes principally factor IX and Factor VII.

Active Factor Xa (a = active) activates other coagulation enzymes. It acts by cleaving prothrombin in two places (an arg-thr and then an arg-ile bond), which yields the active thrombin.

Factor X is synthesised in the liver, then transported into the blood plasma.

Commercial applications

Use in Biochemistry

The Factor Xa protease can be used in biochemistry to cleave off protein tags that improve expression or purification of a protein of interest. Its preferred cleavage site (after the arginine in ile-glu/asp-gly-arg) can easily be engineered between a tag sequence and the protein of interest. After expression and purification, the tag is then proteolytically removed by Factor Xa.

Therapeutic use

Inborn deficiency of factor X is very uncommon (1:500,000) and treatment involves transfusions of human blood plasma.

Inhibitors

The majority of research that is associated Factor Xa is concentrated on the factors that inhibit its action; therefore preventing clotting.

There is such interest in this area, due to the complications that often occur after orthopaedic surgery. An effective inhibitor of Factor Xa, could dramatically decrease the number of deaths associated with orthopaedic surgeries.

Price

Company	Unit	Price	Source	Reference	Source date
MP Biomedicals	1mg	$6268	Bovine	http://www.mpbio.com/product_info.php?cPath=491_1_12&products_id=191396&depth=nested&keywords=factor%20Xa	2008
Haematologic Technologies	1mg	$420	Bovine	http://www.haemtech.com/Enzymes/Factor_Xa_B.htm	2008
Pierce	1mg	$1497	Bovine	http://www.piercenet.com/Products/Browse.cfm?fldID=02040704	2008
Innovative Research	1mg	$239	Bovine	http://www.innov-research.com/innovative/Literature/Catalog-2008.pdf	2008

Market Size

- Currently Factor X and factor Xa are being utilised in the market for research purposes.
- Some of the benefits associated with Factor Xa as a research tool is that it can act to remove histidine tags from fusion proteins, cleaves the last amino acid in the sequence Ile-Glu-Gly-Arg, and has an activity greater than 125U/mg .
- The process for extracting Factor Xa has already been defined, and therefore minimising the difficulty of developing a new extraction procedure.
- The current market price for Factor Xa is approximately AUS $200 for 0.1mg but the prices differ quite dramatically across the market.
- Factor X concentration in plasma is low (10mg/Litre) thus the isolation and purification procedure would be difficult.

Reference

1 Pierce, The Protein People: http://www.piercenet.com/Products/Browse.cfm?fldID=02040704

Factor XIII

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Factor XIII	non-activated citrated bovine plasma: 11.34kg	subunit a ~ 15 mg/L, subunit b ~ 21 mg/L	Transglutaminase, zymogen converted to the active enzyme, Factor XIIIa, by bovine thrombin;	diagnostics, bioconversion	Sigma Aldrich; Haematologic Technologies, Inc.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Factor XIII or fibrin stabilising factor is an enzyme of the blood coagulation system that crosslinks fibrin. Factor XIII is the protein responsible for stabilising the formation of a blood clot. In the absence of Factor XIII, a clot will still develop but it will remain unstable.

In humans with a deficiency of Factor XIII, the tenuously formed clot will eventually break down and cause recurrent bleeds.This condition is perhaps the rarest of all factor deficiencies. The incidence of Factor XIII deficiency is estimated at one in five million births.

Commercial Applications

- No commercial uses for Factor XIII were found.
- Any medical uses for Factor XIII would use human plasma as the source tissue.
- Human fresh-frozen plasma is used to treat factor XIII deficiency.

Price

- No retail suppliers of bovine or human factor VIII.

Market Size

- As there are no commercial applications for purified Factor XIII the market for the enzyme would be limited to research.
- The market size for Bovine Factor XIII would be limited to the scientific research market.
- The lack of commercial suppliers of bovine factor VIII suggests that there is no market demand for bovine factor VIII.

What product processing is involved?

Ammonium sulfate fractionation followed by heparin-agarose, DEAE-Sephadex, CM-cellulose, arginine-agarose, and benzamidine-agarose column chromatography.

Purity: 90%

Fibrinogen

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Fibrinogen	non-activated citrated bovine plasma: 11.34kg	~ 1 g/L	Composed of two sets of three polypeptide chains	Cell culture, microbial culture, tissue adhesion & numerous other "glue" applications	Sigma.; Innovative Research, Inc; Armour Laboratories, Selborne Biological Services,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Fibrinogen is the inactive precursor of fibrin. Fibrin is the principle protein involved in the clotting of blood. It is a fibrillar protein that is polymerised to form a "mesh" that forms a haemostatic plug or clot (in conjunction with platelets) over a wound site.

Processes in the coagulation cascade activate the inactive prothrombin to the protease thrombin, which is responsible for converting fibrinogen into fibrin. Fibrin is then cross linked by factor XIII to form a clot.

The amount of fibrinogen in the plasma can serve as a nonspecific indicator of whether or not an inflammatory process is present in the body. Fibrinogen from any mammalian source will be cleaved by thrombin from any mammalian source.

Role in disease

Excessive generation of fibrin due to activation of the coagulation cascade leads to thrombosis (clotting), while ineffective generation predisposes to haemorrhage (bleeding).

Commercial applications

Food/Nutrition

The thrombin: fibrinogen preparation is applied to meat where thrombin transforms fibrinogen to fibrin that interacts with collagen enabling the binding of meat pieces in re-constituted meat. It can also be applied on poultry, fish and seafood.

Medicine

Fibrinogen and fibrin can be made into a tissue sealant to stop excessive blood loss during surgery or traumatic injury. The product will replace current plasma derived sealants on the market.

An Australian Bioactive company, Bovogen Biologicals' supplies bovine fibrinogen. The company states that it is a premium grade product which is ideal for a variety of applications requiring a product of high protein purity in conjunction with excellent clottability and stability both on dry storage and following reconstitution. Pack Size: 1g – 10kg+

Applications include:

- As a reagent in Baird Parker microbiological media for Staphlococcus aureus identification
- As a reagent for routine blood clotting in Serology laboratories
- General research reagent
- Reagent in fibrin glue products

Price

Company	Amount	Price	Source	Reference	Source date
Innovative Research	25mg	$175	Bovine plasma	http://www.innov-research.com/fibrinogenproductsanimal.htm	2008
Sigma	25g	$1953	Bovine plasma	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/F8630	2008

Market

Plasma based fibrin sealants currently represent a world market of $968AUD million.

The market size for fibrinogen as an intermediate is estimated to be over $97AUS million.

Furthermore, a substantial shortage of human fibrinogen is anticipated because the market demand is expected to increase to 500 kg and more per year, requiring over 1 million litres of donor blood. Fibrin based tissue sealants are used during various surgical procedures and currently represent a world market of $968AUD million.

www.pharming.com/index.php?act=dl&file=Mid_year_report_2004.pdf

What product processing is involved?

Manufacturing process: Fibrinogen is produced from blood. It involves precipitation by adjusting the temperature. The blood is separated into blood cells and blood plasma. The plasma is frozen at -3°C for a maximum of 7 days and then melted at 0°C and centrifuged for the separation of fibrinogen. The level of fibrinogen is standardized to 5% (w/w)

Prothrombin

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Prothrombin	non-activated citrated bovine plasma: 11.34kg Liver 5.1kg	100 mg/mL	The mature single chain protein circulates in plasma as a zymogen and, during coagulation, is proteolytically activated to the potent serine protease _-thrombin.	coagulation	Haematological Technologies, Inc.; Aniara Corp.; Innov-ative Research, Sigma, American diagnostics.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Prothrombin is the inactive precursor of thrombin. Thrombin is produced by the enzymatic cleavage of two sites on prothrombin by activated Factor X (Xa).

Thrombin is a coagulation protein that has many effects in the coagulation cascade. It is a protease that converts soluble fibrinogen into insoluble strands of fibrin, as well as catalysing many other coagulation-related reactions.

Commercial applications

- Prothrombin's commercial applications revolve around its active form, thrombin. Prothrombin is likely to be more stable than thrombin and therefore easier to transport and store until use.
- An Australian company Bovogen Biologicals' sells crude prothrombin from Australian bovine blood plasma using salt precipitation. Once isolated by large scale centrifugation, the product is decanted into plastic pails and stored under frozen conditions. Pack Size: 1kg - 10kg+

Price

Company	Unit	Price	Source	Reference	Source date
American Diagnostica GMBH	2mg	N/A	Bovine	http://www.american-diagnostica.de/Prothrombin_-_Protei.206.0.html?&L=1	2008
Innovative Research	100mg	$1074	Bovine	http://www.innov-research.com/innovative/Literature/Catalog-2008.pdf	2008

Market

As there is a bulk supplier of crude prothrombin from bovine plasma it is likely that there is a market for the product.

The exact size of this market and the final commercial applications for bovine prothrombin were unknown at the time of writing.

What product processing is involved?

Blood fractionation, precipitation, prothrombin extracted by dilute calcium bicarbonate, activation of prothrombin by sodium citrate (25%), prothrombinase or bovine lung thromboplastin to yield thrombin.

Barium citrate absorption, EDTA elution, citrate buffer, DEAE and heparin-Sepharose column chromatography. http://login.american-diagnostica.de/fileadmin/user_upload/datasheets/417bov.pdf

Purity: ≥95%

Thrombin

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Thrombin	non-activated citrated bovine plasma: 11.34kg	100 mg/mL	The mature single chain protein circulates in plasma as a zymogen and, during coagulation, is proteolytically activated to the potent serine protease _-thrombin.	coagulation, veterinary uses, diagnostics	Haematological Technologies; Sigma; Innov-ative Research, Selborne Biologicals Services., Biopur; Bio Pharm Labs

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Thrombin is a coagulation protein that has many effects in the coagulation cascade. It converts soluble fibrinogen into insoluble strands of fibrin, as well as catalysing many other coagulation-related reactions.

Biological function

Thrombin converts fibrinogen to an active form that assembles into fibrin, which forms a blood clot. Thrombin also activates factor XI, factor V, and factor VIII. This positive feedback accelerates the production of thrombin.

Commercial applications

Medicine

Surgeons use bovine-derived thrombin to stop minor bleeding during surgery and to release growth factors from concentrated autologous platelets (platelet gels) inserted into wound sites to accelerate healing of bone and tissue.

However, bovine-derived thrombin can create cross-reactive anti-bovine antibodies that cause allergic reaction and inhibit blood clotting in humans, which can cause post-operative hemorrhage in a subsequent operation.

Research

- Thrombin is used by many diagnostic companies to defibrinate human plasma, producing serum matrixes to be used as controls and standards.
- Thrombin is also used to cleave r-fusion proteins containing a thrombin site for removal of affinity tags as well as certain diagnostic pharmaceutical applications.

Price

Company	Size	Price	Source	Reference	Source date
Bio Pharm Labs	700mg	$2760	Bovine plasma	http://www.bovinethrombin.com/	2008
Sigma	1mg	$436	Bovine plasma	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIAL/T6634	2008
BioPur	2mg	$336	Bovine plasma	http://www.biopur.com/product_info.php?products_id=470	2008

Market Size

- The current thrombin market, estimated at $180 million per year (2006), is dominated by thrombin sourced from bovine blood. (thermogenesis)
- Bayer reported that it sold $500 million of thrombin in European and U.S markets. (Forbes 2008).
- Bayer is a major pharmaceutical company however it is not the sole supplier of thrombin to the world market and the report did not include sales outside of the U.S and Europe.
- The majority of these sales were in the medical industry as thrombin is used as a coagulant in surgery.
- The sales for bovine plasma thrombin make up a substantial proportion of this market, as bovine thrombin has been used for over 30 years in medical procedures.
- The development of recombinant human thrombin may eventually replace bovine thrombin as the main source of thrombin for medical procedures.
- The market growth for thrombin is expected to increase by 15% per annum, so it is likely that there will always be a market for both sources of thrombin especially with the development of advanced wound healing materials and sealants.

What product processing is involved?

Blood fractioning, precipitation, prothrombin extracted by dilute calcium bicarbonate, activation of prothrombin by sodium citrate (25%) or bovine lung thromboplastin to yield thrombin. Thrombin is then purified by ion exchange chromatography and stabilized.

References

(Haemacure Corporation Annual General Meeting April 26, 2005)

Total sales of Thrombin-JMI were $220.6million in 2005 (biopharma book)

2005 = $190 M (US only) (Haemacure Corporation Annual General Meeting April 26, 2005)

by 2008 >$400 million potential worldwide

http://www.thermogenesis.com/newsroom/pdf/042106%20AMSECT%20Release%204-20%20kms%20_2_%20FINAL.pdf

http://www.forbes.com/afxnewslimited/feeds/afx/2008/09/02/afx5377228.html

Glucoseaminoglycans

Chondroitin and Dermatan Sulphate

Chondroitin Sulphate

Heparan Sulphates

Hyaluronic Acid

Hyaluronidase

Keratan Sulphate

Chondroitin and Dermatan Sulphate

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Chondroitin A	Trachea: 2.71kg	~ 1.25 -15 g/Kg depending on source and process	Stimulates chondrocyte synthesis of proteoglycans, especially in aggrecans and numerous other modes of action.	Dietary supplements, antifibtolyic/throbolytic agent and numerous other applications, including separation and manufacturing aids	Numerous suppliers
Chondroitin B (Dermatan)	Intestinal Mucosa: N/A Skin: 31.8kg	~ 1.25 -15 g/Kg depending on source and process	Numerous modes of action	Dietary supplements, antifibtolyic/throbolytic agent and numerous other applications, including separation and manufacturing aids	Numerous suppliers
Chondroitin C	Achilles tendon: 0.19kg	~ 300-600 mg/ Kg depending on process	Numerous modes of action	Dietary supplements, antifibtolyic/throbolytic agent and numerous other applications, including separation and manufacturing aids	Numerous suppliers
Chondroitin D	Bone: 72.8kg	~ 100 mg/ Kg	Numerous modes of action	Dietary supplements, antifibtolyic/throbolytic agent and numerous other applications, including Neuroregatory roles	Numerous suppliers
Chondroitin E	Joint cartilage: N/A	~ 100 mg/ Kg	Numerous modes of action	Use in treatment of collagen fibril diseases; Inhibitor of viral infections	Numerous suppliers

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Chondroitin sulphate is a large polymer molecule produced by the body to provide support, flexibility and resistance to pressure. Chondroitin sulphate belongs to a group of structural carbohydrate polymers called glycosaminoglycans (GAG).

Chondroitin sulphate is most abundant in the skin, cartilage, tendons and connective tissues of animals where it provides much of the resistance to compression. There are 5 types of chondroitin sulphate (A to E) that are found in different parts of the body and are chemically different. Chondroitin sulphate is not only a structural support protein as it can also act as a chemical signalling molecule regulating inflammation.

Chondroitin sulphate has many different pharmaceutical, dietary supplement, cosmetic and veterinary uses.

In the pharmaceutical industry it is used in a wide variety of anti-rheumatic and anti-inflammatory products. It is also used in a range of different ophthalmic products ranging from eye drops to surgical solutions.

In the dietary supplement industry, chondroitin sulphate has found various uses ranging from the treatment of arthritis to the preservation of eyesight. It is used in both capsules and functional drinks.

The major cosmetic use of chondroitin sulphate is in skin creams. Chondroitin sulphate holds up to 30 times its weight in water, an ability which gives it very good moisturising properties.

Structure and Types

Chondroitin sulphate belongs to a group of natural polymers found in the body called glycosaminoglycans (GAG). There are 5 types of chondroitin sulphate (A, C, D, and E) with chondroitin sulphate B now known as Dermatan sulphate.

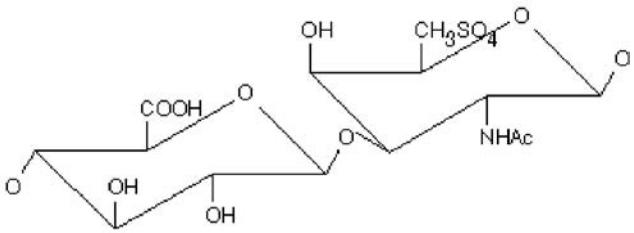
Chondroitin sulphate chains are unbranched polysaccharides of variable length containing two alternating monosaccharides: D-glucuronic acid (GlcA) and N-acetyl-D-galactosamine (GalNAc).

Some GlcA residues are switched into L-iduronic acid (IdoA); the resulting disaccharide is then referred to as dermatan sulphate.

Chondroitin Sulphate - A

GlcUA-GalNAc-6S

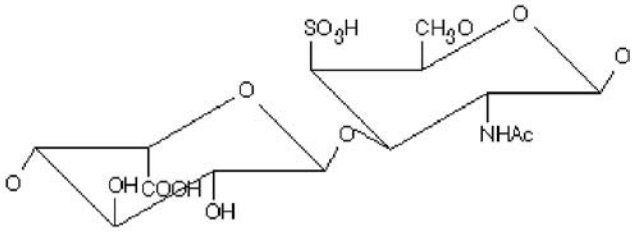
Chondroitin sulphate A is the alternative name for chondroitin 6-sulphate i.e chondroitin sulphate which is sulphated on the C6 position of the GlcNAc.



Chondroitin Sulphate - B / Dermatan sulphate

IdoUA-GalNAc-4S

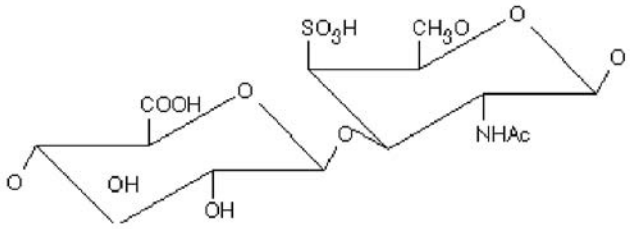
Chondroitin sulphate B is the alternative name for dermatan sulphate. It is sulphated on the C4 position of GlcNAc but the C5 of the uronic acid has undergone epimerisation to Iduronic acid. Note the difference between this molecule and chondroitin 4-sulphate below.



Chondroitin Sulphate - C

GlcUA-GalNAc-4S

Chondroitin sulphate C is the alternative name for chondroitin 4-sulphate i.e CS which is sulphated on the C4 position of the GlcNAc.



Chondroitin sulfate E refers to CS predominantly sulphated at carbons 4 and 6 of the GalNAc sugar (chondroitin-4,6-sulfate).

Terminology

Chondroitin sulphate was originally isolated well before the structure was characterised, leading to changes in terminology with time. Early researchers identified different fractions of the substance with letters.

Letter identification	Site of sulfation	Systematic name
Chondroitin sulphate A	carbon 4 of the N-acetylgalactosamine (GalNAc) sugar	chondroitin-4-sulfate
Chondroitin sulphate C	carbon 6 of the GalNAc sugar	chondroitin-6-sulfate
Chondroitin sulphate D	carbon 2 of the glucuronic acid and 6 of the GalNAc sugar	chondroitin-2,6-sulfate
Chondroitin sulphate E	carbons 4 and 6 of the GalNAc sugar	chondroitin-4,6-sulfate

Function

Chondroitins' functions largely depend on the properties of the overall proteoglycan (protein chain) of which it is a part. These functions can be broadly divided into structural and regulatory roles. However, this division is not absolute and some proteoglycans have both structural and regulatory roles

Structural

Chondroitin sulphate is a major component of extracellular matrix (the "filler" substance existing between cells in an organism), and is important in maintaining the structural integrity of the tissue.

Chondroitin sulphate is a major constituent of cartilage, providing structure, holding water and nutrients, and allowing other molecules to move through cartilage. The latter is an important property, as there is no blood supply to cartilage.

The tightly packed and highly charged sulphate groups of chondroitin sulphate generate electrostatic repulsion that provides much of the resistance of cartilage to compression. Loss of chondroitin sulphate from the cartilage is a major cause of osteoarthritis.

Regulatory

Chondroitin sulphate readily interacts with proteins in the extracellular matrix due to its negative charges. These interactions are important for regulating a diverse array of cellular activities. The lecticans are a major part of the brain extracellular matrix, where the chondroitin sugar chains function to stabilise normal brain synapses. The levels of chondroitin sulphate proteoglycans are vastly increased after injury to the central nervous system where they act to prevent regeneration of damaged nerve endings. Although these functions are not as well characterised as those of heparan sulphate, new roles continue to be discovered for the chondroitin sulphate proteoglycans.

Medical use

Chondroitin is an ingredient found commonly in dietary supplements used as an alternative medicine to treat osteoarthritis and also approved and regulated as a symptomatic slow-acting drug for this disease in Europe and some other countries. It is commonly sold together with glucosamine. Chondroitin and glucosamine are also used in veterinary medicine for similar reasons.

Chondroitin sulphate has been shown, in numerous double-blind trials, to relieve symptoms and possibly slow the progression of, or reverse, osteoarthritis.6

Wound Repair

Dermatan sulphate is the predominant glycoaminoglycan expressed in the skin and levels of dermatan sulphate expression have been shown to increase markedly during wound repair.

Anti-coagulant

Dermatan sulphate has anti-coagulative properties that are similar to those elicited by heparin that may make it a useful agent for the preventative and therapeutic treatment of blood clotting.

Dermatan sulphate could therefore be used as an alternative to aspirin for preventative therapy for thrombosis and as a blood-thinning agent without the risk of the gastrointestinal side effects commonly associated with aspirin.

Mechanisms of Action

The benefit of chondroitin sulphate in patients with osteoarthritis is likely the result of a number of effects including its anti-inflammatory activity, the stimulation of the synthesis of proteoglycans and hyaluronic acid, and the decrease in catabolic activity (breakdown) of cartilage cells inhibiting the synthesis of protein digesting enzymes, nitric oxide and other substances that contribute to damage cartilage matrix and cause death of joint cartilage cells.

The rationale behind the use of chondroitin sulphate is based on the belief that osteoarthritis is associated with a local deficiency in some natural substances, including chondroitin sulphate.

Price

Company	Unit	Price	Source	Reference	Source date
Sigma	100mg	$294	Porcine intestinal mucosa (dermatan)	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C3788	2008
Sigma	5000mg	$73	Bovine trachea CS-A	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C9819	2008
Sigma	5000mg	$369	Bovine Cartilage	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C6737	2008
Sigma	5000mg	$339	Shark cartilage	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C4384	2008

The significantly higher price of dermatan sulphate compared to chondroitin sulphate is due to the different source tissues. Dermatan sulphate is extracted from the intestinal mucosa of animals (in this case pigs), whilst chondroitin sulphate is extracted from trachea and cartilage of animals. Intestinal mucosa makes up a small percentage of total animal weight and requires further processing and extracting compared to cartilage and trachea.

Global market size

Please note that estimated figures have been extrapolated for Chondroitin Sulphate as a whole and have not been disseminated into chondroitin subgroups as this information was unavailable.

Global market is estimated at 3000 tonnes per annum and is worth approximately $5billion. The majority of the worlds' chondroitin is sourced from shark cartilage, bovine cartilage, tendon, bone, trachea and chicken cartilage.

Chondroitin sulphate price per kilogram ranges from $65 to $150 for the higher quality material. (http://www.nutraingredients-usa.com/Industry/Cargill-to-expand-chondroitin-manufacturing)

The Chinese provide 85-90% of the market. The leading producer, Yantai Kangde Biochemical Products produces 30 metric tons of Chondroitin per month.

Forecast for global annual growth is 5% to 7%. Demand is likely to spike in the next 10-20 years as the ageing populations of developed countries tackle degenerative diseases like arthritis. In the UK alone there were approximately 7 million people reported to have long-term health problems associated with arthritis. Around 206 million working days were lost in the UK in 1999-2000, equal to £18 billion ($45 billion Aus) of lost productivity.

San Diego-based *Nutrition Business Journal* put the US for chondroitin and sulphate at $810m (€553m) in 2005. The majority of the world's chondroitin and glucosamine is sourced from China.

The HA market is growing at 15 per cent in the US

French supplier, Diana Naturals, Antrain, France, recently launched a joint health formulation under the Phytonutriance ChondrActiv brand.

The patented ingredient contains a combination of collagen type-2, chondroitin sulfate and HA and is extracted from chicken cartilage.

A clinical trial involving 37 people with "*joint issues*" showed ChondrActiv decreased the consumption of non-steroidal anti-inflammatory drugs (NSAID's) by 73 per cent, while mobility and quality of life was improved.

Growth

Forecast for global annual growth is 5% to 7% annually[1].

Major global players

Retailers

- Nature's own
- Nature's Origin
- LifeWise Naturals
- Cargill Health and Food Technologies
- The Health Company
- Nutra-Life
- Blackmores

Supply

Tissue source

There are two main sources from which Chondroitin may be derived;

- Cattle tracheal cartilage
- Cattle nasal septum

Global supplier and market share

The Chinese provide 85-90% of the market. The leading producer in Asia for Chondroitin Sulfate is Yantai Kangde Biochemical Products Co, LTD.

Kangde produces a capacity of 30 metric tons of Chondroitin per month, and their quality has been highly evaluated by customers

Dr Bruce Lee, director of the Food Futures Flagship, said that the sources to be investigated include grape skins, olive leaves, cartilage and cow hides.

"For example, dermatan sulphate which is extracted from cow hides is reported to have anti-inflammatory properties and inhibit the formation of blood clots," he said.

According to a new report diabetic nephropathy represents a multi-billion-dollar market that is poorly served by existing drugs. All this is set to change as Palosuran, currently in clinical development, could become the first drug in a new class for a condition that may affect as much as 140 million people.

As such, with a large and expanding market the competitive environment is relatively benign.

KRX-101 (suledoxide) is a mixture of two glycosaminoglycans: 80 per cent heparin and 20 per cent dermatan sulphate[2].

Approximately seven million people in the UK alone are reported to have long-term health problems associated with arthritis. Around 206 million working days were lost in the UK in 1999-2000, equal to £18 billion (€26 billion) of lost productivity[3].

Batches of shark cartilage capsules originating from the United States could contain salmonella, the UK Food Standards Agency has warned.

Chondroitin sulphate, one of the most popular supplements for joint health, is extracted from animal cartilage like shark cartilage. The supplement is usually sold in combination with glucosamine and, according to the Nutrition Business Journal, US sales for these combined supplements were $810m (€132m) in 2005[4].

New Zealand's Waitaki Biosciences has launched a bovine collagen ingredient for the growing cosmeceutical and joint health markets.

The ingredient Coll2, rich in biologically active type II collagen and glycosamino glycan (GAG), is prepared by a patented process and is intended for both supplements and functional foods.

"We are extremely excited to launch Coll2 at this year's Vitafoods exhibition in Geneva," said Crag McIntosh, CEO, Waitaki Biosciences. *"Scientific trials have demonstrated the effectiveness of Coll2 both when used to decrease wrinkles and to reduce skin inflammation - key cosmeceutical applications."*

A recent study by Kline & Company valued the global market for what it terms 'nutricosmetics',

supplements aimed at outward appearance, at $1bn. The company forecasts that the market is set to double over the next five years. To date, the trend has been more marked in Europe and Japan, with the North American market not catching on at the same pace.

The collagen ingredient is sourced purely in New Zealand and contains between 55 and 65 per cent type II collagen and 12 to 15 per cent GAG, mostly in the form of chondroitin sulphate.

Steve Caulton, Waitaki's regional export manager for North America and Europe, told NutraIngredients.com in that while other type II collagens are available to formulators, the Waitaki ingredient has the advantage of being sourced in New Zealand - a BSE-free country[5].

References

1 http://www.otd.ou.edu/spinoff/index.html

2 http://www.nutraingredients.com/Industry/Fenchem-affirms-non-GMO-joint-health-status

3 http://www.nutraingredients.com/Research/Glucosamine-ineffective-for-hip-osteoarthritis-study

4 http://www.nutraingredients.com/Industry/Salmonella-fears-for-shark-cartilage

5 http://www.nutraingredients.com/Industry/Waitaki-targets-inner-beauty-with-new-collagen-ingredient

Collagen

Bioactive compound	Source (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Wet Tissue Weight (kg)	Supplier
Collagen, Type 1	Calf dermis, tendons and the organic part of bone.	Collagen I-VI are the main connective tissue proteins in animals and the most abundant protein in mammals, making up about 40% of the total. Depending on tissue source different collagens can be obtained	Most abundant type of collagen, major structural protein in connective tissue; Type I to Type XXVII collagens have been characterised and classified depending on the collagen chain type generating the tropocollagen fibril/structure	Major applications in tissue augmentation and wound healing, food & beverage/ wine industry -- gelatin, processing aid, adhesives, glues; cosmetics; medical and pharmaceutical industries, e.g. sutures, capsules, cell culture; dermal augmentation for cosmetic purposes	?+0.19 +72.8	Biodesign International; USB Corp., , Rockland Immunochemical Inc; US Biologicals; Koken Industries; Kamiya Biomedical Company; Raybiotech, Inc.;
Collagen, Type II	calf joint cartilage	See Above	Structural protein in articular cartilage tissue	Major applications in food industry -- gelatin, adhesives, glues; cosmetics; medical and pharmaceutical industries, e.g. sutures, capsules, cell culture	?	Numerous Suppliers
Collagen, Type III	calf skin	See Above	This is the collagen of granulation tissue, and is produced quickly by young fibroblasts before the tougher type I collagen is synthesised	See Above	9.54	Numerous Suppliers

Collagen, Type IV	calf eyes	See Above	Structural protein of the basal lamina; eye lens	See Above	0.1	Numerous Suppliers
Collagen, Type V	most connective tissue; placental villi	See Above	Associated with type I	Major applications in capsules, cell culture, wound healing	?+?	Numerous Suppliers
Collagen, Type VI	calf connective tissue, heart cartilage	See Above	Most interstitial tissue, assoc. with type I	Major applications in capsules, cell culture	?+?	Numerous Suppliers

**Assume 300kg HSCW. See Appendix A for other HSCW*

Collagen is the main protein of connective tissue in animals and the most abundant protein in mammals, making up about 50% of the whole-body protein content.

It is one of the main constituents of skin, bones, tendons, cartilage, and ligaments, forming fibres that bind together and strengthen these tissues.

Tough bundles of collagen called collagen fibres are a major component of the extracellular matrix that supports most tissues and gives cells structure from the outside, but collagen is also found inside certain cells.

Collagen has great tensile strength, and is the main component of connective tissue, cartilage, ligaments, tendons, bone and skin.

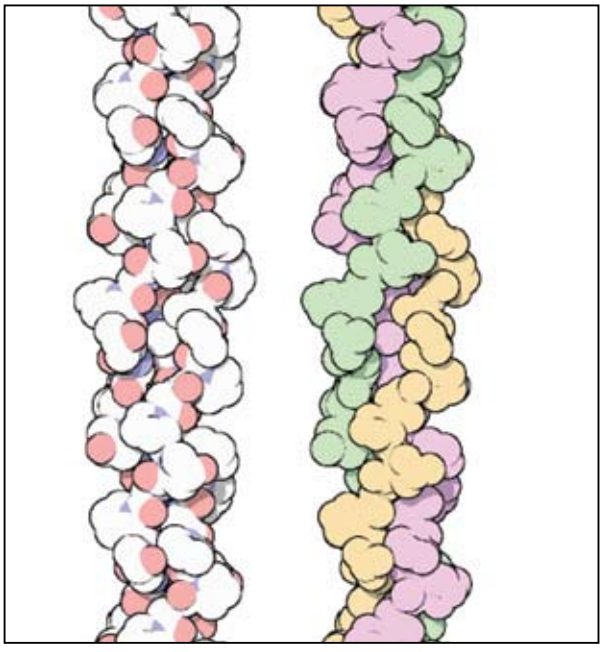
Along with soft keratin, it is responsible for skin strength and elasticity, and its degradation leads to wrinkles that accompany aging.

It strengthens blood vessels and plays a role in tissue development. It is present in the cornea and lens of the eye in crystalline form.

There are several diseases that affect collagen synthesis and degradation. The most commonly know disease involving collagen is scurvy. Deprivation of vitamin C interferes with a step in collagen synthesis; the resulting bleeding, bruising, and poor healing are part of the picture of scurvy.

Conformation and structure

Collagen structure is complex. Its conformation can be considered at the monomeric level (individual) collagen molecules and/or at its aggregate level, how the monomers are arranged i.e. their packing structure (fibrils, networks, etc).



Types

Collagen occurs in many places throughout the body. There are more then 28 types of collagen described in literature. Over 90% of the collagen in the body, however, are of type I, II, III, and IV.

- Collagen One - skin, tendon, vascular, ligature, organs, bone
- Collagen Two - cartilage
- Collagen Three – connective tissue and skin commonly found alongside type I.
- Collagen Four - forms bases of cell basement membrane in eyes
- Collagen Five – connective tissue
- Collagen Six – connective tissue and heart cartilage

Commercial applications

Collagen has numerous commercial applications and it is used in food, medicine, cosmetic and pharmaceutical industries. Collagen or gelatine has been used by humans for over 8000 years as it was used as glue and was produced from the hides and tendons of animals.

Gelatine

Gelatine is made from collagen by-products from the meat and leather industry. The extracted collagen is partially broken down using acids or boiling water. The complex collagen helix separates into separate strands which form random coils that cool and form gelatine.

Gelatine is produced from cow hides, pig skins and bones from the meat industry. Every year over 300, 000 tonnes of gelatine are used worldwide www. gelatine.org/en, the primary use of gelatine is in foods however it is also used in the pharmaceutical industry and photography.

Edible

Gelatine is used extensively in the food industry as a gelling agent, stabiliser, thickener, texturiser and volumiser. Gelatine is added to so many products as it has very little taste and is low in calories.

Gelatine is also used extensively in the beverage industry as a clarifier. Isinglass is a form of gelatine from fish bladders that is used to clarify beers and wines. Although isinglass is currently produced from fish bladders there is no reason why it cannot be produced from bovine sources. The annual sales of isinglass in the Australian beer and wine industry were calculated to be worth approximately $3.6 million[4].

Technical uses

- Gelatine typically constitutes the shells of pharmaceutical capsules in order to make them easier to swallow.
- Animal glues such as hide glue are essentially unrefined gelatine.
- It is used to hold silver halide crystals in an emulsion in virtually all photographic films and photographic papers. Despite some efforts, no suitable substitutes with the stability and low cost of gelatine have been found.
- Used as a carrier, coating or separating agent for other substances, it, for example, makes beta-carotene water-soluble, thus imparting a yellow colour to any soft drinks containing beta-carotene.

Other uses

- Blocks of ballistic gelatine simulate muscle tissue as a standardized medium for testing firearms ammunition as seen on Mythbusters.
- Gelatine is used to make the shells of paintballs, similar to the way pharmaceutical capsules are produced.

Collagen

Medical uses

Collagen has been widely used in cosmetic surgery, as a healing aid for burn patients for reconstruction of bone and a wide variety of dental, orthopaedic and surgical purposes.

The attractiveness of collagen as a biomaterial rests largely on the fact that it is a natural material of low immunogenicity and is therefore considered as a normal constituent rather than as foreign matter by the body.

Immunogenicity relates to the capacity of the material to elicit the production of antibodies in animals or humans. Nearly all studies on collagen have shown that it has very low or no immunogenicity.

Of the 10 collagen types that have been characterised, types I, III and V are the most desirable for biomedical applications because of their high biocompatibility and low immunogenicity.

Most medical collagen is derived from bovine calves from certified BSE free animals. Most manufacturers use donor animals from either “closed herds”, or from countries which have never had a reported case of BSE such as Australia and New Zealand.

Collagen Injections

Collagen injections are a common medical procedure and are primarily carried out to reduce the signs of aging. Collagen is injected to give support and structure to skin, bones, ligaments, and other body parts. The primary source of collagen used for these procedures is obtained from bovine sources.

Collagen Haemostat

Collagen haemostat is an absorbable topical haemostatic (stops bleeding) agent prepared from purified bovine corium collagen and shredded into fibrils. When in contact with a bleeding surface, collagen haemostat attracts platelets which adhere to its fibrils and undergo the release phenomenon. This triggers aggregation of the platelets into thrombi in the interstices of the fibrous mass, initiating the formation of a physiologic platelet plug. The common brand names for haemostats are Avitene®; Avitene® Flour; Avitene® Ultrafoam; Avitene® UltraWrap™; EndoAvitene®; Helistat®; Helitene®; Instat™; Instat™ MCH; SyringeAvitene™.

Value

The worldwide market for collagen as an intermediate in medical devices and aesthetic products is estimated at AUD$500 million[3]. An alternate source estimates global demand for collagen in cosmetic products to reach at least US$170m in 2007[2].

Interestingly, for a breakdown of the US Collagen market, one report from Frost & Sullivan quotes that the US Collagen market for Biomedical and Research uses, 46% of the market is dermal implants, 35% is wound care products and 14% is urinary incontinence implants. The remaining 5% of the market consists of products such as corneal shields and cell/drug delivery vehicles[1].

Growth

The Wound Care market has been used within this report as an indication of market growth. Wound Care is one of the fastest growing markets for collagen-based medical products and is currently worth over US$1.8 billion. The industry is expected to grow at a rate of 10% annually to reach US$3.1 billion by 2011.

Colltech estimates the collagen sector of the cosmetic market to grow to US 1.1 billion in 2009 .

Cosmetics and supplements

Hydrolysed collagen is used in many cosmetics and hair products. Collagen is added to counteract the degradation of the collagen found naturally in the skin.

Collagen is also commonly sold as a dietary supplement. Nutritional supplements containing hydrolysed collagen are typically marketed for osteoporosis, osteoarthritis, rheumatoid arthritis, weight loss, and to assist in recovery from exercise and sports-related injuries, although scientific data in these areas is sparse.

Collagen Price

The following figures were obtained from a number of sources. It appears that price varies greatly with regard to the purification level.

Grade	Quantity	Price AUD$
Cosmetic grade collagen	1 kilogram	$5,000 to $10,000[1]
Research grade	1 kilogram	$27,250 – $82.25[10] [12]
Higher Quality Research grade	1 gram	$624 000 - $10,900 [11]

Global Market Size

Sales of collagen-based products in medical devices, cosmetics and pharmaceutical product are estimated to be US$2 - $3 billion worldwide. 5-10% of this amount is spent on acquiring raw collagen materials.

http://www.ilsi.org.il/companies_life_science_company.asp?ID=936

Other

Recently there has been an emergence of synthetic and recombinant collagens. These have been developed in response to concerns regarding the use of bovine collagens in medical, pharmaceutical and cosmetic products. Although these products are marginally safer than animal sourced collagens they are extremely difficult to produce and cost considerably more than current sources.

Collagen Retail prices

Type 1

Company	Amount	Price	Source	Reference	Source date
Sigma	100mg	$238	Rat tail	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C3867	2008
Worthington Biochem	10000mg	$80	Bovine	http://www.worthington-biochem.com/CL/pl.html	2008

Type 2

Company	Amount	Price	Source	Reference	Source date
Alexis (90%)	5mg	$318	Bovine	http://www.axxora.com/	2008
Sigma	10mg	$744	Bovine	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C4486	2008
Rockland	0.5mg	$225	Bovine	http://www.rockland-inc.com/ccp11514-bovine-collagen-type-ii-28nasal-cartilage29-001-001-104-001-001-104.htm	2008
EMD Biosciences	1mg	$394	Bovine	http://www.emdbiosciences.com/product/234184	2008
GeneTex Inc	0.5mg	$350	Bovine	http://www.genetex.com/commerce/catalog/product.jsp?product_id=1380	2008

Type 3

Company	Amount	Price	Source	Reference	Source date
Rockland	0.5mg	$190	Bovine	http://www.ihcworld.com/mall/Proteins%7CPeptides-Protein-C/c3_82/p171087/Bovine-Collagen-Type-III/product_info.html	2008

Type 4

Company	Amount	Price	Source	Reference	Source date
Rockland	0.5mg	$210	Bovine	http://www.rockland-inc.com/ccc1170-collagen-proteins.htm	2008

Type 5

Company	Amount	Price	Source	Reference	Source date
Abcam	0.5mg	$360	Bovine	http://www.abcam.com/index.html?datasheet=7530	2008
US Bio	0.5mg	$370	Bovine	http://www.usbio.net/Product.aspx?prodSku=C7510-56G	2008
Rockland	0.5mg	$230	Bovine	http://www.rockland-inc.com/ccp11517-bovine-collagen-type-v-001-001-107-001-001-107.htm	2008

Type 6

Company	Amount	Price	Source	Reference	Source date
Rockland	0.5mg	$210	Bovine (Placenta)	http://www.rockland-inc.com/ccc1170-collagen-proteins.htm	2008

Reference

1 http://www.colltech.com.au/assets/18/20061020CAU.pdf

2 CollTech Australia Ltd (2007), BioInternational Convention [Online] Available: http://bio2007.bdmetrics.com/portal/ViewCompany.aspx?id=2992957

3 The New Pharming (2004), Company Mid Year Report [Online] Available: HYPERLINK “http://www.pharming.com/index.php?act=dl&file=Mid_year_report_2004.pdf” www.pharming.com/index.php?act=dl&file=Mid_year_report_2004.pdf

4 Project M218: Collagen Utilisation, MLA 1997

Heparan sulphates

Bioactive compound	Tissue Source & Weight	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Heparan sulphates	Intestinal mucosa: N/A Brain: 0.34kg Lung: 2.85kg Liver: 5.1kg kidney:1.2kg	150-400 mg/Kg	Complex linear sulphated polysaccharides present on the cell surfaces and in the extracellular matrix of most mammalian cells, normally attached to core proteins to form heparan sulphate proteoglycans (HSPGs). Involved in cell migration, growth and differentiation, morphogenesis, immune response, inflammation,	Cell culture, diagnostics, tissue repair, nutraceuticals, pharmaceuticals, slow release agent, cell separation and medical devices	Neoparin Inc.; Sigma Seikagaku America Inc, Vitaflex Inc;

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Heparan sulphate is a complex sugar produced by all cells in the body, including cells in the liver. It is related to the anti-coagulant, heparin. Heparan sulphate is also present in the extracellular matrix; the biological 'glue' that binds cells together into tissues.

Heparan sulphate regulates a wide variety of biological activities, including developmental processes: blood vessel growth, blood coagulation, tumour growth, regulation of thrombosis, growth factor signalling, cell proliferation, adhesion and mobility.

HS structure and differences from heparin

Heparan sulphate is a member of the glycosaminoglycan family of carbohydrates and is very closely related in structure to heparin. Both consist of a variably sulfated repeating disaccharide unit. The main disaccharide units that occur in heparan sulfate and heparin are shown below.

The most common disaccharide unit within heparan sulphate is composed of a glucuronic acid (GlcA) linked to N-acetylglucosamine (GlcNAc) typically making up around 50% of the total disaccharide units. Compare this to heparin where IdoA(2S)-GlcNS(6S) makes up 85% of heparins from beef lung and about 75% of those from porcine intestinal mucosa. Problems arise when defining hybrid GAGs that contain both 'heparin-like' and 'HS-like' structures. It has been suggested that a GAG should qualify as heparin only if its content of N-sulphate groups largely exceeds that of N-acetyl groups and the concentration of O-sulphate groups exceeds those of N-sulphate.

Commercial Applications

The market applications for heparan sulphate are limited to use as research reagents, specifically the analysis of complex carbohydrates.

Heparan sulphate has also been found to reduce fat and cholesterol levels in the liver, though the research is in its early stage and human trials are many years away.

Price

Company	Source	Amount	Price	Reference	Source date
Sigma Aldrich	Bovine kidney	10mg	$930	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/H7640	2008
Sigma Aldrich	Porcine intestinal mucosa	5mg	$431	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/H9902	2008
Neoparin Inc.	N/A	1mg	$200	http://www.heparinoids.com/catalog.htm	2008

Market Size

The market size for heparan sulphate is unknown, but as it has no known commercial applications other that research it is likely that the market is very small.

Opportunity potential: possible "research reagent"

Summary of Commercial Potential for Australian Beef Industry:

Strengths – Currently manufactured from bovine kidney

Weaknesses – Though related to the pharmaceutical heparin there are no major applications for bovine heparan sulphate other than research reagent

Opportunities – Supply to fine chemical companies, potential for bovine intestine (based on murine concentrations) to have 10 fold higher heparan sulphate than current commercial source (kidney)

Threats – no particular reason for market growth

Concentration in Bovine Tissue

Bovine liver

Bovine liver (2.5kg)	
Product	Yield
Purified Heparan Sulphates	22 mg

María J. Hernáiza, Hyun-Ok Yanga, b, Nur Sibel Gunaya, Toshihiko Toidac and Robert J. Linhardt, 'Purification and characterization of heparan sulfate peptidoglycan from bovine liver', Carbohydrate Polymers, Volume 48, Issue 2, 1 May 2002, Pages 153-160

Bovine brain

Bovine brain	
Product	Yield
Purified Heparan Sulphates	3.4ug/g

Y Park, G Yu, N S Gunay, and R J Linhardt, 'Purification and characterization of heparan sulphate proteoglycan from bovine brain', Biochem J. 1999 December 15; 344(Pt 3): 723–730 Bovine lung, heart, kidney, spleen, intestine, skin tissue – Assumption: similar order of magnitude concentration in bovines

Heparan sulphates from murine tissue	
Tissue	Yield (mg/g)
Lung	0.037
Heart	0.025
Kidney	0.018
Spleen	0.027
Intestine	0.22
Skin tissue	0.30

Mohamad Warda et al, 'Isolation and characterization of heparan sulfate from various murine tissues', Glycoconj J (2006) 23:555–563

Reference

1 http://www.anst.uu.se/pje13912/hs.html

2 http://www.sigmaaldrich.com/sigma/general%20information/carbohydrate_analysis_bf8.pdf

Hyaluronic Acid

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Wet Tissue Weight (kg)	Supplier
Hyaluronic Acid	Eyes: 0.05kg Joint fluids: N/A	10 g /Kg depending on source	Negatively charged properties result in the mutual precipitation of cationic proteins such as albumin at low pH	Cell culture, cosmetic, nutraceuticals regen-erative medicine and numerous other uses	?	US Biologicals, Calzyme Labs Inc and numerous other vendors

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Hyaluronic acid is a chain protein glycosaminoglycan (GAG) distributed widely throughout connective tissues, surface cells, the nervous system and the eye.

Hyaluronic acid is a major component of joint fluid as it increases the viscosity of fluids and it is one of the fluid's main lubricating components.

Hyaluronic acid is also a major component of skin, where it is involved in tissue repair especially after sunburn.

While it is abundant in extracellular matrices, hyaluronic acid also contributes to tissue hydrodynamics (water regulation), movement and proliferation of cells, and participates in a number of cell surface receptor interactions

The Hyaluronic acid Industry

Biomedical products

Hyaluronic acid is naturally found in many tissues of the body, such as skin, cartilage, and the vitreous humour of the eye. It is therefore well suited to biomedical applications targeting these tissues. The first hyaluronic acid biomedical product, Healon, was developed in the 1980s for use in eye surgery. Healon is still used today and it is produced from avian sources most probably rooster combs.

Hyaluronic acid has also been used in the synthesis of biological scaffolds for wound healing applications. These scaffolds typically have proteins such as fibronectin attached to the hyaluronic acid to facilitate cell migration into the wound.

Hyaluronic acid is also used to treat osteoarthritis of the knee. HA treatments are administered as a course of injections into the knee joint and are believed to supplement the viscosity of the joint fluid, thereby lubricating the joint, cushioning the joint, and producing an analgesic effect.

Hyaluronic acid is used in cosmetic surgery as a dermal filler. Hyaluronic acid is injected under the skin to temporarily smooth wrinkles by adding volume under the skin, with effects typically lasting for six months. Other common dermal fillers are collagen and botox. In 2003 the FDA approved hyaluronic acid injections for filling soft tissue defects such as facial wrinkles. Restylane is a common trade name for the product.

The majority of hyaluronic acid based biomedical products are produced from HA that is synthesised by Streptococci bacteria. Animal sourced HA is less common as it is more expensive and is more immunogenic.

Medical Use Summary

Intra-articular injection: Treatment of pain in osteoarthritis in knee in patients who have failed nonpharmacologic treatment and simple analgesics

Intradermal: Correction of moderate-to-severe facial wrinkles or folds

Ophthalmic: Surgical aid in cataract extraction, intraocular implantation, corneal transplant, glaucoma filtration, and retinal attachment surgery

Topical: Management of skin ulcers and wounds

Cosmetic applications

Hyaluronic acid is also used in anti wrinkle creams and moisturisers and is sold under the brand name Hyasol-BT. Hyaluronic acid is added to anti aging creams to “help reduce spider veins, deeply hydrates and plumps fine lines in any climate”. There is no proof for or against these claims and the effectiveness of any anti aging creams is difficult to determine. The amount and source of Hyaluronic acid was not stated in any product.

Price

Company	Amount	Price	Source	Reference	Source date
Calzyme	1mg	$1.20	Rooster comb	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1065	2008
Worthington	100mg	$348	Bovine eye	http://www.worthington-biochem.com/vhha/cat.html	2008
Sigma	50mg	$530	Bovine eye	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/FLUKA;53728	2008
Sigma	1000mg	$578	Rooster comb	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/FLUKA;53728	2008
Sigma	10000mg	$432	Streptococcus equi	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/FLUKA;53728	2008

Based upon the prices stated by Sigma it is assumed that bovine eye HA is difficult to obtain or produce. Bovine HA is 20 times more expensive than rooster HA and 200 times more expensive than microbial HA.

Global Market

Worldwide market for hyaluronic acid was $563.9million in 2001. The development of new medical, cosmeceutical and neutraceutical products has doubled the current worldwide market for HA to over $1 billion.(Widner et al. 2005; Chong et al. 2005) (Kogan, Soltes et al. 2007).

Hyaluronic acid is worth approximately $US 100 000 per kilogram, though this price is likely to fall as the world market expands[1].

The majority of the hyaluronic acid used commercially is used in cosmetic surgery as a dermal filler. Hyaluronic acid for cosmetic surgery is sold under the trade names Restylane and Perlane, both these sources use non-animal hyaluronic acid. These products are primarily sold in the U.S and Japan.

Market Share:

- U.S. = 41.8%
- Europe = 11.2%
- Japan = 47%

Restylane and Perlane from microbial sources had sales of $129million[2].

The market for hyaluronic acid is expected to grow 15% annually as consumers in developing countries fight the signs and symptoms of aging.

The market for bovine hyaluronic acid was unattainable as there appears to be no bovine sourced products on the market. There are no clear reasons for this but it is hypothesised that bovine hyaluronic acid is either too expensive to produce or is not suitable for medical products. If bovine hyaluronic acid could be produced cheaply and could be used in medical products there would diffidently be a market for it.

Reference

1 http://www.cheque.uq.edu.au/research/bioengineering/research/Metabolic_Engineering/HA.html

2 http://www.pharmaceutical-business-review.com/article_feature.asp?guid=B402C979-6E01-4C08-90FB-08961D23CD85

Hyaluronidase

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Hyaluronidase	Testis: 0.9kg	~25 mg/Kg	hydrolysis of endo-N-acetylhexosaminic bonds of hyaluronic acid and chondroitin sulfate A and C (but not B)	Ophthalmic surgery; Enzymatic processing	Worthington Biochemical Calzyme Labs Sigma Aldrich

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Hyaluronidase is an enzyme that breaks down hyaluronic acid. Hyaluronidase is found where ever hyaluronic acid is found, for example; joint fluid, the eye and skin.

Commercial applications

Hyaluronidase is used in medicine to increase the effectiveness of local anaesthesia or increase the absorption or distribution of injected drugs.

Hyaluronidase is effective as it modifies the permeability of connective tissue through hydrolysis of hyaluronic acid, one of the chief ingredients of tissue cement which offers resistance to diffusion of liquids through tissues; hyaluronidase increases both the distribution and absorption of locally injected substances.

Hyaluronidase is also used in medicine to treat hyaluronic acid overdoses.

Price

Company	Amount	Price	Source	Reference	Source date
Worthington	1000mg	$120	Bovine testes	http://www.worthington-biochem.com/HSE/cat.html	2008
Sigma	5000mg	$567	Bovine testes	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/H3757	2008
Calzyme	1000mg	$20	Bovine testes	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1066	2008

Market Size

Estimated U.S market size for hyaluronidase is US$750million and is broken up into 3 main categories[1]

a) Spreading agent US$50mil

b) Eye fluid bleeding US$200 mil

c) Diabetic retinopathy US$500mil

Brand names and anima source of hyaluronidase used in medicine include:

1. Hydase (bovine)

2. Vitrase (ovine)

3. Amphadase (bovine)

4. Hylenex (human recombinant)

Hyaluronidase is also produced from sheep testes and Streptomyces hyalurolyticus. The price per gram is equal to that of bovine hyaluronidase and there appears to be no difference in activity or potential uses.

Reference

1 http://library.corporate-ir.net/library/12/121/121179/items/190755/CIBC_ISTA_Presentation040406_1030%20am.pdf

Keratan Sulphate

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Keratan sulphate	Trachea: 2.7kg Eye Cornea: N/A Joint cartilage: N/A	~2.2 g/Kg	Glycosaminoglycan consisting of repeating disaccharide units composed of alternating residues of D-galactose and N-acetyl-D-glucosamine linked b-(1-4) and b-(1-3),	Veterinary therapeutic applications, diagnostics; food additives, nutraceuticals	Seikagaku Products Ltd; AMS Biotechnology Ltd

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Keratan sulfate is any of several sulfated glycosaminoglycans (structural carbohydrates) that have been found especially in the cornea, cartilage, and bone.

Keratan sulfates are large, highly hydrated molecules which in joints can act as a cushion to absorb mechanical shock.

The proportion of keratan sulphate in articular cartilage is about 5-20% of the total glycosaminoglycan content [Bjelle, 1975], and changes in the amount and structure occur during adolescent development [Zirn et al, 1984; Thonar et al, 1986; Brown et al, 1998].

Glucosamine is the monomer unit that makes up the keratan sulphate polymer.

Current and Future Applications

At this point in time there appears to be no commercial suppliers or users of keratan sulphate, however as it is a glycosaminoglycan found in joint cartilage, it is possible that this could be marketed in conjunction with glucosamine or chondroitin sulphate.

Global Market

- The world market for Keratan Sulphate is assumed to currently be small and is likely limited to research.
- If Keratan sulphate were to be sold/marketed as a joint cartilage supplement like glucosamine or chondroitin sulphate market the demand could grow substantially.

What product processing is involved?

Mechanical separation, acid digestion and acetone extraction.

Purity: 95% - Lower for cosmetic purposes.

Hydrolytic Enzymes

Amylase

Carboxypeptidase A

Carboxypeptidase B

Chymotrypsin

Chymotrypsinogen

DNAse 1

Elastase

Lipase

Lipoprotein Lipase

Pepsin

Pepsinogen

Ribonuclease A

Ribonuclease B/C &D

Trypsin Inhibitor

Trypsin

Trypsinogen

Amylase

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Use	Supplier
Amylase	Pancreas: 0.43kg Salivary glands: N/A	2-4 mg/Kg	Polysaccharide hydrolysing enzymes, tannin binding	Calzyme, Biopure, Worthington Biochemical Corp

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Amylase is an enzyme that breaks starch down into sugar. In animals amylase is present in saliva, where it begins the chemical process of digestion. The pancreas also makes amylase (alpha amylase) to break down dietary starch into di- and trisaccharides which are converted by other enzymes to glucose to supply the body with energy.

Amylase is produced by fungi and bacteria to break down starch in products like bread and biscuits. Amylase is also abundant in germinating grains and cereals as it breaks down the starch reserves contained in them.

Commercial applications

- Amylase enzymes are used extensively in bread making to break down complex sugars such as starch (found in flour) into simple sugars. Yeast then feed on these simple sugars and converts it into the waste products of alcohol and CO2.
- While Amylase enzymes are found naturally in yeast cells, it takes time for the yeast to produce enough of these enzymes to break down significant quantities of starch in the bread.
- Modern bread making techniques have included amylase enzymes (often in the form of malted barley) into bread improver thereby making the bread making process faster and more practical for commercial use.
- Bacterial amylase is also used in detergents to dissolve starches from fabrics.
- Amylase is used for the liquidizing process of glucose, cerealose, alcohol, beer, monosodium glutamate, wine, distillate spirits and antibiotic industry, as well as the desizing process of textile industry.

Price

- Commodity amylase costs between $6 and $25/kg. The price is based upon the quality and the activity of the enzyme.http://www.agbios.com/docroot/articles/05-266-001.pdf
- Industrial amylase is produced from microbial sources as it is much cheaper and easier to produce.

Company	Unit	Price	Source	Reference:	Source date
Calzyme	10mg	$14	Porcine pancreas	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1003	2008
Calzyme	1mg	$720	Human pancreas	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1003	2008
Biopure	0.5mg	$2000	Human pancreas	http://www.biopur.com/product_info.php?products_id=487	2008

**Assume 300kg HSCW. See Appendix A for other HSCW*

Market Size

- $ 200million. Underlying assumption:
  - o Among the specific types of industrial enzymes, protease and amylase lead the market with current shares of 25% and 20%, respectively.
  - o Industrial enzyme market = $1,100million[1]
- Market dominated by non animal varieties as extraction and purification are much simpler.
- Bovine amylase suppliers were not found.
- Only potential use of bovine amylase is in research.

Reference

1 http://www.bccresearch.com/editors/RC-147U.html

Carboxypeptidase A

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Carboxypeptidase A	Pancreas: 0.43kg	~50 mg/Kg	Acts preferentially, but not exclusively, on peptide bonds adjacent to the C-terminal aromatic amino acid residues.	Reagent and inter alia protease bioreactor manufacturing	Sigma; Calzyme Labs Inc.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Carboxypeptidase A is a protein digestive enzyme that is produced by animals and microbes.

In animals carboxypeptidase A helps break down proteins that have already been digested by the digestive enzyme chymotrypsin.

Carboxypeptidase A like most carboxypeptidases is initially produced in an inactive form; this precursor form is referred to as a procarboxypeptidase. In the case of pancreatic carboxypeptidase A, the inactive zymogen form, pro-carboxypeptidase A, is converted to its active form - carboxypeptidase A - by the enzyme enteropeptidase. This mechanism ensures that the cells wherein pro-carboxypeptidase A is produced are not themselves digested.

Carboxypeptidase A is an exopeptidase (an enzyme that breaks the last peptide off a protein chain and the attacks the next peptide and so on).

Commercial Applications

Carboxypeptidase A is found in digestive aid supplements.

Carboxypeptidase A from the fungus Aspergillus niger, can be used to accelerate the ripening of cheeses.

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	1mg	$3.33	Bovine Pancreas	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C9268	2008

Market

There were few suppliers of carboxypeptidase A, indicating that there is limited demand for the enzyme.

Any bulk commercial applications involving Carboxypeptidase A would use microbial sources of the enzyme as it is cheaper and easier to produce in bulk.

Carboxypeptidase B

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Use	Supplier
Carboxypeptidase B	Pancreas: 0.43kg	~50 mg/Kg	Hydrolysis at C-terminal basic lysine residues	Worthington Biochemicals Corp, Calzyme Labs Inc. Sigma, Roche

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Carboxypeptidase B (CPB) is a pancreatic exopeptidase which catalyses the hydrolysis of the peptide bonds of the basic amino acids lysine, arginine and ornithine. Because of its high specificity for C-terminal basic amino acids it has found wide use in end-group analysis for sequence determination

Carboxypeptidase B is involved in the degradation of polypeptides in the digestive system. Carboxypeptidase B degrades proteins that have already been degraded by the digestive enzyme trypsin.

Commercial applications

Carboxypeptidase B is used in a number of applications, primarily for research purposes.

- Catalysing the hydrolysis of the basic amino acids L-Lys and L-Arg from the C-terminal position in polypeptides.
- Possible marker for pancreatitis
- End-group analysis for sequence determination and further degradation of products of trypsin digestion.
- Preparation of antibodies.
- The enzymatic decomposition of proinsulin, along with trypsin, in their immobilised forms, in the production of recombinant human insulin.

Recombinant carboxypeptidase B is used for human applications and porcine carboxypeptidase B and rabbit carboxypeptidase B are used predominantly for research applications.

There appears to be very little usage of the bovine carboxypeptidase B and much of the information gathered refers to porcine carboxypeptidase B.

Price

Company	Units	Cost ($US)	Source	Reference	Source date
Worthington	50 mg	$415	Porcine	http://www.worthington-biochem.com/COA/default.html	2008
Sigma	5mg	$196	Porcine	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage?Query=Carboxypeptidase&Scope=NameSearch	2008
Biopur	25mg	$440	Porcine	http://www.biocompare.com/ProductDetails/483454/Carboxypeptidase-B.html	2008
Roche	5mg	$163	Porcine	http://www.biocompare.com/ProductDetails/483454/Carboxypeptidase-B.html	2008
Calzyme	1mg	$3	Porcine	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1036	2008

Market size

Information regarding the market size of carboxypeptidase B is difficult to locate as it is a single compound in a large product line of compounds within the product range of each supplying company. It appears the market size is not significant enough for it to have been quantified.

Competitors

Products

- The recombinant carboxypeptidase B is a major competitor with the potential bovine carboxypeptidase B as it has broader applications in human health, is safer and is a satisfactory substitute.
- Other competing products include porcine carboxypeptidase B and rabbit carboxypeptidase B (used primarily in the production of antibodies).

Other

- Porcine and Bovine carboxypeptidase B seem to be virtually identical[1], however porcine carboxypeptidase has a stronger position in the marketplace and is used almost exclusively over the bovine equivalent.

Recommendations

- The market for bovine carboxypeptidase B is at present virtually non-existent primarily due to competing porcine carboxypeptidase B and recombinant carboxypeptidase B.
- Through the information gathered from this preliminary market analysis we have determined that although there exists no readily available market gap there is potential to establish a market for bovine carboxypeptidase B if the Australian beef industry can break into the existing porcine market.
- This approach may be better executed when the Australian beef industry has established themselves as a reputable provider of bovine bioactives.
- Currently there exist several suppliers, retailers and distributors of porcine carboxypeptidase B.
- The majority of retailers and distributors source carboxypeptidase B from US suppliers, produce the product in the USA and then export to distributors in Australia. Consequently MLA may be able to derive benefit from establishing relationships with either retailers or distributors, however in light of the relatively small and sporadic nature of this particular market it is advisable to attempt to gain a place in the global market as opposed to the local one.
- Obtaining the chromatically purified grade of carboxypeptidase B most commonly accepted is a relatively simple extraction process and it may be possible for producers to undertake these operations themselves.
- However, the potential revenue, although not quantified, does not appear to merit the associated initial production costs on this product alone. Alternatively this particular strategy may have value if isolation and purification facilities were already in existence.

Reference

1 Moshe M. WERBER, Moshe MOLDOVAN, Mordechai SOKOLOVSKY (1975) Modification of Arginyl Residues in Porcine Carboxypeptidase B European Journal of Biochemistry 53 (1), 207–216.

http://www.blackwell-synergy.com/doi/abs/10.1111/j.1432-1033.1975.tb04059.x (April 17th)

Chymotrypsin

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Use	Supplier
Chymotrypsin	Pancreas: 0.43kg	~500-750 mg/ Kg depending on final quality/ crystallinity	Numerous biocatalytic/ biotransformational roles	Sigma, Abd Serotec, Calzyme

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Chymotrypsin is a digestive enzyme that can perform proteolysis (breakdown of proteins).

Chymotrypsin, as a hydrolase type of enzyme (which means it adds a water molecule during the breakdown process) acts by catalysing the hydrolysis of peptide bonds of proteins in the small intestine. It is selective for peptide bonds with aromatic or large hydrophobic side chains on the carboxyl side of this bond. Chymotrypsin also catalyses the hydrolysis of ester bonds.

It is produced by the pancreas as an inactive form called chymotrypsinogen that becomes activated in the small intestine.

Commercial applications

Generally, the primary uses of chymotrypsin are as a digestive aid and as an anti-inflammatory agent.

Chymotrypsin, along with the other pancreatic enzymes, is most often used in the treatment of pancreatic insufficiency.

- Chymotrypsin is used in dietary supplements to help digestion and metabolic function; it is retailed under the names “Medizyme, Digestion Super Enzyme and Peptizyme.”
- Chymotrypsin is used in research for protein sequencing
- Treat inflammation and reduce swelling
- Treat arthritis and other autoimmune diseases.
- Treat ulcerations and abscesses.
- Liquefy mucus secretions.
- Treat worms and other parasites in the digestive tract.

Preparations

Chymotrypsin is produced from porcine and bovine pancreas. It can be taken orally, topically, or by injection (by injection only by a physician in severe life-threatening situations), but is commonly taken orally in tablet form.

Usually chymotrypsin is included in a combination with other enzymes. A typical formulation may include: chymotrypsin (0.5–1 mg), bromelain (a plant protease) (25–45 mg), pancreatin (a mixture of many pancreatic enzymes) (100 mg), papain (a plant protease similar in action to chymotrypsin) (25–60 mg), and trypsin (a pancreatic protease) (24 mg). The price for these supplements range from $7-$25 depending upon the quality and the amount of tablets.

Price

Company	Units	Cost ($US)	Source	Reference	Source date
Sigma	1mg	$2340	Bovine pancreas	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C6423 Note: highly purified for use in protein sequencing	2008
AbD Serotec	100 mg	$120	Bovine pancreas	http://www.serotec.com/asp/datasheet.asp?code=2100-0202	2008
Calzyme	1000mg	$45	Bovine pancreas	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1042	2008

Market Size

- The digestion supplement market is growing substantially each year, especially in the developing economies of Asia.
- Worthington Biochemical a research reagent supplier reported that they sold around 5kg of highly purified Chymotrypsin worldwide.
- Based upon this value and the size of the dietary supplement market it is estimated that around 1000kg of Chymotrypsin is sold in purified and tablet form worldwide each year.

Competition

- The majority of Chymotrypsin is sourced from bovine and porcine pancreas.
- There is no significant difference between the two sources.

Chymotrypsinogen

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Chymotrypsinogen A/B	Pancreas: 0.43kg	500-750 mg/Kg depending on final quality/ crystallinity	Zymogens of chymotrypsin isoenzymes; molecule is inactive and must be cleaved by trypsin, precursor of chymotrypsin	Numerous biocatalytic/ biotransformational roles	Worthington Biochemicals Corp, Sigma

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Chymotrypsinogen is the inactive precursor of the digestive enzyme chymotrypsin.

This molecule is inactive and must be cleaved by trypsin, and then by other chymotrypsin molecules before it can reach its full activity. Chymotrypsin is an enzyme responsible for the break down of proteins into amino acids.

The active site of the chymotrypsinogen is covered by a six-amino-acid-long mask. It is only when this mask is removed - when it enters the lumen of the intestine and comes into contact with chymotrypsin molecules - that the enzyme becomes active.

This is a very useful safety feature for a protein-digesting enzyme. If it were not inactivated in this way, it would digest the pancreatic cells upon synthesis.

Commercial Applications

- Chymotrypsin is used in dietary supplements to help digestion and metabolic function; it is retailed under the names “Medizyme, Digestion Super Enzyme and Peptizyme.”
- Chymotrypsinogen may have better potential as a digestive enzyme in the dietary supplement market compared to Chymotrypsin, because Chymotrypsinogen is activated where it is needed, in the intestine.
- There were no dietary supplements found containing Chymotrypsinogen.

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	5000mg	$404	Bovine pancreas	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C4879	2008
Worthington	5000mg	$180	Bovine pancreas	http://www.worthington-biochem.com/CGC/cat.html	2008

Market Size

- As Chymotrypsinogen is not used as a dietary supplement its market size is significantly smaller than that of Chymotrypsin.
- Chymotrypsinogen would primarily be used for research purposes.

Other

- The potential for Chymotrypsinogen to be used as a dietary supplement may not have been explored.
- The quoted price from research reagent supply companies for purified bovine Chymotrypsinogen is quite cheap.
- This suggests that the extraction and purification process is simple and inexpensive.

DNAse 1

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
DNase 1	Pancreas: 0.43kg and other tissues	~ 1g/Kg	DNase I is a versatile enzyme that nonspecifically cleaves DNA to release 5'-phosphorylated di-, tri-, and oligonucleotide products	DNAse I has become an indispensable reagent in modern molecular biology research and diagnostic methods	US Biologicals; Ambion, Worthington Biochemicals

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

DNase 1 is an endonuclease, which splits phosphodiester linkages preferentially adjacent to a pyrimidine nucleotide yielding polynucleotides with free hydroxyl group at the 3' position and phosphate group at the 5' position[1].

There are four deoxyribonucleases of beef pancreas: A, B, C, and D. They are glycoproteins differing from each other either in carbohydrate side-chain or polypeptide component. DNase A, also known as DNase 1, is the predominant form.

DNase 1 has historically been prepared from bovine pancreas[2] and is also found in trace amounts in most other bovine tissues.

Biological Function

DNase 1 has a role in digestion, particularly DNA degradation in apoptosis and DNA clearance from extra cellular media

Grades/Uses

There are different grades of DNase 1 available depending on use and supplier, some common ones include:

1. Molecular Biology Grade: Chromatographically purified to remove RNase and protease.
2. Tissue Culture Grade: to digest DNA from damaged cells thereby reducing viscosity, and removing membrane bound DNA fragments.
3. Amplification Grade: Low RNase activity[3]

Uses for bovine DNase 1 include: PCR Cloning, Molecular Biology, Tissue Culture.

Uses for recombinant DNase 1 include: pharmaceutical products (Fibrinolysin - drug for the relief of cystic fibrosis symptoms), as a debriding agent (to liquefy pus in order to aid in the removal of necrotic debris from skin surfaces), insulin extraction and HIV gene therapy. Majority of human therapeutic uses utilise recombinant DNase 1

Market

DNase 1 is used extensively in research laboratories and Universities. Without access to resources such as market research reports it is a difficult market to categorize in terms of value and size.

It is likely to have an annual market size ranging from $10-100 million based upon its extensive use and the retail prices reported.

Units

Due to competition within the DNase market, each supplier utilises different product units each of which has different parameters of temperature and pH. These parameters determine the compounds effectiveness and as such there's a distinct lack of uniformity in the volumes and prices of which DNase is sold in.

Price

Company	Unit	Price	Source	Reference	Source date
Sigma	2.5mg	$131	Bovine	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/D4263	2008
Worthington	1000mg	$780	Bovine	http://www.worthington-biochem.com/dnase/cat.html	2008
Calzyme	1mg	$100	Bovine	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1044	2008
Calzyme	1000mg	$60	Bovine	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1044	2008

Growth

Specific Information regarding the growth of the Market for DNase 1 specifically could not be located. Growth of this particular market may very well be dependant on the scientific research industry which has grown approximately 7% over the last 2 years.

Competitors

Products

Recombinant DNase (rDNase) is derived from cloned bovine DNase. It has a distinct advantage over bovine prepared DNase (bpDNase) as it eliminates the risk associated with transmission of bovine spongiform encephalopathy to humans and avoids regulatory restrictions regarding this area of safety[4].

Ambion's pricing of rDNase and bovine derived DNase were compared to ascertain cost differences. The rDNase (1000 U: $149) is slightly more expensive than bpDNase (2000 U: $273)[5]. This information suggests rDNase has benefits primarily in the use of Human therapeutics and as such competes with bpDNase in this market.

Extraction

Bovine pancreatic juice contains DNases that are chromatographically indistinguishable from the A, B, and C components extracted from the tissue. This makes the extraction of DNase 1 (A) slightly more difficult than if it weren't the case[6].

Recommendations

From information gained in this market analysis it seems that although there is a market for Dnase1 there are also multiple suppliers that are able to meet the demand for this product whose reputations and expertise place them in a strong position in maintaining their established market share, both in Australia and globally.

Furthermore it appears that the supply demands for this market are also being met and that a number of the suppliers are more than capable of carrying out their own extraction techniques.

The companies various distribution channels are well established and the increasing use of DNase 1 in human therapeutics does not offer larger market potential as this area of demand is being met with the production of recombinant DNase 1. In terms of value adding to the Australian Red Meat Industry, the supply of DNase 1 does not appear to offer much potential.

Reference

1 Worthington 2006, http://www.worthington-biochem.com/DNASE/cat.html

2 Ambion, 2006, http://www.ambion.com/catalog/ProdGrp.html?fkProdGrp=263

3 Worthington 2006, http://www.worthington-biochem.com/DNASE/cat.htmlWei-Jung, C. et al, 2004

4 http://www.ambion.com/catalog/CatNum.php?AM2235

5 http://www.ambion.com/catalog/CatNum.php?AM2235

6 http://www.jbc.org/cgi/reprint/245/21/5685.pdf#search=%22deoxyribonuclease%20isolation%20from%20bovine%20pancreatic%20juice%22

Elastase

Bioactive compound	Source (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Elastase	White Blood cells: N/A Lung: 2.85kg Serum: 11.34kg Pancreas: 0.43kg and other sources	pancreatic form ~ 200 mg/Kg	Neutral protease that breaks down elastin and outer membrane proteins. Exists in two forms depending on source.	Elastin degradation, Research, Digestive	Sigma, Worthington Biochemical Corp, Calzyme Labs Inc.,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

In molecular biology, elastase is an enzyme from the class of proteases, or peptidases, that break down proteins. Elastase breaks down various proteins however it is most effective against elastin. Elastin is an elastic fibre protein commonly found in the lungs, skin and cartilage.

There are two different types of elastase depending upon the production source and purpose.

1) Pancreatic elastase, for the digestion of elastin in food.
2) Neutrophil elastase, produced by inflammation cells, for the breakdown of pathogens.

Commercial applications

Pancreatic elastase breaks down elastin, an elastic fibre that, together with collagen, determines the mechanical properties of connective tissue. Pancreatic elastase is available as a digestive aid and is sold under the brand names Megazyme and Medi-zyme.

Elastase is also used in the production of leather and other animal fibres.

Research applications

Because elastin in found in highest concentrations in the elastic fibres of connective tissues, elastase is frequently used to dissociate tissues which contain extensive intercellular fibre networks. For this purpose, it is usually used with other enzymes such as collagenase, trypsin, and chymotrypsin. Elastase is the enzyme of choice for the isolation of Type II cells from the lung.

Medical applications

Neutrophil elastase breaks down the outer membrane protein of E. coli and other Gram-negative bacteria that are often causes of disease.

Elastase inhibitors are used in medicine in cases of elastase over activity, like non-healing wounds, even though elastase is designed to kill bacteria in an infected wound and help clean out tissue that is devitalised. In non-healing wounds there is an excess of elastase, more than 20 times the normal level, the elastase then degrades the skin surround the wound keeping it open. To heal these wounds elastase inhibitor has to be applied to let the normal healing process take place.

Price

Company	Unit	Price	Source	Reference	Source date
Worthington Biochemical	1000mg	$924	Porcine pancreas	http://www.worthington-biochem.com/ES/pl.html	2008
Sigma	250mg	$1820	Porcine pancreas	http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/45125	2008
Calzyme	1000mg	$250	Porcine pancreas	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1047	2008

Market Size

The market size for elastase was not determined as elastase is often used in conjunction with other proteases. The primary source of elastase is porcine pancreas and there were no retailers of bovine elastase, the reason for this was not determined.

The potential for bovine pancreas elastase to become a supplier to the market was not determined.

Lipase

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Lipase	Pancreas: 0.43kg and many other tissues	Varies according to type as well as source	Hydrolyzes ester linkages between triglyceride residues, employing a chymotrypsin-like hydrolysis mechanism.	Enzymatic bioconversion delipidation processes, food processing,	Sigma, Calzyme, numerous other vendors

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Lipases perform essential roles in the digestion, transport and processing of dietary lipids (e.g. triglycerides, fats, oils) in most- if not all- living organisms.

There are three main types of lipase found in nature, Pancreatic lipase, lysosomal lipase and microbial lipase.

Pancreatic lipase is an enzyme necessary for the absorption and digestion of nutrients in the intestines. This digestive enzyme is responsible for breaking down lipids (fats), in particular triglycerides, which are fatty substances in the body that come from fat in the diet. Once broken down into smaller components, triglycerides are more easily absorbed in the intestines. Lipase is primarily produced in the pancreas but is also produced in the mouth and stomach.

Lysosomal lipase is produced in the cellular organelle called the lysosome. The lysosome is a compartment of a cell where digestion takes place. Lysosomal lipase breaks down lipids for energy and to convert them into new lipids. Lipase is also used to digest the cell membranes of bacteria and other pathogens.

Fungi and bacteria secrete lipases to facilitate nutrient absorption from the external medium (or in examples of pathogenic microbes, to promote invasion of a new host).

As biological membranes are integral to living cells and are largely composed of lipids, lipases play important roles in cell biology.

Commercial Applications

Lipases from fungi and bacteria serve important roles in human practices as ancient as yogurt and cheese fermentation. However, lipases are also being exploited as cheap and versatile catalysts to degrade lipids in more modern applications.

Biotechnology companies use recombinant lipase enzymes in applications such as baking, laundry detergents and even as biocatalysts in alternative energy strategies to convert vegetable oil into fuel.

Cosmetic

Emollient in personal care products such as skin and sun-tan creams, bath oils etc

Nutrition

Lipase supplements are thought to help the body absorb food more easily, keeping nutrients at appropriate, healthy levels throughout the body

Medicine

Lipase is used by clinicians to treat food allergies, cystic fibrosis, and autoimmune disorders, such as rheumatoid arthritis and lupus

Price

Company	Unit	Price	Source	Reference	Source date
Sigma	1mg	$946	Bovine milk	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/L2254	2008
Sigma	1mg	$1.50	Candida rugosa	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;L2254	2008
Calzyme	1mg	$3	Porcine pancreas	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1073	2008
Calzyme	1mg	$1	Candida rugosa	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1073	2008

Global Market:

Lipases are worth approximately $ 90million world wide. *"proteins biotechnology and biotechnology." Pg362*

The market is supplied with cheap microbial lipases suggesting that there is little opportunity for bovine product.

The difference in price per milligram is almost 1000 fold between bovine sourced lipase and microbial lipase.

Lipoprotein Lipase

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Lipoprotein lipase	Milk: N/A Muscle: N/A	~ 5 mg/mL	Hydrolyses lipids in lipoproteins like those found in chylomicrons into fatty acids and an alcohol.	Diagnostics, bioconversion processes	Sigma

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Lipoprotein lipase is an enzyme produced in fat cells and bound to the walls of capillaries. It breaks down triglycerides into free fatty acids and glycerol, which can enter cells for storage or energy production.

Lipoproteins (lipids attached to a protein) are particles that transport dietary lipids from the intestines to other locations in the body via the blood stream.

As fats cannot be dissolved in water a protein has to be attached so that they are soluble and can move within the water based solution of the blood stream.

Lipoprotein lipase is most abundant in the surface cells of capillaries, where it breaks down the lipoproteins into smaller units which can then be absorbed into the cells.

Commercial applications

Currently lipoprotein lipase is only used for research.

Price

Company	Unit	Price	Source	Reference	Source date
Sigma	1mg	$1085	Bovine milk	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/L2254	2008
Sigma	50mg	$448	*Pseudomonas sp*	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;L9656	2008

Pepsin

Bioactive compound	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Pepsin	Pancreas: 0.43kg Stomach mucosa: N/A	Generated from the zymogen, pepsinogen or from gastric "juice"	Cleaves proteins preferentially at carboxylic groups of aromatic amino acids such as phenylalanine and tyrosine.	Food industry, gelatin manufacture, leather industries; bioconversion biocatalysis, diagnostics,	US Biologicals, Worthington Biochemical Corp

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Pepsin is a digestive protease released by the chief cells in the stomach that functions to degrade food proteins into peptides.

Pepsin is and endopeptidase cleaves proteins preferentially at carboxylic groups of aromatic amino acids such as phenylalanine and tyrosine. It is an enzyme that begins the digestion of proteins in the stomach.

Current Market Applications:

Rennet (Rennet Powder) – is a natural complex of enzymes including pepsin, it is produced in any mammalian stomach (intestinal mucosa). Pepsins are proteolytic enzymes (proteases) that coagulate milk, causing it to separate (synerisis) into solids and liquid. Due to this function it is a useful ingredient in cheese production. The active enzyme in rennet is rennin (chymosin). [1]

Animal rennet is obtained from high-quality bovine stomachs (stated as of controlled origins). Alternatives include microbial coagulants, porcine pepsin and recombinant chymosin. Some products use bovine exclusively, with some selectively obtaining rennet from calves. The older the calf, the more pepsin and less chymosin is in the rennet. Bioren ® obtains their raw materials for rennet manufacture from New Zealand and Australia.[2]

Pepsin content in rennet is between 3%-80%, but depending on the specific use of the products, the ratio between chymosin and bovine pepsin varies.

Nutraceutical – dietary supplements to aid protein digestion.[3] Porcine pepsin is used in some products. Pepsin is an ingredient in supplements used for the treatment of anaemic conditions and gastric digestive problems.

Protein Analysis Research Tool – used in laboratories for research

Current Suppliers & Unit Cost

Company	Source	Amount	Price	Unit Cost	Reference:	Source date
Lab Vision	N/A	125ml	US$207	$1.66/ml	http://www.biocompare.com/ProductDetails/766468/ProductDetails.html	2008
Spring Biosciences	N/A	7ml	*US$19*	$2.71/ml	http://www.biocompare.com/ProductDetails/766468/ProductDetails.html	2008
USB	Porcine stomach	1g	*US$35*	$35/g	http://www.usbweb.com/category.asp?cat=116&id=20010	2008
Sigma Aldrich	Porcine gastric mucosa	500g	*AU$227*	$0.45/g	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIAL/P7125	2008
Sigma Aldrich (high grade)	Porcine gastric mucosa	10g	*AU$577*	$57.70/g	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIAL/P6887	2008

Market

The market size for pepsin is approximately 50 tons a year. http://books.google.com.au/books?id=V-UXYLXyqxAC&pg=PA186&lpg=PA186&dq=pepsin+market+world+litres&source=web&ots=hO9kxUblw3&sig=uxfeBEbJmZZuZQ1zsoe_JicuZBM&hl=en&sa=X&oi=book_result&resnum=9&ct=result#PPA186,M1

Majority of pepsin is extracted from porcine stomachs.

Pepsin is widely used in the food and beverage industry.

Bovine pepsin is not widely available.

The world market for rennin, a mixture of chymotrypsin and pepsin, is around 50 tons.

Rennin is used in cheese production. It is produced from calves. Microbial rennin has been developed and it is the industry standard now due to price and greater reliability

Opportunity potential: possible “bulk reagent”

Summary of Commercial Potential for Australian Beef Industry

Strengths – Australian and New Zealand are well recognised as the best source for natural ingredients particularly relating to dairy products and their constituents

Weaknesses – a proportion of food reagents require the extraction of pepsin from calves and have preference over matured cattle

Opportunities – many existing products on the market including nutraceuticals, supplements and natural cheese coagulants require pepsin

Threats – growing market for non-animal derived pepsin for food reagents. There are also alternative sources of pepsin including other animals and bovine recombinants (do not have the same sanitary risk associated with animal tissue). These substitutes have similar characteristics and exclusive benefits

Competitive Products

Porcine Pepsin: free from sanitary risks associated with bovine derived pepsin

ComfreyAids: herb/alternate medicine claimed by its manufacturers to show properties of healing in respiratory ailments, arthritis, fractures, lung wounds and promotes the secretion of pepsin (general aid to digestion). It is not scientifically acknowledged however herbal remedies are popular alternatives in the market.

Functional Foods: a category of value-added products that provide additional health benefits to complement the food product itself. These often contain added minerals, vitamins, fortification or boast specific health benefits such as reduced cholesterol. Specifically foods which claim to provide digestive aid such as Yakult[7]. Various value-added products including yogurts are potential competitors for pepsin-based nutraceuticals.

Data Monitor Business Unit Research – a strong growing interest in concepts of self-medication and health drivers which provoke consumers to take prophylactic measures has shown positive trends in product innovations that boast health properties. Consumers are looking for products that aim to treat or control medical conditions.

This is can be both a positive and negative signal for pepsin supplements. There may be possible increase in desire for such nutraceuticals to treat digestive disorders but also stimulate competitor growth for other functional products[8].

Reference

1 http://www.hundsbichler.com/php/details_en_5_20.html?PHPSESSID=d0dcccc65782728091b56985bf53a512

2 http://www.ethicalnutrients.com.au/detox&digestion.html

3 http://www.danlac.com/enzymes.shtml

4 http://www.associatedcontent.com/article/143080/dieters_cleanse_natural_weight_loss.html

5 http://www.naturessunshine.com.au/cgi-bin/cat/index.cgi?display=xx8lj2uflk

6 http://www.jigsawhealth.com/products/betaine_hcl_pepsin.html

7 http://www.yakult.com.au/product01.htm

8 Nunny, S. (2001) Growth strategies in dairy. *Business Insights*.

Pepsinogen

Bioactive compound	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Pepsinogen	Stomach mucosa: N/A	~20mg/Kg	Zymogen of pepsin	gastric disorders or helminthosis	Worthington Biochemical Corp., Sigma Aldrich

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Pepsinogen is the inactive precursor of pepsin. Pepsin is a digestive protease that functions to degrade food proteins into peptides.

The hormone gastrin and the vagus nerve trigger the release of both pepsinogen and hydrochloric acid from the stomach lining when food is ingested. Hydrochloric acid creates an acidic environment which allows pepsinogen to unfold and cleave itself in an autocatalytic fashion, thereby generating pepsin (the active form).

At least 8 isozymes of pepsinogen have been identified in gastric epithelial cells, and these have been categorized into two immunologically-separable types (pepsins A and C).

Commercial Applications

- Although pepsin is the active enzyme in digestion, pepsinogen may be a more effective digestive aid.
- This theory is based upon the storage conditions and activation characteristics of the two enzymes. Pepsinogen is inactive until it is unfolded into pepsin by hydrochloric acid in the stomach, once this has occurred pepsin cannot be deactivated. Pepsin stored at room temperature will self cleave and become degraded. It must therefore be stored at very cold temperatures -20oC or under alkaline conditions pH 11. Digestive aids must therefore also be stored under these conditions.
- Pepsinogen would make a more effective digestive aid as it does not need to be stored under these conditions and is just as effective as pepsin once it is in the stomach.

Current Suppliers & Unit Cost

Company	Amount	Price	Source	Reference	Source date
Sigma	1mg	$507	Human	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/P1490	2008
Sigma	100mg	$698	Porcine	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/P4656	2008

Market Size

- Global market size for Pepsinogen is very small (exact size is unavailable).
- The large difference in price quoted for pepsin ($0.74) and pepsinogen ($6984) per gram suggests that difficulties of the extraction and purification process for the two proteins are vastly different.
- Global Market size for Pure Bovine Pepsin is small as it is primarily used in conjunction with other proteases.

Process

- Removal via ammonium sulfate precipitation and chromatography on DEAE and hydroxyapatite.

Purity: 95%

Yield:

- 34 mg/1686g of mucosa
  Or 20.17mg/kg of raw mucosa

Bovine Pepsinogen A: Isolation and Characterization of Isoforms with High Activity Journal of Animal Veterinary Advances 4(11): 894-901, 2005

Djibo Idrissa-Sidikou, Benoit Remy, Nicole Gerardin-Otthiers, Bernard Joris and Jean-François Beckers

Ribonuclease A

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Ribonuclease A	Pancreas: 0.43kg Seminal fluid N/A Brain: 0.34kg And other tissues	N/A N/A ~60 mg / Kg (brain)	An endoribonuclease that specifically cleaves at the 3_-side of pyrimidine (uracil or cytosine) phosphate bonds with the highest activity exhibited with single stranded RNA. Unlike pancreatic RNase A, seminal plasma RNase is a dimer in which the subunits are cross-linked by two disulfide bonds.	molecular biology, diagnostics, biosensors; biocatalysis; large scale down stream processing of plasmids/ DNA vaccines; therapeutic uses	Worthington Biochemical Corp; Calzyme Labs Inc; Fermentas Life Sciences; Pierce Biotechnology

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Market Applications[1]:

Bovine pancreatic RNase A is used in –

- Plasmid and genomic DNA preparation.
- Removal of RNA from recombinant protein preparations.
- Ribonuclease protection assays[2]; a sensitive method used to detect the quantities of specific mRNA transcripts in a mixture of RNA or mRNA molecules.
- Mapping single-base mutations in DNA or RNA.

Bioactive Function: ribonucleases are a class of enzymes that have the capability of degrading nucleic acids. They are classified based on their specific hydrolysis of RNA. RNase A specifically degrades single stranded RNA[3].

Bovine pancreatic ribonuclease A (RNase A) when administered to human patients does not produce any allergic reactions due to a weak antigenic activity. Bacterial and fungal RNases are also suitable[4]. RNase 1 in humans is a pancreatic digestive enzyme, a counterpart to bovine RNase A. In addition, human endothelial cells express large amounts of pancreatic-type RNase that is related/ identical to human pancreatic RNase.

Current Commercial Sources

- Bovine Pancreas
- Polyclonal (Rabbit Host) against bovine ribonuclease A[7].
- Recombinant Bovine RNase A[8]

Price

Company	Source	Quantity	Price	Unit Cost	Reference	Source date
Worthington-Biochem,	Bovine Pancreas	100 mg	US$ 95.00	$0.95/mg	http://www.worthington-biochem.com/RNASE/cat.html	2008
Sigma-Aldrich	Bovine Pancreas	10 g	AU$ 2,244	$224.40/g	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/R5503	2008
AbD Serotec,	Bovine Pancreas	100 mg	US$ 200	$0.20/mg	http://www.ab-direct.com/catalog/datasheet.aspx?ProductCode=8040-0106&SearchType=Simple&SearchString=ribonuclease	2008
GenScript	Bovine Pancreas	5g	US$ 1,320.96	$0.26/mg	http://www.genscript.com/product_001/rec_protein/code/Z02004/category/protein/RNase_A.html	2008
Chem-Impex International,	Bovine Pancreas	250 mg	US$ 191.10	$0.76/mg	http://www.chemimpex.com/product1.asp?P=02109	2008

Market Size

- Bovine RNase is used extensively in the research industry.
- The market size for RNase was not determined
- However the amounts used for research purposes are very small and majority of the costs/ value of RNase are incurred in the purification and processing stages.
- The market for RNase is very competitive as there are numerous retailers and numerous end users around the globe.
- Any new retailer entering into the market would need a competitive advantage to garner any real profits in this industry.

Opportunity potential: possible “research reagent”

Summary of Commercial Potential for Australian Beef Industry

Strengths – intensive analysis of RNase protein and function has been conducted, it is one of the most studied proteins. Considerable information is available regarding its properties.

Weaknesses – preference for ribonuclease A derived from human for human use rather than other sources. Recombinant seminal ribonuclease produced from E. coli has desirable functional properties and is homologous to bovine RNase A.

Opportunities – many research areas involving bovine RNase.

Threats – due to the ubiquitous nature, other sources of RNase are available including bacteria and fungi, which also hold similar properties to bovine RNase. Many suppliers for bovine RNase.

Concentration in Bovine Tissue

Bovine Milk

Bovine Milk (95 Litres) – purified		
Product	Protein (mg)	Specific Activity (mg/mg)
Ribonuclease A	148 mg	1.16

Bingham, E.W. & Zittle, C.A. (1963). Ribonuclease of bovine milk: purification and properties. *Archives of Biochemistry, 106, 235-239, Elsevier Inc.*

Bovine Pancreas – Assumption: similar order of magnitude concentration in bovine

Mouse pancreas (250g) – purified	
Product	Protein (mg)
Ribonuclease A	70 mg

J. A. Lenstra et al, The Amino Acid Sequence of Mouse Pancreatic Ribonuclease, European Journal of Biochemistry, Vol 98 (2) pp 399-408, 1979

Reference

1 http://www.fermentas.com/catalog/modifyingenzymes/ribonucleasea.htm

2 http://www.piercenet.com/Proteomics/browse.cfm?fldID=B7273393-CD71-46C0-A8A7-E702C294529E

3 http://www.accessmedicine.com/resourceTOC.aspx?resourceID=18

4 http://wwwsoc.nii.ac.jp/jbiochem/jb/132-5/5fcbcjtx.htm

5 http://www.freepatentsonline.com/5840840.html

6 http://www.patentstorm.us/patents/5840296-description.html

7 http://www.usbio.net/Product.aspx?ProdSku=R2011-14

8 http://www.mobitec.de/int/products/bio/06_dna_prot_tools/nucleases.html

9 http://www.freepatentsonline.com/5840296.html

10 http://www.jbc.org/cgi/content/abstract/268/23/17392

11 http://www.mesotheliomasos.com/treatmentOnconase.php

Ribonuclease B/C & D

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Ribonuclease B/C & D	Pancreas: 0.43kg Brain: 0.34kg	~5 mg/Kg	Found in greatest quantity in ruminant pancreas. The major component is RNase A and the next most abundant minor component is RNase B which is a glycoprotein	molecular biology, diagnostics, biosensors; biocatalysis; large scale down stream processing of plasmids/ DNA vaccines; therapeutic uses	Sigma, Worthing Biochemicals

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Ribonuclease, abbreviated commonly as RNase, is a nuclease that catalyzes the degradation of RNA into smaller components.

Ribonucelases can be divided into endoribonucleases and exoribonucleases, and comprise several sub-classes within the phosphorolytic enzymes and the hydrolytic enzyme classes.

All organisms studied contain many RNases of many different classes, showing that RNA degradation is a very ancient and important process.

As well as cleaning of cellular RNA that is no longer required, RNases play key roles in the maturation of all RNA molecules, both messenger RNAs that carry genetic material for making proteins, and non-coding RNAs that function in varied cellular processes.

In addition, active RNA degradation systems are a first defence against RNA viruses, and provide the underlying machinery for more advanced cellular immune strategies such as RNAi.

RNase B is identical in protein sequence and conformation to RNase A. The difference between them is that RNase B is a glycoprotein and contains 6 mannose residues and 2 residues of N-acetylglucosamine.

RNase D is an exoribonucleases identified. It is an 3'-5' exoribonuclease and which has been shown to be involved in the 3' processing of various stable RNA molecules. RNase D has homologues in many other organisms.

RNase B, C & D represent about 20% of all RNases extracted.

RNase A >70%.

Commercial Applications

The individual commercial applications for each Ribonuclease B, C, and D were not determined as RNase A dominates the market.

As the ribonuclease types are structurally very similar but differ in there modes of action and optimum conditions it is difficult to determine how often they are used in a crude mixture.

Ribonuclease A is used in research for the following purposes:

- To test for complimentarily between RNA: DNA hybrids.
- RNase protection assays
- Remove unspecifically bound RNA
- Analysis of RNA sequences
- Hydrolyze RNA contained in protein samples

Bovine pancreatic RNase A is used in –

- Plasmid and genomic DNA preparation.
- Removal of RNA from recombinant protein preparations.
- Ribonuclease protection assays; a sensitive method used to detect the quantities of specific mRNA transcripts in a mixture of RNA or mRNA molecules.
- Mapping single-base mutations in DNA or RNA.

Price

Company	Amount	Price	Source	Reference	Source date
Sigma (RNase B)	100mg	$173	Bovine pancreas	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;R7884	2008
Worthington Biochem (RNase B)	100mg	$100	Bovine pancreas	http://www.worthington-biochem.com/RNASE/cat.html	2008
Worthington Biochem (mixture)	2500mg	$500	Bovine pancreas	http://www.worthington-biochem.com/RTC/cat.html	2008

Market

New innovations and pharmaceutical targets will grow as the human genome is mapped.

The oncology industry shows signs of growth but there is also dense competition between biotech companies.

Trypsin Inhibitor

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Trypsin Inhibitor	Pancreas: 0.43kg	~10 U/Kg depending on the process	Inhibits serine protease such as trypsin, kallikrein, chymotrypsin, and plasmin; member of the Kunitz family of inhibitors,	Forms a very stable 1:1 complex with bovine trypsin between pH 3 and 10	Worthington Biochemical Chemicon Inc.,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Trypsin Inhibitors reduce the bio-availability of trypsin as well as other protein digesting enzymes like elastase.

Trypsin Inhibitors serve an important role in inhibiting the immuno-defensive enzymes released by white blood cells. White blood cells (neutrophils) are responsible for attacking and removing foreign objects and pathogens. One method used by white blood cells to attack pathogens is the production and release of digestive enzymes (proteases). The white blood cells secrete the proteases when a pathogen is detected, the proteases dissolve the proteins of the pathogens thus neutralising the threat. The proteases are not very specific and will also degrade the proteins of nearby healthy body cells causing inflammation and puss. Trypsin inhibitors help reduce the collateral damage by inhibiting proteases when they are not needed.

A deficiency of trypsin inhibitors results in enzymes like trypsin and elastase running rampant and degrading vital organs. It is thought that smoking reduces the effectiveness of trypsin inhibitors in the lungs leaving elastase free to break down elastin -- which contributes to the elasticity of the lungs -- resulting in respiratory complications such as emphysema.

The FDA has approved the use of three trypsin inhibitor products derived from human plasma: Prolastin, Zemaira and Aralast to treat trypsin inhibitor deficiency, therapy can cost up to U.S$100,000 per year per patient.

Trypsin inhibitors are found in a variety of sources, such as soybean, human plasma, lima bean, and bovine pancreas. Trypsin inhibitors are found in high concentrations in some beans as they are a defence mechanism. The trypsin inhibitors stop the digestion of the beans if they are eaten, therefore the animal gains little to no sustenance from them.

Soybeans contain high concentrations of trypsin inhibitor and must be heated/cooked to denature the enzyme before they can be eaten. Uncooked soybeans fed to rats, chickens and hamsters reduce their rate of growth as they must produce more digestive enzymes. The pancreas of these animals becomes enlarged and eventually causes pancreatic cancer. The same affect is not seen in other animals like dogs, pigs and calves when fed uncooked soybeans. The affect of trypsin inhibitor on humans has not been tested as the risks are too great.

Commercial Applications

Mostly used in laboratory procedures. Trypsin itself is used on cells to remove them from culture flasks but is toxic and requires the use of a trypsin Inhibitor after a few minutes.

Trypsin inhibitor is used for the treatment of hyperactive conditions of the pancreas and trypsin inhibitor deficiencies.

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	25mg	$1395	Bovine Pancreas	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/T0256	2008
Calzyme	1000mg	$120	Soybean	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1101	2008

Market Size

- The market size for trypsin inhibitors was not ascertained, it is likely to be a small percentage of the trypsin world market as one is often used with the other. The use of trypsin inhibitors as a reagent in cell culturing is an example of this.
- The availability of cheap legume trypsin inhibitors has diminished the market for bovine trypsin inhibitors; the difference in price is significant as bovine TI is approximately 500 times more expensive than soybean TI.
- The primary market for bovine TI would be the research and development sector which would use them only when necessary.

What product processing is involved?

TI is extracted by digesting the bovine pancreas into physiological buffer. The extract is then de-fatted using acetone, and the protein is further purified by employing gel filtration and ion-exchange chromatography. The final CTI preparation appears as a single band by SDS-PAGE analyses under both reducing and non-reducing conditions.

Trypsin

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Trypsin	Pancreas: 0.43kg	> 100 mg/ Kg when affinity methods are used	Serine protease that cleave proteins at the carboxyl side (or "C-terminus") of the basic amino acids lysine and arginine	Widely used enzyme in various applications ranging from proteomic, cell culture through to large scale industrial biocatalysis uses in food and other industries	US Biologicals, Worthington Biochemical AlbioChem; Sigma-Aldrich; CalbioChem; Calzyme Lab

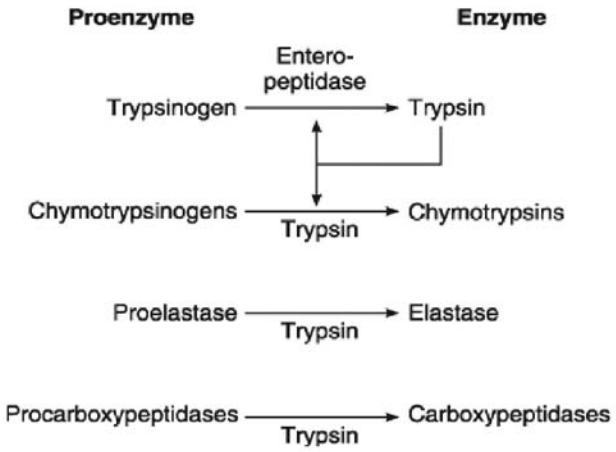
**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Trypsin is a naturally occurring protein that is produced in the pancreas of all animals and breaks down protein chains as part of the digestion process.

Trypsin is secreted into the small intestine, where it acts to hydrolyse peptides into their smaller building blocks, namely amino acids (these peptides are the result of the enzyme pepsin breaking down the proteins in the stomach). This is necessary for the uptake of protein in the food as though peptides are smaller than proteins, they are still too big to be absorbed through the lining of the small intestine.

Trypsin conversion occurs when trypsinogen is activated by enzymes, in turn, trypsin is responsible for the activation of subsequent proteolytic enzymes. Trypsin cleaves bonds between amino acids within a target peptide chain.[4]



Source Ganong WF: Review of Medical Physiology, 22nd Edition
http://www.accessmedicine.com

The pancreas also contains a trypsin inhibitor to prevent enzymes digesting the pancreas when trypsin levels are higher than normal.

Current Market Applications:

Bovine and porcine trypsin are used in numerous applications including:

Food processing – proteolytic enzymes such as trypsin are used in various food applications including cheese making, starch processing and juice production to increase appearance, nutritional value or to generate flavours.[1]

Digestive Aid – trypsin is used as an ingredient in digestive aid supplements. There are numerous digestive aid supplements containing trypsin, the retail prices of these supplements range from $5 to $25 for 90 tablets.

Research reagent and cell culture reagent

It is a critical intermediary in the manufacturing of insulin, where it is used to help cleave the protein into its active form

Trypsin is widely used in cell culture applications for research and production of recombinant proteins for clinical uses.

Another application is its use in the wound care markets as an oral treatment for inflammatory edema, hematoma and pain associated with a wide variety of internal and external wounds. Some brands are Granulex®; Xenaderm.

Current Suppliers & Unit Cost

Company	Source	Quantity	Price	Unit Cost	Reference:
Human sources					
Biopur	Human Pancreas	100ug	US$180	$1.80/ug	http://www.biocompare.com/ProductDetails/621834/ProductDetails.html
Calbiochem	Human Pancreas	50ug	US$154	$3.08/ug	http://www.biocompare.com/ProductDetails/460575/ProductDetails.html
RayBiotech	Human Recombinant	10mg	US$396	$39.60/mg	http://www.biocompare.com/ProductDetails/822804/ProductDetails.html
Yeast sources					
GenScript Corporation	Yeast	10mg	US$400	$40/mg	http://www.biocompare.com/ProductDetails/730218/ProductDetails.html
Bovine sources					
Sigma-Aldrich	Bovine Pancreas	5g	AU$1,135	$227/g	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIAL/T1426
Research Organics	Bovine	1g	US$199	$199/g	http://www.biocompare.com/ProductDetails/456227/ProductDetails.html
Worthington	Bovine Pancreas	100mg	US$16	$0.16/mg	http://www.worthington-biochem.com/TRY/cat.html
Calzyme	Bovine Pancreas	1g	$12	$12/g	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1102

Current Commercial Sources

- Transgenic Trypsin Sources – chemical company Sigma-Aldrich has been marketing trypsin processed from transgenic maize.
- Recombinant Trypsin – bacterial host for trypsin product or modified trypsin.[7]
- Human Pancreas[8]
- Bovine Pancreas[9]
- Porcine Pancreas[10]
- Sheep Pancreas[11]

Competitive Products

Chemically Modified Enzymes/Trypsin – These new proteins can be used for applications outside of their normal function and allow them to carry characteristics that are required for their activity or stability under harsh or unnatural conditions.

Recombinant – the favourability for non-animal trypsin has led to the production of secondary sources of bovine pancreatic-like proteases which have similar function and/or more beneficial actions. [13] Further examples include a trypsin producing strain of Fusarium.[14,15]

Detergents16 – enzyme use in detergents is common in developed countries. This industry is the largest market for enzymes at 25-30% of sales. The enzymes used must be cost-effective and safe to use. Bacillus is the common industrial scale source of these enzymes. The industry is dominated by two companies, Novo Industries and GistBrocades.

Global Market

The worldwide market for all trypsin products was approximately US $120 million in 2004. http://www.springerlink.com/content/43v50uey1ravf5kj/

Proteases represent a US$40 billion industry.[24]

The majority of the world's trypsin is sourced from bovine and porcine pancreas. Trypsin is available in high quantities in animal pancreases and can be purified rather easily. Although there are alternative sources, yeasts and bacteria they are more expensive and less efficient.

The market for trypsin is rapidly growing as a result of the worldwide increase in diabetes as well as the progress of alternative delivery systems for insulin, which, due to low bioavailability, require higher quantities to reach a therapeutic effect

It is estimated that the worldwide demand for trypsin will increase five fold in the next five years.

Opportunity potential: possible "bulk reagent"

Summary of Commercial Potential for Australian Beef Industry

Strengths – bovine sourced trypsin sold commercially by companies such as Sigma Aldrich; growing market.

Weaknesses – bovine/animal derived and risks associated such as BSE in comparison to bacterial models.

Opportunities – heavily studied protein and used as a research reagent.

Threats – prominence of bacterial trypsin-like or proteases in commercial products.

Concentration in Bovine Tissue

Bovine pancreas- Assumption: similar order of magnitude concentration in bovines

Human pancreas (1000g) – purified	
Product	Protein (mg)
Trypsin	16.3 mg

P. A. Mallory and J. Travis, 'Human Pancreatic Enzymes. Characterization of Anionic Human Trypsin', Biochemistry, 1973, Vol. 12, No. 15, 2847

Opportunity potential: possible "bulk reagent"

Summary of Commercial Potential for Australian Beef Industry

Strengths – bovine sourced trypsin sold commercially by companies such as Sigma Aldrich

Weaknesses – bovine/animal derived and risks associated such as BSE in comparison to bacterial models.

Opportunities – heavily studied protein and used as a research reagent.

Threats – prominence of bacterial trypsin-like or proteases in commercial products.

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11 http://books.google.com/books?id=mGZFb_ZLbuMC&pg=RA1-PA147&lpg=RA1-PA147&dq=trypsin+food+processing&source=web&ots=DlAl3HjrtS&sig=wI1NaLGVidi90XENSLGnkf7XPPw

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18 http://www.novozymes.com/en/MainStructure/ProductsAndSolutions/Detergents/Whiteness/Savinase/

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23 http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Cell_Culture/Product_Lines/TrypZean_Solution.html

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http://www.fass.org/FASStrack/news_item.asp?news_id=152

Trypsinogen

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Trypsinogen	Pancreas: 0.43kg	> 100 mg/Kg when affinity methods are used	Inactive zymogen of trypsin	Inactive precursor of trypsin	MP Biologicals Worthington Biochemical Sigma-Aldrich; Calzyme

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Trypsinogen is the precursor form of the pancreatic enzyme trypsin. It is found in pancreatic juice, along with amylase, lipase, and chymotrypsinogen. It is activated by the enzyme enteropeptidase, which is found in the intestinal mucosa, to form trypsin. Once activated, the trypsin can activate more trypsinogen into trypsin. Trypsin cleaves peptide bond on carboxyl side of basic amino acids.

Serum trypsinogen is measured using a blood test. High levels are seen in acute pancreatitis, and cystic fibrosis.

Commercial Applications

- The commercial applications for trypsinogen are limited as it is not an active enzyme.
- There are many commercial uses for trypsin as discussed previously.

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	1000mg	$600	Bovine pancreas	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/T1143	2008
MP Biologicals	1000mg	$478	Bovine pancreas	http://www.mpbio.com/product_info.php?products_id=101195	2008

Market Size

- The market size for trypsinogen would be only a fraction the market size of its activated form trypsin which was worth $120 million in 2004.
- Bovine pancreas is the main production source for trypsinogen and trypsin worldwide.

What product processing is involved?

- Initial ion exchange chromatography step on sulfopropyl (SP)-Sephadex at pH 2.6, affinity column of lima bean trypsin inhibitor-agarose at high ionic strength, affinity chromatography on the same material at low ionic strength, ion exchange chromatography on SP-Sephadex at pH 6.0 (Brodrick, Largman et al. 1978).
- FPLC on a MonoS column at pH 4.5 http://www.jbc.org/cgi/reprint/M011374200v1.pdf
- Chromatography on carboxymethyl cellulose http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=14470138&dopt=Abstract

Purity: 95%

Immunoglobulins

Alpha 2 – Macroglobulin

Gamma-Globulin

IgA

IgG

IgM

Alpha 2 - Macroglobulin

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Use	Supplier
Alpha-2-Macroglobulin	Serum:11.34kg	40-400 µg/mL depending on source and process	Inhibits coagulation; trauma, binding partner, protease regulation	Sigma, Cortex

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Alpha-2-Macroglobulin is a large plasma protein found in the blood stream and is produced in the liver. Alpha 2-Macroglobulin inhibits a wide variety of proteolytic enzymes (enzymes that breakdown proteins), including trypsin, plasmin, thrombin, kallikrein, and chymotrypsin, it does this by entrapping and reducing the accessibility of their functional sites to large molecules.

Alpha-2-Macroglobulin is unusual in that it functions as an inhibitor of coagulation by inhibiting thrombin, whilst also functioning as an inhibitor of fibrinolysis (clot breakdown) by inhibiting plasmin and kallikrein

Commercial applications

There were no bulk commercial applications for Alpha-2-Macroglobulin reported.

Value

Company	Unit	Price	Source	Reference	Source date
Sigma	10mg	Human	$1035	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/M6159?langId=-1&storeId=11001	2008
Cortex Biochem	5mg	Sheep	$370	http://www.cortex-biochem.com/commerce/catalog/product.jsp?tabid=-&product_id=1649&czuid=1220403849810	2008

**Assume 300kg HSCW. See Appendix A for other HSCW*

Market Size

The market size for Alpha-2-Macroglobulin is limited to medical research at this time.

The market size for bovine Alpha-2-Macroglobulin would be very small, there appears to be no supplier of bovine Alpha-2-Macroglobulin at this time.

What product processing is involved?

Process: Chromatography and Gel filtrations.

Purity: 98%

Summary

Alpha-2-Macroglobulin appears to have little economic value for the red meat industry as the protein has no bulk commercial uses.

The primary market is the medical research industry and this industry has a preference for human sourced proteins.

Gamma-Globulin

Bioactive compound	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
g-Globulin	Plasma: 11.34kg	up to 75 g/L	Polyspecific antibodies of the class G	Prevention of infections and various other diagnostic and cell culture uses	Bioactive Technologies, Intl., Bethyl Laboratories; Thermo Electron Corp./Trace Biosciences NZ Ltd

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Gamma globulins are a class of proteins found in the blood and other bodily fluids. The most significant gamma globulins are immunoglobulins (antibodies).

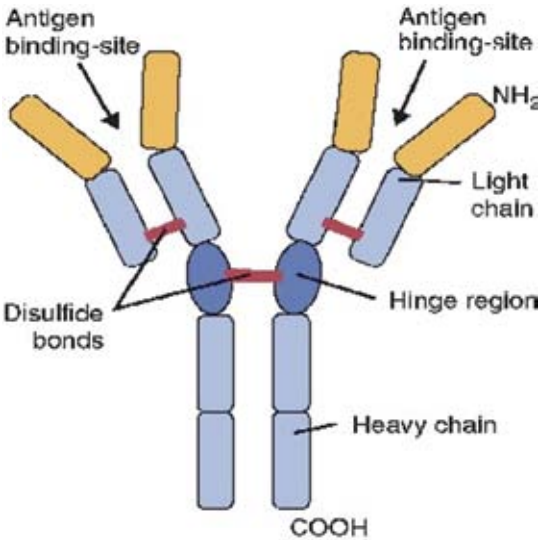
There are five classes of immunoglobulins IgA, IgD, IgE, IgG and IgM. The immunoglobulins (Ig) are classified based upon their structure and they all play different roles in the immune system.

Name	Types	Description
IgA	2	Found in mucosal areas, such as the gut, respiratory tract and urogenital tract, and prevents colonisation by pathogens. Also found in saliva, tears, and breast milk.
IgD	1	Functions mainly as an antigen receptor on B cells that have not been exposed to antigens. Its function is less defined than other isotypes.
IgE	1	Binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy. Also protects against parasitic worms.
IgG	4	In its four forms, provides the majority of antibody-based immunity against invading pathogens. The only antibody capable of crossing the placenta to give passive immunity to foetus.
IgM	1	Expressed on the surface of B cells and in a secreted form with very high avidity. Eliminates pathogens in the early stages of B cell mediated (humoral) immunity before there is sufficient IgG.

Antibodies are used by the immune system to identify and neutralise foreign objects, such as bacteria and viruses. Antibodies are produced by a kind of white blood cell called a B cell.

They are typically made of basic structural units - each with two large heavy chains and two small light chains.

Although the general structure of all antibodies is very similar, a small region at the tip of the protein is extremely variable, allowing millions of antibodies with slightly different tip structures to exist.



Structure of an immunoglobulin molecule
Source Tizard IR, Veterinary Immunology. An Introduction, Saunders, 2001

Commercial Applications

Medicine

Immunoglobulins or antibodies are used extensively in medicine to transfer passive immunity to certain diseases. Passive immunity is achieved through the transfer of ready-made antibodies in the form of human or animal serum (primarily equine), pooled immunoglobulin or monoclonal antibodies, into the affected individual. Immunity from passive immunisation is used primarily in cases of acute infection and poisoning.

Immunity derived from passive immunisation lasts for only a short period of time, and there is also a potential risk for hypersensitivity reactions, and serum sickness, especially from gamma globulin of non-human origin. Passive immunity provides immediate protection, but the body does not develop memory, therefore the patient is at risk of being infected by the same pathogen later.

Today, the preparation and use of human immunoglobulin (Ig) for passive immunisation is standard medical practice. In the United States alone, there is a $1,400,000,000 per annum market for human Ig, and each year more than 16 metric tons of human antibody is used for intravenous antibody therapy. http://www.freepatentsonline.com/7074983.html

Bovine sourced immunoglobulins are not used in human medicine, due to the risk of infection and allergic reactions. However antibodies from healthy adult cattle blood can be injected into calves to boost their immune system.

Research

In research, purified antibodies are used in many applications. They are most commonly used to identify and locate proteins and cells. Research techniques that use antibodies for protein identification are ELISA, Western Blot and ELISPOT.

An Australian bioactive supply company sells bovine immunoglobulins for the domestic and international markets. The catalogue stated that the Igs are sold in 50g to 10kg packs and suitable for the following applications:

- Use as a diagnostic biochemistry standard
- Use as a quenching reagent or co-precipitant in Radio Immuno Assays
- Used to reduce non-specific adsorption of antibodies in immunoassays
- Use as a starting material for isolation of Ig subclass materials
- Use as molecular weight markers
- Use as a standard protein in electrophoretic applications
- Protein stabiliser in analyte preparations
- Immunoglobulins also have applications as an immune supplement for neonatal offspring of domestic animals to help prevent bacterial (mainly pathogenic E.coli or Salmonella) and viral (rotavirus) scours. It's use also decreases postpartum mortality and leads to higher weight gain at weaning

Supplement

The Igs are the principal agents that protect the gut mucosa against pathogenic microorganisms. IgG antibodies express multifunctional activities, including complement activation, bacterial opsonisation and agglutination, and act by binding to specific sites on the surfaces of most infectious agents or products, either inactivating them or reducing infection.

The public's concern regarding their state of immunity is now a major global trend and is reflected in the sales of immune support products. This trend is overwhelmingly founded on the belief that oral consumption of IgG-enhanced products is safe and may provide consumers improved protection from gastrointestinal tract infection. Consumer acceptance of such value-added protein products began in Asia and has now migrated to Europe and the US, targeting sports nutrition, infant formulae, dietary supplements and physiologically functional foods.

Immunoglobulins supplements are sourced primarily from bovine blood and colostrum.

There are several immunoglobulin supplements on the market, the most popular being Proliant's ImmunoLin. ImmunoLin website states that the product contains bovine immunoglobulins, obtained from serum not colostrum.

ImmunoLin website states that "immunoglobulin of bovine origin contains antibodies that are also specific to human pathogens. This includes such pathogens as E. coli (Freedman et al., 1998; Griffiths and Humpheys, 1977), H. pylori (Korhonen et al., 1995), cryptosporidia (Osame et al., 1991), and rotavirus (Acres and Babiuk, 1978; Hess and Bachman, 1981; Ushijima et al., 1990). ImmunoLin® is particularly rich in the immunoglobulins IgG, IgM and IgA. It also contains transferrin, endotoxin-binding proteins and other acute phase proteins that can provide additional immune support benefits."

"It's an all-natural and lactose-free source of immunoglobulin and other immuno-proteins that are very easily added to bars, capsules, tablets or drink mix form."

The retail price for ImmunoLin ranges from $10 to $100 for 60 tablets or 30g of immunoglobulins.

Animal sourced immunoglobulins are may also be added to animal feed as an alternative to antibiotics. Antibiotics in animal feed are currently under scrutiny as they may increase the prevalence of antibiotic resistant bacteria. There is a growing consumer demand for antibiotic free animals and immunoglobulins could be used as an alternative. The effectiveness of immunoglobulins in animal feed compared to antibiotic feed has not been reported.

Value

Company	Amount	Price	Source	Reference	Source date
Calbiochem	100g	$1036	Bovine blood	http://www.emdbiosciences.com/product/345876	2008
Pel-Freez Biologicals	100g	$290	Bovine blood	http://www.pelfreez-bio.com/programs/productdescription.asp?prodid=27005-2	2008
Sigma	10g	$177	Bovine blood	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G7516?LastFive	2008
Sigma	10g	$763	Human blood	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G4386	2008

**Assume 300kg HSCW. See Appendix A for other HSCW*

Market Size

The world market for immunoglobulins is large as they are used extensively in medicine and research. The worldwide sales of immunoglobulins have been reported to be worth from $ 3 billion to $26 billion a year.

The market size for bovine sourced immunoglobulins was reported to be worth $1.4 billion a year. http://www.bizjournals.com/dallas/stories/2008/06/02/story7.html

It is difficult to determine if any of the values stated are correct, however what is certain is that the market is significant.

The market for bovine immunoglobulins is also likely to grow as the demand from research industry, supplement and neutraceutical market is predicted to increase.

Igs derived from bovine lacteal secretions (i.e. colostrum and milk) have been utilised in the immunological supplementation of infant formulae and other foods, yielding sales of approximately US$100 million in 2004 (1-3)

The use of immunoglobulins for commercial applications other than medicine is predicted to expand as the cost of production falls. The development of new extraction and purification techniques and equipment will reduce the costs associated with immunoglobulin production.

The Dutch Bio Affinity Company has introduced two new products for scientists who need to purify hard-to-separate antibodies such as immunogloblins A and M. (http://www.labtechnologist.com/Products/IgM-and-IgA-purification-made-simple)

References

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3 Mehra R, Marnila P, Korhonen H (2006) Int Dairy J 16:1262

IgA

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
IgA	Plasma:11.34kg Mucosal linings : N/A	~300 mg/ mL	prevents adhesion penetration of pathogens	Veterinary therapeutic applications diagnostics	Sigma, American customs chemicals corp.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Immunoglobulin A (IgA) is an antibody playing a critical role in mucosal immunity. In its secreteory form IgA, is the main immunoglobulin found in mucous secretions, including saliva, colostrum, intestinal mucus, and secretions from the respiratory system. It is also found in small amounts in blood.

IgA protein is resistant to the digestive enzymes and acids found in the mucosal linings of the digestive system and respiratory tracts, as it has to provide protection against microbes that multiply in body secretions.

In the gut, IgA binds to the mucus layer on top of the surface cells to form a barrier capable of neutralising threats before they reach the cells.

Commercial Applications

Immunoglobulin A has no known bulk commercial applications. IgA is commonly sold in conjunction with other immunoglobulins for its immune boosting properties.

The primary reason that purified IgA has no commercial applications is that it is difficult and unnecessary to separate it from the other immunoglobulins produced by the body.

In products that contain a mixture of all 5 immunoglobulins, such as the immune boosting supplement ImmunoLin, IgA is vital for boosting the health of the digestive system.

IgA given as a supplement is reported to boost the levels of IgA found in the mucus of the digestive tract, thus decreasing the incidence of disease.

Immunoglobulins added to animal feed, have been proposed as an alternative to antibiotics. The ability of immunoglobulins in feed to reduce the incidence of disease in livestock has not been effectively proven by scientific research.

Price

Company	Unit	Price	Source	Reference	Source date
Sigma	25mg	$1100	Human colostrum	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/I1010	2008

Market

- The market size of IgA was indeterminable as there appears to be no commercial applications for it in a purified form.
- IgA is available for research purposes however it is very expensive and this would prohibit its commercialisation.
- Separating and purifying IgA from other immunoglobulins is expensive; as immunoglobulins are structurally and chemically similar therefore separating them is difficult and costly.
- Separating immunoglobulins into the five types is also unnecessary as immunoglobulins are often used commercially as immune boosters for human and animal health, therefore the broader the range of immunoglobulins the more effective the immune supplement.

IgG

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
IgG	Plasma: 11.34kg	~10 to 90 mg/mL depending on process and animal source	Prevents development of infection	Veterinary therapeutic applications, diagnostics	US Biologicals, Bethyl Inc., Axxora, Inc., VMRD Inc., Protein Factory Inc. Purecaps Inc.,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Immunoglobulin G (IgG) is a monomeric immunoglobulin, built of two heavy chains and two light chains. Each IgG has two antigen binding sites. It is the most abundant immunoglobulin and is approximately equally distributed in blood and in tissue liquids.

IgG molecules are synthesised and secreted by blood plasma B cells.

IgG antibodies are predominately involved in the secondary antibody response, (the main antibody involved in primary response is IgM) which occurs approximately one month following antigen recognition, thus the presence of specific IgG generally corresponds to maturation of the antibody response.

This is the only immunoglobulin type that can pass through the placenta, thereby providing protection to the foetus in utero. Along with IgA secreted in the breast milk, residual IgG absorbed through the placenta provides the neonate with humoral immunity before its own immune system develops.

It can bind to many kinds of pathogens, for example viruses, bacteria, and fungi, and protects the body against them by agglutination and immobilisation, complement activation (classical pathway), opsonisation for phagocytosis and neutralisation of their toxins.

Commercial applications

Intravenous immunoglobulin (IVIG) is a blood product administered intravenously. It contains the pooled IgG immunoglobulins (antibodies) extracted from the plasma of over one thousand blood donors. IVIG's effects last between 2 weeks and 3 months. It is mainly used as treatment in three major categories:

1. Immune deficiencies - Immune deficiencies such as X-linked agammaglobulinemia, hypogammaglobulinemia (primary immune deficiencies), and acquired compromised immunity conditions (secondary immune deficiencies), featuring low antibody levels.
2. Inflammatory and autoimmune diseases
3. Acute infections

IVIG is an infusion of IgG antibodies only. Therefore, peripheral tissues that are defended mainly by IgA antibodies, such as the eyes, lungs, gut and urinary tract are not fully protected by the IVIG treatment.

IVIG cost is climbing and well over $50/g. ($10,000 for a 100kg person at 2g/kg)

Research

Purified bovine IgG may be used as a reference antigen, standard, blocking agent or coating protein in a variety of immunoassays including ELISA, dot immunobinding, Western immunoblotting, immunodiffusion and immunoelectrophoresis. Other applications include starting materials for the preparation of immunogens and solid-phase immunoadsorbents.

- *For more commercial applications of IgG refer to g-globulins (antibodies).*

Price

Company	Amount	Price	Source	Reference	Source date
Serotec	10mg	$105	Bovine	http://www.serotec.com/asp/product.asp?Cat=PBP002&Currency=2	2008
Bethyl Laboratories	100mg	$200	Bovine	http://www.bethyl.com/matrix.asp?catid=19&search=bovine+IgG	2008
Equitech-Bio	100000mg	$1080	Bovine	http://www.equitech-bio.com/immunoglobulin%20Solutions%2097.htm	2008
Fitzgerald	10000mg	$300	Bovine	http://www.fitzgerald-fii.com/view_product.php?product=30-AB44&searchlink=yes&search=BOVINE%20IGG&page=1	2008
Sigma	100mg	$340	Bovine	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/I5506	2008
Sigma	100mg	$429	Human	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/I4506	2008

Market Size

The world market for IgG has grown significantly in the last 30 years as production has increased from 7.4 tons in 1984 to 55 tons in 2005.[1]

The primary market for IgG is IVIG in medicine; the secondary market would be for research applications. Intravenous immunoglobulins are mainly sourced from human blood though equine blood is sometimes used.

There was no market data found relating specifically to bovine IgG. Bovine IgG sales would be worth significantly less than human IgG as bovine IgG is unsuitable for medical use and there is a chronic shortage of human blood whilst there is an abundant supply of bovine/animal blood.

Bovine IgG are widely used in research (refer to antibodies), there are numerous suppliers of bovine IgG and the market appears to be competitive.

What product processing is involved?

The product is manufactured by the Cohn-Oncley cold ethanol fractionation process followed by ultrafiltration and chromatography. The manufacturing process includes treatment with an organic solvent/detergent (S/D) mixture composed of tri-n-butyl phosphate (TNBP) and Triton X-100.[2]

IgG fraction produced in bovine, purified by anion-exchange chromatography.

Purity: _90% (SDS-Page)

Yield: 1.44–2.56 g/dl (Kameyama et al, 2003s)

- 14.4-25.6g/L

Reference

1 http://www.medscape.com/viewarticle/534010

2 http://www.octapharma.com/USA/documents/Octagam%20PI%2020th%20Feb%202007.pdf

IgM

Bioactive compound	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
IgM	Plasma: 11.34kg	~2- 70 mg/mL depending on process and animal source	Modulates recurrent infection	Veterinary therapeutic applications, diagnostics	US Biologicals, Bethyl Inc., Axxora, Inc.,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

IImmunoglobulin M (IgM) is a basic antibody that is present on B white blood cells. IgM constitutes about 10% of serum immunoglobulin and is the first antibody to be produced in response to antigenic stimulation. IgM in normal serum is often found to bind to specific antigens, even in the absence of prior immunization. For this reason IgM has sometimes been called a “natural antibody”.

IgM is by far the largest antibody as it forms a polymer of multiple immunoglobulins that are linked together with disulfide bonds, mostly as a pentamer but also as a hexamer. Because each monomer has two antigen binding sites, a pentameric IgM has 10 binding sites. Typically, however, IgM cannot bind 10 antigens at the same time because the large size of most antigens hinders binding to nearby sites.

Because IgM is a large molecule, it cannot diffuse well, and is found in the tissue fluid only in very low quantities. IgM is primarily found in serum; however, it is also important as a secretory immunoglobulin.

IgM antibodies are mainly responsible for the clumping of red blood cells if the recipient of a blood transfusion receives blood that is not compatible with their blood type.

Commercial Applications

IgM has no bulk commercial applications at this time.

IgM is commonly sold with other immunoglobulins as an immune booster or anti-bacterial. *(see g-globulin for further information)*

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	10mg	$510	Bovine Serum	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/I8135	2008

Market

- The market uses for bovine IgM is limited to research purposes, it is therefore a small market. There are few suppliers of bovine IgM and the price of purified IgM is much greater than a mixture of immunoglobulins.
- The market for bovine immunoglobulin mixtures is much larger, as it is much cheaper.
- It is unlikely that there will ever be a large market for purified bovine IgM

Iron and Oxygen Binding

Apoferritin

Ferritin

Fetuin

Haemoglobin

Haemopexin

Haptoglobin

Holo-transferrin

Myoglobin

Transferrin

Apoferritin

Bioactive compound	Source (kg/head)*	Abundance (per/kg of tissue)	Use	Supplier
Apoferritin	Spleen 0.66kg Liver 5.1kg Intestinal mucosa N/A		iron deficiency anemia and in iron overload	Calzyme Laboratories Inc. and other vendors

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Ferritin that is not bound to iron is known as Apoferritin.

Ferritin is a highly specialised protein whose main function is to store excess iron intracellularly keeping it in a soluble and non-toxic form.

It is widely distributed throughout the animal and plant kingdoms. In mammalian tissues, ferritin is present in concentrated form in spleen, liver and the intestinal mucosa.

Ferritin is a enzyme present in the intestinal mucosa that binds and stores iron by combining with a ferric hydroxide-phosphate compound to form ferritin $Fe^{2+} \rightarrow Fe^{3+}$.

Commercial Applications

- Ferritin has many commercial applications however it is sold primarily as a dietary supplement to treat iron deficiency anemia. Iron deficiency is the most common and widespread nutritional disorder in the world (W.H.O 2008).
- Apoferritin is not sold as an iron supplement as it has no bound iron.
- Apoferritin is primarily used in research as a molecular marker and as a cell culture reagent.
- Apoferritin can be used in the treatment of iron overload however this is a rare human disorder and there are more effective treatment techniques than apoferritin.

Price

Company	Amount	Price	Source	Reference:	Source date
Calzyme	1mg	$1	Equine Spleen	http://www.calzyme.com/commerce/catalog/product.jsp?product_id=1121&czuid=11976 48748215	2008
Sigma	50mg	$50	Equine Spleen	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/A3660	2008

Market Size

- No bulk commercial applications for apoferritin found.
- Only demand for Apoferritin would be for research purposes.
- Bovine sources of Apoferritin were not found
- $1 per mg of protein suggests there is a reasonable market or that the protein is relatively easy to extract and purify.

Ferritin

Bioactive compound	Tissue Source and Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Ferritin	Spleen 0.66kg Liver 5.1kg	~ 5 mg/Kg	Regulates a wide range of oxidative processes because of its iron binding properties (~ 25% of total Fe(III) pool).	Dietary supplement used for the treatment of iron deficiency anaemia.	Calzyme Labs Inc., New Zealand Pharmaceuticals Ltd;

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Ferritin is a globular protein it is the main intracellular iron storage protein, keeping it in a soluble and non-toxic form. Ferritin which is not combined with iron is called apoferritin.

Free iron is toxic to cells as it acts as a catalyst in the formation of free radicals. Hence organisms have evolved an elaborate set of protective mechanisms to bind iron in various tissue compartments.

Under steady state conditions, the serum ferritin level correlates with total body iron levels; thus, the serum ferritin level is the most convenient laboratory test to estimate iron stores.

Commercial Applications

- Ferritin is used as a nutritional supplement to boost iron levels.
- Approximately 30% of the worlds population are iron deficient making iron the most deficient nutrient worldwide.

Price

Company	Amount	Price	Source	Reference:	Source date
New Zealand Pharmaceuticals	1kg	$3100	Equine Liver	http://www.newzealandpharmaceuticals.com/products.php?cid=1&pid=12	2008
Calzyme	1g	$150	Equine Spleen	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1050	2008

Market Size

New Zealand Pharmaceuticals halted manufacturing of bovine ferritin several years ago due to the BSE scare. New Zealand and Australia are both BSE free; retailers and consumers of dietary supplements simply do not want to take the risk when there are alternative products on the market.

New Zealand Pharmaceuticals currently produces Ferritin from equine liver.

Their main export market is in South Korea where they supply tens of kilos of lyophilized ferritin a year. They also sell formulated product into Korea which has a much bigger market, “a couple of tonnes of powder and several thousand litres of a ferritin solution”. These products are only registered in Korea.

The market is turning away from animal derived ferritin to other sources, predominantly plant and recombinant sources due to the BSE scare.

The only way to reverse the downturn in the market is to remind consumers that Australia and New Zealand are BSE free and that our products are safe.

What product processing is involved?

Thermal denaturation, ammonium sulphate fractionation, Sephacryl S-300 gel filtration, DEAE-blue gel affinity chromatography, SDS-gel electrophoresis, gel filtration and ultracentrifugal analysis (Suryakala and Deshpande 1999)

Strengths:

- Readily available tissue source- bovine spleen and liver
- Iron deficiency is a global nutrition problem

Weaknesses:

- Iron supplement market is well established
- The people in need of iron supplements are located in developing countries

Opportunities:

- Ferritin could be incorporated into existing iron supplements to boost their effectiveness.
- Reports suggest that ferritin is required for early childhood development and could therefore be incorporated into infant formula.

Fetuin

Bioactive compound	Tissue Source and Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Fetuin	Serum:11.34k Liver: 5.1kg	~22 g/Litre of serum	Potent inhibitor of systemic calcification. Mediate the transport and availability of a wide variety of cargo substances in the blood stream.	Cell Culture, diagnostics, veterinary applications	Bovogen Biologicals Sigma Aldrich; AbD Serotec, EMD Biosciences, Trace Biosciences NZ Ltd

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Fetuins are blood proteins produced by the liver then secreted into the blood stream. Fetuins belong to a large group of binding proteins mediating the transport and availability of a wide variety of cargo substances in the blood stream.

The best known representative of these carrier proteins is serum albumin, the most abundant protein in the blood plasma of adult animals. Foetal calf serum contains more fetuin than albumin, while adult serum contains more albumin than fetuin.

Fetuin is a mixture of proteins and other compounds containing a wide range of growth and attachment factors normally found in foetal calf serum. The major protein components include _-1-glycoprotein, _-1 and _-2 globulins, ß-globulins and a variety of growth factors such as Insulin Growth Factors and Fibroblast Growth Factors.

Commercial Applications

- Fetuin is a protein which contributes to the attachment and spreading of cells in culture medium. Researchers often add serum that contains fetuin for this purpose.
- Fetuin can be used as a glycoprotein standard for carbohydrate structure in a glycoprotein.
- Fetuin is used extensively in cell culture to increase both cell growth rates and protein expression.
- Fetuin is added to serum-free media to enhance the growth of cells beyond the capacity of insulin, transferrin and selenium.
- Fetuin therefore provides the advantages of foetal calf serum without the need for serum supplementation.

Price

Company	Amount	Price	Source	Reference:	Source date
AbD Serotec	1g	$349	Bovine foetal calf serum	http://www.ab-direct.com/catalog/datasheet.aspx?ProductCode=4430-2204&SearchType=Simple&SearchString=Fetuin	2008
EMD Biosciences	1g	$239	Bovine foetal calf serum	http://www.merckbiosciences.com/product/341506	2008
Sigma	5g	$796	Bovine foetal calf serum	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/F2379	2008

Market Size

Currently the market for Fetuin is centred around its research applications. Fetuin has several applications in research especially in cell culturing. The actual market size for fetuin was not determined though the cell culture market is worth approximately $1 billion annually.

Bovine Fetuin would only make up a small fraction of the cell culture reagent market.

What product processing is involved?

Ammonium sulphate fractionation. Pederson, K.O., *J. Phys. and Colloid Chem*., **51**, 164 (1947).

Purity: >99%

Yield: up to 21.6 mg from 1 ml of FBS (Cartellieri, Hamer et al. 2002)

Haemoglobin

Bioactive compound	Tissue Source and Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Haemoglobin	Red Blood Cells: 4.86kg	~ 150g /L of blood	Iron-containing oxygen-transport metalloprotein, assembled from four globular protein subunits. Each subunit is composed of a polypeptide chain tightly associated with a non-protein heme group.	When polymerised as a blood substitute; pigment; food additive, cell culture, diagnostics	US Biologicals, Worthington Biochemical Corp; Starrate Pty Ltd., Pan Biotech GmbH; Innovative Research Inc.,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Haemoglobin is the iron-containing oxygen-transport protein in the red blood cells of vertebrates. [1]

In mammals, the protein makes up about 97% of the red cell's dry content, and around 35% of the total content (including water of the cells).

Haemoglobin transports oxygen from the lungs or gills to the rest of the body, such as to the muscles, where it releases the oxygen for cell use. It also has a variety of other roles of gas transport and effect-modulation which vary from species to species, and are quite diverse in some invertebrates.

Commercial Applications

Human Haemoglobin has various uses in the medical industry the most important being blood transfusions for the injured and the sick. Animal haemoglobin is not suitable for human medical use as human and animal blood is not compatible and will often cause detrimental side effects.

Bovine haemoglobin is used for research purposes as a cheap substitute for human haemoglobin. Bovine haemoglobin is used for cell culturing and as a substrate for proteases and other enzymes.

An Australian company 'Bovogen Biologicals' sells Bovine Haemoglobin Powder that is ideal for a variety of applications, which include:

- A colouring agent for pet foods.
- A natural source of iron for nutraceutical use.
- A protein source for non-ruminant animals.
- A raw material for pharmaceutical porphyrin derivative production.

Bovogen Biologicals sells bulk bovine haemoglobin ranging from 500kg – 1000kg.

Price

Company	Amount	Price	Source	Reference:	Source date
Sigma	1000 g	$485	Bovine Red Blood Cells	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/H3760	2008
USB corp	1000 g	$64	Bovine	http://www.usbweb.com/category.asp?cat=bio&id=16890	2008
AbD serotec	10 g	$131	Bovine RBC	http://www.ab-direct.com/catalog/datasheet-4870-2002.html	2008

Market Size:

- The market size for human Haemoglobin and bovine Haemoglobin differ significantly due to their different uses.
- The human sourced Haemoglobin market is approximately US $ 11 billion worldwide and there are chronic supply shortages.
- Bovine Haemoglobin sales are substantially less than that as it is rarely used for medical purposes.
- Hemopure corporation manufactures 2 products from chemically stabilised haemoglobin; Hemopure as a substitute for human blood and Oxyglobin blood substitute for veterinary use.
- Hemopure has been approved for human use only in South Africa and it is used very rarely.
- Oxyglobin has been approved in the U.S. and Europe and total 2005 sales of Oxyglobin (for veterinary use) were $U.S 205,000.
- The market size for bovine Haemoglobin as a cheap alternative to human Haemoglobin for research purposes could not be determined.
- The market size of Bovine Haemoglobin for use in the pet food, neutraceutical and animal feed industries was not determined.

Haemopexin

Bioactive compound	Tissue Source and Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Haemopexin	Plasma 11.34kg	~500 mg/L	Plasma protein with the highest binding affinity to heme among known proteins.	sequestration of haem	Gentaur BvbA-Bioxys; Life Diagnostics, Athens Research

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Haemopexin binds heme (an iron protein) with the highest affinity of any known protein. Its function of scavenging the heme released or lost by the turnover of heme proteins such as haemoglobin, protects the body from the oxidative damage that free heme can cause.

Its function is to preserve the body's iron; haemopexin is the major vehicle for the transportation of heme in the plasma, thus preventing heme-mediated oxidative stress and heme-bound iron loss.

Clinical significance

Its levels in serum reflect how much heme is present in the blood. Low levels indicate that there is a lot of it. Therefore, low haemopexin levels indicates that there has been significant degradation of heme containing compounds - the latter being primarily haemoglobin, it indicates haemolysis and low haemopexin levels are therefore one of the diagnostic features of a haemolytic anaemia.

Current and Future Applications

No known commercial applications for bovine haemopexin at this time.

Price

Company	Units	Price	Source	Reference	Source date
Life Diagnostics	1 mg	$1056	Rat Serum	http://www.lifediagnostics.com/acute_phase_proteins.htm	2008
Athens Research	0.5 mg	$45	Human	http://athensresearch.com/protein/hemopexin.html	2008

Market Size

- No bovine haemopexin on the market
- No market data available
- Market for bovine Haemopexin would be limited to very specific research as it very expensive and has no commercial applications or medical applications.

Processing

Bioaffnity chromatography on haeme-Sepharose.

Purity: 95%

Yield:

- 0.5 mg/ml bovine blood (Noiva, Pete et al. 1987)
- 70% from human blood serum (Strop, Borvak et al. 1981)

What is the source of the product?

Plasma, Liver (increased after inflammation)

References

Noiva, R., M. J. Pete, et al. (1987). “Bovine serum hemopexin: properties of the protein from a single animal.” Comp Biochem Physiol B 88(1): 341-7.

Strop, P., J. Borvak, et al. (1981). “Isolation of human haemopexin by bioaffinity chromatography on haeme-sepharose.” J Chromatogr 214(3): 317-25.

Haptoglobin

Bioactive compound	Tissue Source and Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Haptoglobin	Serum: 11.34kg	~50 mg/L	Glycoprotein that binds free hemoglobin, in blood; an excellent acute phase marker in cattle	Diagnostics	Sigma; AbD Serotec; Life Diag-nostics.Inc., Gentaur BVBA -Bioxys Inc

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Haptoglobin is a haemoglobin-binding protein present in the plasma of all vertebrates and believed to participate in haemoglobin transport.

Haptoglobin is an acute-phase protein which means its concentration in blood plasma increases and decreases in response to inflammation.

Haptoglobin is synthesised in the liver, adipose tissue and lung.

Biological Function

Haptoglobins' primary function is to provide antioxidant and antimicrobial activity. Haptoglobin acts as a natural antagonist for receptor-ligand activation of the immune system.

This protein's potent antioxidant ability is due to is affinity to bind haemoglobin, thus preventing oxidative tissue damage. Haptoglobin has also been shown to prevent kidney damage, iron loss, and tissue destruction.

Commercial applications

- Haptoglobin prevents iron loss from ruptured or injured blood vessels.
- Haptoglobin levels dramatically rise after infection or injury; it is usually during this time that Haptoglobin is detectable in the blood.
- In 95% of cattle the levels of Haptoglobin are undetectable. This level dramatically rises following any form of trauma.
- Haptoglobin combines with free plasma oxyhaemoglobulin in an irreversible reaction to form a complex that cannot be filtered by the kidneys and hence helps prevent oxidative kidney damage.
- Additionally, changes in the measured serum concentrations can be used to assess the disease status of patients with inflammations, infections and malignancy.

Price

Company	Units	Price	Source	Reference	Source date
Sigma	5 mg	$569	Human plasma	http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/51325	2008
AbD Serotec	1 mg	$240	Human plasma	http://www.ab-direct.com/catalog/datasheet-4890-0504.html	2008
Life Diagnostics	5 mg	$1320	Bovine plasma	http://www.lifediagnostics.com/acute_phase_proteins.htm	2008

Market Analysis

Bovine and non bovine Haptoglobin is available online from several companies; however the exact size of this market cannot be defined due to limited available data.

Recommendations

Currently, Haptoglobin is predominantly used in research applications, however additional applications are using this bioactive as a biomarker in the detection of injury and infection. This protein has also been utilized in some cases to determine the presence of tissue damage in cattle[1].

The basal level of Haptoglobin is undetectable in the majority of cattle, excluding those that are injured or sick. This indicates that Haptoglobin does not seem to be a suitable candidate for commercial development and value adding to the cattle industry.

Reference

1 Monoclonal antibodies to bovine haptoglobin and methods for detecting serum haptoglobin levels US Patent Issued on September 3, 1996: September 3, 1996

Holo-transferrin

Bioactive compound	Tissue Source and Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Holo-transferrin	Serum:11.34k Liver: 5.1kg		Iron saturated form of transferrin; iron mobilisation;	Cell Culture, biochemistry, cancer therapies	Bovogen Biologicals Pty. Ltd.; SAFC Biosciences; Sigma Aldrich

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Holo-transferrin is the iron saturated form of transferrin. Transferrin is a blood plasma protein for iron delivery. Transferrin is a glycoprotein, which binds iron very tightly but reversibly. Although iron bound to transferrin is less than 0.1% of the total body iron, dynamically it is the most important iron pool, with the highest rate of turnover.

Transferrin is also associated with the innate immune system. Transferrin is found in the mucosa and binds iron, thus creating an environment low in free iron, where few bacteria are able to survive.

Current and Future applications

- As Holo-transferrin is the iron saturated form of transferrin, it would have no affinity for unbound Fe; therefore it would not be an effective bactericide.
- It may be an effective nutritional supplement to help boost iron levels, however no scientific studies have been found relating to this theory.
- The majority of research into holo-transferrin is being done on human holo-transferrin.

Price

Company	Units	Price	Source	Reference	Source date
Sigma	1 g	$870	Bovine	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/T1283	2008
Merck	100mg	$132	Bovine plasma	http://www.merckbiosciences.com/product/616420	2008

Market Size:

As Bovine Transferrin is used extensively in cell culturing the world market is approximately 1000kg a year.[1]

The market size for holo-transferrin was not determined; however as holo-transferrin has fewer commercial applications than transferrin the market would be much smaller.

What product processing is involved?

The protein to be treated can be manufactured according to general methods for fractionating plasma and separating the different plasma proteins, or by recombinant methods.

Isolated from FrIV in “Cohn’s cold ethanol method” and further purified by chromatography[2]

Purity: ≥97.0%[3]

Content

- Human: 1000 mg/L[3] Whole blood
- Human: 1600 to 3700 mg/L blood (0.16 to 0.37 g/dl)[5]
- When iron stores are low, Transferrin levels increase, while Transferrin is low when there is too much iron.

Reference

1 http://www.equitech-bio.com/anim_ser_plasma_main.html)

2 http://www.prospec.co.il/~prospec/cart/catalog/bHTF.html

3 http://v3.espacenet.com/textdes?DB=EPODOC&IDX=US5817765&F=1&QPN=US5817765

4 Schwartz and Granger, 2004

5 http://www.fpnotebook.com/HEM78.htm

Myoglobin

Bioactive compound	Tissue Source and Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Myoglobin	Heart: 1.5kg	~25 g/Kg	Primary oxygen-carrying pigment of muscle tissues	diagnostics;	Hytest Ltd; Calzyme Inc., Sigma Aldrich;

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Myoglobin is the primary oxygen-carrying pigment of muscle tissues.

High concentrations of myoglobin in muscle cells allow organisms to hold their breaths longer

Myoglobin forms pigments responsible for making meat red. The color that meat takes is partly determined by the charge of the iron atom in myoglobin and the oxygen attached to it. When meat is in its raw state, the iron atom has a charge of +2 and is bound to O_2, an oxygen molecule. Meat cooked well done is brown because the iron atom has a charge of +3, having lost an electron, and is now bound to a water molecule (H_2O)

Myoglobin is released from damaged muscle tissue, which has very high concentrations of myoglobin. The released myoglobin is filtered by the kidneys but is toxic to the renal tubular epithelium and so may cause acute renal failure.

Commercial Applications

Myoglobin is a sensitive marker for muscle injury, making it a potential marker for heart attack in patients with chest pain, though this involves its detection rather than its use.

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	5mg	$1525	Sperm Whale (Recombinant)	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/M7527	2008
Sigma	10000	$1535	Equine heart	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/M1882	2008

Market Size

- The only commercial use for Myoglobin is in antigen production for medical diagnostic markers.
- The market for bovine Myoglobin would be limited to research.

What product processing is involved?

Precipitation in buffer solution (w/ 70% of sat. (NH4)2SO4).

Purity: 95%

Transferrin

Bioactive compound	Tissue Source and Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Transferrin	Plasma: 11.34kg	1.2 - 3.5 g /L	Essentially all circulating plasma iron normally is bound to transferrin; iron-binding glycoprotein that controls the level of free iron in biological fluids	Iron transporter with a wide range of uses, ranging from cell culture, diagnostics and biomanufacturing	Equitech-Bio; Bioscienc-es NZ Ltd; Sigma, Proliant Inc., Biosciences

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Transferrin is a blood plasma protein for iron ion delivery. Transferrin is a glycoprotein (a protein attached to a sugar), which binds iron very tightly but reversibly. Although iron bound to transferrin is less than 0.1% (4 mg) of the total body iron, dynamically it is the most important iron pool, with the highest rate of turnover.

Transferrin is also associated with the innate immune system. Transferrin is found in the mucosa and binds iron, thus creating an environment low in free iron, where few bacteria are able to survive.

Deficiency of transferrin can cause iron products to accumulate in the liver resulting in liver damage and possibly liver failure.

Commercial Application

Transferrin acts as an iron carrier transporting essential iron from media to the cell during cell culture. It has been identified as one of the serum proteins critical to the culture of mammalian cells. One of the limitations of serum-free media is the availability of iron in a form that is bioavailable. The di-ferric form of iron in transferrin (2 atoms of iron per transferrin molecule) is the most bioavailable form for cells.

Transferrin also behaves as a scavenger of toxic metal ions and will scavenge harmful cations from solutions. It is also known to behave like a growth factor in cell culture improving the performance of cells in-vitro.

The concentration of transferrin used in cell culture will vary with the media and cell type but as a guide, the concentration should be between 10 – 200mg/L.

Cell culture reagent vendors often supply transferrin in the apo (iron free), holo (iron saturated) or partially saturated forms and are soluble in cell culture media or similar products.

Research application summary

- Bovine Transferrin is a crucial component for the cultivation of mammalian cells in-vitro.
- Bovine Transferrin is critical for long-term cells growth in-vitro.
- Bovine Transferrin is used for detoxification of media by binding contaminating metal ions.
- Bovine Transferrin is often used as a nutrient in fermentation media for recombinant protein and biopharmaceutical production.
- Additional common uses of Bovine Transferrin are: Molecular weight, Affinity purification of anti-human transferrin antibodies and also as receptor mediated transfection of molecules such as DNA, into cells.[1]

Price

Company	Amount	Price	Source	Reference	Source date
Sigma	1000mg	$811	Bovine liver	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/T1428	2008
Sigma	1000mg	$1005	Human	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;T1283	2008

Market

As Transferrin is used extensively in cell culturing the market demand for transferrin is significant. The assumed worldwide market size would be approximately 1000kg a year.[2] Based upon the prices quoted 1000kg would have a retail value of $811 000. Transferrin sales make up a small fraction of the cell culture reagent market which is worth approximately $2 billion worldwide and growing 15% annually.[3]

Although bovine transferrin was the industry standard for the last 30 years, recently there has been a demand from the market for non-animal sources. This demand was met recently with the development of recombinant transferrin produced from yeast. Although recombinant transferrin is more expensive than bovine transferrin it is seen as a more reliable product, as it is disease free and the price does not fluctuate. Sold under the brand name Deltaferrin the price for this transferrin is likely to fall as the market grows and becomes more competitive.

Reference

1 http://www.prospec.co.il/~prospec/cart/catalog/bHTF.html

2 http://www.equitech-bio.com/anim_ser_plasma_main.html

3 http://www.genengnews.com/articles/chitem.aspx?aid=2086&chid=0

Lipids

Ceramides

Conjugated Linoleic acid

Ganglioside

Lysophosphatidylcholine (Lyso-PC or Lysolecithin)

Phosphatidylserine

Ceramides

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Use	Supplier
Ceramides	Brain: 0.34kg	Amounts vary according to extraction process	Skin care cosmetics, cell biology	Very large number of suppliers/users

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Ceramides are a family of lipid molecules. They are found in high concentrations within cell membranes and are important structural lipids and signaling molecules.

Biological Function

Ceramides are involved in essential structures in animal tissues (skin and cell membranes), as signalling molecules between cells, and are the building blocks of complex sphingolipids[3].

Although most tissues synthesise ceramides, they are usually used up quickly and free ceramides rarely accumulate. Ceramides are a major lipid constituent of the skin, as they provide the skin with its water-permeability barrier, and contribute to the skin layers organisation and cohesion[3].

Commercial Applications

There are many different grades of ceramides however their major use is in skin and hair care products.

Many skin disorders (eg psoriasis, acne, dry skin, aging skin and dermatitis) have been linked with lower ceramide levels, and changes in the ceramide composition of the skin (corneum layer)[4].

Formulations containing lipids, amongst them ceramide, in combinations similar to the one found naturally in skin, have been shown to relieve several of these conditions[5].

Ceramides have an obvious role in the barrier properties of the skin, limiting loss of water and solutes and at the same time preventing ingress of harmful substances.

Price

Company	Unit	Price	Source	Reference:	Source Date
Sigma	100mg	$694	Bovine brain	http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/22244	2008
Sigma	1mg	$3520	Human plasma	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;A9141	2008

The cost of the ceramides on the market is very high, about $4000/kg.[1]

Market Size

- Ceramide specific market information has been very hard to locate. As they are used in skincare their major market's growth characteristics were outlined as an indication of the market.
- The skincare market is rapidly increasing largely due to the ageing baby boomer population[7].
- Facial skincare is the most mature market within the skincare industry and was $6.7 billion in 2004 showing growth of 23% between 1999 and 2004.
- The global cosmetics and toiletries market is growing rapidly and is projected to reach $19 billion by 2010[2].
- In the US the skincare industry has showed an average compounded growth of 14.5% over 5 years[6].

Competition

Products

There are a diverse range of sources of ceramides and hence competing products including phytoceramides or plant-derived ceramides[8], sheep obtaining ceramides from wool[9].

Since ceramides can also be extracted from all nerve and skin-tissues, therefore it may be obtained from many other animals, from chicken eggs and the human brain.

Raw Materials

The raw material for cerebrosides in cosmetics comes from cattle, oxen or swine brain cells or other nervous system tissues. Alternatively, the raw material may be isolated from plant sources. Industry cosmetic scientists claim that the use of cerebrosides in skin products results in a smoother skin surface and better moisture retention, effects that translate into marketing claims such as luminosity and ever-improving hydration. It should be noted that the FDA has not evaluated the studies on which these claims are based."[10]

Recommendations

Due to the complex structure and cost of processing ceramides we believe that even though there is a synthetic version of this product it would not threaten the market potential for ceramides sourced from bovine brains.

Also, providing that the global skin care industry continues in its current trend of growth there will be a demand for ceramides.

Reference

1 http://www.tekes.fi/partner/eng/search/nayta_haku.asp?hakuid=37672

2 BCC Research (2006) GLOBAL MARKET FOR COSMETICS AND TOILETRIES WILL APPROACH $19 BILLION BY 2010 [Online]. Available: http://www.bccresearch.com/editors/RCHM019E.html. [Accessed: 10 Oct. 2006].

3 BIOMOL International LP (2006) Product Guide [Online]. Available: http://www.biomol.com/. [Accessed: 27 Sep 2006].

4 Choi, M. J., and H. I. Maibach. 2005. Role of Ceramides in Barrier Function of Healthy and Diseased Skin. Am J Clin Dermatol. 6 (4): 215-223 [Online]. Available: EBSCOHost Database [Accessed: 27 Sep. 2006].

5 Coderch, L., O. López, A. de la Maza, and J. L. Parra. 2003. Ceramides and Skin Function. Am J Clin Dermatol. 4 (2): 107-129 (accessed September 27, 2006, from EBSCOhost database)

6 Feedback.com (2005) US Consumer Skin Care Products [Online]. Available: http://www.feedback.com/mar04ezine.htm. [Accessed: 10 Oct. 2006].

7 Global Information Inc. (2002) Anti-Aging Skincare Treatments - US - October 2005 [Online]. Available: http://www.giichinese.com.tw/chinese/mt33406-skincare.html. [Accessed: 10 Oct. 2006].

8 Kertec Limited (2004) Home [Online]. Available: http://www.keratec.co.nz/keratec/home/. [Accessed: 3 Nov. 2006].

9 Larodan Fine Chemicals (2006) Phytoceramides (N-acyl-Phytosphingosines) NEW [Online]. Available: http://www.larodan.se/prod.asp?ProdGrpID=815. [Accessed: 3 Nov. 2006].

10 U.S. Food and Drug Administration (2002) Cosmetic Ingredients:Understanding the Puffery [Online]. Available: http://www.fda.gov/fdac/reprints/puffery.html. [Accessed: 27 Sep. 2006].

Conjugated Linoleic Acid

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Conjugated Linoleic acid	Muscle Fat: N/A	1.2-10mg/g depending on source		Pharmaceutical and nutraceutical / functional food applications; animal feed additives	Fraken Biochem, Sigma, HungChoy.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Conjugated linoleic acids (CLA) are a family of at least 13 types (isomers) of linoleic acid found especially in the meat and dairy products derived from ruminants. Some are beneficial and some are detrimental to health.

Conjugated linoleic acids are trans fatty acids. Unlike other trans fatty acids, CLA is not harmful, but is in fact claimed to be beneficial. CLA has been proven to reduce fat levels and increase lean body mass and hinder cancer growth.

CLA is produced naturally in the human body however it is only produced in small quantities that are insufficient to gain any significant benefits. The primary source of CLA is from the meat and dairy products of ruminants. Ruminants contain high concentrations of CLA as it is produced as a by-product of microbial fermentation in the rumen.

The production and concentration of CLA found in ruminants is significantly affected by their diet. Cattle grown primarily on pasture grasses have 3-5 time more CLA than cattle raised on grains. Recent studies have found that Australian beef has higher levels of CLA than beef from any other beef producing countries.

Diet and health

Antioxidant and anti-cancer properties have been attributed to CLA, and studies on mice and rats show encouraging results in hindering the growth of tumors in mammary, skin, and colon tissues.

Studies of CLA in human diets show that it tends to reduce body fat, particularly abdominal fat, improves serum lipid profiles, and decreases whole-body glucose uptake. The maximum reduction in body fat was achieved with a daily dose of 3.4g.

Food products from grass-fed ruminants are good sources of CLA, and contain much more of it than those from grain-fed animals. In fact, meat and dairy products from grass-fed animals can contain 300-500% more CLA than those of cattle fed the usual diet of 50% hay and silage, and 50% grain. Australian beef has the highest concentration of CLA with 17mg/g of fat, but one would need to eat 1kg of beef per day in order to absorb the 3.4g of CLA.

Commercial Applications

CLA produced from safflower oil and linseed is sold as a supplement and is widely available in Australia and across the globe. It is often sold as a fat burner and sports supplement and retails for $40 Australian for sixty 100mg tablets.

There are no supplements containing bovine CLA on the market. Oil seeds are the primary source of CLA as they are a much cheaper raw material than beef products and vegetable oils can be transformed into CLA synthetically.

Though CLA can be produced cheaply from vegetable oils, it appears that the CLA so produced is not the correct CLA and has been associated with increases in cardiovascular diseases and insulin resistant diabetes. Therefore if CLA could be produced from ruminants without changing its structure there may be a market for the product.

Price

Company	Amount	Price	Source	Reference:	Source Date
Sigma	1000mg	$189	N/A	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/O5507	2008
Sigma	1mg	$3520	Human plasma	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;A9141	2008

Global Market

The global sales of CLA have been estimated to increase in the future from $30-40 million in 2002 to $2 billion in 2014.

In 2006 the global sales of CLA used for functional foods was estimated at $650 million. With $150 million sold for dietary supplements, $117 million for immunity related products, and $90 million for dietary foods individually.

The present international market demand of conjugated linoleic acid for foods, functional foods, cosmetics and drugs is about 8 000-12 000 tons per year. http://fec2.mofcom.gov.cn/aarticle/cooperativeprojects/200608/20060802950180.html)

Strengths

- Demand for CLA is predicted to increase
- CLA is reported to have several beneficial affects.
- Ample opportunities for CLA to be used in functional foods

Weaknesses

- Primary source of CLA is reported to be bovine muscle.

Threats

- New cheaper sources of CLA have been developed e.g sunflower, fish oil etc.

Reference

"Handbook of Functional Lipids" (2006)

http://www.nutraingredients.com/Research/Obese-children-may-benefit-from-CLA-study

http://www.nutraingredients.com/Research/Science-stacks-up-for-CLAs-weight-management

Ganglioside

Protein	Source (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Asialoganglioside GM1	Brain: 0.34kg	Purified via salt/solvent extraction & HPLC	Differential markers of erythropoietic and granulopoietic cells in bone marrow; gene transfections.	Glycobiology applications; diagnostics; cell culture reagent	Numerous Suppliers
Asialoganglioside GM2	Brain: 0.34kg	Purified via salt/solvent extraction & HPLC	Differential markers of erythropoietic and granulopoietic cells in bone marrow; gene transfection;	Glycobiology applications; diagnostics; cell culture reagent	Numerous Suppliers
Ganglioside GD1a/b	Brain: 0.34 Eyes: 0.05kg Kidney: 1.2kg Pancreas: 0.34kg	~50 mg/Kg	Most of the common range of gangliosides are derived from the ganglio- and neolacto-serine of neutral oligoglycosphingolipids, Their abundance relative to other lipids is usually low	Cell culture, diagnostics, pharmaceutical, cosmetic and various other applications	Numerous Suppliers
Ganglioside GD2	Brain: 0.34 Eyes: 0.05kg Kidney: 1.2kg Pancreas: 0.34kg	Amounts vary from 6-350 nmole/ g dry weight	see Item above	see Item above	Numerous Suppliers
Ganglioside GD3	Brain: 0.34 Eyes: 0.05kg Kidney: 1.2kg Pancreas: 0.34kg	Amounts vary from 6-350 nmole/ g dry weight	see Item above	see Item above	Numerous Suppliers
Ganglioside GM1	Brain: 0.34 Eyes: 0.05kg Kidney: 1.2kg Pancreas: 0.34kg	Amounts vary from 6-350 nmole/ g dry weight	see Item above	see Item above applications	Numerous Suppliers

Ganglioside GM2	Brain: 0.34 Eyes: 0.05kg Kidney: 1.2kg Pancreas: 0.34kg	Amounts vary from 6-350 nmole/ g dry weight	see Item above	see Item above	Numerous Suppliers
Ganglioside GM3	Brain: 0.34 Eyes: 0.05kg Kidney: 1.2kg Pancreas: 0.34kg	Amounts vary from 6-350 nmole/ g dry weight	see Item above	see Item above	Numerous Suppliers
Ganglioside GM4	Brain: 0.34 Eyes: 0.05kg Kidney: 1.2kg Pancreas: 0.34kg	Amounts vary from 6-350 nmole/ g dry weight	see Item above	see Item above	Numerous Suppliers
Ganglioside GT1a/b	Brain: 0.34 Eyes: 0.05kg Kidney: 1.2kg Pancreas: 0.34kg	Amounts vary from 6-350 nmole/ g dry weight	see Item above	see Item above	Numerous Suppliers

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Ganglioside is a component of the cell plasma membrane that modulates cellular interactions. Gangliosides belong to a group of lipids known as glycolipids, which are carbohydrates attached to lipids. Their role is to provide energy and also serve as markers for cellular recognition.

Gangliosides occur where a carbohydrate chain is associated with phospholipids on the outer surface of the cell membrane. The carbohydrates are found on the outer surface of all multi-cellular organism cell membranes.

They extend from the phospholipid bilayer into the aqueous environment outside the cell where it acts as a recognition site for specific chemicals as well as helping to maintain the stability of the membrane and attaching cells to one another to form tissues.

Ganglioside is a compound composed of a glycosphingolipid (ceramide and oligosaccharide) with one or more sialic acids (N-Acetyl-Neuraminic Acid NANA) linked on the sugar chain. The 60+

Location

They are present on cell surfaces, with the 2 hydrocarbon chains of the ceramide chain embedded in the plasma membrane and the oligosaccharides on the extracellular surface.

Gangliosides can amount to 6% of the weight of lipids from brain, where they constitute 10-12% of the total lipid content (20-25% of the outer layer) of neuronal membranes. The main gangliosides of the brain are GM1, GD1a, GD1b and GT1. GM3 is present mainly outside brain tissues.

Function

Gangliosides found on the surface of membranes provide cells with distinguishing surface markers that can serve in cellular recognition and cell-to-cell communication.

In the plasma membrane, it is believed that gangliosides are segregated together with other sphingolipids and cholesterol into raft micro-domains, where the very large surface area occupied by the oligosaccharide chain imparts a strong positive curvature to the membrane. Therefore gangliosides are vital for cells as they help hold the shape of the cellular membrane.

The occurrence of gangliosides in cell nuclei suggests a possible involvement of gangliosides in the expression of genes relevant to neuronal function. Ganglioside GM1 is also important for Calcium homeostasis in the nucleus. Calcium ions are used by cells to send electrical signals and contraction of all muscle types.

The Guillain–Barré syndrome is an acute inflammatory disorder, usually triggered by a severe infection, which affects the peripheral nervous system. Antibodies to gangliosides are produced by the immune system, leading to damage of the axons. It can result in paralysis of the patient.

Impaired ganglioside metabolism is also relevant to Alzheimer's disease, as the aggregated amyloid _-protein deposits that characteristically accumulate are complexed with ganglioside GM1.

In contrast, gangliosides are believed to have a neuro-protective role in certain types of neuronal injury, Parkinsonism, and related diseases.

In experimental systems, gangliosides have been shown to be cell-type specific antigens that control growth and differentiation of cells, and to have an important role in the interactions between cells. In particular, they have key functions in the immune defence systems.

Also, they are involved in pathological states such as cancer, as certain distinctive gangliosides are found in tumours but not in the normal healthy tissue.

They act as receptors of interferon, epidermal growth factor, nerve growth factor and insulin and in this way may regulate cell signalling

Commercial Applications

Human gangliosides contain only N-acetylneuraminic acid residues, whereas bovine gangliosides may contain both N-acetylneuraminic ($C_{18:1}$) and N-glycosylneuraminic acid ($C_{20:1}$) residues at a ratio of 3:2[9]. This difference in bioequivalence has been identified in clinical trials to cause serious adverse side effects when injected into human systems[11]. Therefore bovine derived gangliosides are not widely used as biologicals.

Bovine derived gangliosides are used in some consumer products as a less pure form known as glycosphingolipids[12]. These include dairy products derived from milk[13] and cosmetic products such as lip balm, shower gels, anti aging creams[14].

Potential use as a neutraceutical

Studies with sphingolipids in animal models have shown that consumed sphingolipids are; anti-carcinogens, cytotoxic to tumour cells, reduce serum low-density lipoprotein, and elevate high-density lipoproteins[16]. Glycosphingolipids are current publicly consumed as bovine milk fat within both milk (lyophilized 21.24±2.88_g/g) and fresh cheese (84.40_g/g)[17].

It is widely accepted that sphingolipids are highly biologically active. In several studies (both in vitro and in-vivo on rats) it has been shown that sphingolipids and their digestion products ceramide and sphingosine have a strong cancer inhibiting effect in the large intestine. These metabolites can also have an inhibiting effect on inflammation in the case of chronic bowel disease (colitis ulcerosa, Crohn's disease). Furthermore, sphingolipids have an anti-oxidative and anti-inflammatory effect in test animals, and they lower the absorption of cholesterol. Phospholipids are easily absorbed in the small intestine, and they have a beneficial nutritional effect.

Market

The market size for gangliosides was not determined.

There are no commercial applications for gangliosides purified into individual types, or as a whole family of 60+ types.

Research supply companies like Sigma sell gangliosides for research purposes. Sigma only stocks bovine gangliosides. The prices quoted for gangliosides are steep with prices ranging from $120-$7000 per mg.

The largest current market for bovine derived gangliosides can be extrapolated to be the cosmetics industry. The moisturising properties of gangliosides in the form of glycosphingolipids are highly regarded in the cosmetics industry. Cosmetics market is worth $35 billion annually.

Alternatively to the Neutraceutical and Functional Food markets may be suitable references for market size. The European Functional Food market was estimated to be worth US$12 billion in 2006, and

the US market was estimated to US$18.9 billion[32]. While in 2006 the Phood market, that is foods providing positive pharmaceutical benefit beyond their basic nutrients, is valued at US$25 billion globally and is predicted to grow to $39 billion by 2011[33].

The primary problem with gangliosides is that their primary source is the brain and nervous tissue. All other tissue sources are significantly lower and would be uneconomical to extract from.

Although BSE is not found in Australia there is still a stigma related to bovine brains and products derived from animal brains. Even though gangliosides could be a valuable bioactive in terms of health cognitive health benefits and moisturising cosmetics, consumers would be unlikely to except them if they knew the source.

Gangliosides are abundant in all animal brains and could be extracted from other animals like sheep, pigs and fish.

Other

Gangliosides are not the easiest of lipids to analyse, as they are most 'un-lipid-like' in many of their properties. For example, in the conventional Folch method for extraction of lipids from tissues, the gangliosides partition into the aqueous layer rather than with the conventional lipids in the chloroform layer. Nonetheless, methods have been devised for quantitative extraction, and they can then be sub-divided into the various molecular forms by high-performance thin-layer chromatography (or less commonly by high-performance liquid chromatography). Nowadays, mass spectrometry is the probably main method for structural analysis and especially for identifying and sequencing the carbohydrate chains, with invaluable assistance from nuclear magnetic resonance spectroscopy.

Competitors

There are no competitive products that incorporate ganglioside, glycosphingolipids or glycolipids as a competitive advantage for use as a neutraceutical of functional food.

Other competitive raw materials include that which are found from other tissue sources than bovine. Gangliosides are found in the majority of vertebrates but may differ structurally from system to system. Therefore any large vertebrate has the potential to compete with bovine derived gangliosides.

Price

Asialoganglioside GM1

Company	Amount	Price	Source	Reference:	Source Date
Sigma	1mg	$541	Bovine Brain	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G3018	2008
Axxora	1mg	$305	Bovine Brain	http://www.axxora.com/	2008

Asialoganglioside GM2 (GA2)

Company	Amount	Price	Source	Reference:	Source Date
Sigma	1mg	$7840	Bovine Brain	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G9398	2008
Axxora	1mg	$4980	Bovine Brain	http://www.axxora.com/	2008

Ganglioside GD1a/b

Company	Amount	Price	Source	Reference:	Source Date
Sigma	5mg	$567	Bovine Brain	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G2392	2008

Ganglioside GD3

Company	Amount	Price	Source	Reference:	Source Date
EMD Biosciences	1mg	$367	Bovine Milk	http://www.emdbiosciences.com/Products/ProductDisplay.asp?catno=345752&	2008
Sigma	1mg	$627	Bovine Milk	http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/43683	2008

Ganglioside GM1

Company	Amount	Price	Source	Reference:	Source Date
Sigma	1mg	$119	Bovine Brain	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G9652	2008

Ganglioside GM2

Company	Amount	Price	Source	Reference:	Source Date
Sigma Aldrich	1mg	$420	Bovine brain	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G8397	2008
Spectrum Chemicals	1mg	$580	Bovine brain	https://webstore.spectrumchemical.com/OA_HTML/ibeCCtpItmDspRte.jsp?a=b&item=1&itemGrpNum=G3183&isSupply=0	2008
Axxora	1mg	$251	Bovine brain	http://www.axxora.com/?content=open.php%3FPID%3DALX-302-003	2008

Ganglioside GM3

Company	Amount	Price	Source	Reference:	Source Date
EMD Biosciences	0.5mg	$134	Bovine milk	http://www.emdbiosciences.com/product/345733	2008

Ganglioside GM4

Company	Amount	Price	Source	Reference:	Source Date
EMD Biosciences	1mg	$622	Human	http://www.emdbiosciences.com/product/345748	2008

Ganglioside GT1a / GT1b (GT1)

Company	Amount	Price	Source	Reference:	Source Date
Merck	1mg	$240	Bovine brain	http://www.merckbiosciences.com/product/345744	2008
Sigma	5mg	$818	Bovine brain	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G3767	2008

Lysophosphatidylcholine (Lyso-PC or Lysolecithin)

Protein	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Lysophosphatidylcholine	Brain: 0.34kg Liver: 5.1kg	Varies according to tissue and process	Has one mole of fatty acid per mole of lipid in position sn-1; found in small amounts in most tissues. It is formed by hydrolysis of phosphatidyl-choline by the enzyme phospholipase A2,	Diagnostics, cell culture, functions in cell signalling, stearoyl lysophosphatidylcholine is protective against lethal sepsis; zwitterionic detergent	Avanti Polar Lipids Inc., Fischer Scientific; Sigma Aldrich; Lipoid GmbH

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Lysophosphatidylcholine, is a functional lipid found in small amounts in most tissues. It is formed by hydrolysis of phosphatidylcholine by the enzyme phospholipase A2

Lyso-PC is reported to be involved in detrimental processes such as hardening of the arteries, free radical production, tumour growth, and cancer development.

Recently, it has been found to have some functions in cell signalling, and specific receptors have been identified. Stearoyl lysophosphatidylcholine has been shown to be protective against lethal sepsis in experimental animals by various mechanisms, including stimulation of white blood cells to eliminate invading pathogens.

Current Market Applications

Lyso-PC does not have any commercial market applications except as a research reagent; however its precursor phosphatidylcholine (PC) is available in various products. PC has been extensively applied to various products in the food industry and growing application in pharmaceutical and cosmetic sectors. It is mainly obtained from eggs and soy beans.

As Lyso-PC has been found to be detrimental to human health the majority of research on the lipid is on its reduction in foods and removal from the body.

Price

Company	Source	Amount	Price	Unit Cost	Reference	Source Date
Sigma-Aldrich	Bovine Brain	25mg	$248	$9.94/mg	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/L1381	2008
Sigma-Aldrich	Soy Bean	5ml	$249	$49.80/ml	http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/62963	2008
Sigma-Aldrich	Egg Yolk	250mg	$204	$0.81/mg	http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/62963	2008
Sigma-Aldrich	Egg Yolk	1000mg	$779	$0.78/mg	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/L4129	2008

Market

The world market for Lyso-PC was not determined however it is likely to be insignificant and restricted to research.

As Lyso-PC is detrimental to human health and it has no commercial applications it is unlikely that market demand for lyso-PC will increase.

Lyso-PC can also be extracted and produced from egg yolk at a much lower cost than from bovine brain.

Opportunity potential: possible “research reagent”

Summary of Commercial Potential for Australian Beef Industry

Strengths – bovine sourced lysophosphatidylcholine is sold commercially by companies such as Sigma Aldrich

Weaknesses – ubiquitous bioactive and with limited concentrations in bovine tissue

Opportunities – higher yield of the phospholipid, phosphatidylcholine, than that of its enzymatic generated species

Threats – availability of lysophosphatidylcholine from egg yolk and soy bean sources

Concentration in Bovine Tissue

Bovine adrenal glands

Bovine adrenal glands - purified	
Product	Protein (per kg tissue)
Lyso-PC	72 mg

G. Arthur and A. Sheltawy, ‘The presence of lysophosphatidylcholine in chromaffin granules’, Biochem J. (1980) 191, 523-532

Bovine brain – no data for concentration of Lyso-PC from bovine brain

Reference

1 http://www.lipidlibraryLYSOPHOSPHATIDYLCHOLINEco.uk/Lipids/pc/index.htm

2 Zhang, W., He, H., Feng, Y. and Da. Shilu, (2003). Separation and purification of phosphatidylcholine and phosphatidylethanolamine from soybean degummed oil residues by using solvent extraction and column chromatography. *Journal of Chromatography B, vol 798 (2), 323-331.*

3 http://www.physoc.org/publications/proceedings/archive/article.asp?ID=J%20Physiol%20539PS195

4 http://www.kewpie.co.jp/english/pdf/food/lpl_20.pdf

5 http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=Retrieve&db=PubMed&list_uids=4080786&dopt=Abstract

6 http://www.patentstorm.us/patents/6235336-description.html

7 http://www.ingentaconnect.com/content/els/01410229/2001/00000029/00000010/art00447

8 http://www.freepatentsonline.com/5955327.html

Phosphatidylserine

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Phosphatidylserine	Brain: 0.34kg	>800 mg/Kg	Involved in cell signaling and apoptosis; regulates many metabolic processes including neuronal signalling.	Cell biology; diagnostics; food ingredient, nutraceutical;	Jena Bioscience; Lipoid Inc., Degussa Food Ingredients; Chemi SpA, Sigma

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Phosphatidylserine (PS) is a naturally occurring phospholipid present in biological membranes. It was originally isolated from lipid of cerebral origin, and it is particularly enriched in the brain where it comprises 10–20% of the total phospholipid pool and especially in the synaptic membrane zones.

PS is indispensable for the activation of some of these proteins, such as protein kinase C, a membrane-bound enzyme that seems to play crucial roles in the transmembrane control of cellular functions. PS, among other phospholipids, has been shown to play a key role in the conduction of the nerve stimulus and the response via neurotransmitter biosynthesis and release.

PS is not very abundant in foodstuffs and few foods except for brain provide substantial amount of PS. For this reason, it must be synthesized by the body through a complex series of reactions, which require heavy expenditure of energy.

Health Benefits

PS has been shown to slow cognitive decline in animal models. PS has been investigated in a small number of double-blind placebo trials and has been shown to increase memory performance in the elderly. Because of the potential cognitive benefits of phosphatidylserine, the substance is sold as a dietary supplement to people who believe they can benefit from an increased intake.

Bioavailability after oral administration is excellent: PS peaks in the blood after approximately 30 min. A few minutes later, it is also found in the brain, where it is distributed mainly in the cortex, hypothalamus and hippocampus.

Phosphatidylserine has demonstrated some usefulness in treating cognitive impairment, including Alzheimer's disease, age-associated memory impairment and some non-Alzheimer's dementias. More research is needed before phosphatidylserine can be indicated for immune enhancement or for reduction of exercise stress.

FDA gave a "qualified health claim" status to phosphatidylserine, stating that "Consumption of phosphatidylserine may reduce the risk of dementia in the elderly" and "Consumption of phosphatidylserine may reduce the risk of cognitive dysfunction in the elderly".

Safety

Traditionally, PS supplements were derived from bovine cortex; however, due to the potential transfer of infectious diseases, soy-derived PS has been established as a safe alternative.

Soy-derived PS is Generally Recognized As Safe (GRAS) and is a safe nutritional supplement for older persons if taken up to a dosage of 200 mg three times daily.

Dietary Sources

PS can be found in meat and fish, but is most abundant in the brain and in organs such as liver and kidney. Only small amounts of PS can be found in dairy products or in vegetables, with the exception of white beans.

Commercial applications

Soy bean PS is sold as a cognitive health supplement and it is widely available in Australia and the world. PS is sold primarily in tablet or soft gel capsule and sixty 100mg tablets sell for U.S$32 on average.

The cosmetics industry has expressed interest in PS as an anti-aging and anti wrinkle cream, PS is reported to have great potential. Though the products appear to be still in development as there were no cosmetics containing PS as this point in time.

Price

Company	Unit	Price	Source	Reference:	Source Date
Sigma	100mg	$550	Bovine brain	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/P5660	2008
Sigma	100mg	$470	Soy	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/P5660	2008

Market Size

Phosphatidylserine market size was not determined; however the market for PS is similar to the market of Ginko Biloba another beneficial cognitive product. The annual sales for Ginko are reported to range from U.S $100 million to $1 billion.

The US cognitive market for supplements is estimated to worth around $350mil. Alzheimer care costs around $100 billion a year around the world. The market for PS is growing in developed countries as consumers have become more concerned about their cognitive health.

Phosphatidylserine taken from bovine cortex is usually not sold because it may contain infectious agents inadvertently introduced in the phosphatidylserine product when extracted from the animal brain Australian sources should not suffer from this restriction.

What product processing is involved?

The method is based on (i) the separation of phosphatidylserine from phosphatidylinositol in bovine brain extract by preparative aminopropyl normal-phase high-performance liquid chromatography using methanol-1 M phosphoric acid (90:10, v/v) as mobile phase and (ii) further purification of phosphatidylserine by anion-exchange chromatography. The main advantage of this approach is that polyunsaturated acid-containing molecular species of brain phosphatidylserine are not lost in the preparation procedure

Strengths

- Phosphatidylserine market has not matured yet.
- The market is not as competitive as other bioactive products.
- The demand for cognitive health and brain boosters is likely to increase as degenerative brain disorders become more common.
- Australia can produce PS without the risks associated with BSE.
- Phosphatidylserine is found in bovine liver and kidney at reasonable levels.

Weaknesses

- PS is currently produced from soy
- Market prefers plant based sources compared to animal brain sources.

Other Bioactives

Alpha-Crystallin

Actin

Alpha 1 - Acid Glycoprotein

Calmodulin

Fibronectin

L-Carnosine

Myosins

Osteocalcin

Osteonectin

Thyroglobulin

Troponins

Alpha-Crystallin

Protein	Tissue Source & Weight (kg/head)*	Abundance (per kg of tissue)	Mode of Action	Use	Supplier
Alpha-Crystallin	Eye 0.05kg	Most abundant lens protein	structural protein	diagnostics	Assay Designs & Stressgen Bioreagents Ltd

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

In biology, a crystallin is a water-soluble structural protein found in the lens of the eye, accounting for the transparency of the structure. It has also been identified in other places such as the heart and aggressive breast cancer tumors. Since it has been shown that lens injury may promote nerve regeneration, crystallin has been an area of neural research.

Function

The main function of crystallins at least in the lens of the eye is probably to increase the refractive index while not obstructing light. However, this is not their only function. It is becoming increasingly clear that crystallins may have several metabolic and regulatory functions, both within the lens and in other parts of the body.

Price

Company	Unit	Price	Source	Reference	Source Date
Sigma	25mg	$649	Bovine Eye	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C4163	2008

Market Size

- There are no bulk commercial applications for Alpha crystallin.
- Alpha crystallin is primarily used for research purposes
- Bovine eyes are the primary source of alpha crystallin for use in research
- This market would be small and is believed to be dominated by Sigma

Actin

Protein	Tissue Source & Weight (kg/head)*	Abundance (per kg of tissue)	Mode of Action	Use	Supplier
Actin	Major skeletal muscle: N/A Liver: 5.1kg	~10 mg/g acetone powder	Major structural motor protein;	diagnostics; cell biology molecular probe;	Ambion Inc; Alpha Diagnostic International Inc

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Actin is a protein directly involved in the conversion of chemical energy into mechanical work. Actin is the most abundant protein in the typical eukaryotic cell, accounting for about 15% in some cell types.[3]

Actin is one of the three major components of the cytoskeleton and forms part of the contractile apparatus in muscle cells. Actin participates in many important cellular functions, including muscle contraction, cell motility, cell division, cell signaling, and the establishment and maintenance of cell junctions and cell shape.

Actin binds with myosin to form actomysin microfibrils, which is the main component involved in muscle contraction.

Commercial Applications

Cosmetics - Hydrolysed actin is used in selected cosmetic products (including skin conditioning and hair treatment), though there are some safety concerns regarding its use in such products.[1,2] It has yet to be assessed for safety in cosmetics by relevant industry panels. Although the source of actin used in cosmetics was not stated it is likely that the actin was plant derived.

Research – Actin is used in research for:

- Identification and characterization of cardiac actin binding proteins
- In vitro cardiac actin polymerization studies

Price

Company	Source	Amount	Price	Unit Cost	Reference	Source Date
Sigma Aldrich	Bovine/ porcine/ rabbit muscle	5mg	AU $504	$100.80 /mg	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/A3653	2008
Cytoskeleton	Bovine cardiac muscle	20mg	US $1,095	$54.75 /mg	http://www.cytoskeleton.com/Merchant2/merchant.mvc?Screen=PROD&Store_Code=CIWS&Product_Code=AD99-C	2008
Cytoskeleton	rabbit skeletal muscle	20mg	US $975	$48.75 /mg	http://www.cytoskeleton.com/Merchant2/merchant.mvc?Screen=PROD&Store_Code=CIWS&Product_Code=AKL99-E	2008

Cytoskeleton	Human	5mg		$125 /mg	http://www.cytoskeleton.com/Merchant2/merchant.mvc?Screen=PROD&Store_Code=CIWS&Product_Code=APHL99-E	2008
Alpha Diagnostic International	Human	0.1mg		$10.60/ mg	http://www.4adi.com/commerce/index.jsp	2008

Market

The market size for actin used in cosmetics or for research purposes was not determined. The concentrations of actin used in hair and cosmetic products were not released by the companies that produced them.

The market for actin is likely to be insignificant and will remain that way until actin is proven to be beneficial and non-toxic when added to cosmetic products.

Actin is not a viable product for the red meat industry as the majority of actin found in animals is located in muscle and its extraction for would seriously degrade the high value meat product.

Opportunity potential: Possible "bulk reagent" opportunity for bovine extraction

Summary of Commercial Potential for Australian Beef Industry

Strengths – Highly conserved across species, high concentration in skeletal muscle, high value, hydrolysed actin incorporated as active ingredient in cosmetics (skin) and hair treatments

Weaknesses – Highest concentration in high valued meat product (muscle). Sigma prices are far too high for cosmetics/hair products

Opportunities – Growth in use in cosmetics and hair treatments

Threats – Some concerns as to the safety of hydrolysed actin

Concentration in Bovine Tissue

Bovine blood (erythrocytes)

Bovine blood (6L) - purified	
Product	Yield
Purified actin	2 mg

D. A. Schafer, P. B. Jennings, J. A. Cooper, 'Rapid and efficient purification of actin from nonmuscle sources', Cell Motility and the Cytoskeleton, Volume 39, Issue 2 , Pages 166 – 171

Bovine brain – Assumption: similar order of magnitude concentration in bovines

Chicken brain (200g) - purified	
Product	Yield
Purified actin	3 mg

D. A. Schafer, P. B. Jennings, J. A. Cooper, 'Rapid and efficient purification of actin from nonmuscle sources', Cell Motility and the Cytoskeleton, Volume 39, Issue 2 , Pages 166 - 171

Bovine muscle – Assumption: similar order of magnitude concentration in bovines

Rabbit muscle (1g) - purified	
Product	Yield
Purified actin	0.7-1.4 mg

J. H. HARTWIG AND T. P. STOSSEL, 'Isolation and Properties of Actin, Myosin, and a New Actinbinding Protein in Rabbit Alveolar Macrophages', The Journal of Biological Chemsitry, Vol. 250, No. 14, Issue of July 25, PP. 5696-5705, 1975

Summary

Opportunity potential: Possible "bulk reagent" opportunity for bovine extraction

Summary of Commercial Potential for Australian Beef Industry

Strengths – Highly conserved across species, high concentration in skeletal muscle, high value, hydrolysed actin incorporated as active ingredient in cosmetics (skin) and hair treatments

Weaknesses – Highest concentration in high valued meat product (muscle). Sigma prices are far too high for cosmetics/hair products

Opportunities – Growth in use in cosmetics and hair treatments

Threats – Some concerns as to the safety of hydrolysed actin

Reference

1 http://www.cosmeticsdatabase.com/ingredient.php?ingred_id=902&nothanks=1

2 http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out123cm_en.pdf

3 http://www.bms.ed.ac.uk/research/others/smaciver/Cyto-Topics/actinpage.htm

Alpha 1 - Acid Glycoprotein

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Alpha 1 - Acid Glycoprotein	Serum: 11.34kg	162 (± 63.7) mg/mL	acute phase protein, elevated in trauma, decreased in inflammation.	Immunomodulation	Sigma, Bio-Var.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Alpha-1-Acid-Glycoprotein (AAG) is an acute-phase reactant (the concentration in the blood stream increases significantly after injury or infection/ inflammation). AAG is synthesised in the liver in response to acute phase inflammation and tissue damage.[2] Blood levels of the protein rise 3 to 4 times in periods of acute inflammation and disease.[3]

Tissue Source

Despite being synthesised in the liver, Alpha 1 - Acid Glycoprotein is found primarily in the blood.

Biological Function

The actual biological function of Alpha 1 - Acid Glycoprotein is still unclear. At present it appears to have an anti-inflammatory and immunoregulatory function[4] with anti-neutrophil and anti-complement activity.[5] It has also been suggested that Alpha 1-Acid Glycoprotein has a role in maintaining capillary permeability.[5]

Commercial Application

Due to lack of information the number of purification grades could not be identified, however Alpha 1 -Acid Glycoprotein expression levels can be used as a diagnostic tool of inflammatory disease states. Due to the drug binding property of Alpha 1-Acid Glycoprotein there may be the potential to use it in the clinical management of drug overdoses and drug toxicity.[6]

Currently animal versions of the protein are used in liquid chromatography

Price

Company	Unit	Price	Source	Reference	Source Date
Sigma	250mg	$1095	Bovine plasma	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G3643	2008
Sigma	250mg	$1635	Human plasma	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G9885	2008

Market

The current global market size for Alpha 1-Acid glycoprotein is small pertaining to only the liquid chromatography market[7]. There are next to no indicators of the worth of this market, as neither current supply for Alpha 1-acid Glycoprotein, nor current demand for it in liquid chromatography applications could be determinded.

Currently only 2 companies produce the protein, these are Sigma Aldrich and Bio-Var. Sigma-Aldrich appears to be the largest producer and supplier of the protein and stocks it in 4 forms: the bovine, baboon, human and sheep[8].

Research has indicated that the Alpha 1-Acid Glycoprotein has future potential as a therapeutic as there has been indications of effectiveness in the treatment of diabetes[11], and treating drug toxicity and overdoses[9]. However, although this has potential for the human derived version it holds little future for any animal derived product due to human therapeutic regulations on animal protein treatments.

Extraction/Processing Technologies

There are 2 patents relating to the commercial extraction level of Alpha 1-Acid Glycoprotein. These detail AAG being isolated from a Cohn faction V isolated using column chromatography (Q-Sepharose Big Bead, Q Hyper D and Toyopearl Super Q).[6] The alpha 1-acid glycoprotein is then eluted using a salt or by decreasing the pH of the buffer below 4.1. The molecule is then depyrogenated using conventional methods (eg. soaking in alkali such as 0.5 M NaOH for at least one hour or by heating at temperatures above 200° C for greater than one hour).[9]

Bovine AAG has been gathered from sera by successive ammonium sulfate precipitation, ion-exchange chromatography and gel filtration.[10]

Other

Given the availability of a human form of Alpha-1-acid Glycoprotein, with all the advantages that a human based protein competitor holds in a therapeutic arena, our research suggests that the liquid chromatography market is not in itself sufficient to support a commercial investment into production of bovine Alpha-1-acid glycoprotein.

Executive Summary

- Bovine Alpha-1-acid Glycoprotein's feasibility as a commercial product for the red meat industry is doubtful.
- Despite being a protein that is active in humans in inflammation response and membrane permeability, and having been shown to have a therapeutic affect in treating diabetes and drug overdoses, the human version of the protein is available for sale and distribution.
- Given the restrictions on animal protein therapeutics, especially when the protein must be injected into the blood supply, that particular segment of AAG's market is not one that can be effectively supplied by bovine derived AAG.
- The other segment of its market, liquid chromatography, shows scant levels of usage, and Sigma-Aldrich have an established worldwide supply chain, and presumably, an established customer base.
- Alternative markets for the product may exist, however they were not evident at the time of this report, and are not dealt with here.

References

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2 Fournier T, Medjoubi-N N, Porquet D (2000) Alpha-1-acid glycoprotein. [Accessed online: 18-09-2006) via NCBI Pubmed

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6 Kishino S, Nomura A, Sugawara M, Iseki K, Kitabateke A, Miyazaki K (1995) Purification method for alpha-1-acid glycoprotein with subsequent high-performance liquid chromatographic determination of monosaccharides in plasma of healthy subjects and patients with renal insufficiency. [Accessed online via PubMed: 23/09/2005]

7 Bio-Var biotechnology (2006) Description: Proteins and Enzymes [Accessed online 09/10/2006] http://www.bio-varbiotechnology.am/description.html

8 Sigma-Aldrich Co (2006) G3643 Alpha 1-Acid Glycoprotein from bovine plasma [Accessed online: 07/10/2006] http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G3643

9 More J E, Rott J, Lewin D R (2002) United States Patent 6387877 Purification method [Accessed online via Patent Storm 23/09/2006] http://www.patentstorm.us/patents/6387877.html

10 Tamura K., Yatsu T., Itoh H. &Motoi Y. (1989), Isolation, characterization, and quantitative measurement of serum alpha 1-acid glycoprotein in cattle, [Online], 2006. [Accessed online: 1/10/2006] http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=2607747&dopt=Abstract

11 WO/2005/117937) Alpha-1-Acid Glycoprotein for the treatment of Diabetes (2006) World Intellectual Property Office [Accessed online: 6/10/2006]

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13 Meat and Livestock exporters treated equitably by AQIS (22/03/2004) The Hon. Warren Truss MP [Accessed online: 8/10/2006] http://www.maff.gov.au/releases/04/04066wt.html

14 Kleiber M., Smith A.H. & Black A.L. (1951), Aacetate as a precursor of milk constituents in the intact dairy cow, [Accessed online 3/10/2006] http://www.jbc.org/cgi/reprint/197/1/371.pdf#search=%22litres%20of%20blood%20in%20a%20cow%22

Calmodulin

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Use	Supplier
Calmodulin	Brain: 0.34kg Cardiac muscle: 1.5kg	> 25 mg/Kg depending on tissue source	Research applications	Sigma; Alexis, AbD Serotec

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Calmodulin (CaM) (an abbreviation for CALcium MODULated proteIN) is a calcium-binding protein expressed in all eukaryotic cells. It can bind to and regulate a number of different protein targets, thereby affecting many different cellular functions.

Function

- CaM mediates processes such as inflammation, metabolism, apoptosis, muscle contraction, intracellular movement, short-term and long-term memory, nerve growth and the immune response.
- CaM is expressed in many cell types and can have different subcellular locations, including the cytoplasm, within organelles, or associated with the plasma or organelle membranes.
- Many of the proteins that CaM binds are unable to bind calcium themselves, and as such use CaM as a calcium sensor and signal transducer.

Commercial Applications

- Potential for growth in research fields
- No known bulk commercial applications for Calmodulin.

Price

Company	Amount	Price	Source	Reference	Source Date
Alexis	1mg	$289	Bovine	http://www.axxora.com/	2008
Sigma	1mg	$249	Bovine	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/P2277	2008
AbD Serotec	1mg	$371	Bovine	http://www.abdirect.com/catalog/datasheet.aspx?ProductCode=1740384&SearchType=Simple&SearchString=Calmodulin	2008

Market Size

Market is primarily limited to research purposes at this point in time.

What product processing is involved?

Ion-exchange chromatography followed by gel-filtration.[1]

Purity: >95%

Yield:

- 12mg/100g of goat testes (51%) (Bandyopadhyay and Ghosh 1990)
- The yield of CaM from 900 g of whole bovine adrenal was 150 mg[2]

∴ 166.7mg/kg of bovine adrenal

Reference

1 http://www.hytest.fi/product/Calmodulin+from+bovine+brain.html

2 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=3970528&query_hl=2&itool=pubmed_DocSum

Bandyopadhyay, S. and S. K. Ghosh (1990). "Goat testis calmodulin: purification and physicochemical characterization." J Protein Chem 9(5): 603-11.

Fibronectin

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Fibronectin	Plasma: 11.34kg	~400 mg/L	High-molecular-mass, multidomain adhesion glycoprotein found in a soluble form in blood plasma and in an insoluble form in loose connective tissue and basement membrane	Cell Culture, diagnostics, veterinary applications	ChemiCon; Sigma; AbD Serotec

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Fibronectin is a high-molecular-weight extracellular matrix glycoprotein that binds to membrane spanning receptor proteins called integrins. In addition to integrins, they also bind extracellular matrix components such as collagen, fibrin and heparan sulphate.

Two types of fibronectin are present in vertebrates:

- Soluble plasma fibronectin is a major protein component of blood plasma and is produced in the liver.
- Insoluble cellular fibronectin is a major component of the extracellular matrix. It is secreted by various cells, primarily fibroblasts, as a soluble dimer and is then assembled into an insoluble matrix in a complex cell-mediated process.

Fibronectin plays a major role in cell adhesion, growth, migration and differentiation, and it is important for processes such as wound healing and embryonic development.

Cellular fibronectin is assembled into the extracellular matrix, an insoluble network that separates and supports the organs and tissues of an organism.

Fibronectin is also found in saliva, which helps prevent colonisation of the oral cavity and pharynx by potentially pathogenic bacteria.

Commercial Applications

Research has demonstrated that fibrinogen-based matrices have extremely potent capabilities in attracting, and influencing the adhesion of growth promoting cells (fibroblasts, osteoblasts, chondroblasts, adult stem cells and others). This trait may be employed in numerous applications to promote tissue regeneration, cellular migration and growth and tissue maturation.

Topically applied plasma fibronectin has been reported as being useful for increasing the rate of wound healing such as in corneal wounds and leg ulcers.

The detection of fibronectin is used in medicine to diagnose if a baby is going to be born prematurely, though this is a multi-million dollar industry it doe not require any fibronectin, only its detection.

Fibronectin is used in research as it can be used as a thin coating on tissue-culture surfaces to promote attachment, spreading, and proliferation of a variety of cell types.

The majority of these commercial applications use human fibronectin rather than bovine fibronectin.

Price

Company	Amount	Price	Source	Reference	Source Date
Sigma	5mg	$573	Bovine plasma	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/F4759	2008
AbD Serotec	1mg	$250	Goat plasma	http://www.ab-direct.com/catalog/datasheet-143001.html	2008

Market Size

The worldwide market for bovine fibronectin is estimated to be of the order of $5 million. (MLA)

Bovine fibronectin has no known medical uses however it is used in cell culturing and research.

The market is seen to be mature though the incorporation of fibronectin into wound healing fabrics or gels would significantly boost the world market size. It is not known if bovine fibronectin will ever be used for medical applications, at this point in time it is not.

L-Carnosine

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
L-Carnosine	Skeletal muscle: N/A Cardiac muscle: 1.5kg Brain: 0.34kg and other tissue sources	~ 1.5 g/Kg	The endogenous dipeptide carnosine (beta-alanyl-L-histidine), at 0.1-10 mM, can provoke sustained contractures of smooth muscle with greater efficacy than adrenaline.	Nutraceutical, food additive/ supplement and pharmaceutical applications	DSL, Biosynth, Acrosorganic Ltd, Chemik AG; BenroChem, Inc., Dayang Chem Industries LTD; EuroLabs Ltd,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Carnosine (B-alanyl-L-histidine) is a naturally-occurring di-peptide (a combination of two amino acids), found in muscle, brain and other animal and human tissues. It is formed by a process involving the enzyme carnosine-synthetase which bonds the amino acids alinine and histidine. This process occurs mainly in muscles and brain. It is kept in equilibrium by the carnisinases which are enzymes specifically aimed at inactivating carnosine in the tissues or in the blood.

For a small molecule carnosine performs a remarkable variety of functions -- most notably anti-oxidation, anti-glycation, pH buffering and chelation of divalent metal cations (particularly copper, Cu2+).

High concentrations of carnosine are present in long-lived cells (such as in neuronal tissues). The concentration of carnosine in muscles correlates with maximum lifespan, a fact that makes it a promising bio-marker of aging.

It is high in actively contracting muscles and low in cases of muscular disease such as Duchennes's muscular dystrophy. Its concentration in mammalian muscles possibly decreases with age, a fact which strengthens the case for supplementation.

In cases of cataract in animals, carnosine concentration in the lens was found to be low. The lower the concentration of carnosine, the higher the severity of cataract.

Carnosine has been proven to scavenge reactive oxygen species (ROS) as well as alpha-beta unsaturated aldehydes formed from peroxidation of cell membrane fatty acids during oxidative stress.

Researchers in Britain, South Korea, Russia and other countries have also shown that carnosine has a number of antioxidant properties that may be beneficial.

Typical vegetarian diets are thought to be lacking in carnosine, but whether this has a detrimental effect on vegetarians is controversial.

Commercial applications

According to research, carnosine helps maintain the protective mucosal lining in the stomach and intestines- the natural barrier of our GI track, which protects the stomach and gut from harmful bacteria and acid exposure.

Carnosine is available as a supplement and is sold by numerous health and Well -Being companies. Carnosine supplements have been used in the past by body-builders, athletes and others, but its use has been confined mainly for improving muscular fatigue, and not for longevity.

Recently, eye drops containing carnosine have been developed and used by Russian researchers . The drops were found to be effective in treating human corneal erosions and other corneal diseases. For example, carnosine drops accelerate the healing of ulcers in herpes and bacterial infections of the eye.[1]

Price

Company	Amount	Price	Source	Reference	Source Date
Sigma	100g	$1300	Plant	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C9625	2008

Other Prices:

Vegetarian L-Carnosine is sold as nutritional supplements for US $500/kg (retail)[2]

Retail bulk Price:

25kg = $ 28911[3]

Market Size

An MLA Bioactives report in 2006 stated "Internationally approximately 30 tonnes of carnosine is traded per year. There appears to be demand for more."

The yield of carnosine may conservatively be 1 g per kg of meat off-cuts or boning waste.

Using this value as a guide, approximately 1ton of meat off cuts would yield 1kg of carnosine. Although carnosine can be extracted from meat off cuts, the cost of extraction and purification may prohibit any economic gains, as carnosine is available from plant sources which are a much cheaper raw material.

Reference

1 http://www.smart-drugs.net/ias-carnosine-article.htm

2 http://www.iherb.com/store/ProductsList.aspx?c=Herbs&cid=l_carnosine&gclid=CMbTxcnrvIwCFQvShgodTiBwVw

3 http://www.sciencelab.com/page/S/PVAR/23043/SLC1546

Myosins

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Myosins	Skeletal Muscle: N/A Heart: 1.5kg	> 1mg/Kg depending on source/ process	The most important mechano-enzyme for muscle contraction; hydrolyses the terminal phosphate groups from adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP) and inosine triphosphate (ITP).		US Biologicals, Calzyme Labs Inc; Sigma Aldrich

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Myosins are a large family of motor proteins found in eukaryotic tissues. They are responsible for actin-based motility.

The most important mechano-enzyme for muscle contraction; hydrolyses the terminal phosphate groups from adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP) and inosine triphosphate (ITP).

Commercial Applications

There are currently several myosin supplements marketed to body builders. The effectiveness of these supplements has not been tested.[1]

The supplement market for myosin would be limited by the fact that myosin is found in all muscle tissue (meat).

Price

Company	Unit	Price	Source	Reference	Source Date
Sigma	50mg	$522	Porcine heart	http://www.calzyme.com/commerce/catalog/product.jsp?product_id=1197&czuid=1195829867215	2008

Market Size

- The market size for myosin was unattainable.
- This bioactive is not a viable option for the red meat industry as myosin is tightly bound in muscle.
- The extraction of myosin from bovine muscle would degrade/destroy the properties of meat.

Reference

1 http://www.mdplusstore.com/pdfs/myosin.pdf

Osteocalcin

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Osteocalcin	Bone: 72.8kg Plasma: 11.34kg	~ 10 mg/Kg	Unknown	BGP binds to phospholipid vesicles in the presence of calcium ions and to hydroxyapatite; efficient inhibitor of hydroxyapatite seeded crystal growth	Haematological Technologies, Inc.; Nordic Bioscience Diagnostics; Biodesign International; Kamiya Biomedical Company

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Osteocalcin is a noncollagenous protein found in bone and dentin. It is secreted by osteoblasts (bone cells) and thought to play a role in mineralisation and calcium ion homeostasis. It has been suggested that osteocalcin may also function as a negative regulator of bone formation, although its exact role is unknown.

As osteocalcin is manufactured by osteoblasts, it is often used as a biochemical marker, or biomarker, for the bone formation process. It has been routinely observed that higher serum-osteocalcin levels are relatively well correlated with increases in bone mineral density.

In many studies, Osteocalcin is used as a preliminary biomarker on the effectiveness of a given drug on bone formation

Osteocalcin is a vitamin K-dependent protein and is essential for the body to utilise calcium in bone tissue. Without adequate vitamin K, the osteocalcin remains inactive, and thus not effective.

Commercial Applications

No known commercial applications other than research

Price

Company	Amount	Price	Source	Reference	Source Date
Kamiya Biomedical	0.1mg	$314	Bovine bone	http://www.kamiyabiomedical.com/pdf/BP-010.pdf	2008
Biodesign	0.1mg	$417	Bovine bone	http://www.biodesign.com/spec.asp?catalog=A95020B	2008
Calbiochem	0.1mg	$456	Bovine bone	http://www.emdbiosciences.com/product/499050	2008

Market Size

- No bulk commercial applications
- The current supply source for Osteocalcin is from demineralised bovine bone though the high price per mg of Osteocalcin ($3140) suggests that the extraction and purification process is difficult and that demand is low.

Processing

From demineralised bone by ion exchange chromatography[1]. Molecular exclusion chromatography on Sephadex G-100, ion exchanger chromatography in NaCl linear gradient, on DEAE Sephadex A-25. The purity of the substance obtained was tested by disk electrophoresis in polyacrylamide gel.

Purity: > 95 %

Reference

1 http://www.kamiyabiomedical.com/

Osteonectin

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Osteonectin	Bone: 72.8kg Blood: 18 Litres	Bone, platelets, plasma (0.9 mg/ml), serum (2.6 mg/ml)	Unknown	Bovine bone osteonectin binds type I collagen, calcium and hydroxyapatite; potent inhibitor of hydroxyapatite seeded crystal growth.	Haematological Technologies,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Osteonectin is a glycoprotein in the bone that binds calcium. It is secreted by osteoblasts during bone formation, initiating mineralization and promoting mineral crystal formation. Osteonectin also shows affinity for collagen in addition to bone mineral calcium.

Commercial Applications

Osteonectin is used in research as it inhibits cell spreading and diminishes focal contacts in vitro (anti-adhesive activity).

Research

- May play an important role in the regulation of bone metabolism by binding hydroxyapatite to collagen.
- Reported to play a role in the differentiation and maintenance of dermis.
- Has been shown to be a potent inhibitor of hydroxyapatite seeded crystal growth (6)
- Effects the expression of matrix components and the activity of a variety of enzymes that might act to regulate cellular interactions with ECM.
- Undergoes proteolysis that exposes and releases regions in the protein that correlates with biological activities, such as stimulation of angiogenesis.[1]

Price

Company	Amount	Price	Source	Reference	Source Date
Cortex Biochem	0.05 mg	$371	Bovine Bone	http://www.cortex-biochem.com/commerce/catalog/product.jsp?tabid=-1&product_id=1228&czuid=1177488722263	2008
Merck	0.05 mg	$514	Bovine Bone	http://www.merckbiosciences.com/product/499240	2008

Market Size

- Very small as no data is available and it has limited applications
- Any use would be limited as it is very expensive ($7420)

What product processing is involved?

Processing:

- EDTA-solubilized extracts of adult bovine bone in the absence of denaturants, ion-exchange chromatography using DEAE-Sephadex A-50 and DEAE-Sephadex A-25, followed by gel filtration on Sephadex G-100.

Purity:

- ≥95%

Yield:

- 100 mg of EDTA extracted non-collagenous bone proteins, 2-3 mg of osteonectin[2]
- 10-30% of bovine bone[3]

Reference

1 http://www.sigmaaldrich.com/sigma/datasheet/s5174dat.pdf

2 http://www.jbc.org/cgi/reprint/260/5/2728

3 Romberg et al (1985)

Thyroglobulin

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Thyroglobulin	Thyroid: 0.001kg	~250 mg/kg	Produced by and used entirely within the thyroid gland to generate the thyroid hormones thyroxine (T4) and triiodo-thyronine (T3).	Often used as a carrier protein for the production of antibodies.	Sigma Aldrich; Calzyme Merck, AbD Serotec

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Thyroglobulin (Tg) is a dimeric protein produced by and used entirely within the thyroid gland. Tg is used by the thyroid gland to produce the thyroid hormones thyroxine (T4) and triiodothyronine (T3).

Clinical significance

Thyroglobulin levels in the blood can be used as a tumor marker for certain kinds of thyroid cancer and Graves' disease.

Commercial Applications

- Research
- No bulk commercial applications

Price

Company	Amount	Price	Source	Reference	Source Date
AbD Serotech	100mg	$218	Bovine thyroid	http://www.serotec.com/asp/product.asp?cat=8900-1354	2008
Merck	100mg	$220	Bovine thyroid	http://www.merckbiosciences.com/product/609310	2008
Sigma	5000mg	$2585	Bovine thyroid	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/T1001	2008

Market

- There are no bulk commercial applications for Thyroglobulin at this point in time.
- There are several scientific research retailers of bovine Thyroglobulin.
- Bovine Thyroglobulin appears to be the most commonly sold Thyroglobulin though there are porcine, mouse and human forms on the market.
- The market size was not determined however it is likely to be small and barring some unforeseen scientific breakthrough it is likely to stay that way.

What product processing is involved?

- Saline Digestion
- High Pressure Liquid Chromatography

Purity: 95%

Troponins

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Troponins	Heart: 1.5kg Skeletal muscle: N/A	Heart ~700 mg/Kg Skeletal ~4 g/Kg;	Troponins are calcium-dependent regulatory protein complexes located on the thin actin filaments of muscle & comprise TnC (17.8 kDa), TnI (20.8 kDa), and TnT (30.5 kDa) subunits.	These proteins are involved in the key regulatory mechanism for muscle contraction.	Life Diagnostics Sigma, Merck, Kamiya Biomedical Inc., Bioprocessing Inc.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Troponin is a complex of three regulatory proteins that is integral to muscle contraction in skeletal and cardiac muscle, but not smooth muscle.

Discussions of troponin often pertain to its functional characteristics and/or to its usefulness as a diagnostic marker for various heart disorders.

Diagnostic use

Certain subtypes of troponin are very sensitive and specific indicators of damage to the heart muscle. They are measured in the blood to differentiate between unstable angina and heart attack in patients with chest pain. A patient who had suffered from a heart attack would have an area of damaged heart muscle and so would have elevated cardiac troponin levels in the blood.

It is important to note that cardiac troponins are a marker of all heart muscle damage, not just heart attack. Other conditions that directly or indirectly lead to heart muscle damage can also therefore increase troponin levels.

The world market for Troponin cardiac tests is worth $300 million worldwide.[1]

Price

Company	Amount	Price	Source	Reference	Source Date
Life Diagnostics	1mg	$770	Bovine	http://www.lifediagnostics.com/cardiac%20proteins.html	2008
Sigma	1mg	$101	Porcine	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;T9924	2008
Sigma	0.02mg	$440	Human	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;T9924	2008
Merck	0.1mg	$728	Human	http://www.merckbiosciences.com/product/648480	2008

Market Size

- The market for Troponin would not be significant as it is not directly used for any commercial application.
- The detection of Troponin for heart problems is worth $300 million however only tiny amounts of Troponin are needed for this application.
- Troponin is also extracted from muscle tissue through a destructive process, thus it is not a viable bioactive for Australia.
- The Troponin found in the heart could be extracted however it would not be economically worthwhile due to the costs of extraction and purification and a limited worldwide market.

What product processing is involved?

Process: Isolated by direct separation on DEAE-cellulose

Purity: 95%

Reference

1 http://www.secinfo.com/dqtWy.21b.d.htm

Other Enzymes

Alkaline Phosphatase

Arginase

Carbonic Anhydrase

Catalase

Cathepsin D

Cholesterol Esterase

Cholesterol Oxidase

Deoxynucleotidyl-Transferase

Glutamate Dehydrogenase

Lysozyme

Phosphodiesterases

Protein Kinase-A

Protein Phosphatase 2C

Urease

Alkaline Phosphatase

Protein	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Alkaline Phosphatase	Calf intestine: 7.2kg	~10 mg/Kg	Alkaline phosphatase catalyses the removal of 5' phosphate residues from DNA, RNA and ribo- and deoxyribonucleoside triphosphates	Diagnostics	Calzyme, Sigma, Lee Bio.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation.

As the name suggests, alkaline phosphatases are most effective in an alkaline environment.

Commercial applications

Alkaline phosphatase has become a useful tool in molecular biology laboratories. Alkaline phosphatase is used in DNA analysis and marking and also enzyme immunoassays (a biochemical test that measures the concentration of a substance in a biological liquid). For these purposes, the alkaline phosphatase from shrimp is the most useful, as it is the easiest to inactivate once it has done its job.

The most common alkaline phosphatases used in research are:

- Bacterial alkaline phosphatase (BAP), from *Escherichia coli* C4 cells
- Shrimp alkaline phosphatase (SAP), from a species of Arctic shrimp (*Pandalus borealis*)
- Calf intestine alkaline phosphatase (CIAP), from calf intestine
- Placental alkaline phosphatase (PLAP)

One common use in the dairy industry is as a marker of pasteurisation. This molecule is denatured by elevated temperatures found during pasteurisation, and can be tested for via colour change. Raw milk would typically produce a yellow colouration within a couple of minutes, whereas properly pasteurised milk should show no change.

Price

Company	Unit	Price	Source	Reference	Source Date
Lee Bio	10mg	$420	Calf intestine	http://www.leebio.com/products/details.html?uid=183	2008
Calzyme	25mg	$40	Bovine kidney	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1019	2008
Calzyme	10mg	$400	Bovine Liver	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1019	2008
Calzyme	10mg	$600	Human placenta	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1019	2008
Sigma	5mg	$325	Bovine intestine	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/P6772	2008

Market Size

- The market size for AP used in molecular biology diagnostics is approximately $20million.[1]
- AP and peroxidise are the two most commonly used enzymes in cellular diagnostics and combined they account for 40% of the $100 million worldwide market.
- The market size for AP in other commercial applications like the diary industry was not determined.
- The market share for each source of AP was not determined however it was often reported that calf AP was the main source.

What product processing is involved?

Subcellular fractionation, solubilization with butanol, fractionation with acetone, chromatography on concanavalin A-Sepharose, DEAE cellulose, DEAE-Sephadex, and gel filtration on Sephadex G-200. Affinity chromatography → purified until homogeneous, as judged by polyacrylamide gel electrophoresis[2]

Solubilization with 10 mM Tris-HCl buffer, pH 7.4, containing 0.2 mM MgCl2 and 0.1% Nonidet P-40 and fractionation by sequential chromatography utilizing DEAE-sephacel, Sepharose CL-6B and concanavalin A Sepharose 4B. Purity was established by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)[3]

Purity: 95%

Reference

1 http://www.cheric.org/ippage/e/ipdata/2004/05/file/e200405-1001.pdf

2 http://jds.fass.org/cgi/reprint/75/12/3394.pdf

3 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=2133740&dopt=Abstract

Arginase

Protein	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Arginase	Liver: 5.1kg	~ 1mg/Kg	Amidinohydrolase activity	Axxora LLC.; Sigma, Calzyme Labs Inc.,	Calzyme, Sigma, Lee Bio.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Arginase is involved in the fifth and final step in the urea cycle, a series of biophysical reactions in mammals during which the body disposes of waste nitrogen amino acids. Specifically, arginase converts L-arginine into L-ornithine and urea.

L-Arginase causes the following reaction:

L-Arginase

L-arginine + H_2O ------> L-ornithine + urea

Organisms that cannot easily and quickly remove ammonia usually have to convert it to some other substance, like urea or uric acid, which are much less toxic. Insufficiency of the urea cycle occurs in some genetic disorders, and in liver failure.

Commercial Applications

Cosmetics:

Cosmetic preparations preferably contain between 1 to 100mg of the arginase enzyme per 100 g of the preparation and protect the skin against dryness, roughness and inflammation.

Nutrition:

Arginase inhibitors are used as a dietary supplement for sufferers of benign prostate hypertrophy, hypertension, kidney/bladder dysfunction, edema and male sexual problems.

Medicine:

Arginase is administered for the treatment of hepatitis and is also administered intravenously to raise blood pressure.

Price

Company	Amount	Price	Source	Reference	Source Date
Sigma	125mg	N/A	Bovine liver	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/A8013	2008
Calzyme	100mg	$35	Bovine liver	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1068&czuid=1172922025000	2008

Market Size

- The market size for arginase was difficult to ascertain as the market information found was on arginase inhibitors.
- The use of arginase in cosmetics appears to be limited and information relating to cosmetics is not readily released.
- Arginase is widely available for research applications and is sold by several companies worldwide.
- Bovine liver is the primary source of arginase for research applications, though arginase can be extracted from almost all animal livers.

What product processing is involved?

Arginase from human liver and red blood cells

The purification procedure used acetone precipitation, heat-treatment, (NH4)2SO4 precipitation, DEAE-cellulose chromatography and gel filtration on Sephadex G-200 in the presence of 2-mercaptoethanol. Both enzymes migrated to the anode at pH8.3 on polyacrylamide-gel electrophoresis.[1]

- 27% recovery

From ox red blood cells:

Haemolysis, heat treatment, CM- and DEAE-Sepharose chromatography, arginine AH-Sepharose chromatography and molecular sieving through Biogel P-150, all in the presence of 2-mercaptoethanol.

It afforded (a) elution of arginase as a single peak, (b) almost 2900 fold purification, (c) 30% recovery and (d) electrophoretically homogeneous arginase.[2]

Reference

1 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1186090

2 http://www.springerlink.com/content/x56286588378n314/

Carbonic Anhydrase

Protein	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Use	Supplier
Carbonic Anhydrase	Red blood cells: 4.86kg	~ 200 mg/ Kg		Calzyme, Sigma Aldrich; AbD Serotec

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

The carbonic anhydrases form a family of enzymes that catalyse the rapid conversion of carbon dioxide to bicarbonate and protons, a reaction that occurs rather slowly in the absence of a catalyst.[1]

Carbonic anhydrases are found in almost all organisms on earth and are essential for the regulation of carbon dioxide in cells and the regulation of pH.

Carbonic anhydrase catalyses the reaction:

$$H_2O + CO_2 \rightleftharpoons H^+ + HCO_3^-$$

Commercial applications

Carbonic anhydrases have no known bulk commercial uses.

Carbonic anhydrases are used for research purposes only with bovine red blood cells and human red blood cells being the primary source tissues.

Carbonic anhydrase inhibitors are used in medicine to treat glaucoma, hypertension and altitude sickness.

Price

Company	Amount	Price	Source	Reference	Source Date
AbD Serotech	250mg	$480	Bovine	http://www.biocompare.com/itemdetails.asp?itemid=615369	2008
Sigma	500mg	$887	Bovine	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C3934	2008

Market Size

- Limited to research
- No bulk commercial applications at this time
- Carbonic Anhydrase is sourced from humans and bovine red blood cells
- Bovine Carbonic Anhydrase is approximately 15 times cheaper than human Carbonic Anhydrase.

What product processing is involved?

- Alcohol and chloroform extraction
- Dialysis of alcohol-chloroform extract
- Precipitation with ammonium sulphate

Catalase

Protein	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Catalase	Liver: 5.1kg Various other tissues also	~ 50 mg/Kg depending on source and process	Decomposes hydrogen peroxide; food & cheese manufacture	Reagent and wide-spread use as biocatalyst, including decomposing hydrogen peroxide to water and molecular oxygen and also cleaves small organic hydroperoxides	Worthington Biochemicals Corp, Calzyme Labs Inc.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Catalase is a common enzyme found in nearly all living organisms. Its primary function is to catalyse the decomposition of hydrogen peroxide to water and oxygen. Catalase has one of the highest turnover rates of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second.

The reaction of catalase in the decomposition of hydrogen peroxide is:

$2H_2O_2 \rightarrow 2H_2O + O_2$

Cellular role

Hydrogen peroxide is a harmful by-product of many normal metabolic processes: To prevent damage, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyse the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules.

Commercial Applications

- Catalase is used in the food industry for removing hydrogen peroxide from milk prior to cheese production.
- Another use is in food wrappers, where it prevents food from oxidising.
- Catalase is also used in the textile industry, removing hydrogen peroxide from fabrics to make sure the material is peroxide-free.
- A minor use is in contact lens hygiene - a few lens-cleaning products disinfect the lens using a hydrogen peroxide solution; a solution containing catalase is then used to decompose the hydrogen peroxide before the lens is used again.

Recently, catalase has also begun to be used in the aesthetics industry. Several mask treatments combine the enzyme with hydrogen peroxide on the face with the intent of increasing cellular oxygenation in the upper layers of the epidermis.

Catalase is also used as a research reagent in microbiology; the catalase test is also one of the main three tests used by microbiologists to identify species of bacteria. The presence of catalase enzyme in the test isolate is detected using hydrogen peroxide. This when exposed to a small amount of a bacterial isolate will bubble if the bacteria possess this enzyme.

Catalase has been used to study the role reactive oxygen species play in gene expression and apoptosis.

Price

Company	Amount	Price	Source	Reference	Source Date
Worthington Biochemicals Corp	10g	$180	Bovine liver	http://www.worthington-biochem.com/CTL/default.html	2008
Calzyme Labs Inc	200mg	$10	Bovine liver	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1039	2008
Sigma	10g	$130	Bovine liver	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C9322	2008
Sigma	500ml	$448	*Micrococcus lysodeikticus*	http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/60634	2008

World Market

The world market size for catalase is approximately $50-150 million. This value is based upon the use of catalase in the textile industry being worth $16 million and multiplying this across the other industries that catalase is used in. (food, diary, packaging, cosmetics and research).[1]

The major source of the worlds' catalase is from genetically modified *Aspergillus niger*. The concentration of catalase is higher in bovine liver than in aspergillus however the production costs are much lower using aspergillus.

It is difficult to determine if bovine sourced catalase has any commercial applications other than research, at this point in time.

Bovine catalase is supplied by the majority of chemical reagent companies at competitive prices so there must be a demand for it from the research industry or other industries.

What product processing is involved?

Acetone fractionation and successive chromatographies on DEAE-cellulose, Sephadex G-200, Blue Sepharose CL-6B and Ultrogel AcA-34. The purified enzyme judged by polyacrylamide gel electrophoresis and FPLC.

Purity: 99%[2]

Reference

1 http://akseli.tekes.fi/opencms/opencms/OhjelmaPortaali/ohjelmat/SymBio/fi/Dokumenttiarkisto/Viestinta_ja_aktivointi/Seminaarit/Pentti_Ojapalo_AB_Enzymes.pdf

2 Prajapati, Bhakuni et al. 1998

Cathepsin D

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Wet Tissue Weight (kg)	Use	Supplier
Cathepsin D	Spleen: 0.66kg Uterus: 0.7kg Adrenal glands: 0.024kg	~ 50 mg /Kg depending on source	0.66+10+0.24	Various therapeutical and diagnostic uses, including digestion of collagen	Calzyme Labs Inc.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

A cathepsin is a cysteine or aspartic protease, (a protein degrading enzyme), found in almost all cells. There are approximately a dozen members of this family, which are distinguished by their structure and which proteins they cleave. Most of the members become activated at the low pH found in lysosomes (digestive compartment of cells) .Thus, the activity of this family lays almost entirely within those organelles.

Cathepsins have a vital role in mammalian cellular turnover, e.g. bone resorption. They degrade proteins and are distinguished by their substrate specificities.

Major function of cathepsin D is the digestion of proteins and peptides within the acidic compartment of lysosome.

Commercial applications

- Diagnostic tests for Cathepsins are indications of some cancers. The actual enzyme has very little use outside of the research industry.

Price

Company	Amount	Price	Source	Reference	Source Date
Sigma	8mg	$1100	Bovine spleen	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C3138	2008
Calzyme	1mg	$70	Bovine Spleen	http://www.calzyme.com/commerce/catalog/product.jsp?product_id=1103&czuid=1220508357889	2008

Market Size

The market for cathepsin D is limited to the research industry as there are no commercial applications for this enzyme. The worldwide sales for cathepsin are difficult to determine as sales are likely to be insignificant and annual sales are unlikely to have been quantified.

Cholesterol Esterase

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Use	Supplier
Cholesterol Esterase	Pancreas: 0.43kg Liver: 5.1kg Intestine: 18kg Kidney: 1.2kg Adrenals: 0.24kg Testis: 0.9kg	> 100 mg/ Kg	Biosensors, diagnostics, biocatalytic manufacture	Calzyme Laboratories, Inc., Worthington Biochemicals Corp; PennBio Sigma-Aldrich Corp.

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Cholesterol esterase enzyme is responsible for the hydrolysis of many of the fatty acid esters of cholesterol. The enzyme is found primarily in pancreas and pancreatic juice, but in other tissues as well.

This enzyme belongs to the family of hydrolases, specifically those acting on carboxylic ester bonds. This enzyme participates in bile acid biosynthesis.

Commercial applications

- Cholesterol esterase has clinical applications in the determination of serum cholesterol
- No other commercial uses were found

Price

Company	Amount	Price	Source	Reference	Source Date
Sigma	6.7mg	$305	Bovine	https://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C3766	2008
PennBio	6.7mg	$473	Porcine	http://www.pennbio.com/enzymes.htm	2008
Calzyme	17mg	$20	Candida rugosa	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1041	2008

Current product source

- *Candida rugosa*
- Recombinant *E.coli*[1]
- *Pseudomonas fluorescens*
- Porcine and bovine pancreas

Market Size

Cholesterol esterase primary market is in the medical determination of cholesterol in blood serum though this would be a significant market there was little information on the worldwide sales or total global production.

The market for animal Cholesterol esterase would be limited as Cholesterol esterase can be isolated from various micro-organisms at a significantly lower cost. Cholesterol esterase from animal sources would be used primarily by the research and medical industries.

What product processing is involved?

Sequential precipitation with 35 % acetone, diethylaminoethyl cellulose chromatography, and hydroxylapatite chromatography[2].

Reference

1 http://www.kikkoman.co.jp/bio/j/rinsyou/pdf/09_CHE-XE.pdf

2 Hyun, J., H. Kothari, et al. (1969). “Purification and properties of pancreatic juice cholesterol esterase.” J Biol Chem 244(7): 1937-45.

Cholesterol Oxidase

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Use	Supplier
Cholesterol Oxidase	Brain: 0.34kg	~ 100 mg/ Kg	steroid pharmaceuticals; biosensors; diagnostics	US Biologicals Inc; ToyoBo Enzymes

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Cholesterol oxidase belongs to the family of oxidoreductases that specifically act on the CH-OH group of donor with oxygen as acceptor. Cholesterol oxidase is an enzyme that catalyses the chemical reaction:

cholesterol + O2 $\rightleftharpoons$ cholest-4-en-3-one + H_2O_2

The enzyme participates in bile acid biosynthesis in mammals.[4] It is associated with the lipid bilayer of eukaryotic cells and is also important in bacterial metabolism and pathogenesis.[5]

Cholesterol oxidase has industrial and commercial applications in bioconversions for clinical determination of total or free serum cholesterol[1] and applications in agriculture[2]. Its activity can be determined by following the appearance of the conjugated ketones, the formation of hydrogen peroxide in a coupled test with peroxidase, or by measuring the oxygen consumption polarographically.[3]

Current Commercial Sources

- Bacterial (Cellulomonas sp.; Nocardia erythropolis; Pseudomonas fluorescens; Pseudomonas sp.; Streptomyces sp.)
- Recombinant (*Escherichia coli*)

Current Market Applications

Price

Company	Source	Amount	Price	Unit Cost	Reference	Source Date
Sigma Aldrich	Microbial recombinant	100UN 2mg	AU $148	$1.49 /UN	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/C1235	2008
Calbiochem (Merck)	Nocardia and streptomyces	100UN 9mg	US $175	$1.75 /UN	http://www.merckbiosciences.com/home.asp	2008
Sisco Research Laboratories	Streptomyces	500UN 33 mg	N/A	N/A	http://www.srlchem.com/products/product_details.php?productId=541&searchQS=productName%3Dcholesterol%2Boxidase%26searchScope%3Dall%26searchType%3DbyName	2008

Market

The market for cholesterol oxidase from animal sources is insignificant.

Cholesterol oxidase for medical and research applications is sourced from microbial organisms. Though no reason was given for this phenomenon it is likely that microbial organisms can produce cholesterol oxidase more efficiently and therefore cheaply. The concentration of cholesterol oxidase in bovine brains is also quiet low at 0.1grams per kilogram.

Summary of Commercial Potential for Australian Beef Industry

Strengths – none

Weaknesses – all commercial sources come from bacterial or recombinant sources

Opportunities – none

Deoxynucleotidyl-Transferase

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Deoxynucleotidyl-transferase	Thymus: 0.295kg	~8-10 mg/ Kg	primer dependent DNA polymerase that catalyzes the repeated addition of mononucleotide units from a deoxynucleotide triphosphate to the terminal 3' hydroxyl of single or double-stranded DNA.	Diagnostics, reagents	US Biologicals; Krackeler Scientific, Inc; Worthington Biochemicals

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Terminal Deoxynucleotidyl Transferase, also known as TdT and terminal transferase, is a specialized DNA polymerase. TdT catalyses the addition of nucleotides to the 3' terminus of a DNA molecule.

Current Market Applications

Terminal transferase has extensive applications in molecular biology research. It can be used in RACE (Rapid Amplification of cDNA Ends) to add nucleotides which can then be used as a template for a primer in subsequent PCR (polymerase chain reaction). It can also be used to add nucleotides labelled with radioactive isotopes.

Current Commercial Sources

- Bovine thymus
- Human recombinant
- Bovine recombinant

Price

Company	Source	Amount	Price	Unit Cost	Source Date
Sigma Aldrich	Calf thymus	750UN 0.15mL	AU$261	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/T4427	2008
Biovision	Human recombinant	2500UN 0.125 mL	US$295	http://www.biovision.com/Merchant2/merchant.mvc?Screen=PROD&Store_Code=B&Product_Code=2001-2500	2008
Calbiochem	Calf thymus	0.155mL	US$247	http://www.merckbiosciences.com/product/PF060	2008
Millipore	Recombinant	300ul 0.3mL	US$120	http://www.millipore.com/catalogue/item/S7107?open&cid=P07091007	2008
New England Biolabs	Recombinant	0.125mL (0.060 mg)	US$244	http://www.neb.com/nebecomm/products/productM0252.asp	2008
USB Corp.	Bovine recombinant	2500UN 0.25mL	US$301	http://www.usbweb.com/category.asp?cat=mbe&id=72033	2008

Market Size

TdT has no bulk commercial applications; as such the market for this enzyme is limited to the research industry.

Although TdT is an invaluable research reagent the volumes of TdT needed by researchers are not large.

Emerging Technologies:

Cheaper recombinant sources of terminal transferase are being developed.

Competitive Products:

Recombinant sources of terminal transferase have been developed, which are competing with bovine (calf) sources.

Concentration in Bovine Tissue

Bovine thymus

Calf thymus (30kg)	
Product	Yield
Purified deoxynucleotidyl-transferase	0.12 g

M. Yoneda and F. J. Bollum, 'Deoxynucleotide-polymerizing Enzymes of Calf Thymus Gland. I. LARGE SCALE PURIFICATION OF TERMINAL AND REPLICATIVE DEOXYNUCLEOTIDYL TRANSFERASES', The Journal of Biological Chemistry, Vol. 240, No. 8, August 1965.

Summary

Opportunity potential: possible "research reagent"

Summary of Commercial Potential for Australian Beef Industry

Strengths – Currently manufactured from calf thymus, numerous applications in DNA synthesis

Weaknesses – human and bovine recombinant sources are commercially available. Low concentration in thymus, calves preferred to adults sources.

Opportunities – Supply to fine chemical companies

Threats – Recombinant enzymes have been developed and currently sold by companies such as Invitrogen

Glutamate Dehydrogenase

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Glutamate Dehydrogenase	Liver: 5.1kg Kidney: 1.2kg and other tissues	~5 mg/Kg	NAD+-dependent oxidoreductase that occupies a pivotal position in cellular metabolism for they catalyze the reversible reaction, linking the glycolytic and tricarboxylic acid cycles	A homohexameric enzyme that catalyses the conversion of glutamate to a-ketoglutamic acid/ bioconversions, diagnostics	Sigma; Calzyme Labs Inc; Boehringer-Mannheim

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Glutamate dehydrogenase is an enzyme, present in mitochondria that converts glutamate to α-Ketoglutarate.

Glutamate is a key molecule in cellular metabolism. In humans, dietary proteins are broken down by digestion into amino acids, which serves as metabolic fuel for other functional roles in the body

Glutamate also plays an important role in the body's disposal of excess or waste nitrogen. Glutamate undergoes deamination, an oxidative reaction catalysed by glutamate dehydrogenase, as follows:

$$\text{glutamate} + \text{water} + NADP^+ \rightarrow \alpha\text{-ketoglutarate} + NADPH + \text{ammonia} + H^+$$

Ammonia (as ammonium) is then excreted predominantly as urea, synthesised in the liver. Transamination can thus be linked to deamination, effectively allowing nitrogen from the amine groups of amino acids to be removed, via glutamate as an intermediate, and finally excreted from the body in the form of urea.

Ammonia incorporation in animals occurs through the actions of glutamate dehydrogenase and glutamine synthetase. Glutamate plays the central role in mammalian nitrogen flow, serving as both a nitrogen donor and nitrogen acceptor.

Commercial applications

Bovine sourced Glutamate Dehydrogenase is used in the determination of blood urea in conjunction with urease. This is a commercial analytical diagnostic that is widely used in medicine and for research purposes. Though the technique is widely used in medicine it only requires minute amounts of Glutamate Dehydrogenase in each test.

Price

Company	Amount	Price	Source	Reference	Source Date
Sigma	1000(mg)	$1129	Bovine liver	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/G2501	2008
Sigma (Fluka)	5ml	$84	Bovine liver	http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/49390	2008

Market Size

- The market size for Glutamate Dehydrogenase is approximately 1-10kg a year.[1]
- The ability to purchase a highly refined Glutamate Dehydrogenase and a less refined bulk product reflects the use of this product for commercial diagnostic and research purposes.
- Bovine liver is the sole source tissue for commercial Glutamate Dehydrogenase production found after an extensive search.

What product processing is involved?

Ion-exchange chromatography on DEAE-cellulose and affinity chromatography on GTP-Sepharose

Purity: 90%

Yield: 312.5mg/kg of Bovine Liver

Content: 1420.45mg/kg of Bovine Liver

Purification of glutamate dehydrogenase from ox brain and liver. Evidence that commercially available preparations of the enzyme from ox liver have suffered proteolytic cleavage.[2]

Reference

1 *biotechnology and biochemistry* 2002

2 A D McCarthy, J M Walker, and K F Tipton Biochem J. 1980 November 1; 191(2): 605–611.

Lysozyme

Protein	Tissue Source & Weight (kg/ head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Lysozyme	Immune cells: N/A Serum: 11.34kg Abomasum mucosa: N/A	6 mg/L serum; ~ 25 mg/g of tissue	beta-N-acetyl-muramyl-hydrolase activity against gram+ bacteria	Antibacterial action; preservative in foods, cosmetics, and agricultural products	US Biologicals, Calzyme Labs Inc

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Lysozymes are a family of enzymes which degrade bacterial cell walls and as such are an integral part of the innate immune system.

Lysozymes are abundant in a number of animal secretions, such as saliva, tears and mucus as well as white blood cells. Lysozymes are effective suppressors of bacterial pathogens and are found in high concentrations in colostrum, infants fed baby formula are more likely to contract diarrhoeal diseases, suggesting that incorporation of lysozyme may be beneficial.

Lysozyme enzymes damage bacterial cell walls by catalysing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins.

Lysozymes are currently extracted from egg whites and human lysozyme can be produced by recombinant bacteria.

Commercial applications

- Lysozymes are used as a food preservative agent in cheese, meat and caviar.
- Lysozyme is also used in the fermentation industry fro products like wine and beer. Lysozyme is added to control gram positive bacterial contamination (like lactic acid bacteria) as lysozyme has no effect on yeast.
- Lysozymes are also used in infant formula and animal feed.
- Since lysozymes are natural enzymes they are seen as an organic alternative to the synthetic preservatives used today.

Price

Company	Amount	Price	Source	Reference	Source Date
Calzyme	1g	$5	Egg white	http://www.calzyme.com/commerce/catalog/product.jsp?product_id=1183&czuid=1184036089009	2008
Worthington	10g	$150	Egg White	http://www.worthington-biochem.com/LY/cat.html	2008
Sigma	10g	$354	Egg White	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/L7651	2008
More Wine	1000g	$184	Egg white	http://morewinepro.com/view_product/15500/beerwinecoffee/Lysosyme_-_1_KG	2008

Market Size

In 2005 the worldwide gross annual output of Lysozyme was 300 tons, but the world demand was estimated at 1,000 tons.

Human recombinant lysozyme has an estimated price of $96,000/Kg

Chicken egg white lysozyme has an estimated retail price of $200/kg [1]

There is no bovine lysozyme on the market at this time, and given the low-cost of the egg lysozyme there appears to be no market for bovine lysozyme as the bulk industrial market is driven by price.

The production of bovine lysozyme is likely to be more expensive than the production of egg lysozyme.

What product processing is involved?

- Multistage affinity filtration

Purity: 95%

Referemce

1 http://www.yashenggroup.com/news_yasheng_group_announces_plan_to.htm

Phosphodiesterases

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Phosphodiesterases	Spleen: 0.66kg Eye: 0.05kg Lung: 2.85kg Pancreas: 0.43kg Heart: 1.5kg Brain: 0.34kg Thyroid: 0.001kg Adrenal: 0.24kg	~30 mg/ Kg (Lung); ~ 8 mg/Kg (thyroid); ~50 mg/Kg (brain)	Catalyze the hydrolysis of phosphodiester bonds. There are 11 families of PDEs, namely PDE1-PDE11; broad abundances and specificities depending on tissue source	Diagnostics, cell biology, bioconversions	ChemiCom; Sigma-Aldrich; Euromedex.com; and numerous vendors offer different PDEs

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

A phosphodiesterase is any enzyme that breaks a phosphodiester bond. Usually, people speaking of phosphodiesterase are referring to cyclic nucleotide phosphodiesterases, which have great clinical significance and are described below. This report focuses on the cyclic nucleotide phosphodiesterases:

The cyclic nucleotide phosphodiesterases (PDE) comprise a group of enzymes that degrade the phosphodiester bond in the second messenger molecules cAMP and cGMP. They regulate the localisation, duration, and amplitude of cyclic nucleotide signalling within subcellular domains. PDEs are therefore important regulators of signal transduction mediated by these second messenger molecules.

Classification

The PDE superfamily of enzymes is classified into 11 families, namely PDE1-PDE11, in mammals. The classification is based on:

- Amino acid sequences
- Substrate specificities
- Regulatory properties
- Pharmacological properties
- Tissue distribution.

Clinical significance

- PDE enzymes are often targets for pharmacological inhibition due to their unique tissue distribution, structural properties, and functional properties.
- PDE inhibitors have been identified as new potential therapeutics in areas such as pulmonary arterial hypertension, coronary heart disease, dementia, depression, and schizophrenia.
- Cilostazol (Pletal) inhibits PDE3. This inhibition allows Red Blood Cells to be more able to bend. This is useful in conditions such as intermittent claudication, as the cells can manoeuvre through constricted veins and arteries more easily

Phosphodiesterase 1 is an enzyme that catalyses the hydrolysis of a phosphodiester bond at the 3_ hydroxyl end of ribopolynucleotide to yield a 5_ ribonucleotide.

Commercial applications

PDE-1 is particularly important to food and pharmaceutical industries because the 5_ ribonucleotides produced by PDE-1—mediated cleavage of yeast RNA are useful as flavour enhancers. Barley cells or rootlets are used in the food industry as a source of PDE-1.

The enzyme has been widely utilised as a tool for structural and sequence studies of nucleic acids.

PDE-2 has few commercial applications outside of the research industry. Bovine organs produce PDE-2 not PDE-1 therefore there are no bulk commercial applications for bovine sourced Phosphodiesterases.

Price

Company	Amount	Price	Source	Reference	Source Date
Sigma	5mg	$305	Bovine Spleen	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;P9529	2008
Sigma	0.1mg	$686	Bovine brain	http://www.sigmaaldrich.com/catalog/search/SearchResultsPage/PricingAvailability/SIGMA;P9529	2008

Market

The majority of research and development into PDE-2 enzymes is concentrating on their inhibition it is likely that there are no bulk commercial applications for PDE-2.

Protein Kinase-A

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Protein Kinase-A	Heart: 1.5kg Brain: 0.34kg	~2 mg/Kg	Mediates the majority of the actions of the intracellular second messenger cAMP	cell biology;	ABR Affinity Bioreagents; Biosource International; EMD Biosciences, Promega

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

A protein kinase is a kinase enzyme that modifies other proteins by chemically adding phosphate groups to them (phosphorylation).

Phosphorylation usually results in a functional change of the target protein (substrate) by changing enzyme activity, cellular location, or association with other proteins.

Up to 30% of all proteins may be modified by kinase activity, and kinases are known to regulate the majority of cellular pathways, especially those involved in signal transduction, the transmission of signals within the cell.

Because protein kinases have profound effects on a cell, their activity is highly regulated. Kinases are turned on or off by phosphorylation, by binding of activator proteins or inhibitor proteins, or small molecules, or by controlling their location in the cell relative to their substrates.

Deregulated kinase activity is a frequent cause of disease, particularly cancer, where kinases regulate many aspects that control cell growth, movement and death. Drugs which inhibit specific kinases are being developed to treat several diseases.

Protein kinase A has several functions in the cell, including regulation of glycogen, sugar, and lipid metabolism.

PKA phosphorylates other proteins, altering their function. However, what proteins are available for phosphorylation depends on in what kind of cell the PKA activity is present, since protein composition varies from cell type to cell type.

Protein kinase A is a highly regulated protein which has been studied extensively, in particular, its possible role in memory disorders, cancer and heart failure. In response to stress, PKA levels rises and the heart works harder.

PKA is a common drug target, and its activity often modulated to gain therapeutic effects such as lowering blood pressure.

“Protein kinases (PKs) belong to the largest single family of enzymes, numbering over 500 and accounting for almost 2% of the proteins encoded by the human genome. Recent success in drug discovery demonstrates that PKs are excellent drug targets.” – Protein Kinase Research (2007)

Current Commercial Sources

- Bovine Heart
- Human *E. coli* recombinant
- Porcine Heart

Current Suppliers & Unit Cost

Company	Source	Quantity	Price	Unit Cost
PhosphoSolutions	Bovine Heart	1µg	US$150	$150/µg
Sigma-Aldrich	Bovine Heart	10mg	AU$179.50	$17.95/mg
Sigma-Aldrich	Human Recombinant (*E.coli*)	1µg	AU$551	$551/µg

Market

The market size of protein kinase A is unknown , the market is likely to be to insignificant for market information to be gathered and quantified.

The lack of commercial applications for protein kinase A outside of the medical research industry limits the market demand of this enzyme.

The concentration of protein kinase A in the bovine brain is low at 2mg per kilogram of heart tissue; this would increase the difficulty of the extraction and purification process, resulting in extra costs.

Opportunity potential: possible “research reagent”

Summary of Commercial Potential for Australian Beef Industry

Strengths – Protein kinase A sourced from bovine heart is sold by companies such as Sigma Aldrich

Weaknesses – heavily regulated enzyme with limited knowledge regarding its activity. Its function is often inhibited rather than promoted to derive therapeutic effects

Opportunities – none

Threats – association of protein kinase A with various disease states

Current Market Applications

- Research marker and therapeutic target.

Concentration in Bovine Tissue

Bovine Heart

Bovine heart (2000g) - purified	
Product	Protein
Protein Kinase A	0.07 mg

Bradley C. Wise, Robert L. Raynor, and J. F. Kuo, ‘Phospholipid-sensitive Ca2’-dependent Protein Kinase from Heart I. PURIFICATION AND GENERAL PROPERTIES’, The Journal of Biological Chemistry Vol. 257, No. 14, July 25, pp. 8481-8488, 1982

Bovine Brain

Bovine brain (2033g) - purified	
Product	Protein
Protein Kinase A	58 mg

Miyamoto et al., ‘Cyclic Nucleotide-dependent Protein Kinases III. PURIFICATION AND PROPERTIES OF ADENOSINE 3’) 5’-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE FROM BOVINE BRAIN, THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 244, No. 23, Issue of December 10, pp. 6395-6402, 1969

Emerging Technologies

Developments in PKA or protein kinase family are swayed towards implementing PKA as a drug target. An example is ‘GEM231’ which targets a subunit of PKA and inhibits formation of malignant tumors. Other companies, such as Novartis and Vertex Pharmaceuticals, are currently developing small molecular drugs which directly target the protein kinase family of enzymes.

Protein Phosphatase 2C

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Protein Phosphatase 2C	Spleen: 0.66kg Brain: 1.5kg	50 mg/Kg	1 of 4 four major classes of mammalian serine/threonine specific protein phosphatases	cell biology; diagnostics	US Biologicals; GeneTex Inc., Sigma Aldrich;

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

A phosphatase is an enzyme that removes a phosphate group from its substrate by hydrolysing phosphoric acid monoesters into a phosphate ion and a molecule with a free hydroxyl group

Phosphatases can be categorised into two main categories: Cysteine-dependent Phosphatases (CDPs) and metallo-phosphatases (which are dependent on metal ions in their active sites for activity).

A protein phosphatase is an enzyme that removes phosphate groups from proteins, often functioning to reverse the effect of a protein kinase.

Serine and threonine phosphates are stable under physiological conditions, so a phosphatase has to remove the phosphate to reverse the regulation. There are several known groups with numerous members in each.

Commercial Applications

Protein Phosphatase 2C has no known bulk commercial applications, though it is useful in biochemical research and is sold by various research reagent suppliers.

Price

Company	Amount	Price	Source	Reference	Source Date
Sigma	0.01mg	$532	Bovine Brain	http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/P1743	2008
Gentaur	0.001mg	$342	Bovine Brain	http://www.gentaur.com/phosphatase.htm	2008
GloboZymes	0.001mg	$703	Bovine Brain	http://www.globozymes.com/product_info.php?manufacturers_id=&products_id=48	2008

Current Product Source: Human, recombinant bacteria, rabbit, cow

Market

- The market for protein phosphatase is very small, there are no bulk commercial applications for protein phosphatases.
- The market for protein phosphatase 2C would be even smaller.
- The extremely high price per mg ($5320) is a good indication that the market is small and that the extraction and purification costs are great.

What product processing is involved?

Chromatography on DEAE-cellulose, precipitation with ammonium sulphate, gel-filtration on Sephadex G-100, affinity chromatography on thiophosphorylated myosin-P-light-chain--Sepharose and chromatography on Mono Q.[1]

Purity: >90% by SDS-PAGE.

Reference

1 McGowan CH, Cohen P. Identification of two isoenzymes of protein phosphatase 2C in both rabbit skeletal muscle and liver. Eur J Biochem. 1987 Aug 3;166(3):713–721. [PubMed]).

Urease

Protein	Tissue Source & Weight (kg/head)*	Abundance (per/kg of tissue)	Mode of Action	Use	Supplier
Urease	Rumen: 8.4kg	Varies according to rumen content	Catalyses the hydrolysis of urea to CO2 and ammonia (as hydroxide)	Widely used in diagnostics	Worthington Biochemical Corp; Sigma-Aldrich; CalbioChem; Calzyme Lab Inc.,

**Assume 300kg HSCW. See Appendix A for other HSCW*

Description

Urease is an enzyme that catalyses the hydrolysis of urea into carbon dioxide and ammonia. The reaction occurs as follows:

$(NH_2)_2CO + H_2O \_ CO_2 + 2NH_3$

Urea + Water --^{urease}--> Ammonium Carbonate

Many gastrointestinal or urinary tract pathogens produce urease, enabling the detection of urease to be used as a diagnostic to detect presence of pathogens.

Urease is an example of an enzyme that is produced by the bacteria found in the rumen of cattle.

The micro-organisms that inhabit the rumen of cattle, sheep and other ruminants are currently being extensively researched as they may produce valuable and novel enzymes.

The enzymes produced by bacteria in the rumen could possibly be used to convert plant matter into ethanol for fuel. Researchers are also conducting experiments with bacteria that produce less methane and introducing them into cattle, to reduce bovine methane emissions.

Price

- No bovine urease is sold

Company	Unit	Price	Source	Reference	Source Date
Calzyme	1mg	$0.75	Jackbean	http://www.calzyme.com/commerce/catalog/spcategory.jsp?category_id=1104	2008
Sigma	25000mg	$168	Jackbean	http://www.sigmaaldrich.com/catalog/search/ProductDetail/FLUKA/94280)	2008

Market Size

- The market for urease is currently sourced from Jack Beans.
- The introduction of a bovine derived urease would not be economically viable as it would be more expensive to produce than the current source.
- The main commercial activity involving urease is its detection in the diagnosis of gastrointestinal and urinary tract pathogens.

What product processing is involved?

- Urease (urea amidohydrolase, EC 3.5.1.5) extracted from the mixed rumen bacterial fraction of bovine rumen contents and purified 60-fold by (NH4)2SO4 precipitation, calcium phosphate-gel adsorption and chromatography on hydroxyapatite.[1,2]

Purity:

- 80%[3]

Reference

1 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1164729

2 http://www.ncbi.nlm.nih.gov/pubmed/4420?dopt=Citation

3 http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1164729&blobtype=pdf

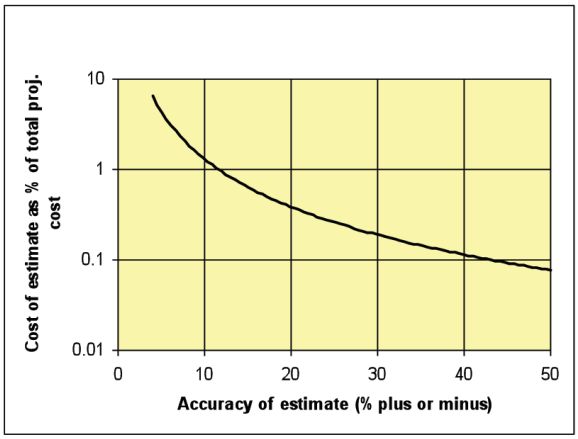
Bioactives cost estimation

Introduction

It is inevitable when one considers entering a bioactives market that an estimate needs to be made of the likely cost of manufacture and the likely capital cost of building and commissioning a bioactive processing plant. Generally one has a generic extraction and purification process or a novel process which has been developed at small scale in a laboratory. Taking the data from a small scale process and extrapolating to predict the performance of a larger scale process is referred to as “scaling up”. In some instances one may have a commercial scale process which was developed in a different country or some years ago and the difficulty in estimating the costs of production and capital arise from currency, inflation over the intervening years, interest rates and cost of labour, energy and materials. There are books written on the subject and there are consultants specialising in cost estimation. Generally the more accurately one requires the estimate, the more expensive will be the fee.

There is an often quoted study from 1973 (WR Park, Cost Engineering Analysis, Wiley , NY, 1973,p133) which investigated the cost and accuracy of cost estimates for a project with a total cost of $1m. The results of this study are shown in Figure 1. This figure shows that for an early stage cost estimate where an error of +/- 30% might be acceptable, $2k (1973 dollars) fee was typical, whereas for final project budgeting where +/- 5% accuracy was required, the fee would be more like($50k (1973 dollars). The point to be made here is not about the absolute cost, but that more accurate estimates require more information and take more time to deliver and are more expensive.

Figure 1: Cost of cost estimates



The aim of this section of the compendium is to give the reader some familiarity with the concepts and strategies in cost estimation, in order to facilitate engagement with such service providers.

Factors impacting cost of goods sold for bioactives.

The types of by-products that can be obtained from animal red meat tissues, organs or fluids are highly diverse, extending from functional proteins and enzymes to polysaccharides, lipids, steroids, amino acid derivatives and many other classes of molecules. Irrespective of the nature of the substance class, molecular size or molecular mass, several common criteria apply to their isolation and purification at scale, including:

- What feedstock source material can be utilised? Will additional treatments be needed to yield the target substances in a crude form suitable for subsequent processing? If so, what are these component steps, and how will they affect subsequent processing steps?
- What yield, productivity, product concentration and purity can be achieved and how many steps are needed to obtain the bioactive product in a form suitable for commercialisation? In particular what technology is best suited to purifying the desired product from many similar compounds or impurities present in the mixture?
- What are the optimal process conditions in terms of temperature, fluid or mass transport, shear or foaming? Should the process be batch or continuous?
- What by-products are generated through the use of a particular process e.g. spent organic solvent, salt stream or even contaminated water, which cannot be recycled or used as a source in other product processing?
- What tools are available to model or simulate the process and its underlying chemistry in order to facilitate scale up and engineering design, or to predict functionality and stability?

Purification costs

In this section, information on the costs of scaling up purification processes is presented using red meat proteins as target molecules, but the general principles also apply to other classes of bioactive substances of significantly lower molecular size. What sets protein apart from many of these other classes of bioactive substances is that proteins are very complex molecules whose intricate structure determines their functionality. Proteins are long and intricately folded chains of amino acids which can be characterised at four levels of structure. (i) the primary structure, which is their amino acid sequence,; (ii) the secondary structure, which is largely defined by the type of intramolecular hydrogen bonding, salt bridges or non-polar interactions that can form; (iii) the tertiary structure that defines the folded state of the protein and the extent of disulphide bonds and their connectivity; and (iv) the quaternary structure which arises from the formation of homo- or hetero-multimeric complexes of the subunits of some, but not all, proteins.

As protein functionality (and therefore value) depends on its shape, charge distribution and size, it also depends on these secondary, tertiary and quaternary structures. This presents a particular challenge to scale-up of recovery and purification processes, since it may be possible to achieve perfect retention of the target molecule but with no functionality if it is damaged by physical mishandling or chemical damage. The thermodynamic and kinetic characteristics of the target molecule need to be understood in designing a process, as they have a bearing on the choice of unit operations in the purification process, the process temperature(s), the process reactor/column size and process tank dimensions and associated capital equipment investment costs. The development of a viable "downstream" purification process for the production of a high-value-added bioactive product requires as a minimum:

a) The identification of the market requirements in terms of purity and stability, either quantified from actual hard market survey information or derived from forecast information for which the assumptions can be specified and to a large extent quantified

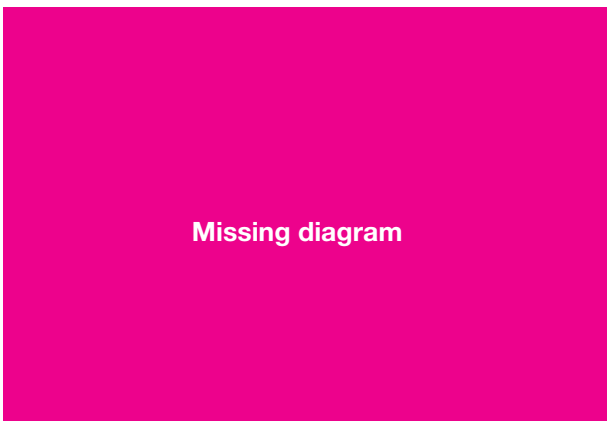
b) Understanding of the quality of the "product supply chain" for the feedstock that will be used as the source of material to produce the desired substance

c) Knowledge of the abundance (the concentration of the target compound in the source tissue/ feedstock) and stability of the bioactive substance in the chosen feedstock and during the process steps

d) Knowledge of the physical and chemical properties of the desired products and other components of the feedstock, and

e) Understanding the nature of the trade-offs that can be tolerated with regard to yield and purity. Yield is the measure of the quantity of the target compound that can be isolated and purified per weight of feedstock. Purity is the measure of how few other non-target compounds are present in a given sample. As a general rule of thumb, high purity arising from high levels of processing is usually associated with lower yields (because each purification step results in some loss of yield) and this in turn has a significant impact on the level of investment in terms of plant, human resources and consumables.

Figure 2: Relationship between process efficiency and product yields per cycle



General Rules of Thumb in process design and cost estimation

Each step in a production process e.g. mincing, extraction, filtration, etc. represents what is known as a "unit operation". Bioactives extraction, separation and purification can typically involve some 10 or so unit operations. At each step, there is inevitably some loss of product, be it due to hold up in pipes, degradation or irreversible adsorption to separation media.

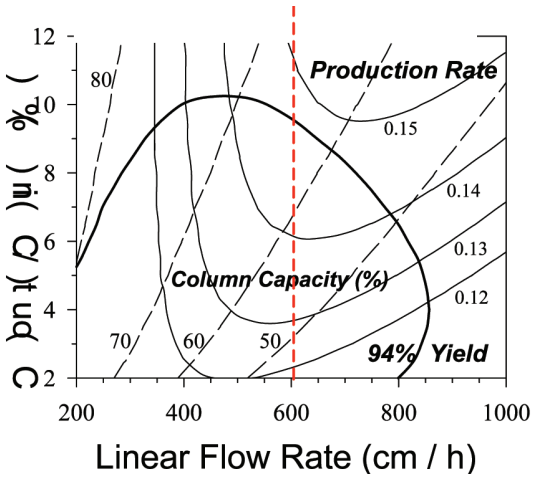
The impact of yield losses on process efficiency is a major determinant in the selection of a viable technology. This relationship between process efficiency and mass yield is illustrated in Figure 2.

Productivity is driven by product yield per unit operation per $ invested. The greater the number of steps in purification, the greater will be the cumulative loss across the purification train and the lower will be the net yield of product. For example in figure 2, a 10-step extraction/purification process that runs with an efficiency of 95% per step would result in only a 60% net yield. A process with an efficiency of 80% per step would result in an even worse net yield of only 10% and would require therefore a plant capacity roughly 6-fold larger (and a commensurately higher capital cost) than that where each step ran at 95% efficiency if the same amount of product were to be made at similar purity. Although there can be no hard and fast rule about the number of steps in a process, many bioactives production processes do go as high as 10 steps. One typically would aim for 90-95% recovery across eachstep. In selecting alternative separation technologies, one should look at the potential to increase yield by minimising the number of processing steps.

Consider the chromatographic fractionation of a complex mixture containing a desired bioactive. Chromatography is a common unit operation which separates chemicals from a mixture based on the strength of their electrical charge or some other physical or chemical property. The majority of the chromatographic methods used in purification of proteins and other high molecular weight biopolymers have exploited one or more attributes of adsorption behaviour. Chromatography refers to the use of special resins in packed columns, onto which the target compounds are preferentially adsorbed. Types of chromatography commonly found in analytical laboratories include ion-exchange, reversed phase, hydrophobic interaction, hydrophilic interaction, multimodal interaction, dipole interaction, immunoaffinity, biospecific affinity, biomimetic affinity and metal ion affinity modes of this technique. For practical process-train reasons, ion-exchange, hydrophobic interaction, biospecific affinity, biomimetic affinity and metal ion affinity chromatographic procedures have come to represent the major versions of this separation technology at production scale, with the other modes used for some specific or niche applications only.

As scale of production has increased, commercial expectations have placed pressure on the vendors of chromatographic materials to ensure that their products have acceptable cycle times, e.g. 100-plus cycles of re-use, clean in place (CIP) capabilities, high resolution attributes, and consistency batch-by-batch. Some, but not all, vendors of chromatographic materials have responded to this challenge. The relationships between cost, loading capacity of the chromatographic sorbent, sample throughput, substance resolution and ease of separation are critical. Usually only three of these five parameters can be optimized simultaneously, necessitating a trade-off against the remaining parameters as a matter of expediency.

Figure 4: Productivity Plot for Large Scale Albumin Fractionation



For example, one might have a relatively easy separation of low cost, low to average resolution, high sample throughput and good sample loading capacity scenario, or one might have a high cost, good loading capacity, good to high resolution but relatively low sample throughput scenario.

Illustrative of these relationships is the plot, shown in Figure 4. C(out)/C(in) is a measure of the column performance. It is the ratio of the amount of product that is lost from the column while it is being loaded with the product. This figure plots the performance

against the linear flow rate. A vertical line at say 600cm/h shows that there is a trade off between production rate and product loss e.g. 3.5% loss at a production rate of 0.13 versus a 6% loss at a production rate of 0.14. The area under the dark curve shows the set of conditions to achieve 94% yield or higher. In this example, a production rate of 0.15 will under all conditions result in yield less than 94%. The key messages from this diagram are that yield, plant capacity utilisation and production rate are interrelated in a complex way, but there are multiple modes of plant operation that will deliver adequate yield. Typically, well-operated systems will achieve a ratio of C(out) to C(in) of 10% or less, i.e. a 10% loss of product across the process.

There is a variety of unit operation options in process chromatography and they can be defined in terms of whether they are based on fixed-bed chromatographic systems, batch (tank) adsorption processes, fluidised-bed adsorption systems or simulated moving bed systems, coupled with a process train that incorporates process integration and intensification into the production strategy.

Such considerations have a direct bearing on the cost burden that may be generated with different operating systems and conditions, as illustrated in Figure 4, from the analysis of the breakdown of the annualized cost of goods per gram for columns of different size run at 75% of their maximum linear flow. Chromatographic resins are the major contributor to annual operating costs of chromatographic columns (figure 5).

Capital Cost is comprised of more than just equipment

In estimating the likely Cost of Goods Sold, it is important to remember that the capital cost of the major pieces of equipment is only one part of the cost of establishing and running a plant. Table 1 illustrates typical capital costs as a fraction of the cost of the major capital equipment. These figures were derived from a real facility producing bioactives and, although providing a valuable insight into the relative impacts of the various factors, can not be taken as a hard and fast rule.

Figure 5: Breakdown of annualised costs of goods for different columns sizes operated at 75% of their maximum adsorptive capacity

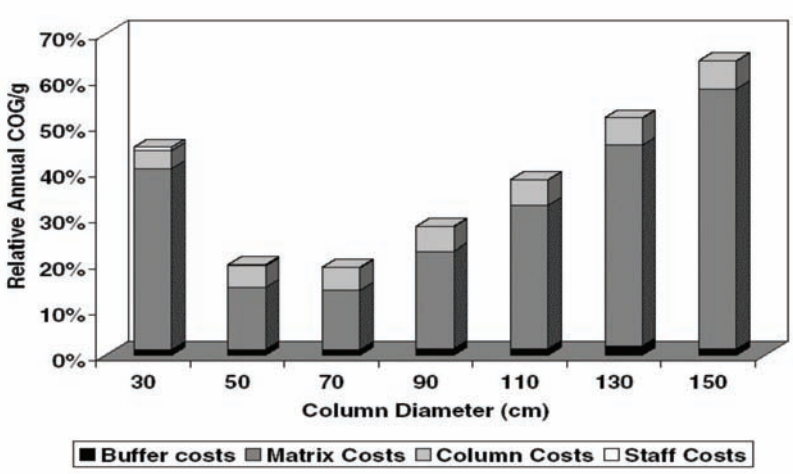


Table 1: Capital costs as a function of major equipment costs

Expense	Percentage of capital equipment costs	Details
Capital equipment	100%	
Installation	15-20%	
Good Manufacturing Practice (GMP)	85-120%	7,500-17,000/m2
Special control (such as temperature/air-conditioning)	30-45%	
Engineering and refurbishment	80-120%,	
Contingency	30-35%	
Project management	10%	

Since the equipment and process format for many recovery/purification systems can be configured to be similar if not identical some rules of thumb can be inferred from this table: plant/equipment installation costs tend to fall into the range of 15-25% of the purchased equipment costs whilst specific fit out costs for a dedicated suite to house the equipment in a Good Manufacturing Practice (GMP)-compatible environment is often between 85-120% of the equipment purchase price. Finally, to house the equipment and process systems, reagent and buffer preparation bays, to provide the necessary level of utilities (power, RO water and temperature control/air-conditioning), a further contribution of between 30-45% of the equipment purchase cost to the direct plant costs as a component of the total fixed capital investment may be required. In many circumstances, the indirect costs will be in the range of 80 to 120 % of the equipment purchase costs, particularly if significant additional engineering and laboratory construction or refurbishment is required, whilst with all process installations prudence demands that a contingency amount of about 1/3rd of the equipment purchase cost is included as part of the budgeted project investment. As noted above, it is rare occurrence for the actual project capital cost to come in at an amount that is within 10 percent of the feasibility study capital estimate.

Operating costs

Operating costs on the other hand are much more product specific. Less flexibility is apparent in areas associated with control over laboratory QC/QA of the product, particularly if GMP-manufacture is entertained. As a consequence, the areas where greatest controls over the operating costs are the cost of the feedstock/raw material, cost of consumables and cost of labour. The latter can be minimized, but not forgotten, by using computer-assisted automation and process control. Many process facilities overseas operate at a scale where labour costs are approximately 5% of the overall operating costs and on a par with utility and waste disposal costs. Facility costs can vary significantly depending on the purity and potential market applications of the bioactive, and can reach a level equal to the cost of the raw materials particularly if extensive storage, sample handling or refrigeration is mandated by the production process. For example in serum manufacture for pharmaceutical media applications, QC and product acceptance by customers can take 6-12 months or more. Such inventory levels represent significant working capital and the lesson here is that the logistics of QC and product release need to be borne in mind when calculating operating costs.

Chromatographic resins and gels represent a significant proportion of consumable costs. As a general rule, the Cost of Goods Sold (COGS) decreases exponentially with the number of recycling procedures that can be achieved, e.g. ≥ 6 fold resin cost reduction may be achievable if a 100-cycling process can be realised. Linked to these considerations is the need to have quantitative information on the cost of cleaning, sterilization, process engineering, system validation, space, labour and human resources charges, waste disposal, utility demands and scale, equipment cost and cost of lost time. A significant consideration also hinges on the type of bioactive to be obtained from the feedstock, with different metrics applied if the substance is a low molecular weight compound rather than a high molecular weight multi-meric protein with enzymatic activity.

Typical components of Cost of Goods Sold (COGS) for bioactives are shown in Table 2. As in Table 1, these percentages are given relative to the capital equipment cost and are meant only to raise awareness of the relative importance of various operating costs. They need to be calculated for the product and plant under consideration when a cost estimation is undertaken.

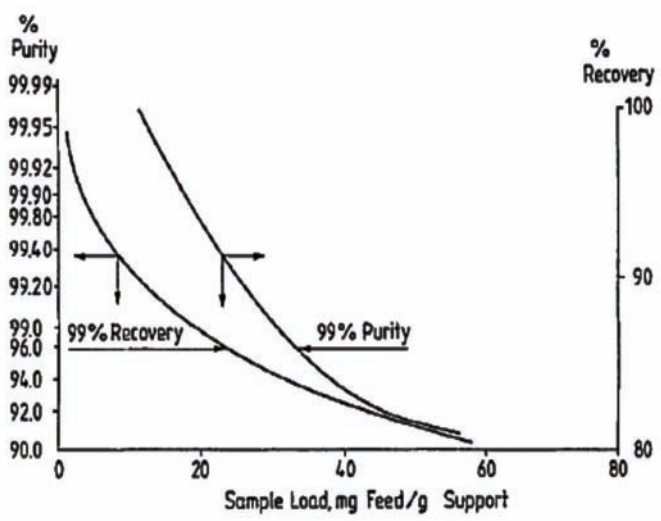
Table 2: Typical components of operating cost

Expense	Percentage of capital equipment costs	Details
Operating expenses	100%	1:1
Raw materials/ feedstock costs	60-65%	
Reagent consumable costs	7.5-12%	
Cost of power water and other utilities	4-6%	
Labour and staff costs	3-10%	
Product quality control and analysis/ validation costs and cost	2-8%	
Waste disposal/ treatment	4-6%	
Space charges	12-15%	

A commonly experienced outcome with process purification is the sensitivity of both product recovery and purity to the sample load. Such a scenario is illustrated in Figure 6. Purity and recovery, although related, are two different concepts. Purity is a measure of the percentage of the product stream that is comprised of the target compound. Recovery is the percentage of the target compound originally present that is recovered. For low molecular weight compounds, the greater the purity in the feed stream, the greater the % recovery is likely to be. Proteins on the other hand present more of a challenge due to conformational changes that are not necessarily reflected in mass changes. One might for example achieve a purity of 90% of enzyme in the final product stream but have lost 50% of the enzyme activity due to damage done to the protein, resulting in only a 45% recovery. Figure 6 shows the impact of sample load on purity and on recovery.

The curve on the left of Figure 6 is the 99% recovery curve, which shows that increasing sample load intensity onto the resin results in the loss of purity. The curve on the right is the 99% purity curve, which shows that increasing loading intensity on the resin results in a loss of product (lower % recovery). Although this data is for a chromatographic column, the same concepts apply to microfiltration.

Figure 6: Relationship between purity, recovery and sample load. Data from K.Jones, Chromatographia, 25 (1988) 487-493. see also A Lyddiatt, Current Opinion in Biotechnology 13 (2002) 95-103.



Scale-up of chromatographic processes

Historically, most purification processes that employ chromatographic systems have been optimized empirically. Although scale up rules for the selection of column size for packed or expanded/fluidized beds can be derived from engineering principles, usually the sorbent choice and operating conditions are determined according to the operator's expertise or bias. Various studies have documented how process efficiencies can be improved through the use of columns of larger diameter operated at higher linear flow velocities of the eluent, as illustrated in Table 3.

Column size thus has a significant impact on the cost of goods per unit amount of product processed. However, it should be noted that an optimum exists in terms of cost of the sorbent (resin) as the column volume is changed, i.e. as the cost of the sorbent starts to dominate cost, the effect of flow rate and cost of eluent components tend to diminish. As single use (disposable) technologies become more prevalent in biomanufacturing, the possibility may arise that higher process yields might be realized. However such developments will require new technologies that are more efficient, faster, less expense and more flexible if they are to be competitive with re-usable process systems. In this context, the use of affinity membrane technology currently offers greater scope than some particulate types of adsorbents Such systems find application in depth filtration and tangential flow filtration devices and so-called membrane chromatographic systems with scale up potential of ~ 1000 litre or 5-6 m^2 capacity. Such systems capture the advantages of convective mass transfer (i.e. pumping and turbulent flow) compared to diffusive mass transport, which in principle translates to higher throughputs, reduced processing time and the ability to handle more concentrated feedstock streams. Current limitations of these technologies relate to "Cleaning in Place" (CIP) constraints and lower loading capacities.

Table 3: Impact of column diameter and linear flow velocity of the eluent on the cycle number required to process a batch of feedstock material. Joseph et al., J Chem Technol. Biotechnol., 81 (2006) 1009-1020.

Linear loading flow rate ($cm\ h^{-1}$)	Column diameter (cm)							
	10	30	50	70	90	110	130	150
50	166	19	7	4	3	2	1	1
100	170	19	7	4	3	2	2	1
150	174	20	7	4	3	2	2	1
200	179	20	8	4	3	2	2	1
250	187	21	8	4	3	2	2	1
300	195	22	8	4	3	2	2	1
350	202	23	9	5	3	2	2	1
400	209	24	9	5	3	2	2	1
450	217	25	9	5	3	2	2	1
500	227	26	10	5	3	2	2	2

From extraction to preservation.

A representative example of the workflow and the types of equipment used and their indicative costs can be found in the industrial production of fructosyltransferase. The work flow employed for the purification of this enzyme is shown in Figure 7. Although this enzyme, which catalyses the transformation of sucrose into fructo-oligosaccharides is now manufactured using genetic engineering and recombinant DNA technologies or culture in *Aspergillus sp*., it nevertheless provides a useful paradigm in terms of design and economic consideration..

Through the judicious use of a series of micro-filtration, chromatographic, dia-filtration, ultrafiltration and lyophilisation steps in purifying this enzyme, a purification factor of up to 65 fold can be achieved with overall mass yield of ~ 54%.

A distinctive feature of the process so developed was that equipment usage of up to 85 % of available operating time could be achieved through appropriate scheduling of the different unit operations leading to an annual production capacity of 80 Kg of the purified enzyme fructosyltransferase, based on an annual operation time of ~7,920h. A number of software packages are commercially available to permit the economic modeling of the process design for such recovery/purification tasks. In this example, the SuperPro-Designer [Intelligen Inc., Scotch plains, NJ, USA; @www.bioprocessguide.com) was employed although other packages are available, e.g. Aspen Batch Plus (Aspen Technologies Inc., Cambridge, MA, USA). The total costs of the equipment use for this process was 2.6 million EUR with a sunk cost for fit out/ equipment installation of a further 465,000 EUR.

As evident from Table 5, the most expensive items in this process were the freeze-drier / lyophiliser and the bioreactors (50% of the equipment capital expenditure), with the remainder being largely associated with the purchase of membrane filtration, chromatographic, centrifugation equipment and process tanks systems. In the case of comparable feedstocks from red meat derived feedstocks (derived from fluids or tissue or organ extracts) bioreactors will typically not be required.

Figure 7: Process flow diagram for the industrial production of fructosyltransferase mapped using the Super-Pro-Designer. Data from Vankova et al., Chem. Pap. 59 (2005) 441-448.

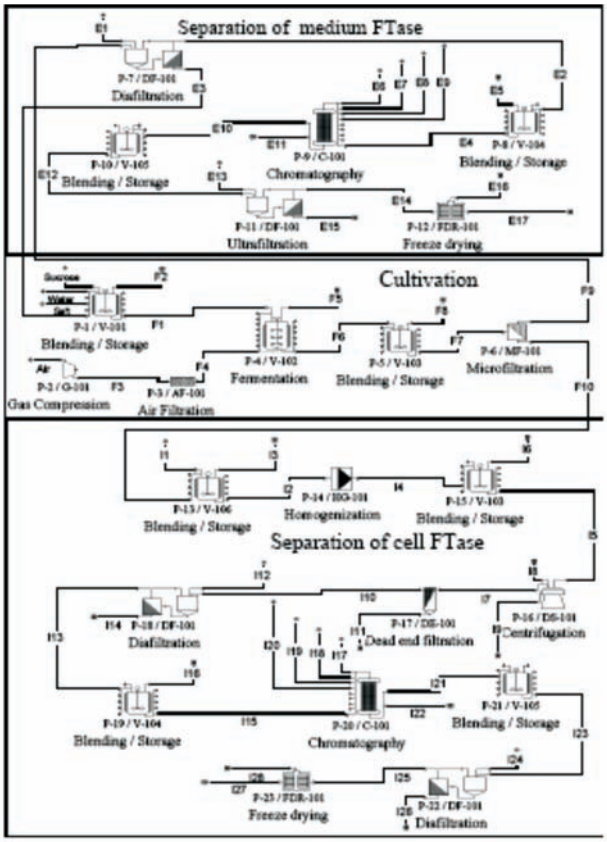


Table 4: List of equipment used in the manufacture of fructosyltransferase. Data from Vankova et al., Chem. Pap. 59 (2005) 441-448.

Name	Description	Cost/EUR
V-104	Blending Tank, Vessel Volume = 1.2 m^3	27 000
DF-101	Diafilter, Membrane Area = 50.0 m^2	95 000
C-101	Chromatographic Column, Volume = 47.7 dm^3, 2 pieces	215 000
FDR-101	Freeze-Dryer, Sublimation Capacity = 283 kg h^{-1}	803 000
V-106	Blending Tank, Vessel Volume = 10.0 m^3	52 000
HG-101	Homogenizer, Rated Throughput = 3.50 m^3 h^{-1}	38 000
DS-101	Disk-Stack Centrifuge, Throughput = 100.00 dm^3 min^{-1}	88 000
DE-101	Dead-End Filter, Filter Area = 15.00 m^2	32 000
MF-101	Microfilter, Membrane Area = 30.00 m^2	44 000
V-102	Bioreactors, Vessel Volume = 10.0 m^3, 2 pieces	506 000
AF-101	Air Filter, Rated Throughput = 0.06 m^3 s^{-1}, 2 pieces	13 000
G-101	Centrifugal Compressor, Power = 400 kW, 2 pieces	63 000
V-101	Blending Tank, Vessel Volume = 10.0 m^3	52 000
V-105	Blending Tank, Vessel Volume = 6.0 m^3	42 000
V-103	Blending Tank, Vessel Volume = 10.0 m^3	52 000

Accounting for change of scale: It is not uncommon to have to estimate the capital cost of a plant based on data for a different sized plant using the same process. One method for estimating this for an early stage estimate is to use the “six tenth’s rule” [see e.g. R.K. Sinnott in Coulson and Richardson’s Chemical Engineering Design, vol. 6 Pergamon Press, Oxford, (1993) pp 209-244; M.S. Peters and K.D. Timmerhaus, Plant Design and Economics for Chemical Engineers, McGraw-Hill, New York, (1991) chapter 6].

The sixth tenth’s rule:

$$\frac{\text{Cost of plant of throughput B}}{\text{Cost of plant of throughput A}} = \left[\frac{\text{Throughput B}}{\text{Throughput A}}\right]^{0.6}$$

Consider the case above: Given this data, one can estimate the capital cost of a smaller plant of, not 80kg/yr but say 8kg/yr.

For a red meat bioactive, the cost of the plant/ equipment for processes operating on an annualised basis with ~140 batches per annum at approximately. 80+Kg overall capacity would be substantially lower than the example in Table 4. The estimated cost of an equivalent facility to process approximately 8 Kg of a specific protein to the level of approximately 98% purity with a purification factor of roughly 100-fold would fall in the range of $800 - 1000K. This level of dedicated equipment expenditure corresponds closely to the cost for the equipment obtained for the recently (2007) established processing facility at the Centre for Green Chemistry at Monash University.

Glossary

Acute-phase proteins are a class of proteins whose plasma concentrations increase (positive acute phase proteins) or decrease (negative acute phase proteins) in response to inflammation. This response is called the *acute-phase reaction* (also called acute phase response).

Adipose tissue or **fat** is loose connective tissue composed of adipocytes. Adipose tissue is derived from lipoblasts. Its main role is to store energy in the form of fat, although it also cushions and insulates the body.

Ammonia is a compound with the formula NH3. It is normally encountered as a gas with a characteristic pungent odour. Ammonia contributes significantly to the nutritional needs of terrestrial organisms by serving as a precursor to foodstuffs and fertilizers. Ammonia, either directly or indirectly, is also a building block for the synthesis of many pharmaceuticals. Although in wide use, ammonia is both caustic and hazardous. In 2006, worldwide production was estimated at 146.5 M tonnes

Angiogenesis is a physiological process involving the growth of new blood vessels from pre-existing vessels. Angiogenesis is a normal process in growth and development, as well as in wound healing. However, this is also a fundamental step in the transition of tumours from a dormant state to a malignant state.

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves.

Apoptosis is a form of programmed cell death in multicellular organisms. It is one of the main types of programmed cell death (PCD) and involves a series of biochemical events leading to a characteristic cell morphology and death, in more specific terms, a series of biochemical events that lead to a variety of morphological changes, including blebbing, changes to the cell membrane such as loss of membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation (1-4). Processes of disposal of cellular debris whose results do not damage the organism differentiate apoptosis from necrosis.

Arthritis is a group of conditions involving damage to the joints of the body. Arthritis is the leading cause of disability in people older than fifty-five years

Osteoarthritis is a clinical syndrome in which low-grade inflammation results in pain in the joints, caused by abnormal wearing of the cartilage that covers and acts as a cushion inside joints and destruction or decrease of synovial fluid that lubricates those joints. As the bone surfaces become less protected by cartilage, the patient experiences pain upon weight bearing, including walking and standing.

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder that causes the immune system to attack the joints, where it causes inflammation (arthritis) and destruction. It can also damage some organs, such as the lungs and skin. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility.

Bile or **gall** is a bitter yellow or green alkaline fluid secreted by hepatocytes from the liver of most vertebrates. In many species, **bile** is stored in the gallbladder between meals and upon eating is discharged into the duodenum where the bile aids the process of digestion of lipids.

Biocatalysis can be defined as utilisation of natural catalysts, such as protein enzymes, to perform chemical transformations on organic compounds.

A biosensor is a device for the detection of an analyte that combines a biological component with a physicochemical detector component

Blood clot is the final product of the blood coagulation step in hemostasis. It is achieved via the aggregation of platelets that form a platelet plug, and the activation of the humoral coagulation system (i.e. clotting factors). A thrombus is physiologic in cases of injury, but pathologic in case of thrombosis.

Carboxypeptidase is a digestive enzyme present in pancreatic juice, will cleave a single amino acid from the carboxylic end of the peptide.

Catabolism is the set of metabolic pathways which break down molecules into smaller units

and release energy. In catabolism, large molecules such as polysaccharides, lipids, nucleic acids and proteins are broken down into smaller units such as monosaccharides, fatty acids, nucleotides and amino acids, respectively. As molecules such as polysaccharides, proteins and nucleic acids are made from long chains of these small monomer units, the large molecules are called polymers.

Cell culture is the process by which prokaryotic, or eukaryotic cells are grown under controlled conditions. In practice the term "cell culture" has come to refer to the culturing of cells derived from multicellular eukaryotes, especially animal cells.

Cholesterol is a lipid found in the cell membranes and transported in the blood plasma of all animals. It is an essential component of mammalian cell membranes where it is required to establish proper membrane permeability and fluidity.

While minimum levels of cholesterol are essential for life, excess can contribute to diseases such as atherosclerosis.

Coagulation is a complex process by which blood forms clots. It is an important part of hemostasis (the cessation of blood loss from a damaged vessel) whereby a damaged blood vessel wall is covered by a platelet and fibrin containing clot to stop bleeding and begin repair of the damaged vessel.

Cytoplasm is the contents of a cell that is enclosed within the plasma membrane. In eukaryotic cells the cytoplasm contains organelles, such as mitochondria, that are filled with liquid kept separate from the cytoplasm by cell membranes.

Electron transport chain couples a chemical reaction between an electron donor (such as NADH) and an electron acceptor (such as O_2) to the transfer of H^+ ions across a membrane, through a set of mediating biochemical reactions. These H^+ ions are used to produce adenosine triphosphate (ATP), the main energy intermediate in living organisms, as they move back across the membrane. Electron transport chains are used for extracting energy from sunlight (photosynthesis) and from redox reactions such as the burning of sugars (respiration).

Endonucleases are enzymes that cleave the phosphodiester bond within a polynucleotide chain, in contrast to exonucleases, which cleave phosphodiester bonds at the end of a polynucleotide chain.

Endopeptidase or **endoproteinase** are proteolytic peptidases that break peptide bonds of nonterminal amino acids (i.e. within the molecule), in contrast to exopeptidases, which break peptide bonds from their end-pieces

Exopeptidase is an enzyme produced in the pancreas that catalyses the removal of an amino acid from the end of a polypeptide chain.

Extracellular matrix (ECM) is the extracellular part of animal tissue that usually provides structural support to the cells in addition to performing various other important functions. The extracellular matrix is the defining feature of connective tissue in animals.

Fatty acid is a carboxylic acid often with a long unbranched aliphatic tail (chain), which is either saturated or unsaturated.

Fibrinolysis is the process wherein a fibrin clot, the product of coagulation, is broken down. Its main enzyme plasmin cuts the fibrin mesh at various places, leading to the production of circulating fragments that are cleared by other proteases or by the kidney and liver.

Free radicals are atoms, molecules or ions with unpaired electrons on an otherwise open shell configuration. These unpaired electrons are usually highly reactive, so radicals are likely to take part in chemical reactions

Glycolipids are carbohydrate-attached lipids. Their role is to provide energy and also serve as markers for cellular recognition. They occur where a carbohydrate chain is associated with phospholipids on the exoplasmic surface of the cell membrane. The carboh ydrates are found on the outer surface of all eukaryotic cell membranes. They extend from the phospholipid bilayer into the aqueous environment outside the cell where it acts as a recognition site for specific chemicals as well as helping to maintain the stability of the membrane and attaching cells to one another to form tissues.

Glycoproteins are proteins that contain oligosaccharide chains (glycans) covalently attached to their polypeptide side-chains.

Glycosphingolipids are a subtype of glycolipids containing the amino alcohol sphingosine. They include:

- Cerebrosides
- Gangliosides
- Globosides

Gram-negative bacteria are those bacteria that do not retain crystal violet dye in the Gram staining

protocol. Gram-positive bacteria will retain the crystal violet dye when washed in a decolorizing solution.

Hemorrhaging/haemorrhaging is the loss of blood from the circulatory system.

Hemostasis (or **Haemostasis**) refers to a process whereby bleeding is halted in most animals with a closed circulatory system.

Hydrolase is an enzyme that catalyzes the hydrolysis of a chemical bond. For example, an enzyme that catalyzed the following reaction is a hydrolase:

$A–B + H_2O \rightarrow A–OH + B–H$

Immunoassay is a biochemical test that measures the concentration of a substance in a biological liquid, typically serum or urine, using the reaction of an antibody or antibodies to its antigen.

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue.

Ligase is an enzyme that can catalyse the joining of the sugar phosphate backbones of Okazaki fragments of DNA. Generally ligase catalyses the following reaction:

$Ab + C–A \rightarrow C + b$

or sometimes

$Ab + cD \rightarrow A–D + b + c$

where the lower case letters signify the small, pendant groups.

Lipophilicity, *fat-liking*, refers to the ability of a chemical compound to dissolve in fats, oils, lipids,

Lyophilized powder is freeze dried powder (also known as lyophilization or cryodesiccation) is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport. Freeze drying works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to gas.

Metric units

Ton	1000 kilograms
Kilogram (kg)	1000 grams
Gram (g)	1000 milligrams
Milligram (mg)	1000 micrograms

Organelle is a specialized subunit within a cell that has a specific function, and is usually separately enclosed within its own lipid membrane.

Oxidoreductase is an enzyme that catalyzes the transfer of electrons from one molecule (the reductant, also called the hydrogen or electron donor) to another (the oxidant, also called the hydrogen or electron acceptor).

Oxidation describes the ***loss*** of electrons by a molecule, atom or ion

Reduction describes the ***gain*** of electrons by a molecule, atom or ion

Peroxisomes are ubiquitous organelles in eukaryotes that participate in the metabolism of fatty acids and other metabolites. Peroxisomes have enzymes that rid the cell of toxic peroxides

Plasmin is an important enzyme present in blood that degrades many blood plasma proteins, most notable, fibrin clots

Polymer is a large molecule (macromolecule) composed of repeating structural units typically connected by covalent chemical bonds. While *polymer* in popular usage suggests plastic, the term actually refers to a large class of natural and synthetic materials with a variety of properties and purposes.

Proteases, also known as proteinases or proteolytic enzymes, are a large group of enzymes. Proteases belong to the class of enzymes known as hydrolases, which catalyse the reaction of hydrolysis of various bonds with the participation of a water molecule.

Peptides (from the Greek “small digestibles”) are short polymers formed from the linking, in a defined order, of α-amino acids. The link between one amino acid residue and the next is known as an amide bond or a peptide bond.

Proteins are **polypeptide** molecules (or consist of multiple polypeptide subunits). The distinction is

that peptides are short and polypeptides/proteins are long. There are several different conventions to determine these, all of which have caveats and nuances.

Proteolysis is the directed degradation *(digestion)* of proteins by cellular enzymes called proteases or by intramolecular digestion.

Satiety the state of being satisfactorily full and unable to take on more

Serine proteases are proteases (enzymes that cut peptide bonds in proteins) in which one of the amino acids at the active site is serine.

Sphingomyelin (SPH) is a type of sphingolipid found in animal cell membranes, especially in the membranous myelin sheath which surrounds some nerve cell axons. It usually consists of phosphorylcholine and ceramide.

Urea is an organic compound with the chemical formula $(NH_2)_2CO$. Urea is, in essence, a waste product. It is found and retracted from urine.

The **vitreous humour** is the clear gel that fills the space between the lens and the retina of the eyeball. The vitreous is the transparent, colourless, gelatinous mass that fills the space between the lens of the eye and the retina lining the back of the eye.

Zymogen (or **proenzyme**) is an inactive enzyme precursor. A zymogen requires a biochemical change (such as a hydrolysis reaction revealing the active site, or changing the configuration to reveal the active site) for it to become an active enzyme.

Appendix A: Cattle organ / tissue weights

	Vealer 70-110 kg	Yearling 140-220 kg	Steer 220-280 kg	Steer 280-350 kg	GF Steer 280-400 kg	Bull 250-420 kg	Cow 150-250 kg
	Weight KG	Weight KG	Weight KG	Weight KG	Weight KG	Weight KG	Weight KG
Liveweight	155.17	310.34	472.41	568.96	603.45	483.87	305.17
HSCW	90	180	274	330	350	300	177
Major components							
Adrenal glands	0.018	0.019	0.021	0.022	0.023	0.024	0.02
Ausmeat trim	4.86	13.33	22	30.36	34	9	9.6
Bible & reed	4.05	5.94	11.23	10.16	10.78	7.8	7.86
Blood	6.3	9.9	19.18	18.15	19.25	18	12.39
Blood red cells	1.7	2.67	5.18	4.9	5.2	4.86	3.35
Blood plasma	3.7	6.24	12.08	11.43	12.13	11.34	7.8
Bone	23.2	37	54	56.8	58.2	72.8	37.1
Brain	0.25	0.3	0.3	0.315	0.325	0.34	0.3
Caul Fat	0.882	1.98	4.4	6.27	6.65	0.44	3.72
Cheek (full cheek)	0.99	1.26	1.57	1.72	1.82	1.86	1.77
Corpus luteum	N/A	N/A	N/A	N/A	N/A	N/A	0.002
Eyes combined	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Fat	4.1	13	29	43.5	61.6	18.8	16.6
Feet	4.5	6.5	8.12	7.5	7.8	9	7
Foetal blood	N/A	N/A	N/A	N/A	N/A	N/A	0.7
Head	5	7.64	11.1	10.91	11.06	14.05	8.97
Heart	0.55	0.988	1.64	1.654	1.75	1.5	1.34
Hide	9.54	19.08	29.04	34.98	37.1	31.8	18.76
Intestine	7.2	12.6	16.71	21.45	22.75	18	13.27
Kidney	0.36	0.54	1.1	1.32	1.4	1.2	0.7
Liver	2.22	4.31	5.75	5.94	6.3	5.1	5.04
Lung	1.1	1.7	2.42	2.289	2.375	2.85	1.95
Mammary tissue	N/A	N/A	N/A	N/A	N/A	N/A	5
Ovary each	N/A	N/A	N/A	N/A	N/A	N/A	0.013
Pancreas	0.3-0.35	0.3-0.36	0.3-0.37	0.3-0.38	0.3-0.39	0.43	0.3-0.39
Paunch = rumen	4.05	6.12	9.48	10.23	10.85	8.4	8.3
Pineal body	0.1-0.2	0.1-0.2	0.1-0.2	0.1-0.2	0.1-0.2	0.1-0.2	0.1-0.2
Pituitary*	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Placenta	N/A	N/A	N/A	N/A	N/A	N/A	0.4
Salivary glands*	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Spinal cord*	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Spleen	0.49	0.72	0.603	0.66	0.7	0.66	0.531
Tail	0.405	0.74	0.93	1.287	1.365	0.96	0.69
Testes	N/A	N/A	N/A	N/A	N/A	0.9	N/A
Tendon	0.06	0.12	0.18	0.21	0.22	0.19	0.11
Tongue	1.65	2.23	3.23	3.012	3.1	3.06	0
Thick skirt	0.234	0.59	0.84	0.825	0.875	0.99	0.55
Thin skirt	0.18	0.58	0.96	0.99	1.05	0.9	0.62
Thymus*	0.295	0.295	0.295	0.295	0.295	0.295	0.295
Thyroid*	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Trachea and trim	0.75	1.44	2.3	2.8	3	2.7	1.7
Weight loss	1.98	3.96	6.02	7.26	7.7	6.6	3.9
Uterus	N/A	N/A	N/A	N/A	N/A	0.6-0.8 normal	10 just after birth

Appendix B: Edible Offal Organ Processing

Processing the brain

The brain is removed from the skull and outer skin. The membrane covering the brain may also be removed if the customer requires it.

Common beef brain products

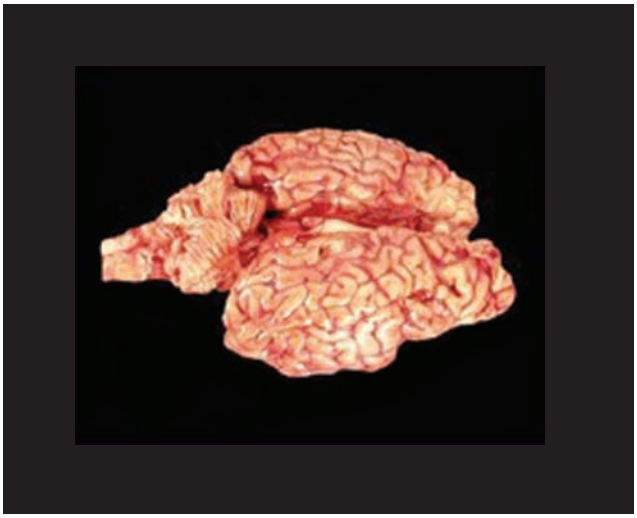
The major beef brain products are detailed below as described by AusMeat. Other brain products may be available that are not listed here.

Brain

The brain is removed from the skull and outer skin but is left in its membrane and consists of the cerebrum, the cerebellum and a small portion of the spinal cord.

Points of specification:

- Degree of detachment of the cerebral hemisphere or cerebellum permitted.



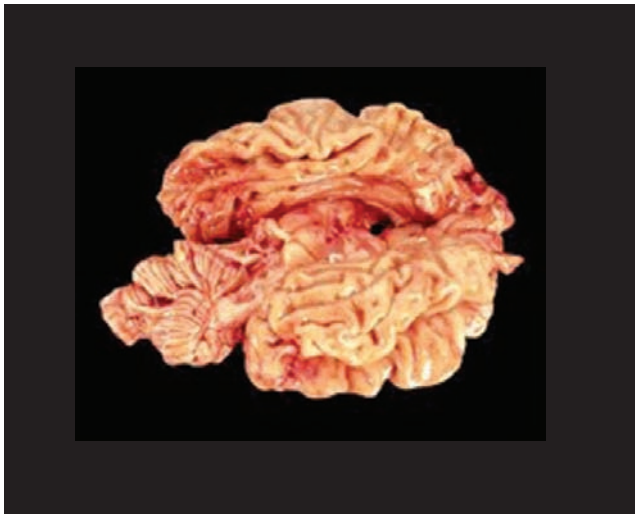
Brain: AusMeat Item No: 6120

Brain - Skinned

Prepared from a brain be the removal of the fine membrane.

Points of specification:

- Degree of detachment of the cerebral hemisphere or cerebellum permitted.



Brain - Skinned: AusMeat Item No: 6130

Processing the heart

The hearts are washed and then trimmed of excess fat according to customer specifications. The hearts may be packed and sold as full hearts or split.

Common beef heart products

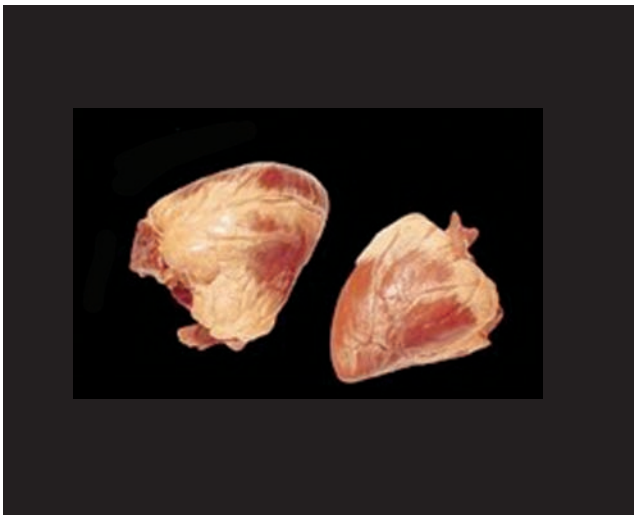
The major beef heart products are detailed below as described by AusMeat. Other heart products may be available that are not listed here.

Heart

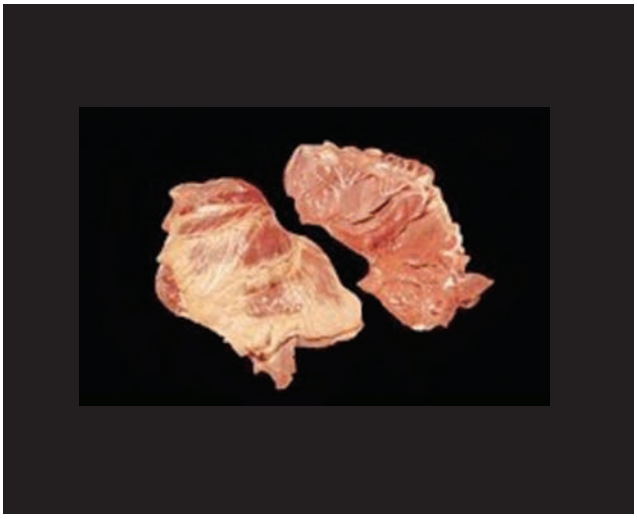
A heart is removed from the pericardium and the arteries and veins trimmed from the base of the heart. It may be purchased whole or cut open.

Points of specification:

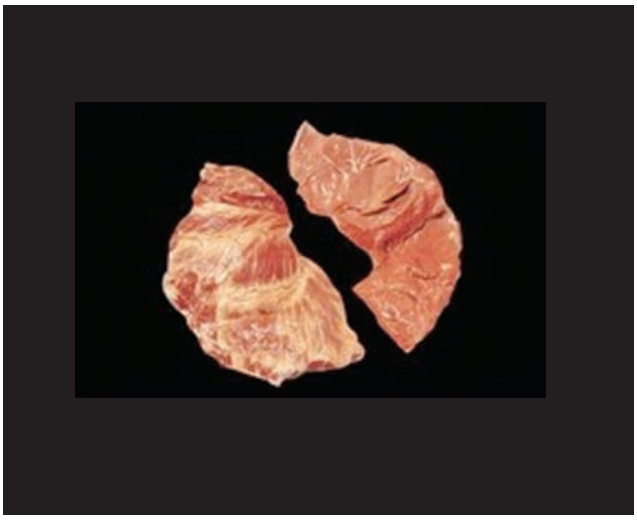
- Method of preparation: whole or cut open.
- Fat coverage required.
- Auricles retained or removed.
- Ossa cordis bones retained or removed.



Full heart: AusMeat Item No: 6100



Full heart (Split): AusMeat Item No: 6100



Full heart (Split and trimmed): AusMeat Item No: 6100

Processing the intestines

The intestines are separated from the paunch and any contents removed. They are then trimmed according to customer specifications and may also be cooked.

Common beef intestine products

The major beef intestine products are detailed below as described by AusMeat. Other intestine products may be available that are not listed here.

Small intestine

The small intestine connects the stomach to the large intestine. It comprises of 3 sections - duodenum, jujenum and the ileum.

Points of specification:

- Cooked
- Amount of fat retained
- Mucous membrane removed
- Length of intestine

Small intestine: AusMeat Item No: 6496

Large intestine

The large intestine comprises of 3 portions:

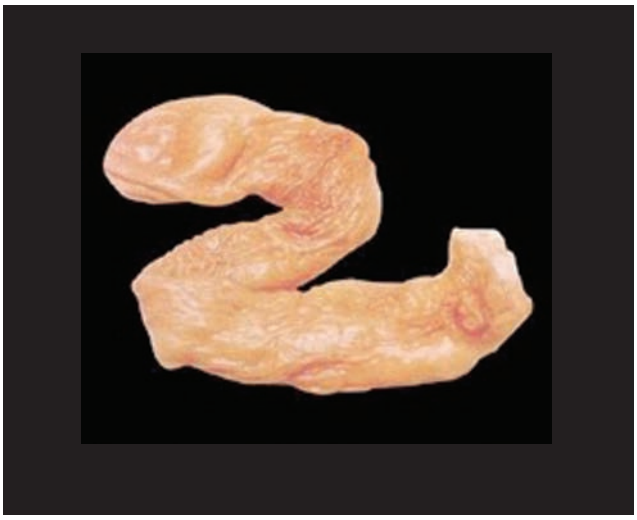
(1) The caecum begins with a blind end and is often referred to as the 'blind gut'.

(2) The colon is arranged in a double elliptical coil between the layers of the mesentery.

(3) The rectum extends from the start of the pelvic channel to the anus.

Points of specification:

- Cooked
- Amount of fat retained
- Mucous membrane removed
- Length of intestine



Large intestine: AusMeat Item No: 6497

Processing the kidney

The kidneys are enucleated (have the kidney capsule removed) and checked for disease on the offal sorting table. They are then washed and if required further trimmed according to customer specifications.

Common beef kidney products

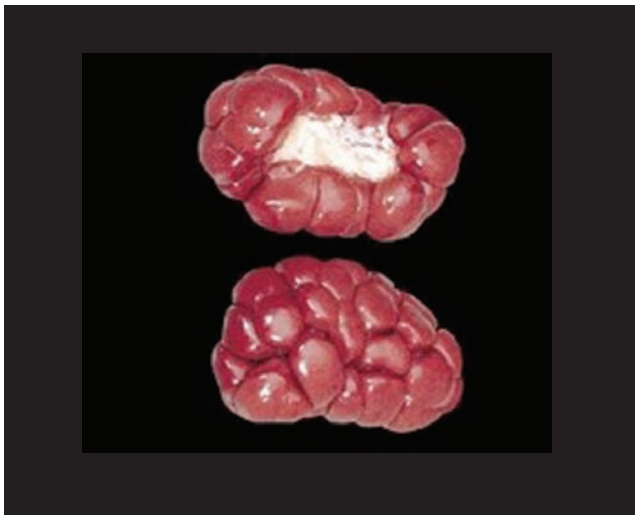
The major beef kidney product is detailed below as described by AusMeat. Other kidney products may be available that are not listed here.

Kidney

The kidney is prepared by the removal of blood vessels and ureter at their points of entry. The kidney capsule is removed and the fat in the renal hilus is partially removed.

Points of specification:

- Fat coverage.



Kidney: AusMeat Item No: 6090

Processing the liver

The liver is washed and trimmed according to customer specification on offal sorting tables. The gall bladder needs to be removed from the liver.

Common beef liver products

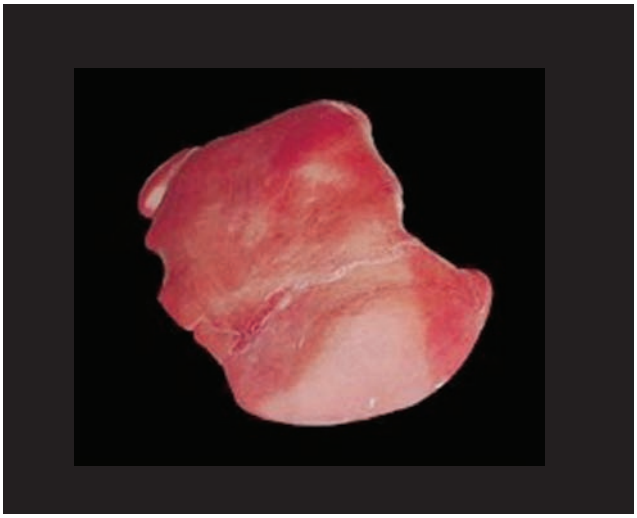
The major beef liver product is detailed below as described by AusMeat. Other liver products may be available that are not listed here.

Liver

Liver is prepared with the hepatic lymph nodes incised and attached. Fat, blood vessels and connective tissue attached to the liver are removed.

Points of specification:

- Skin to be retained
- Vein removal
- Liver required whole or sliced



Liver: AusMeat Item No: 6080

Processing lung

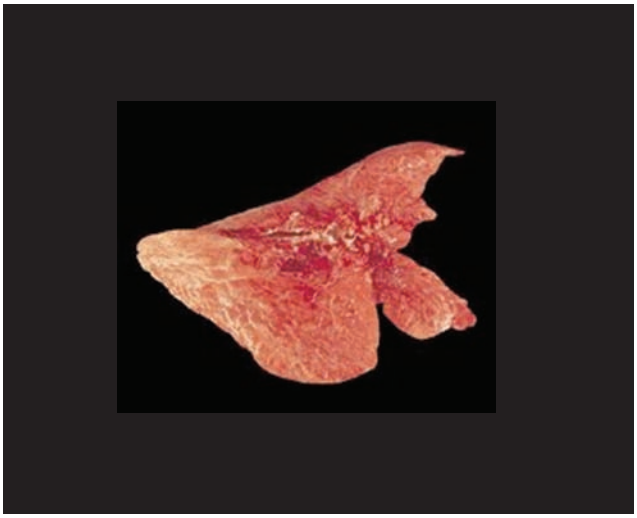
The lungs are separated from the pluck at the trachea. They are then trimmed according to customer specifications.

Common beef lung products

The major beef lung product is detailed below as described by AusMeat. Other lung products may be available that are not listed here.

Lungs

Prepared by the removal of the trachea.



Lungs: AusMeat Item No: 6210

Processing the thymus gland

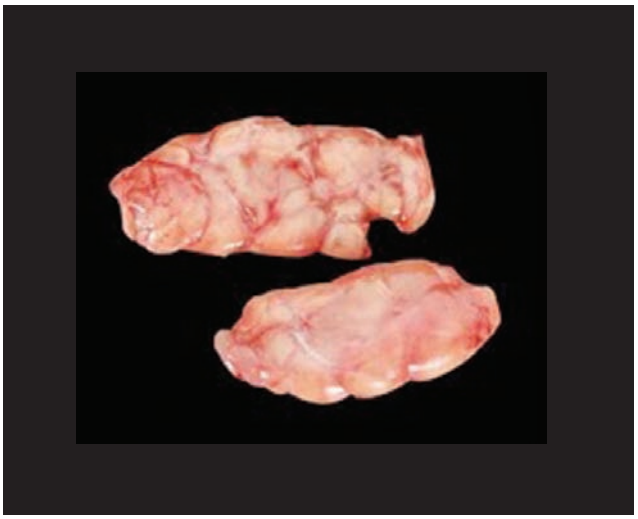
The thymus gland is removed from the carcass and trimmed of surrounding fat and tissue according to customer specifications.

Common beef thymus gland products

The major beef thymus gland product is detailed below as described by AusMeat. Other thymus gland products may be available that are not listed here.

Thymus gland

A thymus gland (sweetbread or throatbread) is derived from young animals and is pale and lobulated. The glands are situated in the neck region, on either side of the trachea. The thymus gland is trimmed of surrounding fat and connective tissue.



Thymus gland: AusMeat Item No: 6110

Processing tripe

The paunch is separated from both the intestines and the weasand. The paunches are then opened using a knife taking care not to damage the rumen pillars. The contents of the paunch is then removed and the paunch washed. Any excess fat and waste is trimmed from the outside of the paunch.

The paunch may then be cut according to customer specifications and sold as trip raw and unscalded or it may be further processed.

Further processing of the paunch involves scalding it with water around 75ºC to 85ºC. This operation is usually performed in an automated machine that

spins the paunches as they are being scalded. After the tripes have been scalded they are usually washed again in cold water.

Common beef tripe products

The major beef tripe products are detailed below as described by AusMeat. Other tripe products may be available that are not listed here.

Tripe (raw unscalded)

Tripe is the paunch (rumen) and the honeycomb (reticulum). The item is carefully prepared to avoid cutting the rumen pillars and then rinsed and cleaned of any paunch content leaving the brown/ black mucous membrane intact.

Points of specification:

- Reticulum retained or removed
- Reticulum only

Tripe (raw unscalded): AusMeat Item No: 6140

Tripe pieces (raw unscalded)

Derived from tripe (raw unscalded) by the removal of the rumen pillars.

Points of specification:

- Scalded
- Cooked
- Cooked and bleached

Tripe pieces (raw unscalded): AusMeat Item No: 6151

Tripe (scalded)

Derived from tripe (raw unscalded) and is scalded by water to remove the mucous membrane.

Points of specification:

- Reticulum retained or removed
- Reticulum only

Tripe (scalded): AusMeat Item No: 6150

Tripe - Honeycomb

Derived from tripe (raw unscalded) and is scalded by water to remove the mucous membrane.

Points of specification:

- Cooked
- Cooked and bleached

Tripe (scalded): AusMeat Item No: 6153

Processing weasand meat

After separation from the paunch any contents of the weasand are removed. The weasand is then washed and trimmed according to customer specifications.

Common beef weasand meat products

The major beef weasand meat product is detailed below as described by AusMeat. Other weasand meat products may be available that are not listed here.

Weasand meat

Weasand meat is the muscular (tunica muscularis) and mucosal portions of the oesophagus.

Points of specification:

- Mucosa retained or removed.

Weasand meat: AusMeat Item No: 6280

Appendix C: Total cattle slaughter assumptions

Constants used in calculations	
Default rendered value ($/kg)	$0.02
Percent healthy animals suitable for processing	70%
Cattle turn off per year	8,854,000
Suitable for processing	6,197,800
Total amount sold constant (reference estimate)	300

Introduction

High value bioactive co-products of red meat processing have the potential to significantly improve the profitability of the red meat industry. Traditional meat products are produced at relatively thin gross margins into a competitive market. Conventional co-products such as hides, offal, tallow and meat and bone meal already make a significant contribution to the value of the carcase, generating up to 20% of the revenue per head. Co-product streams currently going to rendering, derived as they are from mammalian tissue, contain compounds which may be expected to be active on other biological systems. Such bioactive compounds generally attract high prices and sell at high margins and represent a significant opportunity for the industry to increase profitability.

The industry recognises this potential but also recognises that in realising it they will need to enter new supply chains, deal with new technologies and jargon and often engage in different business models. During discussions with the industry, meat processors have expressed a desire for information on products, markets and technologies to be compiled in order to assist them in deciding which bioactive products they should develop.

Meat and Livestock Australia has supported a number of projects over the years on specific technologies, on bioactives markets and supply chains and holds regular bioactives workshops for the red meat bioactives supply chain players. The outcomes have been disseminated via newsletters, workshop presentations and reports and the present compendium aims to draw the available information together in one resource in order to assist those companies interested in red meat bioactives in assessing their opportunities.

There are currently (mid 2009) more than 200 bovine and ovine bioactives on the market. Selection of target bioactives for manufacture involves a series of evaluations of candidate compounds, starting with high level “back of the envelope” calculations and moving towards more detailed market analysis, technical and market R&D and finally, capital expenditure leading to production.

The aim of this compendium is to facilitate the high level screening of the opportunities by processors and value adders. The information presented here is not intended to replace or even be the basis for a technical and market due diligence assessment on a given product or technology. Considering the investment in time and capital often involved in developing a bioactive project the reader should engage suitable professional help to perform such due diligence studies.

How to use this compendium

The compendium is written with three audiences in mind: Meat Processors, Value Adders (supply chain partners in taking refined bioactives to market), and R&D / technical service providers.

Section 1. The Australian Bioactives Industry

Section 1 provides an overview of the bioactives industry in Australia and in particular examines an example of the cost structures of players in the bioactives industry and how to estimate them. It is the intention of this section to alert the reader and would-be investor to the fact that:

- The supply chain is very different to the normal red meat supply chain,
- There is value to the processor in moving even one step down the supply chain
- Adding value can result in improved profit levels.
- “If you don’t add value, you can’t expect to get a share of the value” (This is a constant theme throughout the MLA value adding programs.)

Section 2. Overview of Regulatory Framework for Bioactives

Section 2 aims to introduce the reader to the regulatory issues and regulatory bodies of relevance to bioactives. As bioactive compounds can find their way into food supplements, nutraceuticals, pharmaceutical ingredients or cosmetic products, their safety, need and efficacy are of interest to the regulatory authorities in each area. The section is designed not to be comprehensive but to help the reader navigate through unfamiliar regulatory territory and to illustrate the importance of considering regulatory dimensions in technology and product development.

Section 3. Bioactives Extraction and Purification Technologies

Section 3 is a brief introduction to the major unit operations in the extraction, separation, purification and preservation technologies used in red meat bioactives production. They are presented here to help someone new to bioprocessing to assimilate the information in the product data sheets and

background information. The internet is a rich source of information on such unit operations at this level. Bioprocess engineering texts will provide detailed information.

Section 4. Product Data Sheets

Section 4 contains the core components of the compendium, the two page (and in some cases more) product data sheets, on each compound.

Each sheet contains data on:

- The compound (technical description)
- The tissue source and weight of tissue per animal
- Abundance (the concentration of the target compound in the tissue)
- Mode of action (the basis of the compound's bioactivity, where available)
- Uses / applications of the bioactive
- Existing suppliers
- Market information where available
- Typical processing technology required for purification

Note: The weight of organ tissue per animal varies in a non-linear way with animal size, breed and fat content. For simplicity, average organ weights per animal for a 250 - 400kg carcase have been used, taking 300kg as an average carcase weight. These are listed in an appendix at the end of the compendium. The reader will need to use real data in estimating yields per head.

The data sheets have been organised in sections according to their functionality (e.g. all the coagulants are grouped together), but there are two indexes which allow the reader to find a compound from its name or from its tissue of origin.

- The Alphabetical Index at the front of the manual is an alphabetic listing of compounds and the page at which the data sheet can be found.
- The Organ Index at the front of the manual is a listing of the organs or tissues and the bioactives that can be found in each and the pages on which they can be found.

Section 5. Bioactives Cost Estimation

Section 5 is a compilation of data and rules of thumb that may be of help in assessing the capital cost or operating cost of a bioactives process. It is likely that the reader will find production or capital expenditure data for a given target bioactive based on pilot scale results and equipment costs. It is inevitable that the scale of interest to the reader will be different to that available in the literature or from pilot studies and the need exists to be able to make an estimate of likely costs. Cost estimation is a complex field in its own right and the aim of this section is to help the reader make ball park estimates of costs based on small scale cost data. It is expected that if more accurate estimates are required a potential investor would retain a consultant to calculate them.

6. Glossary

Often the jargon in biotechnology and bioactives is more difficult to deal with than the concepts. The glossary is a ready reference to help the reader cut through to the key issues and not be blinded by the jargon.

Appendices

Yields: As mentioned above, the weight of organ tissue per animal varies in a non-linear way with animal size, breed and fat content. For simplicity, average organ weights per animal for a 250 - 400kg carcase have been used in the product data sheets, taking 300kg as the average carcase weight. The appendix contains a listing of organ weights for animals in seven weight ranges. The reader will eventually need to use real data in estimating yields per head.

Limitations

This compendium is designed to help meat processing and value adding companies identify candidate bioactives for further evaluation. It is not designed to replace due diligence in project evaluation. Figures on yields and abundance are based on available literature figures and are necessarily averages and sometimes estimates. Market information is particularly difficult to obtain and estimates can vary widely. Wherever possible the source of estimates are referenced and assumptions are stated.

It is anticipated that the next step in project selection would be the development of business cases for the most attractive compounds. This would then lead potentially to research and development designed to develop and prove technology and get more accurate technical and market data, which would form the basis of a commercialisation plan.

Appendix D: BIOREG Matrix

An Australian Government Initiative

BioRegs Online

MAPPING AUSTRALIA'S BIOTECHNOLOGY REGULATIONS

www.bioregs.gov.au

Australian Biotechnology Regulation Matrix

This overview map lists the government agencies involved in the regulation of biotechnology in Australia, and outlines their regulatory roles across the major biotechnology research and product categories.

Customised regulatory and development pathways for specific biotechnology products can be navigated using the BioRegs Online web tool at **www.bioregs.gov.au**.

✔ **KEY REGULATOR(S)**
Primary regulator or regulators for each product category. Contact early in product development process.

GMO **GENETICALLY MODIFIED ORGANISMS**
The OGTR is a primary regulator for any dealings or processes involving a GMO.

◎ **POSSIBLE REGULATOR OR COMPLIANCE REQUIREMENT**
Depending on nature of product, process, use, etc.

	GOVERNMENT AGENCY	BIOTECHNOLOGY RESEARCH OR PRODUCT CATEGORY	TREATMENT OF PESTS AND/OR PLANTS	ANIMAL THERAPEUTIC	HUMAN THERAPEUTIC: OTC MEDICINES	HUMAN THERAPEUTIC: COMPLEMENTARY MEDICINES	HUMAN THERAPEUTIC: PRESCRIPTION MEDICINES	HUMAN THERAPEUTIC: MEDICAL DEVICES	HUMAN THERAPEUTIC: BLOOD & TISSUE	HUMAN THERAPEUTIC: CELLULAR THERAPIES	HUMAN FOOD CONSUMPTION	INDUSTRIAL CHEMICALS	ENVIRONMENTAL / BIOFUELS	AGRICULTURAL
OGTR	Office of the Gene Technology Regulator www.ogtr.gov.au	// Accredits organisations to work with GMOs. // Certifies physical containment facilities. // Licenses dealings with GMOs (contained and intentional release).	GMO ◎	GMO ◎	GMO ◎	GMO ◎	GMO ◎		GMO ◎	GMO ◎	GMO ◎	GMO ◎	GMO ◎	GMO ◎
APVMA	Australian Pesticides and Veterinary Medicines Authority www.apvma.gov.au	// Assesses and regulates research, production and sale of pesticides and veterinary medicines.	✔	✔							◎			
TGA	Therapeutic Goods Administration www.tga.gov.au	// Assesses and registers human therapeutic goods. // Licenses manufacturers of medicines. // Includes labelling, health claims, packaging.			✔	✔	✔	✔	✔	✔	◎			
NHMRC	National Health and Medical Research Council www.nhmrc.gov.au	// Licenses human embryo research and approves human cellular therapies. // Advises on human and animal ethics committees.								✔				
FSANZ	Food Standards Australia New Zealand www.foodstandards.gov.au	// Assesses and regulates the production, handling and sale of food in Australia; includes labelling and packaging.									✔			
NICNAS	National Industrial Chemicals Notification and Assessment Scheme www.nicnas.gov.au	// Assesses and regulates the import and production of industrial chemicals in Australia. // Registers importers and manufacturers of industrial chemicals.										✔	◎	
DEH	Department of the Environment and Heritage www.deh.gov.au	// Regulates access to and benefit sharing for native genetic and biochemical resources in Commonwealth areas. // Regulates the import and export of wildlife, wildlife specimens and products. // Regulates research and associated activities involving threatened species and ecological communities. // Regulates the import and export of hazardous wastes, including medical and clinical wastes. // Regulates the quality of fuel supplied in Australia including biofuels such as biodiesel and ethanol.	◎	◎	◎	◎	◎	◎	◎	◎	◎		◎	◎
AQIS	Australian Quarantine and Inspection Service www.aqis.gov.au	// Inspects and assesses, for quarantine risk only, imported plants, parts of plants, plant products, animals and microbial-derived products. // Regulates the export of primary products.	◎	◎		◎	◎		◎	◎	◎	◎	◎	◎
CUSTOMS	Australian Customs Service www.customs.gov.au	// Controls the import and export of prohibited and restricted goods, including therapeutic goods, industrial chemicals, human and animal biologicals, radioactive substances, endangered animals and plants.	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎
DTCC	Defence Trade Control and Compliance www.defence.gov.au/strategy/dtcc	// Controls the export of "Defence and Strategic Goods List" goods, including chemicals, micro-organisms and toxins.	◎	◎			◎	◎	◎			◎		◎
ASNO	Australian Safeguards and Non-Proliferation Office www.asno.dfat.gov.au	// Provides permits for producers, processors or consumers of chemicals under the Chemical Weapons Convention (CWC), including saxitoxin and ricin. // Regulates import of chemicals under the CWC including saxitoxin and ricin.	◎					◎				◎		
ARPANSA	Australian Radiation Protection and Nuclear Safety Agency www.arpansa.gov.au	// Licenses Commonwealth entities working with radioactive facilities and sources. // Regulates the land transport of radioactive substances.					◎	◎	◎					
	State and Territory Governments	// Regulate research, trials and cultivation of GM canola and food crops within State/Territory jurisdictions. // Enforce national regulations such as Food Standards. // Regulate workplace safety, environment protection, dangerous goods transport, handling of radioactive materials and other aspects of business.	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎	✔	◎

Appendix E: Potential Bioactives Candidates and their relevant animal source

Abomasum
Lysozyme Other Enzymes 10.1

Achilles tendon
Chondroitin C Glucoseaminoglycans 1.1

Adrenals
Cholesterol Esterase Other Enzymes 6.1

Phosphodiesterases Other Enzymes 11.1

Cathepsin D Other Enzymes 5.1

All non-muscle tissue
Calmodulin Other Bioactives 4.1

Articular cartilage
Chondroitin E Glucoseaminoglycans 1.1

Bile Acids
Taurine Cell Nutrients and Growth Factors 4.1

Bone
Chondroitin D Glucoseaminoglycans 1.1

Osteocalcin Other Bioactives 8.1

Osteonectin Other Bioactives 9.1

Catalase Other Enzymes 4.1

Brain
Asialoganglioside GM1 Lipids 3.1

Asialoganglioside GM2 Lipids 3.1

Ceramides Lipids 1.1

Coenzyme Q10 Antioxidants 1.1

Cholesterol Oxidase Other Enzymes 7.1

Calmodulin Other Bioactives 4.1

Ganglioside GD1a/b Lipids 3.1

Ganglioside GD2 Lipids 3.1

Ganglioside GD3 Lipids 3.1

Ganglioside GM1 Lipids 3.1

Ganglioside GM2 Lipids 3.2

Ganglioside GM3 Lipids 3.2

Ganglioside GM4 Lipids 3.2

Ganglioside GT1a/b Lipids 3.2

FGF-1, 2, ….. Cell Nutrients and Growth Factors 1.1

Malate dehydrogenase Catabolic Enzymes 6.1

Lactate dehydrogenase Catabolic Enzymes 5.1

Heparan sulphates Glucoseaminoglycans 3.1

Pyruvate kinase Catabloic Enzymes 8.1

Ribonuclease B/C & D Hydrolytic Enzymes 13.1

Ribonuclease A Hydrolytic Enzymes 12.1

L-Carnosine (D-alanylhistidine) & anserine (D-alanyl-1-methylhistidine) Other Bioactives 6.1

Phosphodiesterases Other Enzymes 11.1

Gangliosides Lipids 3.1

Phosphatidylserine Lipids 5.1

Calf caritlage
Collagen, Type II Glucoseaminoglycans 3.1

Collagen, Type VI Glucoseaminoglycans 3.2

Calf dermis
Collagen, Type I Glucoseaminoglycans 3.1

Calf eyes
Collagen, Type IV Glucoseaminoglycans 3.2

Calf Heart
Collagen, Type VI Glucoseaminoglycans 3.2

Calf interstitial tissue
Collagen, Type VI Glucoseaminoglycans 3.2

Calf intestine
Alkaline Phosphatase Other Enzymes 1.1

Calf skin
Collagen, Type III Glucoseaminoglycans 3.1

Cardiac muscle
L-Carnosine (D-alanylhistidine) & anserine (D-alanyl-1-methylhistidine) Other Bioactives 6.1

Cheek
Coenzyme Q10 Antioxidants 1.1

Colostrum
g-Globulin Immunoglobulins 2.1

Corpus luteum
Glycogen phosphorylase isoenzymes Catabolic Enzymes 4.1

Cholesterol Esterase Other Enzymes 6.1

Erythrocytes
Carbonic Anhydrase Other Enzymes 3.1

Haemoglobin Iron and Oxygen Binding 4.1

Superoxide Dismutase Antioxidants 4.1

Glutathione peroxidase Antioxidants 3.1

Eyes
Phosphodiesterases Other Enzymes 11.1

Ganglioside GD1a/b Lipids 3.1

Ganglioside GD2 Lipids 3.1

Ganglioside GD3 Lipids 3.1

Ganglioside GM1 Lipids 3.1

Ganglioside GM2 Lipids 3.2

Ganglioside GM3 Lipids 3.2

Ganglioside GM4 Lipids 3.2

Ganglioside GT1a/b Lipids 3.2

Eyes lens
a-Crystallin Other Bioactives 1.1

Granulocytes
Lysozyme Other Enzymes 10.1

Heart
Myosins Other Bioactives 7.1

Malate Hydrolase Catabolic Enzymes 7.1

FGF-1, 2, ….. Cell Nutrients and Growth Factors 1.1

Superoxide Dismutase Antioxidants 4.1

Creatine kinase Catabolic Enzymes 1.1

cytochrome C Antioxidants 2.1

Myoglobin Iron and Oxygen Binding 8.1

Protein Kinase-A Other Enzymes 12.1

Malate dehydrogenase Catabolic Enzymes 6.1

Glycogen phosphorylase isoenzymes Catabolic Enzymes 4.1

Lactate dehydrogenase Catabolic Enzymes 5.1

Troponins Other Bioactives 11.1

Phosphodiesterases Other Enzymes 11.1

Coenzyme Q10 Antioxidants 1.1

Intestine
Heparin Anticoagulants 3.1

Chondroitin B Glucoseaminoglycans 1.1

Cholesterol Esterase Other Enzymes 6.1

Intestinal mucosa Heparan sulphates Glucoseaminoglycans 3.1

Apoferritin Iron and Oxygen Binding 1.1

Kidney
Ganglioside GD2 Lipids 3.1

Ganglioside GD1a/b Lipids 3.1

Ganglioside GD3 Lipids 3.1

Ganglioside GM1 Lipids 3.1

Ganglioside GM2 Lipids 3.2

Ganglioside GM3 Lipids 3.2

Ganglioside GM4 Lipids 3.2

Ganglioside GT1a/b Lipids 3.2

Superoxide Dismutase Antioxidants 4.1

Glutamate Dehydrogenase Other Enzymes 9.1

Pyruvate kinase Catabloic Enzymes 8.1

Cholesterol Esterase Other Enzymes 6.1

Liver
Cholesterol Esterase Other Enzymes 6.1

Superoxide Dismutase Antioxidants 4.1

Glutathione peroxidase Antioxidants 3.1

Heparan sulphates Glucoseaminoglycans 3.1

Arginase Other Enzymes 2.1

Enolase Catabolic Enzymes 2.1

Glutamate Dehydrogenase Other Enzymes 9.1

Actin Other Bioactives 2.1

Prothrombin Coagulants 7.1

Fetuin Iron and Oxygen Binding 3.1

Holo-transferrin Iron and Oxygen Binding 7.1

Apoferritin Iron and Oxygen Binding 1.1

Ferritin Iron and Oxygen Binding 2.1

Catalase Other Enzymes 4.1

Lung
Heparan sulphates Glucoseaminoglycans 3.1

Heparin Anticoagulants 3.1

Aprotinin Anticoagulants 2.1

Elastase Hydrolytic Enzymes 7.1

Phosphodiesterases Other Enzymes 11.1

Taurine Cell Nutrients and Growth Factors 4.1

Major skeletal muscle
Actin Other Bioactives 2.1

Mammary gland
Glutathione peroxidase Antioxidants 3.1

Milk
Ganglioside GD1a/b Lipids 3.1

Ganglioside GD2 Lipids 3.1

Ganglioside GD3 Lipids 3.1

Ganglioside GM1 Lipids 3.1

Ganglioside GM2 Lipids 3.2

Ganglioside GM3 Lipids 3.2

Ganglioside GM4 Lipids 3.2

Ganglioside GT1a/b Lipids 3.2

Lipoprotein lipase Hydrolytic Enzymes 9.1

Cathepsin D Other Enzymes 5.1

Monocytes
Lysozyme Other Enzymes 10.1

Most interstitial tissue
Collagen, Type V Glucoseaminoglycans 3.2

Mucosa
Lysozyme Other Enzymes 10.1

Pepsin Hydrolytic Enzymes 10.1

Muscle
Lactate dehydrogenase Catabolic Enzymes 5.1

Enolase Catabolic Enzymes 2.1

Lipoprotein lipase Hydrolytic Enzymes 9.1

Pyruvate kinase Catabloic Enzymes 8.1

Protein Phosphatase 2C Other Enzymes 13.1

Muscle Fat
Conjugated Linoleic Acid Lipids 2.1

Neutrophils
Elastase Hydrolytic Enzymes 7.1

Non-activated citrated bovine plasma
Antithrombin III Anticoagulants 1.1

Factor V Coagulants 1.1

Factor VIII Coagulants 2.1

Factor XIII Coagulants 5.1

Fibrinogen Coagulants 6.1

Plasminogen Anticoagulants 4.1

Thrombin Coagulants 8.1

Prothrombin Coagulants 7.1

Organic part of bone
Collagen, Type I Glucoseaminoglycans 3.1

Other tissues
Catalase Other Enzymes 4.1

Glutamate Dehydrogenase Other Enzymes 9.1

Pyruvate kinase Catabloic Enzymes 8.1

Elastase Hydrolytic Enzymes 7.1

DNAse 1 Hydrolytic Enzymes 6.1

Ribonuclease A Hydrolytic Enzymes 12.1

L-Carnosine (D-alanylhistidine) & anserine (D-alanyl-1-methylhistidine) Other Bioactives 6.1

Lysophosphatidylcholine Lipids 4.1

Lipase Hydrolytic Enzymes 8.1

Pancreas
Ganglioside GD1a/b Lipids 3.1

Ganglioside GD2 Lipids 3.1

Ganglioside GD3 Lipids 3.1

Ganglioside GM1 Lipids 3.1

Ganglioside GM2 Lipids 3.2

Ganglioside GM3 Lipids 3.2

Ganglioside GM4 Lipids 3.2

Ganglioside GT1a/b Lipids 3.2

Elastase Hydrolytic Enzymes 7.1

Carboxypeptidase A Hydrolytic Enzymes 2.1

Carboxypeptidase B Hydrolytic Enzymes 3.1

Chymotrypsin Hydrolytic Enzymes 4.1

Chymotrypsinogen A/B Hydrolytic Enzymes 5.1

Trypsin Hydrolytic Enzymes 15.1

Trypsin Inhibitor Hydrolytic Enzymes 14.1

Trypsinogen Hydrolytic Enzymes 16.1

DNAse 1 Hydrolytic Enzymes 6.1

Ribonuclease B/C & D Hydrolytic Enzymes 13.1

Cholesterol Esterase Other Enzymes 6.1

Ribonuclease A Hydrolytic Enzymes 12.1

Pepsin Hydrolytic Enzymes 10.1

Pepsinogen Hydrolytic Enzymes 11.1

Phosphodiesterases Other Enzymes 11.1

Amylase Hydrolytic Enzymes 1.1

Lipase Hydrolytic Enzymes 8.1

Pituitary
FGF-1, 2, ….. Cell Nutrients and Growth Factors 1.1

Placental villi
Collagen, Type V Glucoseaminoglycans 3.2

Plasma Malate Hydrolase Catabolic Enzymes 7.1

Osteonectin Other Bioactives 9.1

Osteocalcin Other Bioactives 8.1

Factor IX Coagulants 3.1

Factor X Coagulants 4.1

Fibronectin Other Bioactives 5.1

Haemopexin Iron and Oxygen Binding 5.1

Lipoprotein Deficient Serum, LPDS Cell Nutrients and Growth Factors 2.1

Serum albumin Cell Nutrients and Growth Factors 3.1

Transferrin (as apo- or holo-forms) Iron and Oxygen Binding 9.1

g-Globulin Immunoglobulins 2.1

IgA Immunoglobulins 3.1

IgG Immunoglobulins 4.1

IgM Immunoglobulins 5.1

Platelets
Osteonectin Other Bioactives 9.1

Purified from fresh citrated bovine plasma
protein C anticoagulants 5.1

Rumen
Urease Other Enzymes 14.1

Salivary glands
Amylase Hydrolytic Enzymes 1.1

Seminal plasma
Ribonuclease A Hydrolytic Enzymes 12.1

Serum
Osteonectin Other Bioactives 9.1

Osteonectin Other Bioactives 9.1

Lysozyme Other Enzymes 10.1

Elastase Hydrolytic Enzymes 7.1

a1-Acid glycoprotein Other Bioactives 3.1

a2-Macroglobulin Immunoglobulins 1.1

Haptoglobin Iron and Oxygen Binding 6.1

Vitronectin Cell Nutrients and Growth Factors 5.1

Fetuin Iron and Oxygen Binding 3.1

Holo-transferrin Iron and Oxygen Binding 7.1

Plasminogens Anitcoagulants 4.1

Skeletal muscle
L-Carnosine (D-alanylhistidine) & anserine (D-alanyl-1-methylhistidine) Other Bioactives 6.1

Myosins Other Bioactives 7.1

Skin Chondroitin B Glucoseaminoglycans 1.1

Smooth cardiac muscle cells
Calmodulin Other Bioactives 4.1

Spleen
Glycogen phosphorylase isoenzymes Catabolic Enzymes 4.1

Phosphodiesterases Other Enzymes 11.1

Protein Phosphatase 2C Other Enzymes 13.1

Cathepsin D Other Enzymes 5.1

Apoferritin Iron and Oxygen Binding 1.1

Ferritin Iron and Oxygen Binding 2.1

Stomach musosa
Pepsinogen Hydrolytic Enzymes 11.1

Tendons
Collagen, Type I Glucoseaminoglycans 3.1

Testis
Cholesterol Esterase Other Enzymes 6.1

Deoxynucleotidyl-transferase Other Enzymes 8.1

Phosphodiesterases Other Enzymes 11.1

Thyroglobulin Other Bioactives 10.1

Trachea
Chondroitin A Glucoseaminoglycans 1.1

Uterus
Cathepsin D Other Enzymes 5.1

Various tissues

Glucose oxidase Catabolic Enzymes 3.1

Lactate dehydrogenase Catabolic Enzymes 5.1

Vitreous humor of the eye Hyaluronic Acid

Appendix F: Bioactives Compounds

No.	Bioactives Compounds	Tissue Source	Page Reference
1	*Actin*	Skeletal muscle	Other Bioactives 2.1
2	*Alkaline Phosphatase (AP)*	Intestine	Other Bioactives 1.1
3	*Alpha 1 Acid Glycoprotein (AAG)*	Blood	Other Bioactives 3.1
4	*Alpha-Crystallin*	Eyes	Other Bioactives 1.1
5	*Alpha 2 - Macroglobulin*	Serum	Immunoglobulins 1.1
6	*Amylase*	Pancreas	Hydrolytic Enzymes 1.1
7	*Antithrombin III (ATIII)*	Plasma	Anticoagulants 1.1
8	*Apoferritin*		Iron & Oxygen Binding 1.1
9	*Aprotinin*	Lung	Anticoagulants 2.1
10	*Arginase*	Liver	Other Bioactives 2.1
11	*Calmodulin (CaM)*	Adrenal glands	Other Bioactives 4.1
12	*Carbonic Anhydrase*		Other Bioactives 3.1
13	*Carboxypeptidase A*	Pancreas	Hydrolytic Enzymes 2.1
14	*Carboxypeptidase B*	Pancreas	Hydrolytic Enzymes 3.1
15	*Catalase*	Liver	Other Bioactives 4.1
16	*Cathepsin D*		Other Bioactives 5.1
17	*Ceramide*	Brain	Lipids 1.1
18	*Cholesterol Oxidase*	Not available	Other Bioactives 7.1
19	*Cholesterol Esterase*		Other Bioactives 6.1
20	*Chondroitin Sulphate A (CS-A)*	Cartilage - Trachea	Glucoseaminoglycans 1.1
21	*Chondroitin Sulphate B (CS-B)*	Cartilage - Trachea	Glucoseaminoglycans 1.1
22	*Chondroitin Sulphate C*		Glucoseaminoglycans 1.1
23	*Chondroitin Sulphate D*		Glucoseaminoglycans 1.1
24	*Chondroitin Sulphate E (CS-E)*	Cartilage - Trachea	Glucoseaminoglycans 1.1
25	*Chymotrypsin*	Pancreas	Hydrolytic Enzymes 4.1
26	*Chymotrypsinogen*		Hydrolytic Enzymes 5.1
27	*Coenzyme Q10*		Antioxidants 1.1
28	*Collagen, Type I*	Not available	Glucoseaminoglycans 3.1
29	*Collagen, Type II*	Cartilage	Glucoseaminoglycans 3.1
30	*Collagen, Type III*	Intestine	Glucoseaminoglycans 3.1
31	*Collagen, Type IV*	Eyes	Glucoseaminoglycans 3.2
32	*Collagen, Type V*	Placenta	Glucoseaminoglycans 3.2
33	*Collagen, Type VI*	Intestine	Glucoseaminoglycans 3.2
34	*Conjugated Linoleic Acid*		Lipids 2.1
35	*Creatine Kinase (CK)*	Uterus	Catabolic Enzymes 1.1
36	*Cytochrome C*	Heart	Antioxidants 2.1
37	*Deoxynucleotidyl-transferase*	Thymus	Other Bioactives 8.1

No.	Bioactives Compounds	Tissue Source	Page Reference
38	*DNAse 1*	Pancreas	Hydrolytic Enzymes 6.1
39	*Elastase*	Pancreas	Hydrolytic Enzymes 7.1
40	*Enolase*		Catabolic Enzymes 2.1
41	*Factor V*	Plasma	Coagulants 1.1
42	*Factor VIII*	Plasma	Coagulants 2.1
43	*Factor IX*	Plasma	Coagulants 3.1
44	*Factor X*	Plasma	Coagulants 4.1
45	*Factor X111*	Plasma	Coagulants 5.1
46	*Ferritin*	Liver	Iron & Oxygen Binding 2.1
47	*Fetuin*	Plasma	Iron & Oxygen Binding 3.1
48	*Fibrinogen*	Plasma	Coagulants 6.1
49	*Fibroblast growth factor (FGF)*	Heart	Cell Nutrients & Growth Factors 1.1
50	*Fibronectin*	Plasma	Other Bioactives 5.1
51	*Gamma-Globulin (g-Globulin or Ig)*	Not applicable (Milk)	Immunoglobulins 2.1
52	*Ganglioside GD1a/b*		Lipids 3.1
53	*Ganglioside GD2 (GDD2)*	Brain	Lipids 3.1
54	*Ganglioside GD3*	Brain	Lipids 3.1
55	*Ganglioside GM1*	Brain	Lipids 3.1
56	*Ganglioside GM2 (GGM2)*	Brain gray matter	Lipids 3.2
57	*Ganglioside GM3*		Lipids 3.2
58	*Ganglioside GM4*		Lipids 3.2
59	*Ganglioside GT1a/b*	Brain	Lipids 3.2
60	*Glucose Oxibase*	Various	Catabolic Enzomes 3.1
61	*Glutamate Dehydrogenase (GD)*	Liver	Other Bioactives 9.1
62	*Glutathione Peroxidase (GP)*	Non available	Antioxidants 3.1
63	*Glycogen phosphorylase (isoenzymes)*	Heart	Cetabolic Enzymes 4.1
64	*Haemoglobin*	Blood	Iron & Oxygen Binding 4.1
65	*Haemopexin*	Plasma	Iron & Oxygen Binding 5.1
66	*Haptoglobin*	Plasma	Iron & Oxygen Binding 6.1
67	*Heparan sulphate*	Skin Tissue	Glucoseominoglycans 4.1
68	*Heparin*	Lung	Anticoagulants 3.1
69	*Holo-transferrin*	Blood	Iron & Oxygen Binding 7.1
70	*Hyaluronic Acid (HA)*	Cartilage	Glucoseominoglycans 5.1
71	*Hyaluronidase*	Testes	Glucoseominoglycans 6.1
72	*IgA*		Immunoglobulins 3.1
73	*IgG*	Plasma	Immunoglobulins 4.1
74	*IgM*	Plasma	Immunoglobulins 5.1
75	*Keratan Sulphate*		Glucoseominoglycans 7.1
76	*Lactate Dehydrogenase*	Heart	Catabolic Enzymes 5.1
77	*L-Carnosine (D-alanylhistidine)*	Not available	Other Bioactives 6.1

No.	Bioactives Compounds	Tissue Source	Page Reference
78	*Lipase*	Pancreas	Hydrolytic Enzymes 8.1
79	*Lipoprotein Deficient Serum (LPDS)*	Plasma	Cell Nutrients & Growth Factors 2.1
80	*Lipoprotein Lipase*		Hydrolytic Enzymes 9.1
81	*Lysophosphatidylcholine (Lyso-PC)*	Adrenal glands	Lipids 4.1
82	*Lysozyme*	Plasma	Other Bioactives 10.1
83	*Malate dehydrogenase*	Heart	Catabolic Enzymes 6.1
84	*Malate Hydrolase*		Catabolic Enzymes 7.1
85	*Myoglobin*	Heart	Iron & Oxygen Binding 8.1
86	*Myosins*	Heart	Other Bioactives 7.1
87	*Osteocalcin*	Bone	Other Bioactives 8.1
88	*Osteonectin*	Plasma	Other Bioactives 9.1
89	*Pepsin*	Gastric juice	Hydrolytic Enzymes 10.1
90	*Pepsinogen A*	Not available	Hydrolytic Enzymes 11.1
91	*Phosphatidylserine (PtdSer)*	Brain	Lipids 5.1
92	*Phosphodiesterases*	Spleen	Other Bioactives 11.1
93	*Plasminogen*	Plasma	Anticoagulants 4.1
94	*Protein C*	Plasma	Anticoagulants 5.1
95	*Protein Kinase-A*	Brain	Other Bioactives 12.1
96	*Protein Phosphatase 2C*	Liver	Other Bioactives 13.1
97	*Prothrombin*	Plasma	Coagulants 7.1
98	*Pyruvate Kinase (PK)*	Neck muscle	Catabolic Enzymes 8.1
99	*Ribonuclease A*	Pancreas	Hydrolytic Enzymes 12.1
100	*Ribonuclease B,C & D*	Pancreas	Hydrolytic Enzymes 13.1
101	*Serum Albumin*	Plasma	Cell Nutrients & Growth Factors 3.1
102	*Superoxide Dismutase*	Blood	Antioxidants 4.1
103	*Taurine*	Lung	Cell Nutrients & Growth Factors 4.1
104	*Thrombin*	Plasma	Coagulants 8.1
105	*Thyroglobulin (Tg)*	Thyroid	Other Bioactives 10.1
106	*Transferrin*	Blood	Iron & Oxygen Binding 9.1
107	*Troponins*	Heart	Other Bioactives 11.1
108	*Trypsin*	Pancreas	Hydrolytic Enzymes 15.1
109	*Trypsin Inhibitor*		Hydrolytic Enzymes 14.1
110	*Trypsinogen*	Pancreas	Hydrolytic Enzymes 16.1
111	*Urease*	Not available	Other Bioactives 14.1
112	*Vitronectin*	Plasma	Cell Nutrients & Growth Factors 5.1

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No.	Bioactives Compounds	Tissue Source	Page Reference
1	*Actin*	Skeletal muscle	Other Bioactives 2.1
2	*Alkaline Phosphatase (AP)*	Intestine	Other Bioactives 1.1
3	*Alpha 1 Acid Glycoprotein (AAG)*	Blood	Other Bioactives 3.1
4	*Alpha-Crystallin*	Eyes	Other Bioactives 1.1
5	*Alpha 2 - Macroglobulin*	Serum	Immunoglobulins 1.1
6	*Amylase*	Pancreas	Hydrolytic Enzymes 1.1
7	*Antithrombin III (ATIII)*	Plasma	Anticoagulants 1.1
8	*Apoferritin*		Iron & Oxygen Binding 1.1
9	*Aprotinin*	Lung	Anticoagulants 2.1
10	*Arginase*	Liver	Other Bioactives 2.1
11	*Calmodulin (CaM)*	Adrenal glands	Other Bioactives 4.1
12	*Carbonic Anhydrase*		Other Bioactives 3.1
13	*Carboxypeptidase A*	Pancreas	Hydrolytic Enzymes 2.1
14	*Carboxypeptidase B*	Pancreas	Hydrolytic Enzymes 3.1
15	*Catalase*	Liver	Other Bioactives 4.1
16	*Cathepsin D*		Other Bioactives 5.1
17	*Ceramide*	Brain	Lipids 1.1
18	*Cholesterol Oxidase*	Not available	Other Bioactives 7.1
19	*Cholesterol Esterase*		Other Bioactives 6.1
20	*Chondroitin Sulphate A (CS-A)*	Cartilage - Trachea	Glucoseaminoglycans 1.1
21	*Chondroitin Sulphate B (CS-B)*	Cartilage - Trachea	Glucoseaminoglycans 1.1
22	*Chondroitin Sulphate C*		Glucoseaminoglycans 1.1
23	*Chondroitin Sulphate D*		Glucoseaminoglycans 1.1
24	*Chondroitin Sulphate E (CS-E)*	Cartilage - Trachea	Glucoseaminoglycans 1.1
25	*Chymotrypsin*	Pancreas	Hydrolytic Enzymes 4.1
26	*Chymotrypsinogen*		Hydrolytic Enzymes 5.1
27	*Coenzyme Q10*		Antioxidants 1.1
28	*Collagen, Type I*	Not available	Glucoseaminoglycans 3.1
29	*Collagen, Type II*	Cartilage	Glucoseaminoglycans 3.1
30	*Collagen, Type III*	Intestine	Glucoseaminoglycans 3.1
31	*Collagen, Type IV*	Eyes	Glucoseaminoglycans 3.2
32	*Collagen, Type V*	Placenta	Glucoseaminoglycans 3.2
33	*Collagen, Type VI*	Intestine	Glucoseaminoglycans 3.2
34	*Conjugated Linoleic Acid*		Lipids 2.1
35	*Creatine Kinase (CK)*	Uterus	Catabolic Enzymes 1.1
36	*Cytochrome C*	Heart	Antioxidants 2.1
37	*Deoxynucleotidyl-transferase*	Thymus	Other Bioactives 8.1

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41	*Factor V*	Plasma	Coagulants 1.1
42	*Factor VIII*	Plasma	Coagulants 2.1
43	*Factor IX*	Plasma	Coagulants 3.1
44	*Factor X*	Plasma	Coagulants 4.1
45	*Factor X111*	Plasma	Coagulants 5.1
46	*Ferritin*	Liver	Iron & Oxygen Binding 2.1
47	*Fetuin*	Plasma	Iron & Oxygen Binding 3.1
48	*Fibrinogen*	Plasma	Coagulants 6.1
49	*Fibroblast growth factor (FGF)*	Heart	Cell Nutrients & Growth Factors 1.1
50	*Fibronectin*	Plasma	Other Bioactives 5.1
51	*Gamma-Globulin (g-Globulin or Ig)*	Not applicable (Milk)	Immunoglobulins 2.1
52	*Ganglioside GD1a/b*		Lipids 3.1
53	*Ganglioside GD2 (GDD2)*	Brain	Lipids 3.1
54	*Ganglioside GD3*	Brain	Lipids 3.1
55	*Ganglioside GM1*	Brain	Lipids 3.1
56	*Ganglioside GM2 (GGM2)*	Brain grey matter	Lipids 3.2
57	*Ganglioside GM3*		Lipids 3.2
58	*Ganglioside GM4*		Lipids 3.2
59	*Ganglioside GT1a/b*	Brain	Lipids 3.2
60	*Glucose Oxibase*	Various	Catabolic Enzomes 3.1
61	*Glutamate Dehydrogenase (GD)*	Liver	Other Bioactives 9.1
62	*Glutathione Peroxidase (GP)*	Non available	Antioxidants 3.1
63	*Glycogen phosphorylase (isoenzymes)*	Heart	Cetabolic Enzymes 4.1
64	*Haemoglobin*	Blood	Iron & Oxygen Binding 4.1
65	*Haemopexin*	Plasma	Iron & Oxygen Binding 5.1
66	*Haptoglobin*	Plasma	Iron & Oxygen Binding 6.1
67	*Heparan sulphate*	Skin Tissue	Glucoseominoglycans 4.1
68	*Heparin*	Lung	Anticoagulants 3.1
69	*Holo-transferrin*	Blood	Iron & Oxygen Binding 7.1
70	*Hyaluronic Acid (HA)*	Cartilage	Glucoseominoglycans 5.1
71	*Hyaluronidase*	Testes	Glucoseominoglycans 6.1
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73	*IgG*	Plasma	Immunoglobulins 4.1
74	*IgM*	Plasma	Immunoglobulins 5.1
75	*Keratan Sulphate*		Glucoseominoglycans 7.1
76	*Lactate Dehydrogenase*	Heart	Catabolic Enzymes 5.1
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82	*Lysozyme*	Plasma	Other Bioactives 10.1
83	*Malate dehydrogenase*	Heart	Catabolic Enzymes 6.1
84	*Malate Hydrolase*		Catabolic Enzymes 7.1
85	*Myoglobin*	Heart	Iron & Oxygen Binding 8.1
86	*Myosins*	Heart	Other Bioactives 7.1
87	*Osteocalcin*	Bone	Other Bioactives 8.1
88	*Osteonectin*	Plasma	Other Bioactives 9.1
89	*Pepsin*	Gastric juice	Hydrolytic Enzymes 10.1
90	*Pepsinogen A*	Not available	Hydrolytic Enzymes 11.1
91	*Phosphatidylserine (PtdSer)*	Brain	Lipids 5.1
92	*Phosphodiesterases*	Spleen	Other Bioactives 11.1
93	*Plasminogen*	Plasma	Anticoagulants 4.1
94	*Protein C*	Plasma	Anticoagulants 5.1
95	*Protein Kinase-A*	Brain	Other Bioactives 12.1
96	*Protein Phosphatase 2C*	Liver	Other Bioactives 13.1
97	*Prothrombin*	Plasma	Coagulants 7.1
98	*Pyruvate Kinase (PK)*	Neck muscle	Catabolic Enzymes 8.1
99	*Ribonuclease A*	Pancreas	Hydrolytic Enzymes 12.1
100	*Ribonuclease B,C & D*	Pancreas	Hydrolytic Enzymes 13.1
101	*Serum Albumin*	Plasma	Cell Nutrients & Growth Factors 3.1
102	*Superoxide Dismutase*	Blood	Antioxidants 4.1
103	*Taurine*	Lung	Cell Nutrients & Growth Factors 4.1
104	*Thrombin*	Plasma	Coagulants 8.1
105	*Thyroglobulin (Tg)*	Thyroid	Other Bioactives 10.1
106	*Transferrin*	Blood	Iron & Oxygen Binding 9.1
107	*Troponins*	Heart	Other Bioactives 11.1
108	*Trypsin*	Pancreas	Hydrolytic Enzymes 15.1
109	*Trypsin Inhibitor*		Hydrolytic Enzymes 14.1
110	*Trypsinogen*	Pancreas	Hydrolytic Enzymes 16.1
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