final report

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Red meat protein snack

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PSH.0812 Red Meat Protein Snack

August 2018

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Purpose: Using Xinova's open innovation and technology review process, develop insights and innovative solutions to develop uses for red meat protein sources in snacking, that can assist the Australian red meat industry to gain greater competitive advantage and price premiums over the next 3-5 years. Also assist MLA and the industry to commercialise the new innovative red meat-based snacks.

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Abstract

Snack products are a dietary mainstay of a large proportion of the population. While consuming some snack products are discouraged because of its high sugar and fat content, there are a number of snack products where protein levels can be low and could benefit from protein fortification.

Proteins are vital to growth and development as they are key mechanical and structural components of our bodies. They can also be broken down further to produce energy in times of hunger. The popularity of protein as a positive nutrient has seen its audience and consumer base grow to become a mainstream trend. Much attention has been paid to replacing dietary energy from carbohydrates and fats with that derived from protein due to evidence for weight loss.

Protein fortification uses isolated protein from single sources. Traditionally, the majority proteins that have been used to fortify products have been from animal sources, such as whey and casein from cow's milk, but these are now being replaced by plant sources. Over the past few years there has been a proliferation of lesser-known proteins, including those from algae, hemp, cranberry and pea, as well as isolated animal proteins from beef, chicken and salmon.

Beef protein isolates as a food ingredient is largely missing in food market, especially in snack market. The common processing method of preparing beef protein isolates is enzymatic hydrolysis, which considers with high solubility and easy extractability. However, this extraction method compromises beef proteins' nutritional value by destroying their functional structural. Additionally, enzymatic hydrolysed proteins give bitter flavour which leads to a negative effect on the usage in food industry. Therefore, the technical challenges of extraction beef proteins in their native forms have been persistently challenging. A novel, simplified and native extraction method for beef protein is a definite need in food market.

The aim of this project was to use leading edge science in meat protein isolate extraction to use red meat derivates as an ingredient in snack (or other) food products that are aligned to insights trends for improved convenience, health (from "naturally healthy" ingredients) and indulgent tastes thereby delivering new high value offerings for the Australian Red meat industry.

MLA and Xinova explored the development of novel applications of isolated beef proteins as a food ingredient, for example in snack products and pet foods, where benefit could be gained from increasing protein content. This project covered the following phases;

Phase 1 - Opportunity Analysis

Phase 2 - Innovation Opportunity Workshop

Phase 3 - Red-Meat Protein Isolate Laboratory Research

Phase 4 - New Product Concepts

Phase 5 - Value Proposition Evaluation and Final Report

Executive summary

Beef protein isolates as a food ingredient is largely missing in food market, especially in snack market. The common processing method of preparing beef protein isolates is enzymatic hydrolysis, which considers with high solubility and easy extractability. However, this extraction method compromises beef proteins' nutritional value by destroying their functional structural. Additionally, enzymatic hydrolysed proteins give bitter flavour which leads to a negative effect on the usage in food industry. Therefore, the technical challenges of extraction beef proteins in their native forms have been persistently challenging. A novel, simplified and native extraction method for beef protein is a definite need in food market.

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Contents

1 Background

Snacking is one of the biggest trends in food and health, affecting every category of every type of food and beverage. Unfortunately, although snacks are unquestionably convenient, versatile and great tasting, traditional snacks are typically classified as sweet or savoury and often not particularly healthy. They are generally high in carbohydrates (especially sugars) and fat and contain ingredients that are not appropriate for higher protein diets. Accordingly, there is a clear need to offer snack-lovers a tasty alternative that fills them up with real and healthy nutritious ingredients that is high in protein, low in fat and refined carbohydrates, has great taste and texture. Further, changing consumer lifestyles and behaviours are seeing the need for snack offerings that can be readily produced with prolonged shelf-life, easy storage and consumption.

Protein has the benefit of being an easily recognised nutrient whose benefits are readily understood and are underpinned by scientific evidence, especially for weight control, building muscle mass and decreasing muscular degeneration. US sales of protein-based food and beverages have been rising steadily, with a 30% increase in new-product launches claiming "high" or "source of" protein in North America in 2014. The growth of protein as a key ingredient in snack foods is slower in Europe and Asia, but the market is increasing with 30% of Asia-Pacific buyers and 11% of European buyers increasing their purchase of protein 2014-2016.

Currently, protein isolates, concentrates and hydrolysates are available to consumers and the food industry as functional ingredients. The key difference between these substrates is that isolates are 90%+ pure protein, whereas concentrates contain other dissolved solids (e.g. carbohydrate, fats and therefore 10-15+% less protein content. Hydrolysates are produced by acid/heat/enzymatic processing that partially breaks the protein molecule, resulting in a more readily digested, but bitter tasting end-product. Consequently, consumers are turning to natural whole protein sources. As added-protein products proliferate and start to look faddish, consumers are opting for "naturally-functional" protein sources such as meat.

The conventional protein isolates/supplements include whey, (milk) casein, egg, soy, rice, hemp and pea protein. Whey, casein, egg, soy proteins are included in the eight major food allergens group which pose a risk to induce adverse immune response. Additionally, other issues might be associated with them, such as high sugar content in whey isolate, slow absorbability in casein protein, low cost-effectiveness in egg protein and genetic modification risks in soy protein. Plant derived proteins (e.g. those derived from rice, hemp and peas) are deficient in several amino acids and are not recommended as a primary source of dietary protein. Specific plant proteins need to be consumed together in order to obtain all essential amino acids. Furthermore, plant foods usually contain protein-limiting agents (e.g. phytates) which bind to protein decreasing their bio-accessibility (release from the food matrix} reducing absorption by the body. Hence, they have limited market share. Conversely, whole protein obtained from animal-based foods contain all essential amino acids and are readily bioavailable.

When developing protein supplements or high protein food and drink products, the protein content is often boosted by incorporating a protein isolate, concentrate or hydrolysate. These are all highly-refined and processed forms of their original protein sources that provide a

concentrated source of protein with the absence or reduction of other nutrients, such as fats and fibre.

Whole protein rich products are available in the form of 'whole' foods such as Greek yoghurt, cottage cheese dips, boiled eggs, tinned tuna. Other foods being utilised for their high protein content includes beans, seeds and nuts. Some of the newer sources of protein being used include combinations of ancient grains such as quinoa, chia and teff, while pulses, grains and seeds such as peas, maize, rice, hemp, lupin and linseed are also being used more for their protein content.

Protein products have now proliferated across many categories. Innovation around new high protein products has been driven by the snack and dairy segments, with snack bars and yogurts accounting for the bulk of new high protein products. However, work to spread protein consumption more evenly across a whole day occasions and usages presents opportunity for breakfast products and snacks to include protein in their formulations to help achieve a more balanced intake of protein. Easy to consume protein rich products could also find popularity among the growing aging population and those part of the flexitarian movement due to protein's health properties in building and maintaining muscular strength. The ease of consuming small portion sized meals is of particular importance to this population.

Technical Challenges

Proteins used in the food industry are derived from either animal or plant sources, which vary significantly in their lipid, carbohydrate and protein content. Proteins of interest maybe separated using dry or wet processing techniques. Dry processing primarily involves air classification and is frequently applied to plant materials such as cereals and grain legume containing high amounts of starch and protein. Wet processing has several processing steps. Deviations to these operations may occur depending on the starting material and the desired end-product. Therefore, the material preparation step for wet processing will vary depending on the source material and desired functionality for further use.

Animal-based proteins are known for their difficulties of protein extraction and isolation. Technically beef protein has not found its way into the mainstream protein ingredient markets because of:

- significant quality issues faced when using traditional processing methods
- strong fat-based flavour volatiles which may have increased susceptibility to oxidation (e.g. rancidity); and
- the inherent high value of meat cuts and their use in main meal options.

Innovation opportunity

Dairy proteins (whey proteins) and plant origin proteins are the main source of protein supplements currently used in snack products. However, meat derived proteins, and in particular red meat proteins are arguably the best source of dietary protein. According to a report published by the Food and Agriculture Organization of the United Nations, beef protein scores highly on the biological value scale (i.e. the proportion of protein from food source that is utilized by the body after digestion), only surpassed by fish and milk. Beef contains all nine essential amino acids, making it one of the richest sources of protein available. However, beef protein has been largely absent from healthy-protein snacking, and as such offers opportunity for growth for Australian Red Meat industry in this product category.

Single-serve products that combine the "naturally healthy" and high value qualities of red meat with the convenience of snacks would appear to be aligned with consumers' desires for ease of consumption, health (from "naturally healthy" ingredients) and premium indulgence. They offer the possibility of a sustained energy marketing message, an increased satiety eating experience and better absorption options.

Australian red meat has key strengths that can be leveraged, including high nutritional value, guaranteed safety, traceability and great taste and flavour. The option of using Australian red meat protein in snackified food products has the potential to provide high nutritional value and to create premium products that command a greater price per serving and grow overall demand for Australian red meat industry by adding value. As the most likely source of red commodity meat input in this protein extraction process will be low grade trim, blood, offal etc., increasing value x2-5 times is possible.

The purpose of this project is to:

a) Conduct an opportunity analysis, including a technical landscape review of protein snacks;

b) Using the technical landscape information together with "snacking" insights delivered by MLA, determine design criteria for new red meat high protein snacks which; match consumer's desires, fall within Xinova and/or MLA's capabilities to find a commercial partner, and are financially sound;

c) Using beef protein as a model system, determine whether it is possible to produce a shelf- stable and useful red meat protein isolate for use in snack (or other) food products;

d) Prototype high protein snack foods, based on the design criteria (from b)) using beef protein isolate for testing purposes and assess consumer acceptability;

e) Define the value proposition and benefits for Australian Red meat industry for future adoption and commercialisation - include technical feasibility, commercial viability and desirability with identified target market; and

f) Take appropriate measures to protect intellectual property arising from the project, either by trade secret or filing of patent applications.

To achieve the technical aims of the project, Xinova intends to partner with Matis Ltd http://www.matis.is/english/, a food and biotech R&D institute based in Iceland. Matis have already developed and are currently commercialising a novel and patented method for providing chicken protein isolates and have further developed a method for manufacturing tortilla wraps which include at least 50% chicken protein. Xinova proposes to partner with Matis, using beef protein as a model system, determine whether it is possible to produce a shelf- stable and useful red meat protein isolate for use in snack (or other) food products and to develop concept high protein snack foods using Matis beef protein isolate for testing purposes and assess consumer acceptability.

2 Methodology

This project was undertaken using the following methodology;

2.1 Phase 1. Opportunity analysis:

Literature & IP landscape search and analysis, and review of market insight research

In this phase, Xinova undertake IP, technology and product scanning as well as a literature review to identify technology trends, new products as well as existing solutions in the area of "red meat derived protein snacks". The scope of this review included both,

a) generation of meat derived protein isolates and

b) available snacks of which protein is considered a fundamental ingredient.

The protein snacks were considered in the context of the entire snack market. The aim of this phase of the project was to provide a pathway for the red meat sector to understand and assess what technology and products (friendly or competing) already available and what opportunities for the red meat sector this may generate.

This study also identified challenges to red meat protein snacks but also suggested areas that have traditionally not been of focus and identified the reasons why.

MLA provided Xinova with insight reports on snacking that had been commissioned under previously funded projects to help orient the project scope by identifying highly relevant opportunity spaces that may transform and create value for the industry.

The outcome of this phase has been included in a report tabling the findings of the landscape analysis, including listing potential opportunity areas for innovation.

2.2 Phase 2: Red-meat Protein Isolate.

Using their knowledge and demonstrated skills and know-how in deriving chicken-based isolates, Matis (under the direction of Xinova) conducted research & development into developing and optimising red-meat (as a model system) protein isolation for use as a food ingredient in snack foods, taking into account the design criteria determined from Phase 2 work. Matis' work included technical development and determination of pH shifts for red meat and improving beef protein quality. Xinova also conducted an intellectual property audit to determine ‘freedom to operate’ and identify if there was any defendable IP position within the Matis work.

The outcome of this phase was a demonstration of the production of a stable red meat protein isolate (technical feasibility).

2.3 Phase 3. Innovation Opportunity Workshop.

It is essential to start the process of sourcing innovative solutions by having a clear definition of the opportunities, problems and issues to be overcome. Following Phase 1, an Innovation Opportunity Workshop involving staff from MLA, Xinova, a number of selected domain experts and inventors was undertaken. The outcomes from Phase 1, literature review, IP

landscape analysis and market insight research were used to inform the workshop discussion.

2.4 Phase 4: New Product Concepts.

Matis (under the direction of Xinova), undertook the development of two potential snack foods using the red meat protein isolate derived during Phase 3 and taking into account the Design Criteria from Phase 2. Development of the concept snack products included sensory testing, proof of concept product development, consumer testing and feedback to ascertain likely target market (desirability) and considered as part of an early adopter what the possible pain point/needs state, size of the market and ability to change the targeted consumer/market the product concepts address. Xinova also conducted an intellectual property audit at the end of Phase 4 to recommend appropriate IP protection as necessary.

3 Results and Discussion

3.1 Phase 1. Opportunity analysis:

Innovation around high protein products has been driven by the snack and dairy segments, with snack bars and yogurts accounting for the bulk of high protein launches. Protein enhancement is also present in specialty products, such as protein powders and foods for body builders, athletes wanting to increase muscle volume, and outdoor enthusiasts. However, work to spread protein consumption more evenly across a whole day, especially in more general food consumption markets presents an opportunity for breakfast products and snacks to include protein in their formulations to help achieve a more balanced intake of protein. Enhanced protein foods could also find favour among the growing ageing population.

Examples of potential uses for isolated beef protein include:

- Beef protein snack: Single-serve products that combine the “naturally healthy” virtues of beef and snacks would appear to be aligned with consumers’ desires for convenience, health (from “naturally healthy” ingredients) and indulgent tastes. They offer the possibility of a sustained energy marketing message, a more filling eating experience and better absorption options.

- Savoury crunchy and/or chewy “sensory-intense” high-protein snacks could be developed for consumption 30 minutes prior to a meal in order to improve satiety and potentially decrease overall calorie consumption. Thickened high-protein beverages could be used for similar purposes as savoury snacks but could be designed for individuals who find sweet foods more acceptable than savoury ones. A focus on convenient high- protein breakfast foods is warranted, because a high-protein breakfast has been reported to decrease craving over the rest of the day. In addition, breakfast is the meal most likely to be skipped and where the least amount of protein is consumed.

- Animal Protein isolates from low value meat portions could be used in pet food to improve the nutritional value and texture for animal well-being. Current pet foods are typically formulated using protein from grains or grain by-product sources, such corn gluten meal, brewer’s rice and wheat, rather than from meat sources. They may also contain poultry by –products, which typically consists of the leftovers unfit for human consumption, such as feet, beaks, undeveloped eggs, and intestines - everything but clean meat. Animal protein is hugely important to pets throughout their entire lives. High quality protein from actual meat sources contains important amino acids that pets need to thrive.

- Animal protein fortified food, such as protein fortified rice, could help counter protein deficiency in developing countries. Protein deficiency diseases occur in developing countries due to poverty as well as from lack of knowledge about nutritional requirements. Animal protein, for example beef protein, contains all essential amino acids; chronic inadequacy of any of these essential amino acids can also cause specific abnormal and harmful functioning. Benefiting from the nature of animal protein isolates, it has an inherently long shelf life and is therefore also suitable for long distance shipping.

3.2 Phase 2: Red-meat Protein Isolate.

The work undertaken with Matis has been completed. It is recommended that no further work be conducted as the process appears to be uneconomic. The economic model for the pH shift protein isolation process doesn't factor in the costs associated with waste water disposal/management. The process requires significant water use, 1:4 at least, plus prewashes, and generates high BOD/COD effluent, which would be bordering on environmentally unsustainable unless untreated. If the process waste stream does undergo treatment, significant costs are added to the process.

Having said that, Matis proposes a protein recovery process from the wash water. Matis claim they can clean up the water for reuse or direct discharge and recover additional proteins (see page 87-90 of the Matis report). Matis have done the majority of the research and development work with fish (not this project) but haven't conducted any trial on beef to support this claim.

The most important but unanswered question is they haven't done enough work to show that the protein isolates or the marinade from their process can be stabilized. They conducted very limited experiments on microbial analysis, freezing, and freeze drying, without any further proper shelf-life evaluation or exploring other drying methods.

They contemplated other possible products e.g. collagen from the process but no actual work performed.

The economic model also doesn't take Capex into consideration. The work is bench scale using laboratory separation equipment some of which may be unscalable to pilot plant.

The Matis process has also failed to provide any technical advantage over other red meat protein isolation process and the process has an inherently complex intellectual property position that would make it difficult to license and protect. Isolated red meat protein process was developed by CSIRO in the early 1970's and may have ramifications in relation to prior-art. The outcomes from the work undertaken by Matis is included as an Attachment.

3.3 Phase 3. Innovation Opportunity Workshop & Phase 4. New Product Concepts.

Several challenges facing meat-protein snack development are flavour / taste, odour and appearance. Novel forms and unique processing methods will assist in creating snacks high in red-meat-derived proteins for improved nutrition and convenience.

The option of using Australian red meat protein in snackified food products has the potential to provide high nutritional value and to create premium products that command a greater price per serving and grow overall demand for Australian red meat industry by adding value. As the most likely source of red commodity meat input in this protein extraction process will be low grade trim, blood, offal etc., increasing value x2-5 times is possible.

Xinova conducted a bespoke Red Meat Snack Workshop on 12th & 13th February 2018. The bespoke workshop consisted of industry stakeholders and representative innovators and MLA team members. The purpose of the workshop was to seek input on the initial red meat snack concepts.

The workshop consisted of representatives from MLA, Xinova and Food Innovators. Attending were **Xinova & Partners** Scott Needham, Yi Lan Chen, Evelyn Miles, Russel Rankin, David Ireland, Greg Caire, Nick Hazell, **MLA/MDC** Christine Pitt, Allister Watson, Michael Lee, Duncan Veal, Rachel Cofrancesco, Emily Walker, **Innovators** Maxime Bilet (Imagine Food Innovation Group), Steve Weaver, Bradley Wardrop-Brown (BluOak Innovation), Bob Hamilton (Earlee Foods), Brett McMullen (Earlee Foods), Trish Linderman (Earlee Foods)

The purpose of the workshop was for selected innovators from Xinova's network to prepare and present 4 to 6 meat protein snack concept prototypes each, for tasting and assessment providing feedback to inform the development pathway and understand value proposition to consumers for market positioning. The workshop was framed as a design thinking process where concept products were reviewed and assessed in terms of desirability, feasibility and viability.

Concepts products presented and tasted were as follows;

Innovator	Taste the Snack	Dream the Snack
Earlee Foods	Collagen Chips	Meat based cracker
	Shelf stable snack sausage	Hi-protein low carb muesli/trail mix
	Breakfast Bar	Chocolate with beef fat
		Hi protein lactose free ice cream
		Protein enriched beverage
BluOak Innovation	Beef chips with inulin fibre	Dual textured coextruded beef or lamb bar
	Fortified beef bars	
	Pre/probiotic collagen recovery drink	
	Beauty bars	
Maxime Bilet	Reimagined Jerky	Jerky and force meats
	Beef offal Fish sauce	Crisps with side of beef
	Thin meat crisps	Crispy crunchy foam
	Meat based starch chips	Coextruded meat pockets
	Plant chips impregnated with protein isolate	Fried rice clusters with meat bits
	Tender Puff V1	

	Tender Puff V2	

The 2 days were broken up into the following stages; "*Taste the Snack*" – Hard Concepts and food experience, "*Dream the Snack*" – Soft Concepts and "*Share the Snack"* - Creative thinking and sharing of ideas. Taste the Snack was focused on taste and evaluation of prototype snacks, Dream the Snack was snack concept ideas and Share the Snack sharing ideas and creating improved snack concepts.

Concept samples were tasted and evaluated against the following criteria, the snack concept, the taste, top 3 challenges and an assessment of Value versus complexity. The workshop was organized and executed over a period of around 12 weeks – from concept, to innovator identification, contractual engagement, creative thought and prototyping, to final delivery of concepts.

The red meat snacking workshop provided a unique and creative insight into the types of red meat-based snacks that may be possible, but further work needs to be done to sort through the concepts presented and identify the most promising snacking business opportunities.

The Projects recommends that a new proposal for the development of these concepts be prepared and submitted to MLA. This new proposal would utilize design thinking methodology to undertake development of the 'best' red meat snack concepts to come out of the Workshop. This methodology would consider the perspectives of the market (viability), the technology (feasibility), and the user (desirability) providing a rigorous framework to identify the most appropriate concepts to navigate through ever-present innovation uncertainties. This will allow the project to short list concepts down to 3 preferred validated snack product concepts.

1) Consumer desirability validation.

A consumer engagement methodology will be designed, that begins by broadly understanding any unmet needs of consumers in Australia, US, and agreed Asian markets in relation to; eating habits, lifestyles, dietary requirements, disease limitations, nutrition understanding/knowledge, current category data (market access). Previous studies commissioned by MLA such as P.PSH.0822 Identifying Snacking Occasions within Red Meat will be used to inform consumer engagement in relation to short listed product concepts. Reports related to consumer and market snacking trends such as protein consumption, satiety/dieting etc will also inform the development of the consumer engagement phase.

Food Innovators will be engaged to produce further specific product concept samples, incorporating any changes in product specifications such as saltiness, fat content, protein content, taste, mouthfeel etc. identified in the first workshop.

2) Validation of Business Viability

Food Marketing engagement – Understanding of opportunities and limitations. Develop the compelling story and understand who the product will be targeting.

Develop an indicative business model using Business Model Canvas tool. This work will begin to identify the path to market for any individual product concepts, potential partners or commercial exits. Identify the need for more funding with estimates of timing and quantum. Investigate the competitive environment and pricing, understand any regulatory issues/timing/costs. Commence discussions with potential business partners and value chain participants.

3) Assessment of Opportunity Feasibility

Choose and reconnect with Food innovator(s). Map the mass production or productization steps required for each of the product concepts short-listed. Develop costing models and understand ingredients, supply and costs (including alternatives). Re-iterate the original prototype using consumer feedback and re-test with consumer groups. Nutrition analysis and conduct any early stage testing confirm bioavailability if functional ingredients, if required.

Xinova will also assist MLA in the commercialisation of the ‘best’ new red meat-based snack concepts, including:

- Formation of partnership and collaboration;
- Project management and delivery.
- Clearly define potential value creation to processors
- Participation in roadshow and/or workshop with potential collaborators, including processors to pitch the opportunity and what is required by different value chain participants to create and capture value and to understand current capability to execute.

4 Recommendations

The Project recommends that a new proposal be developed and submitted to MLA to undertake further work to sort through the concepts presented and identify the most promising snacking product opportunities. The proposal would cover work associated with development and commercialisation of a new red meat snack concept product against consumer snacking attributes within the context of design thinking criteria of desirability, feasibility and viability and detail a commercialisation pathway to launch.

The proposal would include validation of 3 red meat protein-based snacks and a clear commercial pathway to launch, covering;

- Formation of partnership and collaboration,
- Clearly defined value creation to producers and the industry,
- Participation in roadshow and/or workshop with potential collaborators and partners, to pitch the opportunity and what is required by different value chain stakeholders to create and capture value and to understand current capability to execute.

Attachment 1: The Use of isolated beef protein as a food ingredient – technical and intellectual property review

Table of contents

1 Background

MLA and Xinova are considering innovations in obtaining and using isolated beef proteins as a food ingredient, for example in snack products and pet foods, where benefit can be gained from increasing protein content.

Proteins are complex and diverse molecules made of linked chains of 21 types of amino acids. Once eaten, the proteins are broken down into individual amino acids, which are used by the body to make new proteins. Proteins are vital to growth and development as they are key mechanical and structural components of our bodies. They can also be broken down further to produce energy in times of hunger.

The popularity of protein as a positive nutrient in a balanced diet is growing to become a mainstream trend. For example, some attention has been paid to replacing dietary energy from carbohydrates and fats with protein due to evidence for weight loss.

Protein fortified foods typically use isolated protein from a single source. Traditionally, the majority of proteins that have been used to fortify products have been from animal sources, such as whey and casein from cow's milk, but these are now being replaced by plant sources, such as soybeans. Over the past few years there has been an increase in the availability of other protein sources, including those from algae, hemp, cranberry and legumes, as well as, to a lesser extent, isolated animal proteins from beef, chicken and salmon.

Beef protein isolates as a food ingredient is largely missing in the food market, especially the snack market. The most common processing method of preparing beef protein isolates is enzymatic hydrolysis, which works with high solubility and easy extractability. However, this extraction method compromises beef proteins' nutritional value by destroying their functional structural. Additionally, enzymatic hydrolysed proteins give a bitter flavour which leads to a negative effect on the usage in food industry. Therefore, the technical challenge of extracting beef proteins in their native form has been persistently challenging. A novel, simplified and native extraction method for beef protein does not appear to have been explored in the food industry.

This report discusses currently commercially available protein sources, reviews the profile of current protein isolates from animal and plant origins and their applications as an innovative means to recover functional protein isolates from low-value sources. It also covers recent attempts to extract protein from their original forms, as noted in the patent literature. A market overview of protein ingredients and protein fortified products is discussed, as well as opportunities for new protein ingredients and markets, with a particular emphasis on beef protein.

2 Protein isolate

Protein isolates are the most refined form of protein products containing the greatest concentration of protein yet contain no dietary fibre. Isolates originated from the United States around 1950s (Jay and Michael 2004). They are very digestible and easily incorporated into different food products. Protein isolates are nowadays believed to have played a major role in the development of a new class of formulated foods. The high concentration of protein with the advantage of colour, flavour and functional properties make isolated protein an ideal raw ingredient for use in beverages, infant foods, textured protein products and certain types of specialty foods (Olaofe *et al.,* 1998).

2.1 Source of protein

There are two sources of protein isolates - animal and plant. Animal products, such as meat, milk, milk products, egg, poultry and fish are rich sources of protein containing a balanced level of amino acids. Animal protein is generally associated with high fat content and, because of this, when consumed in large amounts, it leads to higher risk of disease, including high blood pressure and heart disease. Animal protein has a balanced combination of all amino acids and hence it is called complete protein. In contrast, with the exception of soy protein, plant protein is incomplete protein.

Protein quality, and thus nutritional value, is determined by amino acid composition and the digestibility of the protein fraction of food. The 20 proteinogenic amino acids are classified as either indispensable or dispensable. Nine amino acids are classified as essential for humans (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) as they cannot be synthesized in the human body from naturally occurring precursors at a rate that meets metabolic requirements. The remaining dietary amino acids are dispensable (alanine, arginine, cysteine, glutamine, glycine, proline, tyrosine, aspartic acid, asparagine, glutamic acid, and serine). Among the 9 essential amino acids, lysine and threonine are strictly indispensable since they are not transaminated and their deamination is irreversible. In contrast, the 7 remaining indispensable amino acids can participate in transamination reactions. In addition, some of the dispensable amino acids that can be synthesized by the body under normal physiological conditions can become limiting under special physiological or pathological conditions. One example is in premature neonates. When the metabolic requirement cannot be met, these amino acids must be supplied in adequate amounts with the diet. They are then called conditionally indispensable amino acids (arginine, cysteine, glutamine, glycine, proline, and tyrosine). The last parameter that needs to be considered for the correct assessment of protein quality is the ratio between essential (E) and nonessential (N) amino acids. According to FAO/WHO criteria, E/(E + N) has to reach about 40% with E/N = 0.6 (FAO 1989). FAO/WHO/EFSA dietary criteria state that each adult must consume 0.66 g/kg of body weight of protein per day (EFSA 2012).

2.1.1 Animal protein

Protein from animal sources contains essential amino acids needed for an adult's diet. A summary of important protein examples are given below, with more detail provided in Appendix 1.

2.1.1.1 *Whey*

Whey is the liquid by-product of cheese making which can further be processed into spray dried products such as whey protein concentrates (WPC), whey protein isolate (WPI) or whey protein hydrolysate (WPH) (Brucic *et al.*2009). It represents about 20% of milk proteins, which remain in the serum phase during processing of cheese (Kimberlee 2012).

2.1.1.2 *Casein*

Casein makes up about 80% of the proteins in cow's milk. It has a wide variety of food and industrial uses. Food uses include protein supplements, cheese, food binding agents, artist's paint medium, glue, plastics, fibre and also medicinal and dental uses. In its uses as a protein supplement, it tends to gel in the stomach, meaning it has an additional use as slowing nutrient release. Caseins are heat stable because they are proline rich.

2.1.1.3 *Egg*

Egg proteins are nutritionally complete with a good balance of essential amino acids which are needed for building and repairing the cells in muscles and other body tissues (Watkins B.A. 1995). Egg proteins are distributed in all parts of the egg, but most of them are present in the egg white and egg yolk amounting to 50% and 40%, respectively. The remaining amount of protein is in the egg shell and egg shell membranes. The major portion of egg yolk exists as lipoproteins, which can be separated by centrifugation into a plasma fraction (which remains soluble) and a granular fraction (which precipitates).

2.1.1.4 *Fish Protein isolates (FPI)*

Fish protein isolate is a protein concentrate which is prepared from fish muscle without retaining the original shape of the muscle. It is not generally consumed directly but used as raw material for production of other value-added products. It is normally utilized as an ingredient for the production of value added products. It is still a good source of protein for the production of ready to eat fish products.

2.1.1.5 *Meat proteins*

The meat protein ingredients discussed are a class of high protein products that are derived from either animal by-products or lean tissue components (Table 1), and they are used primarily as ingredients in meat and other food products.

Table 1 Major Sources and types of meat protein ingredients

Source	Ingredients
Lean tissue	Finely textured meat/poultry
	Mechanically separated meat/poultry
	Meat protein isolates
Bone	Gelatin (type B)
	Edible bone collagen (ossein)
	Bone collagen hydrolysates (stocks and broths)

Pig skin	Gelatin (type A) Stocks and broths
Beef hides	Gelatin (type B)
Poultry skin (chicken, turkey)	Concentrated collagen Stocks and broths
Collagen-rich tissues	Concentrated collagen Collagen hydrolysates
Blood	Blood plasma (liquid, frozen, dried) Whole blood (liquid and dried) Red cell protein (decolorized) Plasma transglutaminase

Sarcoplasmic, stromal and myofibrillar are types of meat protein. Sarcoplasmic proteins contain enzymes myoglobulin and cytoplasm. Collagen and elastin are the content of stromal proteins while myosin, actin, tropomysin and troponins are the content of myofibrillar proteins. Stromal and myofibrillar proteins, soluble in salt solutions, are used for making edible films and coatings. Collagen, a fibrous stromal protein extracted from connective tissue, tendons, skin, bones and the vascular system, is a waste product of meat processing. Collagen is a superhelical structure formed by a combination of three parallel alpha-chains, and forms gelatine (Haug *et al.,* 2004). Collagen exposed to mild heat treatment under acidic or alkaline conditions forms gelatine (Badii and Howell, 2006).

Stromal proteins (connective tissue), primarily collagen, represent as 10-15% of total muscle protein, being the most abundant protein in the animal body (skin, sinews, tendons, etc.). Stromal proteins are not soluble in water regardless of pH, temperature, or ionic solubility. Stromal proteins hold fibres together and are therefore generally tough and inert. Increased crosslinking occurs as animal age increases, increasing toughness. Stromal proteins are not very valuable in processed meats because of their little binding ability. They shrink when heated to 140 °F and convert to gelatine at 160-180°F. But if heated when dry, collagen becomes very hard and impermeable. This character is important in the handling of collagen and/or natural casings. The main application of collagen is to make gelatine, contact lenses, and pharmaceuticals.

Sarcoplasmic proteins have been shown to form low quality gels, have low water holding capacity, and interfere with myosin cross-linking during formation of gel network; therefore, sarcoplasmic proteins may have a negative impact on food texture. (ASCL, 2014)

2.1.1.6 *Others (e.g. insects)*

Insect protein content is very high, with many species ranging above 60%. For example, Finke and others (1989) reported that the house cricket (Acheta domesticus), when fed to

weanling rats, was superior to soy protein as a source of amino acids at all levels of intake. One of the most widely eaten insects in China is the silkworm, whose protein content is comparable to other animal proteins.

2.1.2 Plant protein

Vegetables, legumes and fruits are good sources of protein. Legumes have a higher content of protein than vegetables and fruits (Creighton TE. New York: Freeman WH; 1993). Different parts of plants are sources of proteins as given in Table 2.

Table 2 Parts of plants as source of proteins with examples

Protein source as part of plant	Examples
Legume	Garbanzo beans, kidney beans, lentils, lima beans, navy beans, soybeans, split peas
Grain	Barley, brown rice, buckwheat, millet, oatmeal, quinoa, rye, wheat germ, wheat, wild rice
Vegetable	Kales, broccoli, mushrooms, sweet corn, spinach
Fruit	Apple, banana, cantaloupe, grape, grapefruit, honeydew melon, orange, papaya, peach, pear, pineapple, strawberry, watermelon
Nuts and seeds	Almonds, cashews, filberts, hemp seeds, peanuts, pumpkin seeds, sesame seeds, sunflower seeds, walnuts

2.1.2.1 *Peanut protein isolates (PPIs)*

The peanut contains 26-29% protein with good nutritional quality. Peanut proteins are used for their functional properties (emulsification, forming) or for their nutritional properties in different food products. They are also used for human nutrition in developing countries to supplement cereals, beverages and skim milk.

2.1.2.2 *Soy protein isolates (SPIs)*

Soy protein isolate is a common isolate. It has high protein content of about 90%. It is usually combined with other food ingredients such as vitamins, minerals and flavour in preparation of soy protein shake powder (Seyam 1983).

The problem of poor flavour, mouth feel, texture, dryness and flavour associated with the use of soy flour and soy concentrate above 10% has meant soy isolate is typically used in sausage-type products for their emulsion-stabilizing effect, gelation, and moisture retention and improved effects on texture (Kinsella 1976). Soy protein is regarded as textured protein products use both in meat and vegetarian meat analog industry and thus, has good water holding capability (Riaz 2006). It is often used as meat extenders in comminute meat products such as patties, fillings, meat sauces, meat balls, etc (Berk 1992).

2.1.2.3 *Canola protein isolates (CPIs)*

CPIs have a good amino acid profile with a well-balanced amino acid composition although it has found only marginal use in the food industry, due to the presence of anti-nutritional factors. CPI thus possesses generally unacceptable food-functional properties including poor water holding, gelling and oil-binding, foaming and emulsification properties. Many studies have been carried out to modify the properties of this isolate e.g. by succinylation, acylation and enzymatic hydrolyses. (Alashi 2011).

2.1.2.4 *Chick pea protein Isolate*

Chick pea is the world's third largest pulse crop in term of area, grown mostly in West Asia and the Mediterranean region. It is one of the major vegetable proteins. Many functional properties of this protein isolate have been studied whereas information on gelation properties of chick pea protein isolate is scarce (Zhang 2007).

3 Protein extraction

3.1 General Methodology

A wide variety of extraction and fractionation tools for proteins and peptides are available based on their physicochemical and structural characteristics such as solubility, hydrophobicity, molecular weight, isoelectric point (pI), and so on. Generally, different technologies focus on cell disruption and solubilisation/precipitation, and enrichment systems are needed to obtain the protein fraction of interest. This section describes the state-of-the-art of extraction and fractionation techniques for food proteins and peptides.

3.1.1 Cell disruption methods

The preparation of any food requires homogenization. Proteins are usually contained in protein bodies inside cell walls, so cell disruption is required before they can be totally solubilized and extracted. The general procedure for sample preparation in this case strongly depends on the food type. Generally, disruption of the cell wall and protein release is crucial for extraction success. Various chemical and physical techniques can be used to destroy the cell wall. These techniques can be grouped into five major categories: mechanical homogenization, ultrasound homogenization, pressure homogenization, temperature treatments, and osmotic and chemical lysis.

3.1.2 Protein Solubilisation/Precipitation

Protein solubilisation is considered one of the key steps in sample preparation procedures. It is generally employed to separate proteins in the sample selectively from different substances (Berkelman and Stenstedt 1998). The solubilisation/precipitation process strongly affects the quality of the final results and thus determines the success of the entire extraction. Taking into account the immense variety of proteins and the huge number of interfering contaminants present in food-derived extracts, simultaneous solubilisation of all proteins remains a great challenge. Each food sample requires a specific protocol that needs to be optimized to minimize proteolysis and modification of proteins (Bodzon-Kulakowska *et al.* 2007). For animal tissues, which have higher protein yields, various protein solubilisation buffers are used, including chaotropic agents, detergents, reducing agents, buffers, and ampholites (Pedreschi *et al.* 2010). The proper use of these additives avoids protein modifications, aggregation, or precipitation that may result in the occurrence of artefacts and the subsequent lowering of protein yield (Gorg *et al.* 2004).

3.1.2.1 *Organic Solvents*

The main organic solvents and additives used to extract proteins from food sources. Many studies performed in the last few years aimed to compare different protein solubilisation methods (Jiang *et al.* 2004; Natarajan *et al.* 2005; He and Wang 2008). The most common method used for the extraction of plant proteins is tricholoracetic acid (TCA)/acetone precipitation as proposed by Damerval *et al.* (1986). This method has been used to extract proteins from different tissues of cereals, legumes, and fruits. However, a disadvantage of TCA precipitated proteins is that they are difficult to redissolve (Nandakumar *et al.* 2003). In the last decade, the phenol extraction procedure has been widely used because of its high clean-up capacity. In contrast to its strong solvent action on proteins, phenol has little predisposition to dissolve polysaccharides and nucleic acids. However, phenol shows the

disadvantages of being more time consuming than other sample precipitation procedures and of being toxic. The alcohol extraction process after de-hulling and conventional de-oiling has a high efficiency of protein recovery. Aqueous alcohols (ethanol, isopropyl alcohol, butanol) are widely used on a commercial scale to remove phenolics, oligosaccharides, or inhibitors from defatted meals and seeds (Moure *et al.* 2006). Recently, extractions with different organic solvents, such as n-hexane, 2-methyl pentane, diethyl ether, acetone, 2-propanol, and ethanol were compared regarding effectiveness, suitability, and protein solubility of the full-fat and defatted lupin (Bader *et al.* 2011).

3.1.2.2 *Aqueous Solutions*

In recent years, because of the growing environmental concerns over the use of organic solvents to extract oil/protein from oil-bearing food materials, aqueous extraction is gaining attention. Water is also operationally advantageous over alcohols because it is non-flammable and neither explosive nor toxic. Commercially, the production of protein concentrates (48–70% protein) or isolates (85–90% protein) consists of an aqueous solubilisation of protein and carbohydrates at acid, neutral, or alkaline pH and the selective recovery of the solubilized protein, separation, and, optionally, washing and neutralization before drying. The protein extraction yield and properties are influenced by the type of extraction process and by different factors such as pH, salts concentration, the ionic strength of the medium, net charge, and electrostatic repulsions (Tan et al. 2011). A number of acid and alkaline protein extraction protocols have been published from various plant and animal tissues. In the last decade, different studies have focused on evaluating the effect of extraction methods on the functional and rheological properties of proteins recovered from by-products of the meat and fish industry (Liang and Hultin 2003; Chaijan *et al.* 2006; Hrynets *et al.* 2010, 2011; Moayedi *et al.* 2010; Omana *et al.* 2010). Recent studies report the use of mainly sodium and calcium salts to extract proteins from different vegetal foods (Ghaly and Alkoaik 2010; Horax *et al.* 2010; Lestari *et al.* 2010; Karaca *et al.* 2011; Nadal *et al.* 2011). These extraction methods are simple because the agents required are easily available. However, as a result of the degradation at high pH conditions, the protein yield is generally low. Also, the protein quality can be altered by alkaline processing due to undesirable reactions involving racemization of amino acids, formation of toxic compounds such as lysinoalanine, reduction of digestibility, loss of essential amino acids, and decrease in nutritive value.

3.1.2.3 *Aqueous Enzymatic Extraction*

An alternative approach combining aqueous and enzymatic extraction is attracting attention. Enzymes can aid in the extraction of proteins in several ways. Carbohydrases, which can attack the cell wall components, may increase protein yield by liberating more protein from the matrix source (Ansharullah *et al.* 1997; Wang *et al.* 1999; Tang *et al.* 2002). A combination of cell wall-hydrolyzing enzymes (i.e., Viscozyme L) has been used to cleave linkages within the polysaccharide matrix effectively and hence liberate more intracellular protein from oat bran (Guan and Yao 2008). In the last few years, different proteases, alone or in combination, have been used to partially hydrolyse proteins to peptides, increasing their solubility and making them more easily extractable. Recently, De Moura and co-workers (2011) developed a two-stage counter current aqueous enzymatic extraction process for soybean, significantly reducing the amount of water used. They achieved slightly higher oil and protein extraction yields than those from standard single-stage aqueous as the long time required and the high cost of enzymes that makes this strategy uneconomical. The use of

immobilized enzyme in protein extraction may reduce the overall cost by allowing the reuse of enzymes.

3.1.2.4 *Ultrafiltration method*

This is a potential membrane processing for extraction of protein, a solution of protein is placed in a cell containing a semi-permeable membrane, and pressure is applied. Small molecules pass through the membrane, whereas the larger ones remain in the solution. The semipermeable membranes with cut-off points of between 500 and 300,000 are mostly available (McClements 2013). The extraction of soy protein isolate by this method was first reported over twenty years ago. The Ultrafiltration system provides a commercially feasible alternative to the existing isoelectric precipitation method of soya beans production. Subsequently, many manufacturers were believed to have implemented the ultrafiltration process for soya isolate. However, very few details are available due to commercial confidentiality (Smith et al. 1939). Ultrafiltration can also be used to concentrate protein solution and can be used in laboratory and commercial scale (McClements 2013).

3.1.3 Protein Enrichments Methods

Once the protein fraction has been isolated from the rest of the constituents and the interference substances have been eliminated, there are still some other steps that are needed prior to further processing. The purpose of fractionation and enrichment methods is to obtain distinguishable fractions and increase the concentration of the proteins of interest.

3.1.3.1 *Centrifugation*

The use of centrifugation is one of the simplest methods used for isolation and enrichment/fractionation of proteins. Centrifugation can be used for different purposes. It can be a first step to separate different cell substructures where our proteins of interest are locally concentrated, for instance, mitochondria, membrane, or nucleus. This process involves multiple centrifugation steps and, as a result, the cellular homogenate is separated into different layers based on the molecular weight, size, and shape of each component.

3.1.3.2 *Precipitation*

It is recognized that among the different precipitants the most widespread is ammonium sulphate (Bodzon-Kulakowska *et al.* 2007). The addition of high amounts of this salt or other such as sodium chloride into a protein solution provokes an increase of protein interactions followed by protein aggregation and finally precipitation. This is known as a salting-out process and, as the salt concentration needed for protein precipitation varies from one protein to another, it allows selective protein separation. Another type of protein enrichment is immunoprecipitation, based upon the binding of the antigen to its specific antibody to form the antigen–antibody complex. In general it offers high recoveries of the proteins and it is widely used for food allergens (Pastorello and Trambaioli 2001). Additionally, using the isoelectric point (pI) is a common method to precipitate protein from solution.

3.2 Methods used for protein extraction from meat/muscle

Muscle proteins are extracted from animal sources using chemical and enzymatic methods. Chemical extraction methods include: 1. buffer extraction which only uses chemicals in neutral pH to solubilize protein; 2. pH shift extraction which adjust pH to acid or alkali to

extract and precipitate them down later using an isoelectric point. Protein hydrolysates-peptides obtained from enzymatic extraction process are also used in the food industry.

3.2.1 Buffer extraction in neutral pH

The sarcoplasmic proteins are readily soluble in water or dilute salt-containing solutions. Extraction of muscle with strong salt solutions dissolves the major portion of the sarcoplasmic plus myofibril proteins (Bate-Smith 1934, 1935). Most of the strong salt soluble proteins can be brought out of solution by diluting the ionic strength to ~0.05. Robinson extended (1952) these studies by using a higher pH extracting solution (pH 8.5) and a further extraction with 0.1 M NaOH. The residue after these sequential extractions was referred to as the connective tissue or stromal fraction. Perry (1952) found that preparations of myofibrils had very low quantities of proteins soluble in 0.08 M Borate buffer, pH 7.1. A common scheme for the separation of the various solubility classes of muscle proteins is shown in Fig 1.

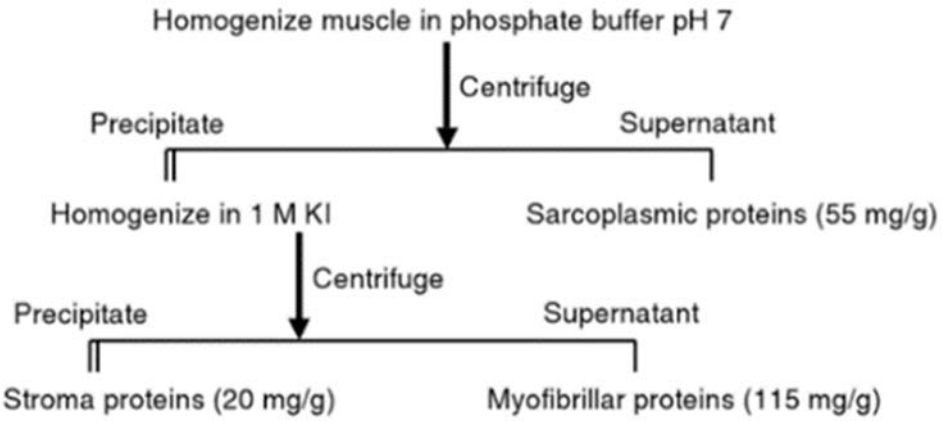


Fig 1 Fractionation of muscle proteins

The diagram in Figure 1 is a simplification of procedures necessary to accurately estimate the true content of each of these protein classes. An extensive study by Helander (1957) compared a number of different salts in extraction, and his results showed that the most efficient extractant was KI-0.1M potassium phosphate, pH 7.4.

3.2.2 Enzymatic Hydrolysis

The enzymatic hydrolysis of various biopolymers in foodstuffs, such as polysaccharides, proteins and pectins, is an important process which is used to improve the physical, chemical and organoleptic properties of the original food in relation to the nutritive value and the intestinal absorption characteristics. The enzymatic hydrolysis of protein is carried out under acid or alkaline controlled conditions without degrading their nutritional qualities for the acceptance in the food industry and broad spectrum of products can be produced for a wide range of applications (Shahidi *et al.*, 1995). Many protein hydrolysates are subjected to enzymatic hydrolysis to produce special diets for babies and sick adults. This is possible only when the hydrolysates are low in bitterness, osmotically balanced, hypoallergenic and

have good flavour. Most of these diets are composed of peptides and are rich in amino acids (Tello *et al*., 1994).

3.3 Isoelectric solubilisation/precipitation (ISP)

3.3.1 Overview

The isoelectric point (pI) of a protein is the pH where the net charge on the protein is zero. Proteins tend to aggregate and precipitate at their pI because there is no electrostatic repulsion keeping them apart. Proteins have different isoelectric points because of their different amino acid sequence, and therefore, they can be separated by adjusting the pH of a solution. When the pH is adjusted to the isoelectric point of a particular protein it precipitates leaving the other protein in the solution (McClements 2013). ISP processing may be a useful technology to recover nutritious and functional protein isolates for development of nutraceutical food products destined for direct human consumption from underutilized fish resources such as krill and invasive nuisance species like Asian carp, fish and meat processing by-products (i.e., frames, heads, etc.), and other low-value animal protein sources that otherwise may be discarded. The effect that pH has on water solubility of muscle proteins has long been known (Meinke &Mattil, 1973; Meinke, Rahman & Mattil, 1972); however, the process of separating proteins and lipids using pH-shifts was proposed by Hultin and Kelleher (1999, 2000).

3.3.2 Previous attempts

The ISP process efficiently recovers high quality protein isolates in terms of nutritional quality and functional properties from sources difficult to process such as krill, fish, chicken, and beef processing by-products (Chen & Jaczynski, 2007a; Gigliotti, Jaczynski, & Tou, 2008; Jaczynski, 2010; Nolsoe & Undeland, 2009; Taskaya, Chen, Beamer, *et al.,* 2009b; Taskaya, Chen,& Jaczynski, 2009c). While proteins are dissolved, they are separated from lipids and other insoluble materials such as skin, bones, scales, etc. Following separation, a subsequent pH-shift induces protein precipitation (Fig. 2 and 3).

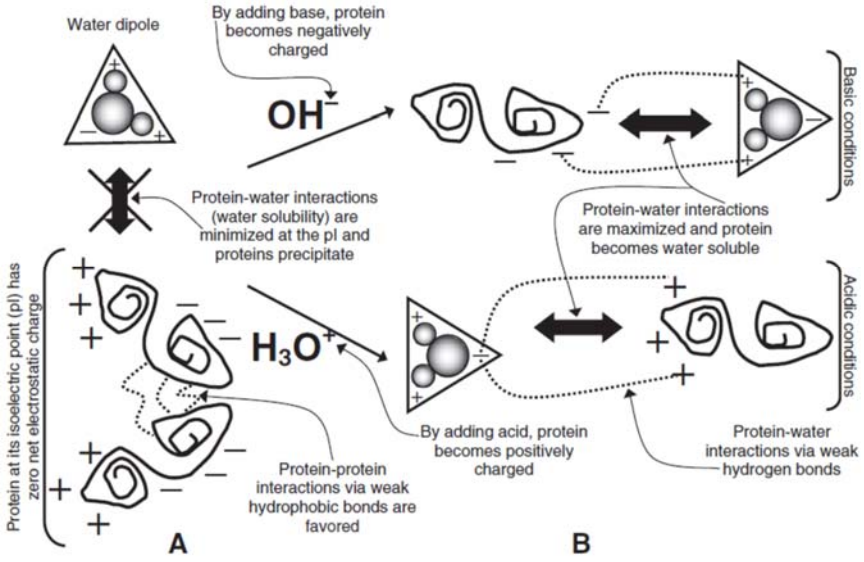


Fig. 2 A protein at its isoelectric point (pI) has a zero net electrostatic charge. A. At its pI, protein–water interactions are at its minimum, while protein–protein interactions via weak hydrophobic bonds are at its maximum, causing protein precipitation. B. Protein–water interactions prevail under acidic or basic conditions far from the pI, resulting in protein solubility in water.
Source: adapted from Gehring et al., 2011.

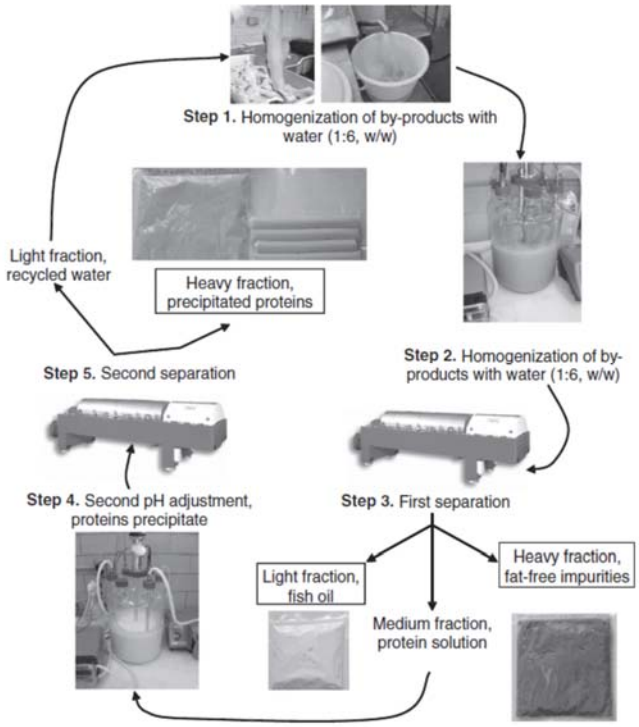


Fig. 3 Diagram of the isoelectric solubilisation/precipitation technology with concurrent oil separation proposed for fish processing by-products.
Source: adapted from Tahergorabi, Hosseini, & Jaczynski, 2011b.

During the first pH-shift meat proteins are dissolved at either acidic or alkaline pH. Centrifugation is typically used to separate lipids by floatation (light fraction) and other insoluble materials (bones, skin, and stromal proteins) by sedimentation (heavy fraction) from dissolved meat proteins (middle fraction). The protein solution (middle fraction) is then adjusted to pH 5.5, the isoelectric point (pI) of meat proteins (Fig. 3). At the pI proteins lose their water solubility; and therefore, precipitate out of the solution. Finally, the precipitated proteins are separated from the process water (i.e., de-watering) typically by centrifugation or ultrafiltration. Since proteins dissolve at very high (pH 10.5–13.0) or very low pH values (pH 1.5–3.0), ISP processing results in mild microbial reduction (Lansdowne, Beamer, Jaczynski, & Matak, 2009a, 2009b). ISP is a useful technology to extract protein isolates from muscle food processing by-products and low-value meat that could be used as a main, bulk ingredient in nutraceutical/functional food products; and therefore, be destined for direct human consumption.

3.3.3 Comparison with other methods

Protein extraction methods can vary widely in reproducibility and in representation of the total proteome, yet there is limited data comparing protein isolation methods. Different extraction procedures have been established and the commonly used methods include chemical and enzymatic hydrolysis extraction. Both of these three protein extraction procedures have their advantages and limitations, depending on factors such as the type of muscle tissue, protein yield and downstream analysis of the extracted proteins.

The most common extraction method used for animal proteins is the buffer extraction method. Separation of muscle proteins soluble in neutral salt solutions of ionic strength lower than 0.6 into two fractions can be achieved by diluting the sale extract with distilled water (volumetric ration of 1:10 to 1:12) and keep the mixture overnight in a refrigerator. Another way to extract both proteins is to run two separate muscle protein extractions with extractants of ionic strength of 0.8 and 0.05 and additionally to precipitate the proteins in extracts with 5% trichloroacetic acid, followed by separation of the liquid by filtration. No myofibrillar protein extraction should be carried out after the preliminary extraction of sarcoplasmic proteins with low ionic strength buffer. Such a procedure would result in reduced solubility of myofibrillar proteins, most probably due to their hydrophobic interactions. Therefore, using buffer extraction will accommodate extra preliminary extraction and type of salt, salt concentration usage could be a concern in food industry.

The pH-shift method is used in the food processing industry for separating proteins. Because of its outstanding functional properties, protein isolates can be used as a protein additive. It has been found that functional proteins can be extracted from muscle using acid-or alkali-aided solubilisation and recovered with ISP. A higher level of proteins can be extracted with high compared with low pH because of higher protein solubility at high pH. From the perspective of developing functional protein ingredients, the pH-shift method can efficiently recover the muscle protein, including myofibrillar protein and the water-soluble proteins. The recovered proteins have a good colour and excellent functional properties, and are safe for consumption. As a result, the use of pH-shift as a method for recovering muscle protein is worth further evaluation.

Enzymatic hydrolysis of the by-products is another method of protein recovery from animal processing industries (Bhaskar *et al.*2008). The isolate is mostly used for production of dried nutritional, flavouring and emulsifying ingredients. In the hydrolysis process, only a minimum amount of water is added and temperature is raised to activate (55-60°C) the enzymes. By using different enzymes and by controlling temperature, time and pH, different end-products can be produced.

Advantages of enzymatic hydrolysis:

- Very high yield of Nutritional Protein
- High Protein and High Nitrogen
- Contains all Essential Amino acids
- Soluble

Disadvantages of enzymatic hydrolysis

- Low emulsification activity index and emulsion stability due to extensive hydrolysis.
- Poor product quality
- Lack of functionality
- A rancid odour/taste

3.3.4 ISP isolated protein nutritional value

In general, the ISP-recovered protein isolate contains 87–95% crude protein, 1–5% lipid, and 2–6% ash. Proximate composition of protein isolates recovered using ISP processing varies depending on protein source, pH as well as the type of acid and base used during ISP. For example, when ISP processing was used to recover protein isolate from whole gutted silver carp and carp fillets, crude protein was concentrated to between 89 and 95%, with the essential amino acid content higher than in the starting material (i.e., whole gutted carp and carp fillets) and control (Alaska pollock surimi) (Taskaya *et al.*, 2009b).

Protein isolates recovered using ISP processing is of high quality, meaning they contain all of the nine essential amino acids (EAAs) in adequate amounts. For example, protein isolate recovered from fish by-products (frames and heads) using the ISP process contained adequate concentrations of the EAAs to support human or animal health (Chen *et al.,* 2007). Because of its high nutritional quality, egg protein is typically used as a reference protein when determining biological value (BV). The quality of protein isolate recovered by ISP was not of as high quality as egg protein; however, the ISP-recovered protein isolate had higher quality than soybean protein concentrates and similar quality to the milk protein casein. It should be noted that protein isolate recovered from fish processing by-products with ISP had a similar concentration of lysine to whole egg, which is important because lysine is often considered a limiting EAA. ISP-recovered krill protein isolate had considerably more EAAs than recovered fish protein isolate. The concentrations for most EAAs were closer to those of whole egg, with lysine values exceeding that of whole egg (Chen *et al.*, 2009).

Although ISP processing efficiently recovers high quality protein isolates, attempts at commercializing food products developed from the ISP-recovered protein isolates have been limited. Results from laboratory-scale product development demonstrate the potential for the use of ISP-recovered protein isolates from seafood processing by-products and low-value meat as a main, bulk ingredient in the development of nutraceutical food products.

3.3.5 ISP isolated protein application

Considerable amount of information exists in the literature describing potential applications for a variety of protein isolates. This review focuses only on literature where ISP-recovered protein isolates were used. Early studies sought to determine whether or not protein isolates recovered with ISP retained their functional properties. In one such study, Taskaya, Chen, Beamer, and Jaczynski (2009a) recovered muscle protein isolates with ISP from whole gutted silver carp and carp fillets and compared their functional properties to Alaska pollock surimi. Functional additives such as potato starch and transglutaminase (TGase) improved texture of ISP protein gels that were comparable to Alaska pollock surimi gels. The ISP-recovered protein isolates retained their functional properties (thermal denaturation, viscoelasticity, and texture properties) performing comparably to Alaska pollock surimi.

Retention of functional properties is critical if the protein isolate is to be used in the development of restructured food products (Taskaya *et al.,* 2009c).

Based on the findings of Taskaya et al. (2009a, 2009b), Tahergorabi, Beamer, Matak, and Jaczynski (2012a) formulated a fish stick product using ISP protein isolate recovered from whole gutted trout with added nutraceutical ingredients. The main, bulk ingredient providing technological functionalities such as gelation, water holding capacity, and fat binding/emulsifying was the ISP protein isolate, while nutraceutical properties were provided by ω-3 PUFAs oils (flaxseed, algae, fish, and krill oils) and KCl-based salt substitute. This study revealed that heat-induced protein gelation as assessed with dynamic rheology was improved by the addition of ω-3 PUFAs, while it was not affected by salt substitute. Whiteness was also improved except when krill or algae oil were added. The sodium content was greatly reduced when salt substitute was used. However, residual sodium, likely from NaOH and HCl used in the ISP process, was retained in the ISP protein isolate and therefore, in the nutraceutical fish stick.

Another approach to improve health aspects of food products is to reduce their sodium content. Therefore, sodium was reduced in the ISP-recovered protein isolate by substituting NaOH with KOH in the ISP process (Tahergorab *et al.,* 2012b). Then, nutraceutical ingredients (dietary fibre, ω-3 PUFAs, and KCl-based salt substitute) were added to the protein isolate from striped bass recovered with the ISP using KOH, subjected to cold- or heat-gelation, and compared to commercial Alaska Pollock surimi gels. Texture properties of the ISP gels were generally better than surimi gels. Although the ISP protein isolate and surimi developed similar final gel elasticity, they had different gelation pattern. The colour of the ISP gels was whiter and brighter than surimi gels; however, this was due to the addition of titanium dioxide, a common food whitening agent. A reduction of sodium and simultaneous increase in potassium content in the ISP gels was also achieved. Although cooking of the ISP gels generally increased lipid oxidation, TBARS values were much below rancidity levels. These trials indicate that KOH can replace NaOH in the ISP process to recover fish protein isolates. The ISP protein isolates in combination with nutraceutical ingredients such as dietary fibre, ω-3 PUFAs, and reduced sodium can be used to develop nutraceutical seafood products targeting reduction of the diet-driven cardiovascular disease (CVD).

Chicken meat has gained popularity with consumers for several reasons and currently it is a major source of dietary animal protein worldwide. However, the increased production of value-added products inevitably generates a large quantity of chicken-meat processing by-products that contain residual meat left on bones, skin, etc. (Dawson *et al.,* 1988). Therefore, these chicken processing by-products present a challenge and opportunity to develop a technology to recover proteins and other nutrients for development of food products for direct human consumption instead of rendering them for chicken meal and subsequent recycling as animal feedstock.

Tahergorabi, Sivanandan (2012c) demonstrated that functional protein isolate can be recovered from skin-on bone-in chicken drumsticks (i.e., model dark chicken-meat processing by-products) with ISP. The ISP-recovered chicken protein isolate formed good quality gels with colour and texture similar to chicken breast meat gels (control) (Tahergorabi *et al.,* 2011a).

These bench-top trials demonstrated a feasibility to develop nutraceutical food product prototype made of chicken protein isolate recovered with ISP from low-value dark chicken meat processing by products. The prototype was enriched with CVD-beneficial nutrients, while retaining quality attributes comparable to respective products made of chicken breast meat.

3.3.6 Challenges

ISP is a technology that efficiently recovers functional and nutritious protein isolates from sources difficult to process through conventional means. These sources include under-utilized and difficult to process aquatic species such as krill and carp, fish processing by-products such as frames and heads, as well as low-value meat sources such as dark chicken meat processing by-products. Although high quality protein isolates can be efficiently recovered with ISP, the key challenge is to develop marketable food products for direct human consumption. Cardiovascular disease (CVD) has been the number one cause of death in several developed countries for many years. Therefore, developing nutraceutical food products tailored to improve cardiovascular health may be a potential venue for successful application of the ISP protein isolates. Nutraceutical food prototypes whose main, bulk constituent are the ISP protein isolates enriched have been developed and tested at a laboratory scale. It is suggested that storage stability as well as nutritional assessment studies should be conducted. In addition, marketing and financial feasibility studies are recommended.

4 IP review

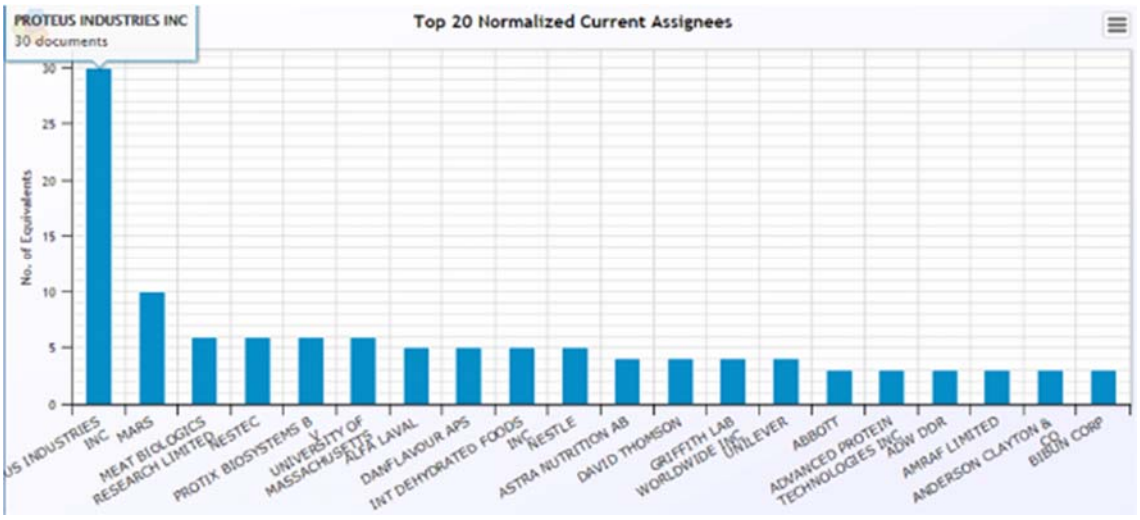
A patent search was performed on IP online search database, Relecura (www.relecura.com). Two search strategies were used to find patents and patent applications relating to protein extracted from meat, and products using extracted animal protein. Results from each search are discussed in general below, with more data in Appendix 2.

4.1 Search 1: Protein extracted from meat

The first search methodology employed the use of the following International Patent Classification (IPC) or Cooperative Patent Classification (CPC) codes:

A23J 1/02: HUMAN NECESSITIES > FOODS OR FOODSTUFFS; THEIR TREATMENT, NOT COVERED BY OTHER CLASSES > PROTEIN COMPOSITIONS FOR FOODSTUFFS; WORKING-UP PROTEINS FOR FOODSTUFFS; PHOSPHATIDE COMPOSITIONS FOR FOODSTUFFS > Obtaining protein compositions for foodstuffs; Bulk opening of eggs and separation of yolks from whites > from meat

The search returned ~480 patent families. The top 20 assignees based on numbers of equivalents[1] are:



[1] An equivalent refers to a set of patent filings with the same priority documents, representing a single invention

The results were reviewed to determine which hits were directly related to protein extracted from meat. The results were grouped generally into 5 categories. Descriptions of each category, with examples of each are described below Figure 10 below shows the distribution of relevant hits across the 5 categories.

- Enzymatic hydrolysis (examples: WO2014083215; CN102106873)

Protein hydrolysis is the breakdown of protein into smaller peptides and free amino acids. Patentee Specialty Enzymes has patent applications directed to the use of animal-based proteases like trypsin, plant based proteases like papain and a large variety of microbial source proteases.

- Chemical hydrolysis (examples:PL219629 and FR2795919)

Using strong acid solution to hydrolyse meat/or meat by-products to extract peptides with improved solubility.

- Organic solvent extraction (examples: WO2008146942 and JPS60118148)

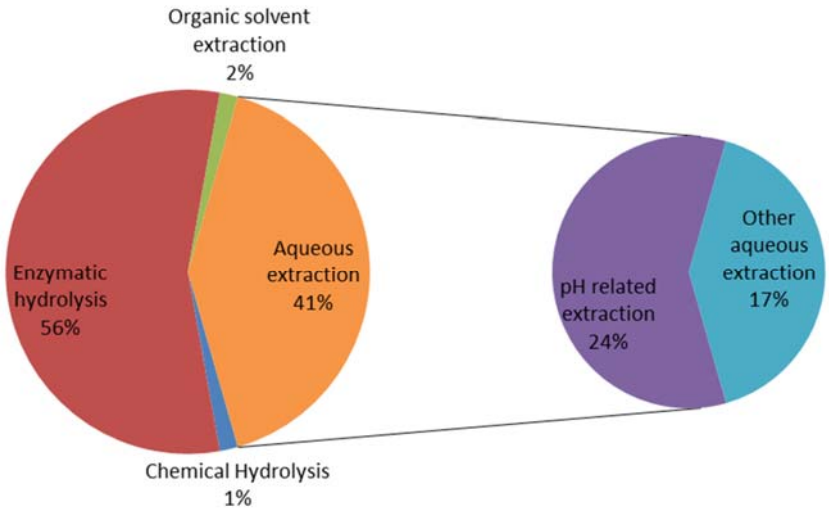
A suitable extraction buffer could be prepared to solubilize proteins in meat. Organic solvents were used to extract protein from meat, which includes alcohol, phenol. But the extracted proteins have low solubility due to denaturation induced by solvent buffer.

- Aqueous solutions extraction (examples: RU252454-buffer extraction and EP0848911-pH shift)

Aqueous solution extraction is one of the most common methods used to extract protein from meat. Due to the high solubility of muscle protein, it can be easily achieved by neutral pH value. The buffer used in extraction is considered as environmental friendly. Using acid or alkaline pH assists with the selective recovery of the solubilized protein.

- Ultrasonic extraction (examples: KR100965479 and RU2204910)

Sonication uses high intensity sound waves to disrupt cells, and extract proteins from tissue that has broken open. Sonication works with varying efficiency, depending on the cell type. For instance, cells walls can be a lot more resistant to sonication disruption than cell membranes alone. The major advantage of sonication is its adaptability to different sample volumes and ease of use. tubes on ice between each sonication cycle, which can really eat



up time.

Fig 10: the distribution of methods used to extraction meat protein

Among the isoelectric precipitation-based methods, alkali extraction has been largely carried out. Using isoelectric point for protein precipitation has been stated in several patents (US9161555; US7033636; US9066562; US8021709; KR20130066920; US20120276277; CA2834241; BE1016630A3; CN1620887; FR2247166; JPS5743643; JPS5743642;

PT94729; EP0848911). Multiple pH adjustments for protein extraction also included EP3117717; WO2010136894; JPS575646; CN101828627; EP2108266; JPH0331414. Additional processing as thermal (US4473589), filtration (GB174433), organic solvent (US4260644) are also conjugated with pH for an optimal result.

The earliest patent was published in 1909, and more than 60% of pH related patents were filed after 2000. The distribution of patents related to pH extraction over these years is shown in Figure 11 (note the final column relates to fewer years than the earlier columns, skewing the result).

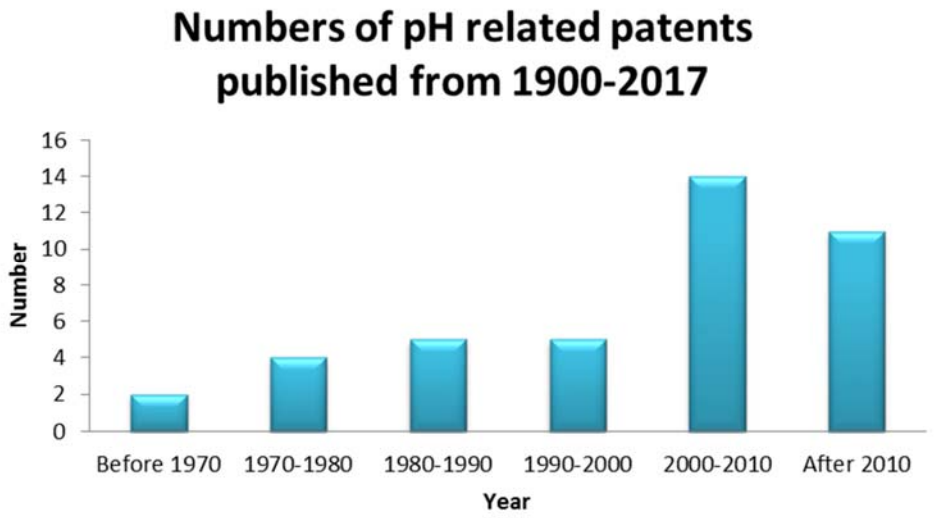


Figure 11 Distribution of pH extraction related patents published since 1900

Notably, Herbert Hultin and Stephen Kelleher of University of Massachusetts have been issued a number of patents related to the “pH-shift” method for protein extraction and compositions. Kelleher has worked with Advanced Protein Technologies and later founded Proteus Industries Inc. (http://www.proteusindustries.com/). The company has a portfolio of protein extraction and compositions. Other entities that have filed in this space include MPF INC, SCHLUMBERGER CIE N, GENESIS GLOBAL LIMITED, AGRICULTURE AND FOOD DEV AUTHORITY (TEAGASC), BUMBLE BEE FOODS LLC, DMV INTERNATIONAL NUTRITIONALS GMBH, LILLY CO ELI, OBSHCHESTVO S OGRANICHENNOJ OT, UNIV DALIAN FISHERIES, BIBUN CORP, UNIV ALBERT. Some representative patent documents are listed in Appendix 2.

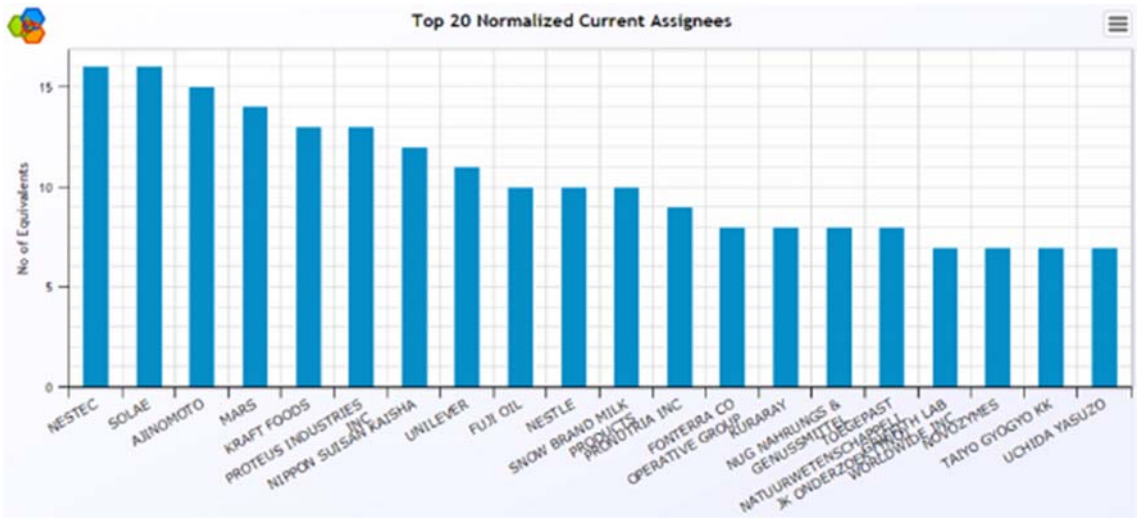
4.2 Search 2: Products using extracted animal protein

A further patent search was performed on Relecura using a combination of the following International Patent Classification (IPC) or Cooperative Patent Classification (CPC) codes:

A23J 3/04: HUMAN NECESSITIES > FOODS OR FOODSTUFFS; THEIR TREATMENT, NOT COVERED BY OTHER CLASSES > PROTEIN COMPOSITIONS FOR FOODSTUFFS; WORKING-UP PROTEINS FOR FOODSTUFFS; PHOSPHATIDE COMPOSITIONS FOR FOODSTUFFS > Working-up of proteins for foodstuffs > Animal proteins

AND the following keywords in Title/Abstract/Claims: meat OR "animal meat" OR "red meat*" OR "red-meat*" OR "redmeat*" OR beef OR cattle OR lamb OR mutton OR sheep OR goat OR pork OR muscle* OR muscular OR poultry OR chick* OR duck* OR turkey

The search returned ~700 patent families and revealed additional patent documents related to further processing of meat-derived protein for food products. The results were reviewed to determine which hits were directly related to products using extracted animal protein. The top 20 assignees based on numbers of equivalents are:



It is worth noting major international food companies Nestec, Mars, Kraft and Unilever are among the top assignees.

Some relevant patent documents, in particular related to meat protein processing, are listed in Appendix 2.

Of particular note from this search are the following examples:

- US20070092616 describes the use of proteins from meat, meat by-products and dehydrated meat material to develop high protein puffed snack products.
- US20050013917 describes an invention utilizing chicken protein to produce protein-rich tortillas.
- Notably, inventor “Musson” and his team invented three meat protein enriched food products for multinational food company “Mars”.
- Also, Solae LLC (a Dupont company) has two patent families related to high protein food applications.

5 Applications of protein isolate

5.1 Sports nutrition

Protein isolates as supplements are popular among trained athletes, particularly bodybuilders and strength athletes. The supplements are marketed as powders, premixed drinks, and bars. Obtaining protein from supplements may be more convenient because of portability and preparation. Protein supplements typically contain whey, casein, egg and soy proteins, egg whites and edamame.

Animal feed Proteins are an essential nutrient required for the proper nutrition of all animals. Animal protein supplement derived from muscle is also commercially available. However, whey proteins hold the largest market share of protein supplements. The significance of protein influences on the immune system, as antigenic factors and anti-nutrition agents, was also stressed, in addition to animal nutrition effects.

Sources of protein for animal feeds are many and varied, with considerable opportunities for further diversification and substitutions.

5.2 Human Food Ingredient

Protein isolates are the acceptable ingredients for dairy application due to their fine particle size and dispensability, emulsification, emulsion stability, colour and flavour are critical in dairy application. Isolates (especially soy proteins) are being used to fortify all type of pasta products such as macaroni, spaghetti, to improve the nutritional value etc (Sipos 2013). Soy protein is regarded as textured protein products use both in meat and vegetarian meat analog industry and thus, has good water holding capability (Riaz 2006).

The problem of poor flavour, mouth feel, texture, dryness and flavour associated with the use of soy flour and soy concentrate above 10% has been resolved by using soy isolate in meat loaves, sausage-type products for their emulsion-stabilizing effect, gelation, and moisture retention and improved effects on texture (Kinsella 1976). It is often used as meat extenders in comminuted meat products such as patties, fillings, meat sauces, meat balls, etc (Berk 1992). The firm-forming ability of soy protein isolates is important in meat products. When heat and pressure are applied the protein films fuse together to form a firm, continuous, textured mass that can be sliced and used as meat substitutes. They also vary in their ability to form gels. Some are designed to form a gel while others will not form gel at 14% solid content. Different protein products such as whey protein, soy protein isolates, wheat gluten, rice bran protein, peanut protein, and cottonseed proteins where investigated for film development (Rhim 1998). Soy proteins are being used to fortify all types of pasta products such as macaroni and spaghetti, to improve the nutritional value (Sipos 2013).

Protein isolates are important sources of protein with high lysine content. Isolates from different legumes varied slightly in physiochemical and thermal properties. They are used as proteaceous ingredients in many food products such as salad dressing, meat products and dessert. Whey proteins are mainly used in beverage applications, due to their health benefits (Kudre *et al.,* 2013).

In food systems, myofibrillar proteins exhibit functional properties that are critical contributors to the final food product quality. These functional properties include gelation, water holding capacity, and fat binding/emulsifying which affect final quality, and therefore, consumer acceptability. The functional properties and potential applications for myofibrillar proteins are impacted by source species (terrestrial vs. marine, cold water fish vs. warm water fish, etc.), age and season of harvest, how fresh the source species is, and also processing parameters such as protein concentration, pH, ionic solubility, and temperature (Suzuki, 1981).

5.3 Pharmaceuticals

Protein isolates are used in drug and gene delivery systems as protein-based nanocarriers. Extended applications include use in controlled delivery, as a film coater, as hydrogels, composites, albumin-based nanoparticles, microparticles and as beads. Some examples are:

- Whey proteins used as hydrogels, nanoparticle systems for encapsulation and controlled delivery of bioactive compounds (Yuko et al., 2008)
- As anti-hypertensive use, like genetically modified soybean seeds accumulating novokinin (Esmaili et al., 2011)
- As solubility enhancer of curcumin in the food industry due to protein–micelle structure (beta-casein), acting as a nano vehicle (Silva and Malcata. 2005)
- As vehicles for bioactives, like milk proteins
- As a source of bioactive peptides, e.g., casein-derived four main bioactive peptides act on the cardiovascular system, nervous system, immune system and nutrition system (Silva and Malcata. 2005)
- As a novel antifungal, e.g., Pisumin proteins obtained from Pisum sativum. Sugar snap pea legumes (Ye et al., 2005).
- In microencapsulation, e.g., vegetable proteins – soy proteins, pea proteins, wheat proteins, rice proteins, oat proteins and sunflower proteins (Yuko et al., 2008)
- As pest control: proteinaceous cysteine proteinase inhibitor, an insecticidal protein found in pulses used to control the proteolytic activity of endogenous digestive cysteine proteinase in the mid-gut of some insects

5.4 Animal Feeds

5.4.1 Soybean

Soybean remains the most important and preferred source of high quality vegetable protein for animal feed manufacture. Soybean meal, which is the by-product of oil extraction, has a high crude protein content of 44 to 50 percent and a balanced amino acid composition, complementary to maize meal for feed formulation. A high level of inclusion (30-40 percent) is used in high performance monogastric diets.

A measure of success of this crop is the increase in production of 50 to 60 percent between 1985 and 2000, with most grown in the United States, Brazil and Argentina. Over half of the crop is now, however, genetically modified (GM) mainly for herbicide tolerance. The potential of soybeans for further nutritional quality enhancement was emphasized by Hard and there are prospects for considerable feed benefits, assuming acceptance of GM sources in the marketplace. Currently, Argentina and Brazil are reported to export 60 percent of their

production and the USA about 16 percent. The market for non-GM soya seems to be growing and may be increasingly important in the future. In the European Union soybean dominates the protein supply for animal feed and the ban on meat and bone meal has resulted in further imports, reportedly of up to 1.5 million t in 2001.

5.4.2 Other oil meal crops

There are many different potential oil crops in addition to soybean, each with strengths and weaknesses for protein meal supply. Local adaptation to growing conditions and local availability provide distinct advantages for feed production in many developing countries. A continuous supply of protein meal of known quality can be made available, as is the case with palm kernel cake, the by-product of palm oil production (e.g. in Malaysia and Indonesia).

Prospects continue to be good for future oil meal crop production (FAO, 2002). Global projections show increasing demands for vegetable oils of 2.1 percent per annum for the next 20 years, and a significant increase in demand for oil meals and cakes. Predictions of future land use suggest that the area of oil crops will increase substantially in some developing countries. Oil palm, sunflower and oilseed rape, in addition to soybeans, will dominate and provide much of the future increase. Currently, the major net exporters in the developing world are Malaysia, Indonesia, the Philippines, Brazil and Argentina, but more oil and protein meal may be retained in future years for their own domestic use.

To what extent such crops as oil palm, coconut, sunflower, sesame, crambe or cotton (seed) can be utilized for meal inclusion in animal feeds depends to a large extent on what price the processor is able to obtain for the extracted oil. With the exception of soybean, the demand for these particular meals is markedly influenced by their vegetable oil price. This is important for the profitability of intensive livestock enterprises such as poultry production, working on low margins. Protein-rich meal inclusion from oilseed crops currently remains the key; however, to high quality feed supply for intensive enterprise performance.

5.4.3 Legumes

Legumes are a traditional source of plant proteins for animal feed and their production can provide a range of benefits both on farms and for feed manufacturers. The exploitation of soybean is a classic example of successful development and use. Peas, beans and lupins are exploited as grain crops in temperate farming systems and their production for home-grown protein supply is encouraged (and supported) in the European Union to reduce dependency on imported proteins. Each has strengths and weaknesses for quality protein provision. Lupins, for example, can yield high levels of crude protein but produce grain which is often low in lysine and sulphur-containing amino acids.

5.4.4 Animal Protein Sources

Fish protein isolates/concentrates and hydrolysates have been used in animal feed stocks and pet food as a protein source (Kristinsson and Rasco, 2000). Following fish meal, fish silage is the second largest feed product made from underutilized low-value fish and fish processing by-products.

6 Market review

6.1 Snack market

Snack foods are typically designed to be portable, quick, and satisfying. Processed snack foods, as one form of convenience food, are designed to be less perishable, more durable, and more portable than prepared foods. They often contain substantial amounts of sweeteners, preservatives, and appealing ingredients such as chocolate, peanuts, and specially-designed flavours (such as flavoured potato chips). Traditionally almost solely eaten between regular larger meals of breakfast, lunch and dinner, there is an increasing trend to eat snacks as a meal replacement.

Snacks sales totalled USD$374B in the year ending March 2014-an increase of 2% year-over-year, according to Nielsen retail sales data (Nielsen report, 2014). Europe (USD$167B) and North America (USD$124B) made up the majority of worldwide snack sales, with sales flat in Europe, and growing at a rate of 2% in North America, compared to the previous year. While annual snack sales in Asia-Pacific (USD$46B), Latin America (USD$30B) and the Middle East/Africa (USD$7B) are significantly lower than in the other two regions, annual growth in these largely developing regions increased more over the past year—4% in Asia-Pacific, 9% in Latin America and 5% in the Middle East/Africa.

Snacks generally can be divided into salty snacks, fruit/vegetables, indulgent, refrigerated snacks, confections, bars and others. Different types of snacks are more popular in different parts of the world. For example, confections—which include sugary sweets, such as chocolate, hard candy and gum—comprise the biggest sales contribution to the overall snack category in Europe (USD$46.5B) and the Middle East/Africa (USD$1.9B). Salty snacks contribute more than one-fifth of snack sales in North America (USD$27.7B), refrigerated snacks comprise almost one third of snacks in Asia-Pacific (USD$13.7B) and cookies and snack cakes make up more than one quarter of total snacks in Latin America (USD$8.6B). Smaller, but fast growing segments are showing great potential for growth In terms of trends, the Nielson Report (2014) notes that while sweet snacks are generally the most popular in this category, sales of savoury snacks are rising. For example, sales of savoury snacks, which include crackers, rice cakes and pita chips, increased 21% in the last year in Latin America. Meat snacks, which include jerky and dried meat, grew 25% in the Middle East/Africa and 15% in North America. Refrigerated snacks, which include yogurt, cheese snacks and pudding, jumped 6.4% in Asia Pacific, while dips and spreads, which include salsa and hummus, rose 6.8% in Europe (Nielsen report, 2014).

The Nielsen Report (2014) also stated that over 60% of North American consumers ate chips/crisps as a snack in 2014. Consumers either eat snacks to satisfy hunger or to replace a meal. If North American respondents eat snacks for hunger satisfaction, 83% indicated that they want to satisfy a craving. Importantly for this report, of those participants eating snacks, 68% consider it to be moderately to very important for snacks to be high in protein (Nielsen report, 2014).

Considering the increasing trends for desirability of savoury snacks, together with recognition of high protein as important, we believe this presents an opportunity in the snacks market for a beef protein ingredient. This needs to be tempered with the other finding

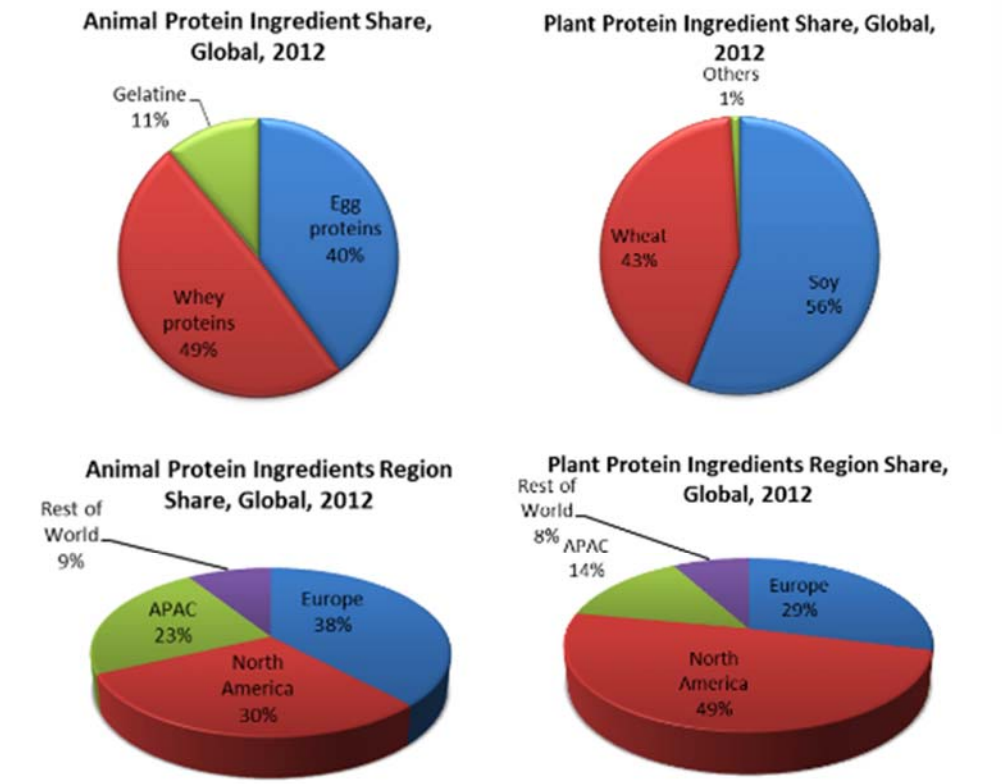
from the Nielsen Report (2014) that consumers prefer snacks that stick with the basics, rather than having introduced ingredients.

6.2 Protein ingredient market

The protein ingredient market, which includes concentrated, hydrolysed and isolated proteins, generated over USD$22B in 2015, of which about half was used in the food and beverage industry (Global Market Insights 2016). It is expected to reach USD$58.5B by 2022 at a CAGR of 6% (Marketsandmarkets.com 2017). It is difficult to break these numbers down further into market share held by isolated protein. However, one report valued the global whey protein market at USD$6.1B in 2016, of which it estimates whey concentrate makes up about 25%, hydrolysed whey protein about 60% and therefore isolated whey protein about 15%, which values the global isolated whey protein market at about USD$1B in 2016 (Mordor Intelligence, 2017).

The protein ingredients products space is highly fragmented, where there is a lot of competition for a limited number of end applications. This makes the importance of quickly identifying and addressing opportunities and threats critical. However, as noted above, the global market for protein ingredients is growing and expected to continue to grow, making it an interesting market place.

Protein ingredients are divided based on their origins: animal protein; and plant protein ingredients. Dairy proteins, egg proteins and gelatine are the three main animal protein sources, whereas dairy proteins represent a great proportion of animal proteins, including milk protein concentrate, whey protein isolate, whey protein concentrates, whey protein hydrolysate and casein. Soy protein, wheat protein, pea protein, rice protein, potato protein and canola protein are considered as plant protein. Protein fortification in food and beverage is a key imperative to meet global challenges in nutritional deficiencies (Shanahan C. 2013). Meat protein isolates have been in competition to plant proteins. With plant protein ingredients, the soy industry has enjoyed success by proactively positioning itself as a sustainable food/protein source.



Source: Frost & Sullivan analysis

According to the market research conducted by Frost & Sullivan analysis ion 2012, animal protein ingredients was taken up to 2.3 M Metric tons and showed a clear trend of increasing, whereas plant protein volume is 1.7 M Metric tons. The competition to plant protein is still carrying out, which indicates a shift to plant devised protein. Functional role of meat protein is minimal and nutritional role gains precedence. Meanwhile, price fluctuations impact market growth and stability. Low consumer awareness of non-soy protein restrains growth of other key plant proteins. The plant protein has advantages on cost competitiveness but is low in nutritional benefits.

6.3 Pet food market

Global pet food retail sales grew 4% in 2015, to a total of roughly US$70 billion, of which the dry food component was 22.59 million metric tons (Phillips-Donaldson 2016).

In Australia, the Pet Food Production industry is projected to post annualised growth of 2.9% over the five years through 2015-16, to reach $1.6 billion. Currently, a third of the industry's revenue is earned from exports and this segment is expected to grow steadily in line with increasing demand in Asian markets for quality pet food. Half the pet food industry's revenue is still earned via supermarket sales. Veterinary practices may have enjoyed the benefit of recent increasing demand for premium, private label and gourmet pet food products in the local market. This trend is expected to continue. Online pet food sales is the fastest moving segment in the industry, allowing small businesses to market gourmet and niche products direct to consumers. (Balzer 2017).

Key players in the market are BASF SE (Germany), Archer Daniels Midland Company (U.S.), E.I. du Pont de Nemours and Company (U.S.), Koninklijke DSM N.V (The Netherlands), and Ingredion Incorporated (U.S.). These companies are adopting growth strategies to gain global market share and increase geographical presence in emerging markets such as China, Brazil, and India.

6.3.1 Pet food trends

Awareness of benefits of high quality pet food, increase in pet population, and concern for the health of pets due to the rise in trend of pet humanization and increase in palatability of pet food are the factors which have resulted in the growth of pet food ingredients market. Stringent regulations supporting the use of high quality and nutritious food for pets and others animals is expected to drive demand over the next few years. Owners are focusing on buying functional and organic products for their pets to improve their health. Most of the manufacturers are reacting to the increasing demands of owners for products with nutritive and organic ingredients, thus driving the pet foods market.

The growth of certain pet food segments includes healthy treats, specialty pet foods and other more premium options. Increasingly, pet owners are moving from expectations of "high quality (for pets)" to "humanized"; that is, they desire pet food options that address the same

health concerns currently influencing human food production, such as unnatural preservatives and genetically modified ingredients—and they're serious about these preferences. Impressively, at least 55% of American and French pet owners claimed that, if they were on a strict budget, they'd be willing to give up chocolate in order for their pet to have high-quality food with the features that are important to them. Additionally, 43% of American pet owners with a Netflix subscription said they'd be willing to trade it for the same.

6.3.2 Protein trends in pet food

Protein is considered as the foundation for a quality pet food. When evaluating a pet food, real, named meat and fish protein sources are more preferable than processed or generic sources. Real named proteins, like beef, salmon, and chicken and not "meat", "poultry", or "fish", are the least processed of all the proteins and the ones that we recommend are the first ingredient in pet's food. Some of the more common named animal proteins include: chicken, beef, salmon, lamb, whitefish, duck, venison, bison, turkey and pork. The common sources of protein in pet food also include meal, by products, unnamed proteins and non-meat sources of protein such as corn, wheat, or pea protein.

The trends in marketing claims regarding the quantity of meat, type of meat, "need" for meat and the value of selecting specific meats in pet food may face significant challenges in the near future. The expanding demand for meats and novel proteins puts ingredient supply at the pinch-point for future growth and new product introduction. To overcome these challenges, there is a clear need to expand our perception of where these proteins come from, develop technologies to convert them into desirable pet foods, understand what the nutritional values are and communicate them to the market ethically, and do so in a safe and effective manner. In the latest Petfood Innovation Workshop 2016, novel proteins in pet food were a hot topic. The high-meat pet food trend has gone global.

6.3.3 Advantages and disadvantages of beef protein isolate vs whey protein isolate (as an example of other protein sources)

Price: Beef protein isolates are commercially available for AU$ 45 per kg whereas whey protein isolates are selling for AU$36 per kg

Nutritional benefits:

- **High purity:** Protein sourced from beef is higher in protein than whey based alternatives - the raw product contains up to 95% pure protein with zero fats and carbohydrates. These benefits makes beef protein isolate perfect for those wanting uncompromisingly clean protein.
- **High biological value:** 1 gram of protein always has an energetic value of 4 calories, regardless of the protein source. However, the body can't always use 100% of its protein intake to build muscle mass. The biological value is a measurement scale used to determine the percentage of protein the body effectively uses. The protein source with the highest biological value is the egg, since the body is able to use up to 94% of the amino acids that are found in this food source. The most interesting aspect about beef protein is that, provided that the ideal nutritional conditions are gathered, this protein source's biological value can be equivalent to the egg's. In turn, cow milk protein's biological value is, on average, 60%.

- **High absorption:** There is the fact that most sugars found in whey come from lactose, a type of sugar most people can't fully digest, or can't digest at all. Moreover, unlike whey that is digested in the intestine beef protein's digestion takes place in the stomach. This avoids the swelling sensation that is commonly associated to whey consumption.
- **Low allergenicity:** Plant proteins could induce severe allergic reaction to certain population, especially for children. For example, cow's milk allergy is one of the most frequent and severe allergies in children, affecting 1.9-2.8% of infants (Wal *et al.* 1995). Whereas meat protein allergy is very uncommon.
- **High nutritional quality:** Adding to the previously mentioned aspects, most beef protein formulas are further enriched with vitamins and minerals, thus improving their nutritional value.

6.4 Commercially Available Protein Products for Human Consumption

6.4.1 Existing meat/beef protein isolates as an ingredient

Several bioengineered beef protein isolates are commercially available. Examples are shown in Figure 4. All these examples employed a hydrolysis technique to isolate the protein from beef. They have been formulated as a highly anabolic muscle building protein.

Fig 4: A, MuscleMeds Carnivor Bioengineered Beef Protein Isolate; B, MuscleTech Platinum 100% Beef protein; C, Olympian Labs Beef Protein; D, Adaptogen Science 100% Beef

One company that provides hydrolysed beef protein isolate to the market is True Nutrition (https://truenutrition.com) (Figure 5). They make similar claims about their product that are made about the products shown in Figure 4, stating that "beef protein isolate has an amino acid profile that is comparable to standard whey protein. It is naturally high in the amino acids alanine, arginine, glutamic acid, glycine, and proline, and serves as a significant source of leucine, isoleucine, and valine, as well. A single

standard serving of 30 grams will contain an astounding 29.5 grams of protein, with zero carbs and less than 1 gram of fat. This gives the Beef Protein Isolate one of the highest concentrations of functional protein out of any of the materials that are currently available on the market … As a soy-free, gluten-free, and dairy-free protein powder, it is also a hypoallergenic source of protein for individuals that may suffer from any number of dietary limitations."

Figure 5: Grass-Fed Beef Protein Isolate, Truenutrition.

The price per kg for the above supplements ranges from about AUD$35-AUD$50.

6.4.2 Meat/Beef Snacks

Beef Jerky (salted and dried beef) is widely consumed as a form of beef snack around the world. On average, one ounce (about 28g) contains about 70 calories and one gram of fat, but delivers 11g protein. It has been considered as a natural substitute for protein shakes, powders and other carn meals.

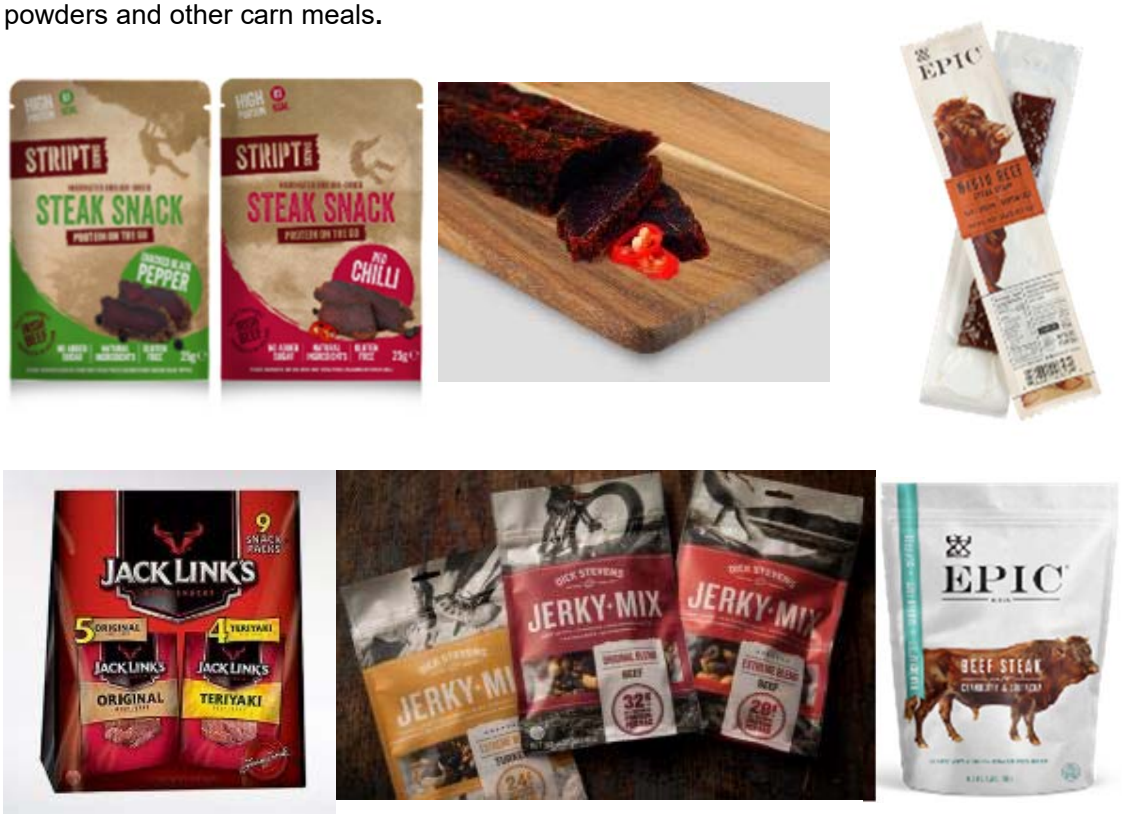


Fig 6: A collection of commercially available beef jerky products: 1. Stript Steak snack 2 Dick Stevens Jerky Mix 3 Epic Wagyu Beef Steak Strip 4, Jack Links Beef jerky 5 Epic Beef Steak Bites

Similar products are shown below in Figure 7, but in bar form, where pureed meat has been combined with dried fruits and grains to create protein meal bars. Note these products contain non-isolated protein.

Fig 7: Chief beef protein bar (77% Australian grass fed beef) and Epic beef protein bar (a savoury high protein snack, low in sugar, gluten free, and absent of both soy and dairy. It contains 11g protein in 43g of products)

Fig 8: Beef chips and Krave meat bars

Other dried meat products are also available on the market. One example is German company “NUD” which makes NUD Beef Chips (https://get-nud.com), which are made from 100% organic meat. NUD advertise that they can eaten from the packet, cooked as a topping (eg on pizza) or used in soup. NUD also claim that their “beef chip is also a great

and easy way to add protein to many meals". The claimed protein content in NUD beef chips is 63%. The price for this product is AU$6 for 30 g package. Another example is Krave Bars, from company Krave Jerky (www.kravejerky.com) which produce a range of bars combining meat jerky bars with fruit, seeds and grains..

6.4.3 Existing protein fortified snacks

One relatively new snack product space is protein fortified snack products. For example, Quest Nutrition (www.questnutrition.com) produce Quest protein bars and Protein Chips, both of which are protein fortified snack foods. Their protein is sourced from milk protein isolate and whey protein isolate as protein additives. There are 22 g protein in each serve (32 g) and selling for AU$3.3 per serve (Fig 8).

Fig 8: Quest protein chips and SimplyProtein chips

SimplyProtein (www.simplyprotein.com) also produce protein fortified bars and "chips" using pea protein isolates. Each packaging contains 50% protein and sells for AU$ 2.7 per serve

(33g).

Fig 9: Meat chips-Meat Chips contains more than 28% protein (chicken protein isolates) in each serve.

Offerings in the meat snack market using isolated meat proteins appears to be small. One example is US based “Meat Chips” (www.meatchips.com) which uses isolated chicken protein mixed into a corn meal masa to produce isolated chicken protein fortified corn chips. The US based company is selling the chips for AU$ 3.40 (75g).

6.5 Current status and future application of beef protein as a food ingredient

Currently, beef protein has been used as a protein source adding into food product, for example, snacks. In this way, the product will be carrying enriched protein content which delivers health benefits and additional nutritional value. The target market for beef protein enriched product could be bodybuilder, people who are prone to weight gain, people with a very sugary, carny, crappy diet and people in middle age.

Beef protein isolates extracted in native form should not been limited to use as protein supplement. There is a great opportunity to use beef protein as carriers for flavour enhancement. The example could be that protein act as carriers of gravy flavours – they could be carried by isolated beef protein powder as a “natural” ingredient.

7 Challenges and Innovation opportunities

7.1 Challenges

7.1.1 Extraction methods challenges

The most common method for preparing beef protein isolates is enzymatic hydrolysis – a method that is technically straight forward to perform. Hydrolysed protein products are high in solubility, but their protein functionality is significantly diminished by the enzymatic hydrolysis process. This is the key reason that restricts beef protein's application to purely protein isolate products. Therefore, there is a need of developing a novel, simplified, native meat extraction method to produce red meat proteins in original form with reasonable solubility while maintaining protein functionality. The extracted meat proteins in their native form are highly likely retaining their flavour and colour, which might need to be considered in future processing.

7.1.2 Food product design challenges

Unlike hydrolysed peptides, proteins are usually intolerant to processing conditions, which means they can easily denature when the condition changes, especially with heat. Also, proteins as a whole retain their functionality of emulsification, gel formation and etc. Therefore, the design of a protein enriched food product needs to be sophisticated and well-planned.

7.2 Innovation opportunities

Innovation around high protein products has been driven by the snack and dairy segments, with snack bars and yogurts accounting for the bulk of high protein launches. Protein enhancement is also present in specialty products, such as protein powders and foods for body builders, athletes wanting to increase muscle volume, and outdoor enthusiasts. However, work to spread protein consumption more evenly across a whole day, especially in more general food consumption markets presents an opportunity for breakfast products and snacks to include protein in their formulations to help achieve a more balanced intake of protein. Enhanced protein foods could also find favour among the growing ageing population.

Examples of potential uses for isolated beef protein include:

- Beef protein snack: Single-serve products that combine the "naturally healthy" virtues of beef and snacks would appear to be aligned with consumers' desires for convenience, health (from "naturally healthy" ingredients) and indulgent tastes. They offer the possibility of a sustained energy marketing message, a more filling eating experience and better absorption options.

- Savoury crunchy and/or chewy "sensory-intense" high-protein snacks could be developed for consumption 30 minutes prior to a meal in order to improve satiety and potentially decrease overall calorie consumption. Thickened high-protein beverages could be used for similar purposes as savoury snacks, but could be designed for individuals who find sweet foods more acceptable than savoury ones. A focus on convenient high- protein

breakfast foods is warranted, because a high-protein breakfast has been reported to decrease craving over the rest of the day. In addition, breakfast is the meal most likely to be skipped and where the least amount of protein is consumed.

- Animal Protein isolates from low value meat portions could be used in pet food to improve the nutritional value and texture for animal well-being. Current pet foods are typically formulated using protein from grains or grain by-product sources, such corn gluten meal, brewer's rice and wheat, rather than from meat sources. They may also contain poultry by –products, which typically consists of the leftovers unfit for human consumption, such as feet, beaks, undeveloped eggs, and intestines - everything but clean meat. Animal protein is hugely important to pets throughout their entire lives. High quality protein from actual meat sources contains important amino acids that pets need to thrive.

- Animal protein fortified food, such as protein fortified rice, could help counter protein deficiency in developing countries. Protein deficiency diseases occur in developing countries due to poverty as well as from lack of knowledge about nutritional requirements. Animal protein, for example beef protein, contains all essential amino acids; chronic inadequacy of any of these essential amino acids can also cause specific abnormal and harmful functioning. Benefiting from the nature of animal protein isolates, it has an inherently long shelf life and is therefore also suitable for long distance shipping.

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9 Appendices

9.1 Appendix 1 – More detail on Protein Isolates discussed in Section 2

9.1.1 Whey

Whey is the liquid by-product of cheese making which can further be processed into spray dried products such as whey protein concentrates (WPC), whey protein isolate (WPI) or whey protein hydrolysate (WPH) (Brucic *et al.*2009). It represents about 20% of milk proteins, which remain in the serum phase during processing of cheese (Kimberlee 2012).

During the production of whey protein isolates, significant amounts of fat and lactose get removed, as a result of which an individual with lactose-intolerant can safety consume these products. Whey proteins are widely used as ingredients in different foods (dairy, meat and bakery products) due to their unique functional and nutritional properties (Brucic et al. 2009). One specific aspect of whey protein that may be challenging for some formulations is sensitivity to heat. Consequently, the Dairy Research Institute, established under the leadership of America's dairy farmers through the dairy check off program, has supported a variety of research that aims to improve whey protein's performance in higher heat processing (Kimberlee 2012). Beta-lactoglubin and α-lactalbumin are the major whey proteins responsible for heat stability characteristics of ingredients such as WPC and WPI (Vardhanabhuti and Foegeding 2008).The relationship between sugar addition and heat stability of whey protein isolate have been studied by many researchers. Sucrose addition was found to increase gelation temperature and gel strength of WPI and bovine serum albumin (Rich and Foegeding 2000). Adding glycerol improves heat stability of WPI and decreases turbidity and protein gelation (Kulmyrzaev et al. 2000). Thermal denaturation temperature of WPI was also found to be increased by addition of sorbitol more effectively than glycerol (Paredes-Lopez 2006). Many food applications contain sugars or sugar alcohols, and their presence can help to improve the heat stability of whey protein ingredients by preventing the formation of large aggregates and providing better clarity in applications such as beverages (Kimberlee 2012).

9.1.2 Casein

Casein makes up about 80% of the proteins in cow's milk. It has a wide variety of food and industrial uses. Food uses include protein supplements, cheese, food binding agents, artist's paint medium, glue, plastics, fibre and also medicinal and dental uses. In its uses as a protein supplement, it tends to gel in the stomach, meaning it has an additional use as slowing nutrient release. Caseins are heat stable because they are proline rich.

There are four main subunits: s1 α, s2 α casein, β-casein and κ-casein, which make up 38%, 10%, 36% and 13% of the casein composition, respectively, and has a unique property to form films. s1 α casein is amphipathic due to the charge between the hydrophobic N- and C-terminals. It has 8 phosphorylated serine clustered with glutamine residues possessing calcium-binding sites and hence is Ca 2 + sensitive, 17 proline, 25 glutamine residues and no cysteine residues. Here, the Ca 2 + sensitivity means aggregation and precipitation in low ion concentrations. Caseins are heat stable because they are proline-rich, which interrupt alfa-helix and beta strands, resulting in the absence of disulfide bridges in the structure. It

has relatively little secondary or tertiary structure. These undergo proteolytic cleavage due their open structure imparted due by the high proline content. This characteristic, along with acid-soluble calcium–phosphate bridging, makes an excellent target-activated release mechanism for unloading drug in the stomach (Swaisgood H.E. 1996; Rosemberg M. and Young S.L. 1993).

9.1.3 Egg

Egg proteins are nutritionally complete with a good balance of essential amino acids which are needed for building and repairing the cells in muscles and other body tissues (Watkins B.A. 1995). Egg proteins are distributed in all parts of the egg, but most of them are present in the egg white and egg yolk amounting to 50% and 40%, respectively. The remaining amount of protein is in the egg shell and egg shell membranes. The major portion of egg yolk exists as lipoproteins, which can be separated by centrifugation into a plasma fraction (which remains soluble) and a granular fraction (which precipitates).

Lipovitellenin, lipovitellin, phosvitin, livetin, yolk immunoglobulins (IgY), and some minor components have been isolated and identified in egg yolk. Egg white contains approximately 40 different proteins. Well-known biological functions of egg white proteins are the prevention of microorganisms' penetration into the yolk and supply of nutrients to the embryo during the late stages of development. Most of the egg white proteins appear to possess antimicrobial properties or certain physiological functions to interfere with the growth and spread of invading microorganisms. Most of egg white proteins are soluble and can easily be isolated. Egg white proteins possess unique functional properties, such as antimicrobial, enzymatic and anti-enzymatic, cell growth stimulatory, metal binding, vitamin binding, and immunological activities (Ibrahim H. R., 1997).

9.1.4 Fish Protein isolates (FPI)

Fish protein isolate is a protein concentrate which is prepared from fish muscle without retaining the original shape of the muscle. It is not generally consumed directly, but used as raw material for production of other value added products. It is normally utilized as an ingredient for the production of value added products. It is still a good source of protein for the production of ready to eat fish products.

To solve the problem of utilisation of unconventional raw material (dark muscle fish, fatty fish) and also fish by-products (fish trims, fish frames etc.), a process was developed to economically develop functional protein isolates from these kinds of raw materials. This technology uses the pH dependant solubility properties of fish muscle proteins for their separation and recovery from other components of muscle that are not desirable in a final product. The overall processes involved are simple. The proteins of the muscle tissue are first solubilised. The solubilisation can be accomplished by addition water with alkali added to approximately pH 10.5 or higher, or with acid added to about pH 3.5 or lower. It is usually necessary to choose the pH at which the consistency of the solution decreases to a value that allows the removal of undesirable material. The mixture is then centrifuged, and due to density differences the oil rises to the top and can then be removed. Other insoluble impurities such as bone or skin are also sedimented at this stage. The muscle protein is then precipitated and collected by a process such as centrifugation (Shaviklo 2006).

9.1.5 Meat proteins

The meat protein ingredients discussed are a class of high protein products that are derived from either animal by-products or lean tissue components (Table 1), and they are used primarily as ingredients in meat and other food products.

Table 1 Major Sources and types of meat protein ingredients

Source	Ingredients
Lean tissue	Finely textured meat/poultry Mechanically separated meat/poultry Meat protein isolates
Bone	Gelatin (type B) Edible bone collagen (ossein) Bone collagen hydrolysates (stocks and broths)
Pig skin	Gelatin (type A) Stocks and broths
Beef hides	Gelatin (type B)
Poultry skin (chicken, turkey)	Concentrated collagen Stocks and broths
Collagen-rich tissues	Concentrated collagen Collagen hydrolysates
Blood	Blood plasma (liquid, frozen, dried) Whole blood (liquid and dried) Red cell protein (decolorized) Plasma transglutaminase

Sarcoplasmic, stromal and myofibrillar are types of meat protein. Sarcoplasmic proteins contain enzymes myoglobulin and cytoplasm. Collagen and elastin are the content of stromal proteins while myosin, actin, tropomysin and troponins are the content of myofibrillar proteins. Stromal and myofibrillar proteins, soluble in salt solutions, are used for making edible films and coatings. Collagen, a fibrous stromal protein extracted from connective tissue, tendons, skin, bones and the vascular system, is a waste product of meat processing. Collagen is a superhelical structure formed by a combination of three parallel alpha-chains,

and forms gelatine (Haug *et al.,* 2004). Collagen exposed to mild heat treatment under acidic or alkaline conditions forms gelatine (Badii and Howell, 2006).

Myofibrillar proteins make up to 55% of total muscle protein but contribute to more than 70-80% of water holding capacity and binding properties. Myofibrillar proteins are from myofibrils, the basic cellular unit of muscle tissue. It is salt soluble with an ionic strength of over 0.3. It is absolutely critical to processing properties, such as emulsion/batter products and heat-set gelation which control binding and texture. Myofibrillar proteins are composed of myosin (~55%), actin, troponin and tropomyosin (40-45%), desmin, synemin, alpha-actinin, nebulin and numerous structural proteins (1-5%). The majority of myofibrillar proteins can be isolated from comminuted muscle tissue using a salt solution of above 0.6 ionic strength, but they are not soluble at low ionic strength. Myosin is generally considered the most important because of its own characteristics including the long filamentous molecule, amino acid composition and large quantity in lean muscle. It is a 520 kDa hexamer consisting of two 220 kDa polypeptides referred to as myosin heavy light chain. The isoelectric point of myofibrillar proteins is 5.5. Myosin, the thick filament in muscle fibres, bind together with actin to form actomyosin during muscle contraction and relaxation. When dissolved in saline solutions, myosin and actin spontaneously form this complex, and therefore it is the main form of salt-soluble muscle protein. Thermal stability of myofibrillar proteins is influenced by pH and ionic strength. In general, myofibrillar proteins from terrestrial animals are more thermally stable than the proteins isolated from seafood sources. Interestingly, when myofibrillar proteins are isolated from cold water aquatic species (for example Antarctic krill, Euphausia superba), they are less thermally stable than warm water species (for example Asian carp). Other proteins are also important. Structural proteins can have a large influence on “release” of myosin/actin and “opening” protein structure to water.

Stromal proteins (connective tissue), primarily collagen, represent as 10-15% of total muscle protein, being the most abundant protein in the animal body (skin, sinews, tendons, etc.). Stromal proteins are not soluble in water regardless of pH, temperature, or ionic solubility. Stromal proteins hold fibres together and are therefore generally tough and inert. Increased crosslinking occurs as animal age increases, increasing toughness. Stromal proteins are not very valuable in processed meats because of their little binding ability. They shrink when heated to 140 °F and convert to gelatine at 160-180°F. But if heated when dry, collagen becomes very hard and impermeable. This character is important in the handling of collagen and/or natural casings. Meanwhile, collagen is highly resistant to enzymes, so enzyme tenderizers are generally ineffective. Chemically, collagen is considered as a unique protein with 33% glycine and 10% hydroxyproline. Therefore collagen is characterised by very nonpolar noncharged molecules with an isoelectric point of about pH 7.2. By far it is the only protein that contains large amount of hydroxyproline, therefore hydroxyproline measurement is the most common method used to determine collagen content in meat. The main application of collagen is to make gelatine, contact lenses, and pharmaceuticals. Higher concentrations (0.1M) of NaOH during extraction result in some losses of collagen and in its structural modification, for which reason its use is discouraged for this purpose. (ASCL, 2014)

Sarcoplasmic proteins represent 30% of total muscle protein contributing to 20% of binding ability. Their isoelectric points are generally between pH 6- pH 7. Sarcoplasmic proteins are soluble in solution of low salt concentration (ionic strength lower than 0.1), but not in water.

As these proteins have been sometimes extracted with pure water, they have become commonly referred to as “water-soluble proteins”. Most are relatively low molecular weight proteins. Sarcoplasmic proteins are rich in enzymes that aid in tenderization, post-mortem glycolysis and potential favour contributions. Myoglobin as the key protein in sarcoplasmic proteins is responsible for all meat colour variations. Where haemoglobin is used in blood as an oxygen carrier, myoglobin is used within cells (especially muscle cells) for oxygen storage. Depending on the oxidation state of the iron and whether the ligands are ionically or covalently bonded to it, the myoglobin changes colour. Because of the high concentration of myoglobin in muscle cells, these colour changes correspond to colour changes of the meat as a whole. Myoglobin is a “conjugated” protein, consisting of a typical amino acid protein chain and a non-protein heme molecule. The heme portion is responsible for all colours while the protein portion is colourless, but it is important to heme stability and affects colour indirectly. Sarcoplasmic proteins have been shown to form low quality gels, have low water holding capacity, and interfere with myosin cross-linking during formation of gel network; therefore, sarcoplasmic proteins may have a negative impact on food texture. (ASCL, 2014)

9.1.6 Others (e.g. insects)

Insect protein content is very high, with many species ranging above 60%. For example, Finke and others (1989) reported that the house cricket (Acheta domesticus), when fed to weanling rats, was superior to soy protein as a source of amino acids at all levels of intake. One of the most widely eaten insects in China is the silkworm, whose protein content is comparable to other animal proteins. The ratio of essential to nonessential amino acids is about 0.6 in silkworm pupae (Longvah *et al.*, 2011). Insect proteins are highly digestible (between 77% and 98%) (Ramos-Elorduy *et al.*, 1997), although insect forms with an exoskeleton have lower values, due to the presence of chitin. Chitin removal increases the quality of insect protein to a level comparable to that of products from vertebrate animals (DeFoliart 2002).

9.1.7 Peanut protein isolates (PPIs)

The peanut contains 26-29% protein with good nutritional quality. Peanut proteins are used for their functional properties (emulsification, forming) or for their nutritional properties in different food products. They are also used for human nutrition in developing countries to supplement cereals, beverages and skim milk. Peanut protein isolate can be prepared from the defatted peanut cake or powdered by macerating with high salt phosphate buffer, centrifuging and then supplementing the supernatant with $(NH_4)_2SO_4$ to 90% saturation. After centrifuging the pellet can be dialysed against distilled water overnight at 4^{o}C and freeze-dried (Mouecoucou 2004).

9.1.8 Soy protein isolates (SPIs)

Soy protein isolate is a common isolate. It has high protein content of about 90%. It is made of defatted soy meal by removing most of the fat and carbohydrates (Seyam 1983). Soybeans are crushed into oil and defatted meal. The meal is usually used as animal feed, while a smaller amount is further processed into food ingredients including soy flour, protein concentrate, protein isolates and textured protein (Kinsella 1976). Soy protein isolate is usually combined with other food ingredients such as vitamins, minerals and flavour in preparation of soy protein shake powder (Seyam 1983). Advances in food technology

resulted in the development of a variety of soy product such as concentrates, isolate and extruded-expanded products, this consequently has led to increased utilisation by technically developed regions of the world (Young 1979).

The problem of poor flavour, mouth feel, texture, dryness and flavour associated with the use of soy flour and soy concentrate above 10% has meant soy isolate is typically used in sausage-type products for their emulsion-stabilizing effect, gelation, and moisture retention and improved effects on texture (Kinsella 1976). Soy protein is regarded as textured protein products use both in meat and vegetarian meat analog industry and thus, has good water holding capability (Riaz 2006). It is often used as meat extenders in comminute meat products such as patties, fillings, meat sauces, meat balls, etc (Berk 1992). Many soy protein isolates have been developed for providing different functional or physical properties to meet the requirement of various food systems. Soy protein isolates form firm, hard, resilient gels, unlike soy flour and concentrates that form soft and fragile gels (Riaz 2006). The firm-forming ability of soy protein isolates is important in meat products. When heat and pressure are applied the protein films fused together to form a firm, continuous, textured mass that can be sliced and used as meat substitutes. They also vary in their ability to form gels. Some are designed to form gel while others will not form gel at 14% solid content.

The production of soy protein isolate involves solubilising the protein and carbohydrate at neutral or alkaline pH and the recovery of the solubilised protein, separation and optionally washing and neutralization before drying (Moure *et al.,* 2006).

Three steps are involved in the processing of soy protein isolates (SPI)
(1) The soy flakes are slurried with water under alkaline conditions (pH 6.8-10 at 27-66^{o}C using sodium hydroxide and other alkaline substances approved for food use) so that the protein and the oligosaccharides can dissolve into the solution. The protein solution is then separated from the insoluble residue by centrifugation,
(2) the supernatant containing the protein and sugars is then acidified to pH 4.5 (where the solubility of proteins is minimal), using hydrochloric acid (HCl). This leads to the precipitation of protein as curd, and
(3) the solubility of the precipitated protein is restored by neutralizing to alkaline pH of 6.5-7.0 after re-diluting with fresh water or being spray dried in its acidic form and packed in multilayer paper bags (Lusas *et al.* 1995, and Anon 2008).

9.1.9 Canola protein isolates (CPIs)

According to USDA 2010, Canola meal is the second largest feed meal after soybean meal. It has a good amino acid profile with a well balanced amino acid composition although it has found only marginal use in the food industry, due to the presence of anti-nutritional factors. The vast majority of canola protein isolates are prepared by alkaline extraction method followed by isoelectric precipitation. This extraction method generates high yield of nitrogen, but the isolate produced by this method has been found to have poor solubility and digestibility, this is most probably due to the nature of proteins constituent of canola meal which consists of an alkaline-soluble fraction that can be easily denatured during the extraction process. CPI thus possesses generally unacceptable food-functional properties including poor water holding, gelling and oil-binding, foaming and emulsification properties. Many studies have been carried out to modify the properties of this isolate e.g. by succinylation, acylation and enzymatic hydrolyses. (Alashi 2011).

9.1.10 Chick pea protein Isolate

Chick pea is the world's third largest pulse crop in term of area, grown mostly in West Asia and the Mediterranean region. It is one of the major vegetable proteins. Many functional properties of this protein isolate have been studied whereas information on gelation properties of chick pea protein isolate is scarce. Chick pea protein isolate dispersed with sodium and calcium salts showed different rheological behaviour at different ionic strength and pH. Increasing the ionic strength of dispersion could strengthen the gelation properties of CPI under acidic conditions, however reduced the elastic parameters of CPI at pH of 7.0 (Zhang 2007).

9.1.11 Cashew nut protein isolate

Cashews have considerable economic importance because their components have numerous economic uses. The kernel has high food value with about 40-57% oil and 21% protein content. A cashew kernel meal contains about 42% crude protein, a low crude fibre and 0.5% and 0.2% calcium and phosphorous, respectively, which is comparable to that of peanut composition, which has been used for peanut protein isolate and concentrate. Protein isolates and concentrates can be obtained from defatted cashew nut powder by both alkaline extraction-isoelectric precipitation (IP) and alkaline extraction-methanol precipitation (MP). Cashew nut protein isolates have water and oil absorption capacities of 2.20ml/g and 4.42ml/g respectably, emulsifying stability index (447%), foam capacity and stability (45% and 55%, respectively), and low gelation capacity of (13.5%) (Ogunwolu 2009).

9.2 Appendix 2 – Highlights from Patent Search 1 – Protein extracted from meat

Publication Number	Title	Abstract	Current Assignees	Inventors	Family Members
US6136959	High efficiency alkaline protein extraction	A process for isolating edible protein from animal muscle by solubilizing the protein in an alkaline aqueous solution is disclosed.	University of Massachusetts	HULTIN HERBERT O|KELLEHER STEPHEN D	[NA]
EP0848911	Process for isolating a protein composition from a muscle source and protein composition	A process is provided for isolating a protein component of animal muscle tissue by mixing a particulate form of the tissue with an acidic aqueous liquid having a pH below about 3.5 to produce a protein rich solution. A protein rich aqueous solution is separated from solids and lipids, including membrane lipids. The protein rich aqueous solution can be treated to effect protein precipitation, followed by protein recovery.	ADVANCED PROTEIN TECHNOLOGIES	HULTIN HERBERT O|KELLEHER STEPHEN D	CA2217669A1|CA2217669C|CA2301854A1|CA2301854C|CA2514342A1|EP0848911A2|EP0848911A3|EP1019433A1|EP1019433A4|EP1019433B1|EP1597268A2|EP1597268A4|WO1999011656A1|WO2004073415A2|WO2004073415A3|AR013855A1|AR016646A1|AT330965T|JP2001514262A|AU754892B2|AU2004212884A1|JP2006518378A|BR9705105A|BR9705105B1|BR9813010A|JP3152291B2|JP3554845B2|CN1071100C|CN1189300A|CN1203086C|CN1277615A|CN1751062A|JPH10179038A|DE69727039D1|DE69727039T2|DE69835022D1|DE69835022T2|DK1019433T3|ES2213793T3|ES2270528T3|ID19203A|ID24817A|IL121894A|IL121894D0|IL136802A|IL136802D0|IL134699A|IL134699D0|IN184327B|IN187725B|IS2015B|IS2595B|IS4589A|IS5386A|MX9708100A|MY129210A|MY123177A|NO20000978A|NO20000978D0|NO316051B1|NO317583B1|NO974671A|NO974671D0|NZ328927A|NZ503150A|NZ541447A|PL189495B1|PL198138B1|PL322703A1|PL339667A1|PT1019433E|RU2005125876A|RU2225694C2|RU2252601C2|TW391863B|TW552114B|ZA200505984A|ZA9709405A|ZA9807661A|US7473764B2|US6005073A|US6451975B1|US6288216B1|US20030124239A1|US20070276127A1|US20020183488A1|DK0848911T3|KR100430830B1|PE1099A1|PE110499A1|NZ328927B|NZ503150B|NZ541447B|AU8760998A|RU97117606A|ZA9709405B|ZA9807661B|ZA200505984B
US7033636	Low cholesterol, functional, animal muscle protein composition and process	A low cholesterol protein composition derived from animal muscle tissue is provided. The low cholesterol protein composition is added to meat or fish prior to cooking to retain moisture during cooking in the fish or meat.	PROTEUS INDUSTRIES INC	Stephen D. Kelleher	CA2522824A1|CA2522824C|CA2586065A1|CA2616041A1|CA2661722A1|CA2661722C|EP1617735A2|EP1617735A4|EP1841324A2|EP1915060A2|EP1915060A4|WO2004093563A2|WO2004093563A3|WO2004093566A2|WO2004093566A3|WO2006080956A2|WO2006080956A3|WO2007018585A2|WO2007018585A3|AU2006277014A1|AU2005325755A1|AU2004232300B2|AU2004232300A1|JP2008527994A|JP2009502916A|CN101389640A|CN101535333A|MX2007007945A|MX2008000935A|NZ542820A|RU2007131732A|US7956081B2|US20080076150A1|US20040224079A1|US20050233060A1|US20050255228A1|US20050129815

					A1|NZ542820B|CN101535333B|EP1617735B1
US7556835	High efficiency protein extraction	The invention relates to a process for isolating edible protein from animal muscle by solubilizing the protein in an alkaline aqueous solution.	UNIVERSITY OF MASSACHUSETTS	Herbert O. Hultin|Stephen D. Kelleher|Yuming Feng|Mark P. Richards|Hordur Kristinsson|Ingrid Undeland|Shuming Ke	CA2421515A1|CA2421515C|EP1328163A2|EP1328163A4|EP1328163B1|WO2002020720A2|WO2002020720A3|AT419755T|JP2004508038A|JP4647881B2|CN1294832C|CN1547437A|DE60137351D1|DK1328163T3|ES2320736T3|HK1070790A1|IS2686B|IS6738A|NO20031027A|NO20031027D0|NO326973B1|PT132816 3E|RU2253288C2|TWI271155B|US20040067551A1|AU9062201A|RU2003109619A
US9161555	Process for isolating a protein composition and a fat composition from meat trimmings	A protein fraction and an oxidation stable fat fraction are recovered from meat trimmings. The trimmings are comminuted, mixed with a food grade acid at pH 3,6 to 4.4 to form a liquid protein fraction and a solid fat fraction. The liquid fraction is mixed with a food grade alkali to precipitate the protein. A myoglobin rich fraction is recovered from the protein fraction and mixed with the precipitated protein.	PROTEUS INDUSTRIES INC	Stephen D. Kelleher|William R. Fielding	CA2794649A1|AR084760A1|AU2012362541A1|AU2011353509A1|AU2011353509B2|WO2012093988A2|WO2013101789A1|EP2688415A4|US20120171345A1|US20120171352A1|WO2012093988A3|CR20130329A|EP2688415A2|NZ599111A|CA2861801A1|CN104066339A|CR20140315A|EP2797425A1|US20150099866A1|WO2015051353A1|BR112012016726A2|HN2012000620A|RU2014131078A|EP2797425B1|US20160088860A1|GT201400137A|CA2926239A1|AU2014331558A1|ES2574504T3|CN105792662A|EP3050436A1|EP3054780A1|AU2012362541B2|CR20160153A|PL2797425T3|CA2794649C|US20170013858A1|PH12016500599A1
US20120276277	Protein product and process for making protein product from uncooked meat purge	A precipitated purge protein is obtained from animal muscle tissue purge. Animal muscle tissue purge is mixed with a food grade acid to form an aqueous acidic solution of animal muscle tissue purge. The acidic solution is mixed with a food grade base to precipitate the protein in the solution. The precipitated protein then is recovered.	PROTEUS INDUSTRIES INC	Stephen D. Kelleher|William R. Fielding	AU2012249152A1|WO2012148490A2|CA2834241A1|CR20130577A|WO2012148490A3|EP2725921A2|CN103889244A|EP2725921A4|RU2013152808A

US9486006	Protein product and process for preparing injectable protein product	Moisture is retained in cooked or thawed food by adding to the food an aqueous suspension of animal muscle protein obtained from animal muscle tissue. The aqueous suspension is obtained by mixing comminuted animal muscle tissue with a food grade base to form an aqueous basic solution of animal muscle protein. The basic solution is mixed with a food grade acid to precipitate the protein in an aqueous composition. The precipitated protein then is comminuted to form an aqueous suspension of comminuted animal muscle protein.	PROTEUS INDUSTRIES INC	Stephen D Kelleher|William R. Fielding|Wayne S. Saunders|Peter G. Williamson	CA2762969A1|CA2762663A1|EP2461706A1|EP2555629A1|WO2011126469A1|WO2011126470A1|AU2010350709A1|AU2010350709B2|AU2010350708B2|AU2010350708A1|CN102639013A|CN102724881A|CR20110651A|CR20110652A|ECSP11010922A|ECSP11010923A|MX2011013727A|MX2011013728A|US20110244092A1|US20110244093A1|US20140023759A1|NZ596433A|NZ596434A|RU2012146624A|RU2012146625A|HN2011003341A|HN2011003342A|EP2461706A4|CA2762663C|EP2555629A4|RU2548994C2|AU2010350709C1|BR112012014149A2|BR112012014151A2|RU2565220C2|ZA201108290B|ZA201108291B|US9491956B2|MX339046B|US20170006904A1|US20170013867A1
US8871291	Methods for separating proteins from connective tissue	Methods and systems for separating muscle tissue from connective tissue are provided, in which animal tissue containing both muscle tissue and connective tissue is subjected to stress, and muscle proteins are separated from the connective tissue. Slurries of separated myofibrillar protein are also provided.	M P F INC	Herbert O. Hultin|Christopher Riley	CA2616562A1|CA2616562C|EP1909602A2|EP1909602A4|WO2007046891A2|WO2007046891A3|AU2006302846C1|AU2006302846B2|AU2006302846A1|JP2008544761A|CN101252849A|KR20080031932A|NO20080609A|NZ565492A|RU2008103181A|RU2413433C2|US8021709B2|US20110236547A1|US20080214792A1|EP1909602B1|TWI405542B|NZ565492B|MY151330A|NO337521B1|TW200715985A
US20050287285	Edible products with reduced oxidation and spoilage	The invention is based, in part, on the discovery that the addition of cations, such as calcium or magnesium ions, to muscle tissue before solubilisation of the muscle proteins enhances removal of membranes, which reduces oxidation and spoilage of the muscle tissue.	University of Massachusetts	Herbert O. Hultin|Yong Liang	WO2003086085A2|WO2003086085A3|AU2003226057A1|AU2003226057A8
US4350624	Method for recovering meat proteins remaining attached to the bones after the boning operation	The installation comprises substantially: a chopper for breaking up raw bones with meat still attached to them, an extraction tank containing an alkaline solution with a pH of the order of 10 to 12 for retaining the proteins, a precipitation tank for the proteins by supplying an acid solution bringing back the pH of the liquid phase to a value of the order of 6, and a separation device for the proteins using physical means such as centrifugation, filtration or decantation means.	SCHLUMBERGER CIE N	HERUBEL JEAN-FREDERIC	DE3000938A1|DE3000938C2|FR2446073A1|FR2446073B1

US20090269440	COMPOSITIONS INCREASING MOISTURE CONTENT AND DISTRIBUTION IN MUSCLE-DERIVED FOOD PRODUCTS	Methods for making dry protein powder or aqueous functional protein suspension compositions which provide increased moisture content and moisture retention in meats and other animal muscle tissue-based products have been developed. An important aspect is the use of alkali rather than an acid or a series of acid and alkaline treatments to dissolve the protein in the starting material. This includes both muscle and connective tissue proteins and fats, yielding a product in higher yield than acid based and other processes in which fat and connective tissue is removed and then only the remaining muscle dissolved in the acid. The connective tissue and fat increases water retention in meat into which it is injected, as compared to meat extracts containing only muscle proteins.	GENESIS GLOBAL LIMITED	Richard B. Hardin	WO2009132297A1
EP3117717	ISOELECTRIC SOLUBILISATION OF ANIMAL MATTER	A method of sequential isoelectric solubilisation of animal by-product comprising the steps of isoelectric solubilisation of the animal by-product in one of an acid or alkali solution to provide a first solubilised protein fraction, separating the first solubilised protein fraction from unsolubilised animal by-product, isoelectric solubilisation of the unsolubilised animal by-product to provide a second solubilised protein fraction, and separation of the second solubilised protein fraction from unsolubilised animal by-product. The protein from the first and/or second solubilised protein fractions is recovered by drying or precipitation. In one embodiment, the acid and alkali soluble protein fractions are proportionally combined to neutralise the composite fraction and precipitate protein.	AGRICULTURE AND FOOD DEV AUTHORITY (TEAGASC)	TIWARI BRIJESH K|ALVAREZ GARCIA CARLOS|TROY DECLAN	WO2017013043A1

US20090123615	COMPOSITION DERIVED FROM A MEAT SOURCE AND PROCESSES FOR MAKING AND USING COMPOSITION	A composition is derived from animal muscle may be added to a substrate animal muscle for improving water-binding capacity of the substrate animal muscle. A process for making a composition in accordance with one embodiment may include the steps of providing a slurry of animal muscle and water; increasing the pH of the slurry to an alkaline level sufficient to solubilize at least a portion of the animal protein in the animal muscle so as to form an alkaline slurry; and maintaining the pH of the alkaline slurry at a level sufficient to prevent coagulation of the animal protein. The alkaline slurry may then be dried to form a substantially dry particulate animal muscle product. The particulate animal muscle product may then be reconstituted to form a marinade, and the marinade may be applied to a substrate animal muscle.	BUMBLE BEE FOODS LLC|MPF INC	HUDSON HEATHER|BADER DEREK RAY	CA2705795A1|EP2222186A1|EP2222186B1|AT527892T|WO2009064487A1|US20090123615A1
US5726033	Process for preparing proteins from protein-containing substance	In a process for preparing proteins from a protein-containing substance, the substance is dispersed in an alkaline solvent with a pH of over 11.5 and at a temperature of under 30 DEG C. The proteins in the substance are thus dissolved. The resulting solution is then neutralized and the proteins in it are concentrated. To improve the profitability of the process in large-scale manufacture, the protein-containing substance is treated with a protease before dispersion in the alkaline solvent.	DMV INTERNATIONAL NUTRITIONALS GMBH	NEUMUELLER WALDEMAR	CA2176951A1|EP0730412A1|EP0730412B1|WO1995014394A1|AT183055T|JPH09505472A|DE4339743C1|DE59408624D1|ES2137487T3|DK0730412T3
CA1043775	PROCESS FOR RECOVERING GLUCAGON	ABSTRACT OF DISCLOSURE The invention provides a process for the recovery of glucagon by A. isolating glucagon-containing protein from insulin process alkaline crystallization super-natant; B. separating the glucagon from other proteins; and C. purifying the glucagon obtained from step B.	LILLY CO ELI	JACKSON, RICHARD L.	GB1494704A|JPS51100071A|DE2505308A1|DE2505308C2|JPS5725024B2|FR2300074A1|FR2300074B1|NL7501265A|SE424406B|SE7501278L|US3875138A

RU2075944	METHOD OF PROTEIN FOOD ADDITION PREPARING	FIELD: food technology. SUBSTANCE: method involves the preparing food addition by extraction of the prepared animal raw (milled frozen organs and tissue of mammalian) using 1-2.5% sodium carbonate solution at pH = 10.4-10.8 for 2-4 h with addition of 0.1-0.5% magnesium chloride. Ratio of volumes of extractable tissue and soda solution is (1:8)-(1:20). Then deposit is separated by filtration or centrifugation followed by extract acidification to pH = 3.8-5.5, inert matters removing, precipitate washing with organic solvents and precipitate drying at temperature 55 C, not above. EFFECT: improved method of preparing. 5 tbl	OBSHCHESTVO S OGRANICHENNOJ OT	MOROZOV VYACHESLAV G|KHAVINSON VLADIMIR KH	RU96104838A
CN101828627	Extract method of muscle protein isolate	The invention discloses an extract method of muscle protein isolate. The invention is characterized in that the method is carried out by the following steps: animal muscle is smashed into chopped meat; nine times volume of distilled water is added and homogenization is carried out by a homogeniser; NaOH or HCl is used for adjusting pH value of minced meat to be 10.8-11.5 or 2.5-3.5, reaction is carried out for 1-4 hours, centrifugation and impurity removal are carried out and supernatant is separated; and pH method is used for adjusting the supernatant to isoelectric point pH 5.1-6.0 of protein, then centrifugation is carried out and sediment is obtained. The produced muscle protein isolate can be used as edible protein base material to make various meat paste products and has the advantages of high purity, good gel property and strong functionality. Compared with the traditional meat paste processing method, the invention has the advantages that, the protein yield can be improved from about 60% to 70-90%, total solid content, total nitrogen and chemical oxygen demand in waste	UNIV DALIAN FISHERIES	JUNRONG LIU|TAO WANG	[NA]

		water are accordingly reduced, and difficulty and cost of waste and waste water treatment are reduced.			
JPS5743643	PROTEIN RECOVERER FROM WASTE FROM MEAT TREATMENT	PURPOSE: A pair of the upper sectioning frame and the lower one that forms vertical tunnel paths is demountably set in the tank with a drain pipe on the bottom to precipitate and recover the protein efficiently.CONSTITUTION: The tank for precipitating protein 1 is provided with the drain pipe 3 having a valve 2 and a pair of parallel-cross frames 6, 7 are piled in the tank so that their vertical tunnels 4, 5 can get through. The waste, treated with acid and alkali, is poured into the precipitation tank 1, then the above frame 6 is removed and the supernatant is taken out of the drain pipe 10 and the pipe 11. The fraction containing the precipitate is taken out of the drain pipe 3 on the bottom and centrifuged to recover the protein.COPYRIGHT: (C)1982,JPO&Japio	BIBUN CORP	NISHIOKA FUJIO|SHIMIZU HIROSHI|KURIHARA YOSHINORI	JPS5823054B2

WO2010136894	PROTEIN COMPOSITIONS AND METHODS OF MAKING AND USING THEREOF	Described herein are the isolation and use of meat proteins and their applications thereof. In one aspect, meat proteins such as, for example, fish, poultry, bovine, or porcine can be used to make films, meat binders or extenders, and extrudable food articles.	UNIV ALBERTA	BETTI MIRKO|XU YAN	CA2801040A1
US7763717	Continuous protein and lipid recovery from food animal processing byproducts	A process and system for recovering protein and lipid from food animal byproducts, and the products thereof, involves homogenizing animal byproducts with water to form a homogenate, solubilizing the homogenate by adjusting the pH of the homogenate to form a first pH adjusted composition, separating the first pH adjusted composition forming a light fraction containing lipids (oil), a medium fraction containing protein in solution, and a heavy fraction containing fat-free impurities, separation by first centrifugation, adjusting the pH of the medium fraction to about the isoelectric point of the proteins thereby precipitating the medium fraction forming a second pH adjusted composition, and separating the second pH adjusted composition forming a light fraction containing water and a heavy fraction containing precipitated proteins. The water may then be recycled and used in the homogenization of further byproducts.	Jacek Jaczynski	WEST VIRGINIA UNIVERSITY RESEARCH CORPORATION	[NA]

US20070281349	Industrial bioreactor and method of use in continuous protein and lipid recovery system	The present invention provides for an industrial scale bioreactor and for a use of the industrial scale bioreactor in a continuous protein and lipid recovery system. The industrial scale bioreactor comprises a pH-resistant container able to hold at least 150 gallons wherein said pH-resistant container further comprises, a means to add influent to said pH-resistant container, a means to remove effluent from said pH-resistant container, one or move thermocouples able to measure the temperature within the pH-resistant container, a mixer reversibly attached within said pH-resistant container, and a means to monitor and control the pH within said pH-resistant container. The continuous protein and lipid recovery system comprises a homogenizer, a means to connect said homogenizer to a first bioreactor wherein said first bioreactor is maintained at a programmed pH level away from the isoelectric point of the protein so it is water-soluble, a separator, a means to connect said first bioreactor to said separator, a second bioreactor maintained at a programmed pH level at the isoelectric point of the protein so it is water-insoluble, a means to connect said separator to said second bioreactor, a second separator, a means to connect said second separator to said second bioreactor, and a means to monitor and control the temperature throughout the protein and lipid recovery process.	Jacek Jaczynski	UNIV WEST VIRGINIA	[NA]

9.3 Appendix 3 – Highlights from Patent Search 2 – Products using extracted animal protein

Publication Number	Title	Abstract	Current Assignee	Inventors	Family Members
US20070092616	Puffed snack products and processes for producing the same	The present disclosure generally relates to high protein puffed snack products including vegetable protein materials and a non-vegetable protein materials and processes for making high protein puffed snack products. More particularly, the present disclosure relates to high protein puffed snack products including soy protein materials and protein from meat, meat by-products, or dehydrated meat material.	Solae	Philip A. Witte|Matthew K. McMindes|Luping Ning	EP1965661A1|WO2007050306A1|BRPI0619305A2|JP2009512444A
US20050013917	Novel high protein tortillas	The subject invention provides novel protein-rich tortillas. In a specific embodiment, the subject invention provides flat, thin tortillas that comprise chicken protein. Other meats can also be used to provide the protein component. Advantageously, the tortillas of the subject invention can be used as a replacement for traditional flour or corn tortillas.	FLORIDA RESEARCH FOUNDATION INC UNIVERSITY OF	Lauren April O'Kelley|Meghan Lea Meller|Michael Shaun Madden|Hordur G.. Kristinsson	WO2005006886A1|US7125576B2
WO2011035403	PROTEIN MIXTURE OF MEAT EXTRACT AND COLLAGEN	A protein mixture of meat extract and collagen from bovine muscle takes the form of a soluble powder composed of macronutrients such as carbohydrates, lipids and proteins, of micronutrients such as calcium, iron and sodium, and of saturated fat. It is therefore a balanced formulation that preserves all essential amino acids, represents a source of iron and calcium and exhibits a low sodium and saturated fat content. The protein mixture can be used with any type of edible liquid or paste, in the form of food supplements, enteral or parenteral fortifying diets, and is particularly	JBS	BERTIN FERNANDO	EP2484226A1|BRPI0903597A2|US20120269932A1

		recommended for patients or persons suffering from some form of deglutition disorder.			
US20090269455	PROTEIN-BASED FOOD PRODUCT AND ASSOCIATED PRODUCTION METHOD	The aim of the invention is to obtain a food product, in particular a snack product, which has a favourable nutrient physiology and a crispy, brittle texture, and an associated production method. To achieve this, the food product is substantially devoid of starch and has a foamed structure and the solid content has a protein fraction of at least approximately 25% by weight, at least 65% by weight of the protein fraction consisting of gelatin and/or collagen hydrolysate.	GELITA AG	Jutta Hoffmann|Michael Ahlers	EP2117347A2|WO2008083802A2|WO2008083802A3|JP2010515431A|CN101616601A|DE102007002295A1|MX2009007362A|BRPI0720798A2
US20150044334	SYSTEM AND METHOD FOR PRODUCING AN EXTRUDED PROTEIN PRODUCT	The present disclosure relates to systems and methods for producing an extruded protein product. In particular, a system for making an extruded protein product using a system that includes a die including channel having a transverse cross section that is a continuous loop along at least a portion of the length of the die is disclosed.	General Mills	Goeran Walther|Bernhard H. van Lengerich|Steven C. Robie|James N. Weinstein	JP2016532447A|WO2015020660A1|WO2015020873A1|CA2920387A1|AU2014304978A1|CN105530820A|EP3030092A1|KR20160068733A

US5071665	PROCESS FOR PREPARING A PROTEINACEOUS FOOD PRODUCT	A process for preparing a proteinaceous food product comprises passing a wet dough of a mammalian and/or avian meat protein, at least part of which is functionally inert protein, between a pair of oppositely rotating rollers to form a sheet of said food product. The functionally inert protein may have been cooked or otherwise treated to impart to the protein one or more characteristics of cooked protein and/or may comprise inert scleroprotein.	Mars	BUCKLEY KEITH|WILLS GARRY D|MUSSON GARY D|SPEIRS CHARLES|PRIM ROSE DAVID|BEECH JOHN|GAYWOOD PAUL	CA1334904C|EP0328349A1|EP0328349B1|GB8802934D0|GB8818941D0|GB8820829D0|GB8901399D0|AT84942T|AU2968589A|AU621339B2|JPH025827A|DE68904569D1|DE68904569T2|DK57289A|DK57289D0|ES2038824T3|GR3007268T3|IE62059B1|IE890410L|NO173683B|NO173683C|NO890540A|NO890540D0|NZ227806A|PT89648A|PT89648B
WO2010136894	PROTEIN COMPOSITIONS AND METHODS OF MAKING AND USING THEREOF	Described herein are the isolation and use of meat proteins and their applications thereof. In one aspect, meat proteins such as, for example, fish, poultry, bovine, or porcine can be used to make films, meat binders or extenders, and extrudable food articles.	University of Alberta	BETTI MIRKO|XU YAN	CA2801040A1
WO2001005251	EDIBLE ANIMAL MUSCLE PROTEIN GELS	The invention relates to commercial grade food protein gels containing low amounts of animal muscle protein and salt, and methods of producing them.	University of Massachusetts	HULTIN HERBERT O|FENG YUMING	CA2375933A1|EP1196048A1|AU6082800A|CN1373638A|RU2002103868A

RU2476079	EXTRUDED FOOD PRODUCT, ITS PRODUCTION METHOD AND EXTRUDER FOR PRODUCT MANUFACTURE	FIELD: food industry.SUBSTANCE: invention is intended for usage in food industry and refers to production of an extruded food product containing from nearly 25% to nearly 77% of meat and/or vegetal protein. The method envisages continuous introduction of the food product components into the extruder, such components including meat and/or vegetal protein, their mixing in the extruder to produce a mixture, the mixture heating in the extruder, the food product extrusion through the forming die and the food product cooling. The product may additionally contain plastifiers and, essentially, may not contain compounds forming cross links. The extruder has a high value of "length to diameter" ratio and contains a row of cylinders, twin screws rotating in the cylinders round parallel axes and heating devices mounted on the cylinders.EFFECT: inventions group ensures production of a product being a meat analogue or substitute and having a fibrous and texturised appearance specific to meat.62 cl, 1 dwg, 3 ex	NESTEK S A	BIZHER FANNI|KART E KATRIN|LESPANOL LJUS EN OGJUST|PIBARO PATRIK|REJNE PER	CA2691884A1|EP2011404A1|EP2170097A1|WO2009003721A1|AU2008271478B2|AU2008271478A1|JP2010532659A|CN101686707A|RU2010103685A|US20100136201A1|CN101686707B|BRPI0812828A2|CA2691884C
KR20120050453	AMORPHOUS PROTEIN EXTRUDATES	This invention relates to an amorphous material and a method for producing food containing a significant amount of protein. In particular, the invention relates to an amorphous protein extrudate , these protein extrudate production method , and a food ingredient such as protein extrudates containing high concentrations of protein applications .	Solae	SOLORIO SANTIAGO	CA2767907A1|EP2456322A2|WO2011011456A2|WO2011011456A3|AU2010276327A1|CN102480993A|US20120171351A1|EP2456322A4|CN102480993B|AU2016200062A1

US20050214420	Protein-containing food product and method of preparing same	A method of manufacturing a protein-containing food product by means of heat-treating a protein and water-containing carrier material suitable for pumping in a turboreactor which has a cylindrical reaction chamber with a rotor equipped with blades in order to centrifuge the carrier material in the form of a dynamic, turbulent layer against an inner wall of said reaction chamber, heat-treating, drying to AW less than 0.6 and granulating the carrier material, advancing the carrier material in the direction of an outlet from the turboreactor, and forming individual food products from the carrier material; a protein-containing food product made by press molding of a carrier material that has been granulated and dried to an AW value of less than 0.6 and that is microbiologically stable, the carrier material being free of gelatinized starch.	Mars	Siegfried Schmidt|Marinus Pannevis	EP1489920A1|EP1489920B1|WO2003079808A1|AT381891T|AU2003216874A1|AU2003216874B2|JP2005520530A|DE10213280A1|DE60318304D1|DE60318304T2|ES2297137T3|US7838057B2
US5827561	Process for producing meat strips or proteinaceous strips	In a process for producing a food product based on meat, animal proteins or vegetable protein, an emulsion is prepared from base components by first comminuting and emulsifying the base components. In the next processing step, the emulsion is loaded into a stuffing device, from which the emulsion then exits in the form of a single strand or numerous parallel strands. In a further step, the strands of the emulsion are cooked. The cooked strands of the emulsion are then cut by a cutting device into shorter strips, preferably having a length of a few centimetres, and the strips are subsequently deep-frozen and then packaged.	DUVE MANFRED	DUVE MANFRED	EP0624320A2|EP0624320A3|EP0624320B1|AT197117T|DE4316235A1|DE59409556D1|ES2153396T3|US5482730A

GB2373707	Concentrated hydrolysed animal protein feed	A method of foodstuff production comprises hydrolysing animal protein to form a slurry and removing water from the slurry. The protein is preferably obtained from raw meat which is pre-ground. Muscle meat, fish muscle, poultry heads, feet, liver, viscera, connective tissue and feathers are possible protein sources. The foodstuff is preferably a moist pet food such as an extruded kibble comprising at least 20% water, most preferably 45% to 55% water. Hydrolysis is preferably achieved by adding enzymes or by autolysis. The method may also include mixing with flour and the addition of an antioxidant to the slurry. The addition of vitamins can be avoided by selection of appropriate raw materials. Fat may be removed by centrifugation or by the addition of chemicals. Water may be removed using falling film evaporation.	Mars	RUSSELL DAVID PAUL|STEIN VON KAMIENSKI BOTHO|LANGE EIKE|SIREL TASUJA	EP1359814A1|GB0103879D0|WO2002065848A1|US20040131750A1
WO1996012413	EXTRUDED HIGH SOLUBLE PROTEIN ANIMAL FEED AND METHOD OF PREPARING SAME	An extrusion process for the production of animal feeds having high soluble protein contents is provided wherein respective starch-bearing and proteinaceous ingredient fractions are differentially processed so as to obtain an extruded final product containing soluble protein. In the process, a starch-bearing fraction is preferably preconditioned and introduced into an extruder; a proteinaceous fraction is introduced into the extruder barrel, preferably adjacent the extrusion die, for mixture and extrusion with the starch-bearing fraction. This yields an extruded feed having a starchy matrix with protein of high solubility carried by the matrix.	WENGER MFG	ROKEY GALEN J	AU3513195A|US5480673A

WO1997011610	FOODSTUFF	The invention provides a canned foodstuff comprising discrete chunks, the chunks comprising a food material and being substantially free of intervening liquid, gel or other matrix material. Preferably, the chunks are of a leavened foodstuff comprising a gelled mixture comprising meat protein. The invention also provides a method of making a foodstuff comprising forming a mixture of meat protein and a gelling system and leavening the mixture while gelling it.	Mars	MUSSON GARY DAVID|WALKER RICHARD JAMES|HARFORD STEPHEN|SPEIRS CHARLES IVIE	CA2232958A1|EP0866661A1|EP0866661B1|GB2308289A|GB2308289B|GB2333942A|GB2333942B|AT264067T|GB9519555D0|GB9708754D0|GB9909449D0|AU7137296A|AU720840B2|BR9610621A|DE69632200D1|DE69632200T2|NZ319199A|NZ501949A|NZ319199B|NZ501949B
US20140030387	MICRO-ENCAPSULATED ANIMAL PROTEIN CONCENTRATE	The invention relates to an animal protein concentrate, fit for the human consumption, elaborated on basis of a fish protein. More specifically, this invention relates with micro-particle matrix or multicore of fish protein and gelatin, optionally coated, fit for the elaboration of processed food for human consumption.	MAP CHILE SPA	Cristian Cifuentes Larios|Simón Salosny Hübner	[NA]
AU746380	Foodstuff	The invention relates to a novel foodstuff, more particularly, a leavened foodstuff comprising a gelled mixture comprising meat protein. The invention also relates to a method of making a foodstuff comprising, forming a mixture of meat protein and a gelling system and leavening the mixture while gelling it.	Mars	MUSSON GARY DAVID|WALKER RICHARD JAMES|HARFORD STEPHEN|SPEIRS CHARLES IVIE	AU2780900A

BE903819	Stable food prods. from animal protein and starch - made continuously by extruding and cooking the mixt. contg. moisture and humefactant	A continuous process for the prodn. of a durable food prod. comprises mixing ingredients in a ratio of 5-60wt.% animal proteins and 20-40% starch, extruding the mixt. in a screw extruder and subjecting it to an elevated temp., mechanically compressing and cutting the mixt. to cook it before and/or after the food has passed through the extruder, and controlling the moisture content of the mixt. to 15-28wt.% and adding a humefactant agent to the mixt., extruding the cooked mixt. through a die to form separate pieces and cooling these pieces. - In a further claimed embodiment, the starting mixt. comprises 45-100wt.% animal protein and 0-40% starch and the cooled pieces of the prod. are opt. dried.	RAINBOW TRADING LTD	QUINN N|O'CONNOR C|TUCK K	[NA]
JPH025827	FOOD	PURPOSE: To provide protein food suitable for human being or animal like pet food by forming the humid dough of meat of the mammals or birds functionally inactivating one part of protein into sheet through rolling. CONSTITUTION: The humid dough of meat protein of the mammals and/or birds such as inactive hard protein, for example, of which one part of protein is functionally inactive and the gel strength value shows 0-400g through boiling or the other treatment as needed, is passed between a pair of rollers at least mutually oppositely rotated by force from 7×10Kg/m<2> to 7×10Kg/m<2> and the one-dimensional food sheet having a length longer than 3cm, preferably, longer than 10cm is formed.	NADORUFU LTD	KIISU BATSUKUREI|GARII DEBITSUTO UIRUZU|GARII DEBITSUTO MATSUSON|CHIYAARUZU SUPEIRISU|DEBITSUTO PURINROOZU|JIYON BIICHI|POORU GEIUTSUDO	CA1334904C|EP0328349A1|EP0328349B1|GB8802934D0|GB8818941D0|GB8820829D0|GB8901399D0|AT84942T|AU2968589A|AU621339B2|DE68904569D1|DE68904569T2|DK57289A|DK57289D0|ES2038824T3|GR3007268T3|IE62059B1|IE890410L|NO173683B|NO173683C|NO890540A|NO890540D0|NZ227806A|PT89648A|PT89648B|US5071665A

JPS62278950	PREPARATION OF COMPOSITE ANIMAL AND VEGETABLE PROTEIN FOOD MATERIAL	PURPOSE:To prepare a protein food raw material having meat-like texture and palatability and free from characteristic smell of raw material, by mixing animal and vegetable protein raw materials rich in sulfur-containing amino acid and treating the mixture with an extruder. CONSTITUTION:An animal protein rich in sulfur-containing amino acid, taurine and taurocholic acid such as gut meat of domestic animal, domestic fowl, fish or shellfish or head meat of fish or shellfish is mixed with a vegetable protein rich in sulfur-containing amino acid such as defatted soybean flour, marine algae rich in red algae, etc. The obtained mixture is supplied to an extruder, heated at a high temperature under high pressure while adding water to the mixture and subjected to shear deformation to effect the mutual modification of the animal protein and the vegetable protein.	SANYO COCA COLA BOTTLING KK	MORI YASUSHI|HIEDA FUKUJI|MATSUKAWA TAKASHI|NAEDA YUJI|MATSUYAMA KIKUO	JPH0365742B2

Attachment 2: Outcomes from Phase 3: Red-meat Protein Isolate.

Xinova worked with Matis to refine the scope of their engagement to formulate new high and low moisture protein isolates from their patented pH shift process using red meat source material. The refined project tasks are detailed below.

Matis project scope

pH shift separation process with beef

1. Working with initial raw material: (75VL (Sausage trim)) and/or 75VL MDM
2. Working with other select raw product types: 50 VL
3. pH shift optimization

Protein isolate forms for formulation

1. High moisture protein isolate creation and assessment
2. Low moisture protein isolate creation and assessment
3. Marinade creation and assessment (eye round and topside cuts vs standard phosphate-based marinade from 75VL and 50VL)
4. Economic assessment of processes and modelling

Creation of Prototypes.

Matis refined the pH shift process to enable them to isolate the protein and produce some samples to review and assess functionality in prototype products.

A mini-report on the results of Matis pH Shift Separation Process and Protein Isolate functionality is included as Attachment 1, including the sample products.

Recap and update

Meeting 17.1.18

- Key Treatments (45 and 75 CL ground beef) – Getting to know the material
  - Alkaline adjustment with filtering
  - Alkaline adjustment with pre-wash and filtering
  - Initial marinade trials with top-round

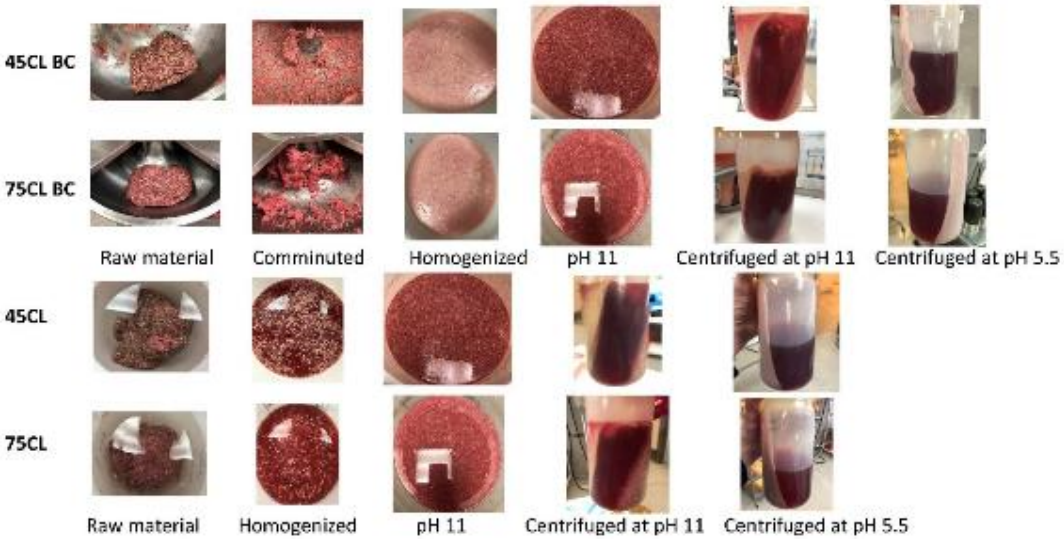
Meeting 8.2.18

- Key Treatments (45 and 75 CL ground beef)
  - Alkaline adjustment with homogenization and centrifugation
  - Alkaline adjustment with bowl cutter (BC), homogenization and centrifugation
  - Alkaline adjustment with two pre-washes and centrifugation
  - Marinade trials with centrifugation and mincing (topside and eye round)
  - Stir fry marinade preparation and assessment
  - Protein isolate gelation (BC, centrifugation, and prewashes)
  - Protein isolate freeze drying (BC and centrifugation=

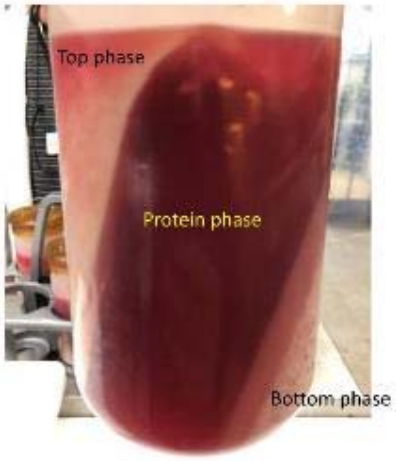
Centrifugation trial 1

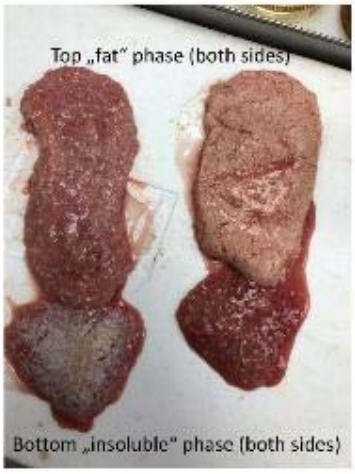
With or without bowl cutter

Process overview



Centrifugation 1: solubilization phases





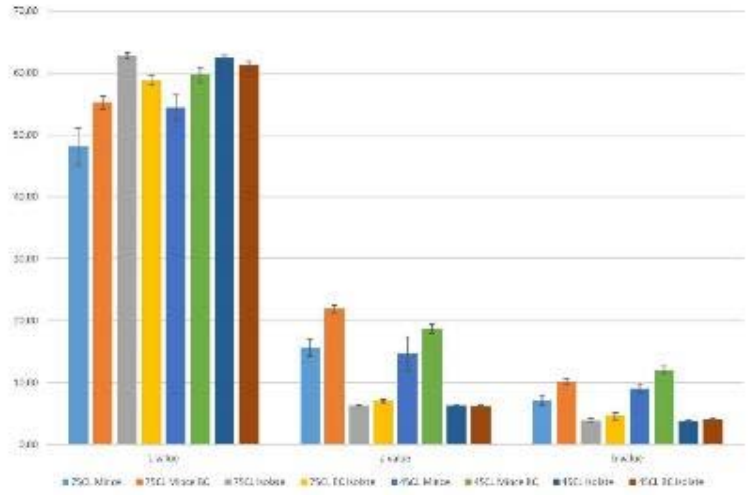
Centrifugation 2 – Precipitation phases

Significant fat reduction

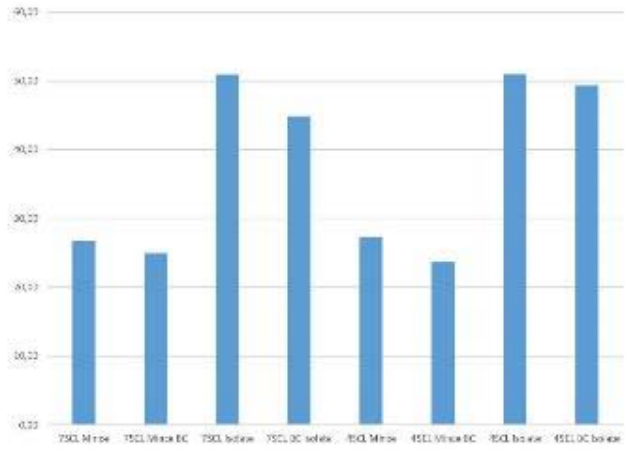
Bowl cutter		No bowl cutter	
75CL Isolate	45CL Isolate	75CL Isolate	45CL Isolate
99.7%	99.8%	98.3%	99.8%

Previous non centrifugation trial: 35-57% fat reduction

Color

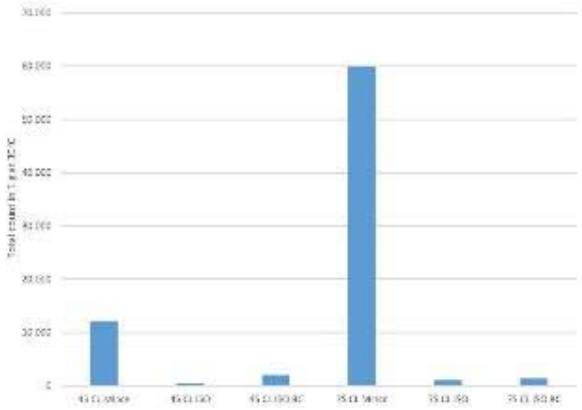


Whiteness



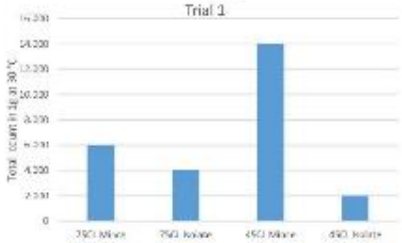
Microbial reduction

Significant Log reductions:
0.8- 1.8 log

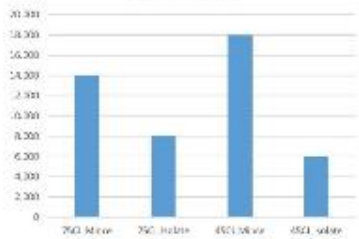


Comparison from previous trials

Trial 1

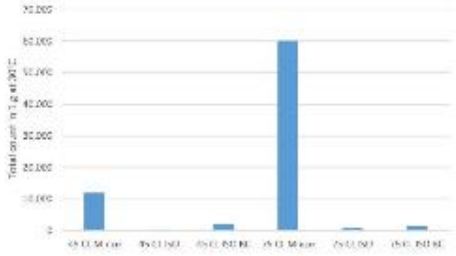


Trial 2- Prewash



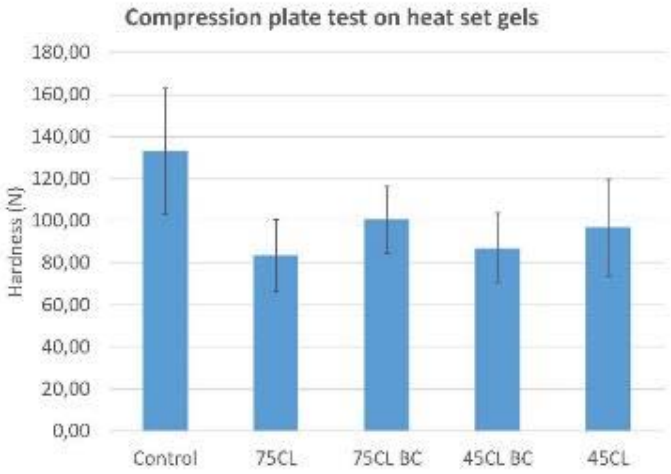
Centrifugation trial

Significant Log reductions:
0.8- 1.8 log reduction



Gels


Control
75CL
45CL
75CL BC
45CL BC


Compression plate test on heat set gels
Hardness (N)
180,00
160,00
140,00
120,00
100,00
80,00
60,00
40,00
20,00
0,00
Control
75CL
75CL BC
45CL BC
45CL

Protein isolate freeze dried powders

45 CL BC ISO

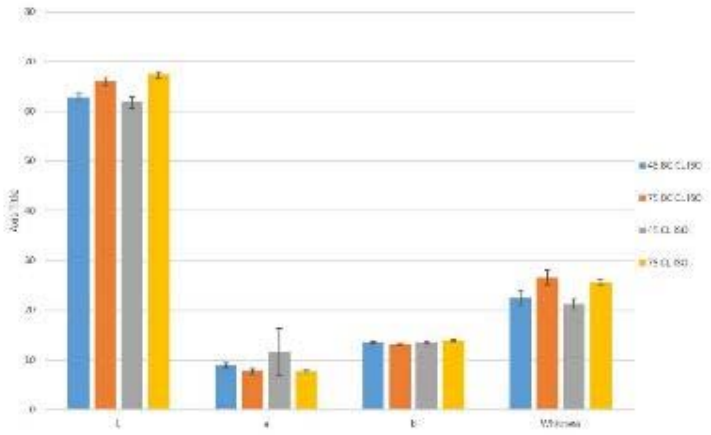
45 CL ISO

75 CL BC ISO

75 CL ISO

Average moisture: 3-4%

Color of freeze dried powders

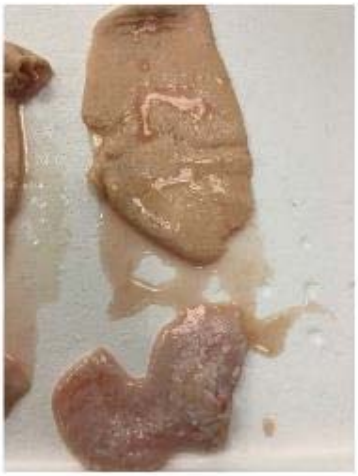


Centrifugation trial 2

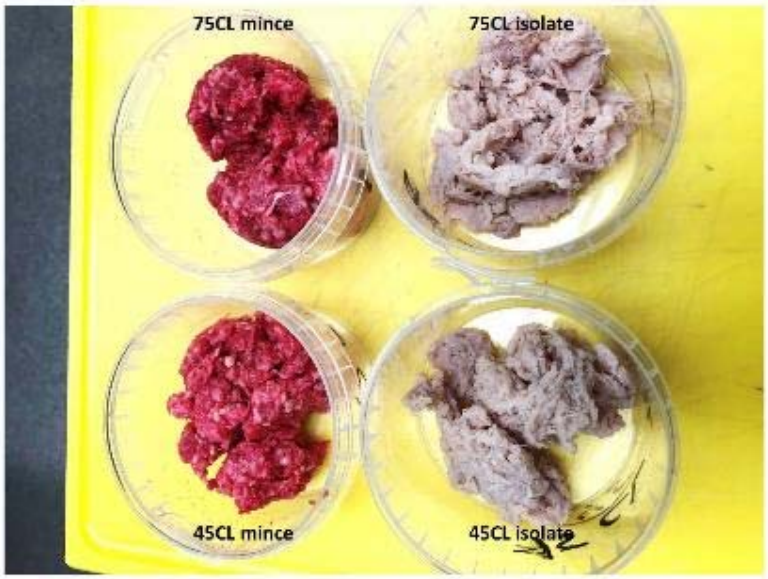
Effect of prewash

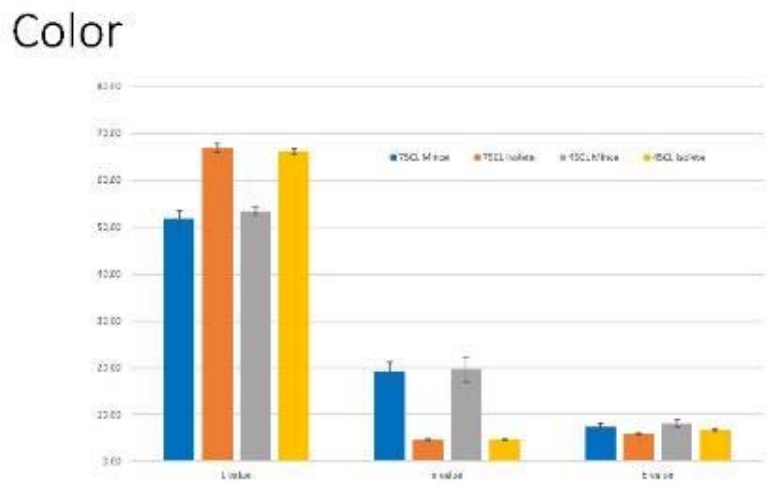
Process overview

Fat and insoluble phases after first centrifugation

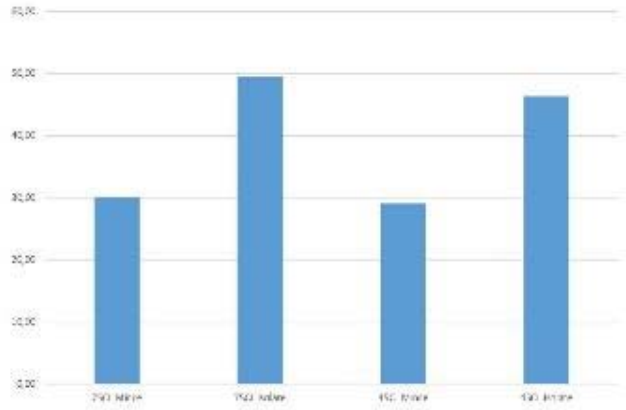


Phases after second centrifugation


75CL mince
75CL isolate
45CL mince
45CL isolate


Color

Whiteness



Protein isolate pastes before cooking

Cooked gels

45CL not pH adjusted 45CL pH adjusted 75CL pH adjusted

Folding test – 45CL no pH adjustment

Folding test – 45CL

Folding test – 75CL

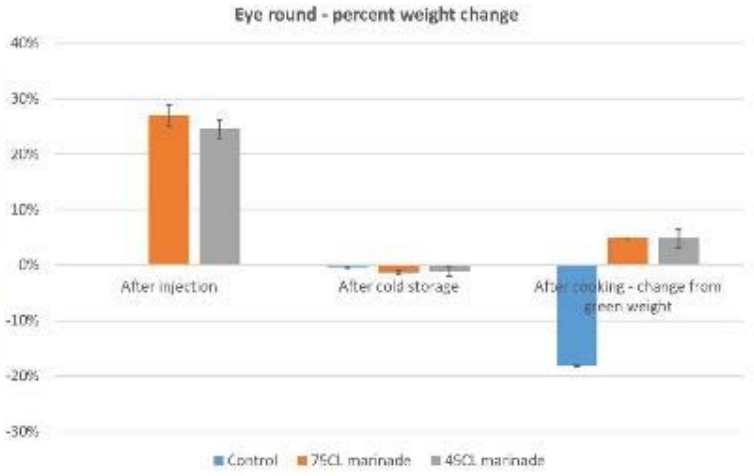
Injection of protein marinades

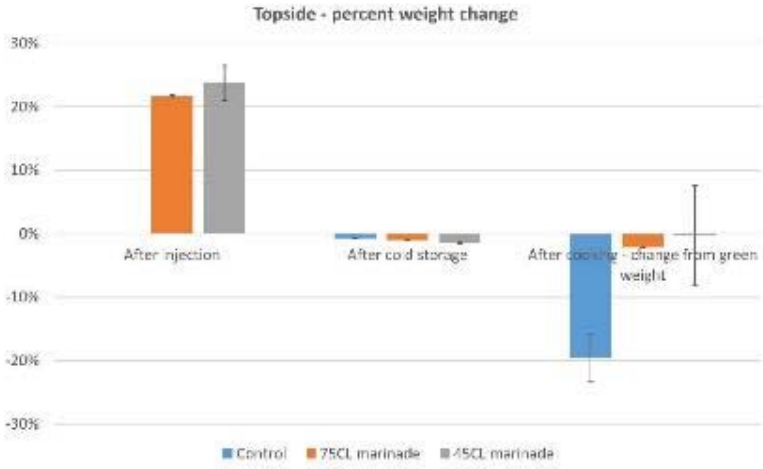
Eye round

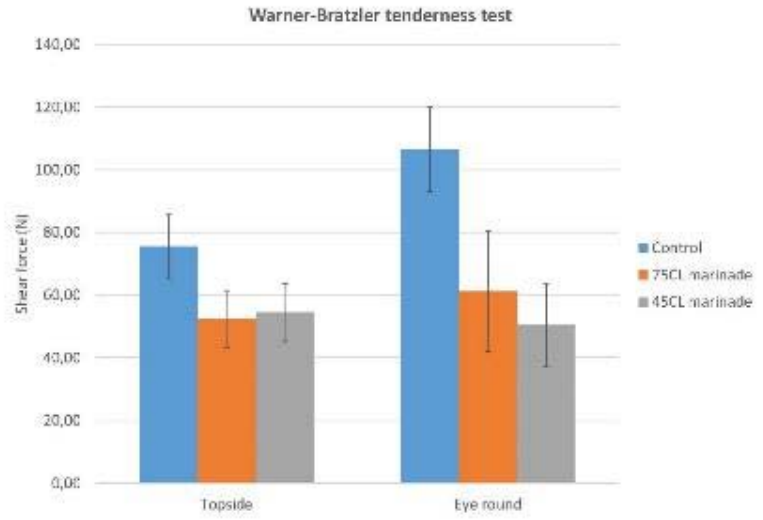
Control 45CL injected 75CL injected Control 45CL injected 75CL injected

Topside

Control 45CL injected 75CL injected Control 45CL injected 75CL injected




Topside - percent weight change
30%
20%
10%
0%
-10%
-20%
-30%
After injection
After cold storage
After cooking - change from green weight
Control
75CL marinade
45CL marinade


Warner-Bratzler tenderness test
140,00
120,00
100,00
80,00
60,00
40,00
20,00
0,00
Shear force (N)
Topside
Eye round
Control
75CL marinade
45CL marinade

Sensory assessment of steaks (5 expert panelists)

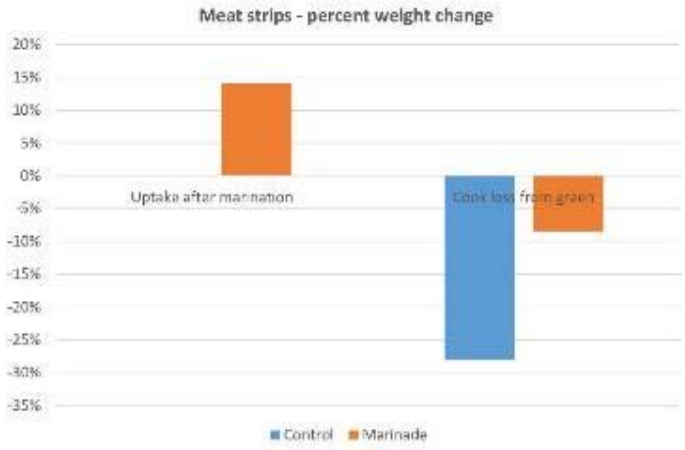
Scale 1-5
Tenderness: 1 tough - 5 very tender
Juiciness: 1 dry - 5 very moist

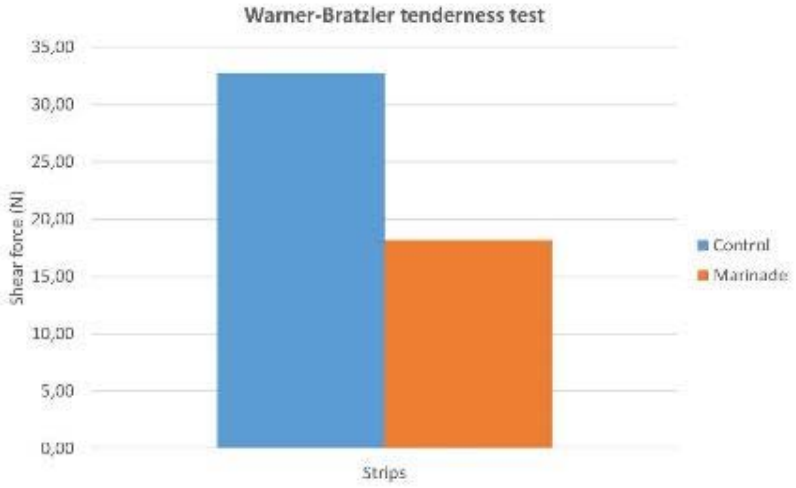
Topside	Average Tenderness	Average Juiciness
Control	2,625	1,625
45 CL	2,75	2,75
75 CL	4,5	4,375

Eye round	Average Tenderness	Average Juiciness
Control	1.625	1.5
45 CL	3.5	3
75 CL	4	4.125

Marinated strips

Marinated strips





Sensory assessment of steaks (5 expert panelists)

Scale 1-5
Tenderness: 1 tough - 5 very tender
Juiciness: 1 dry - 5 very moist

Stir Fry	Average Tenderness	Average Juiciness
Control	2	2.2
75 CL	3.9	4

* Treatments were superier right off of the skillet compared to control (5)

Next Steps

- **Complete Wash and Centrifuge Assessment**
  - Proximate, yields, micro, dried products, etc.
- **Fresh material focus moving forward**
- **Further optimization**
  - Smaller scale runs
  - Optimize pH values for yield and quality improvements
  - Complete and compare phosphate vs. alkaline marinades (eye round and topside)
  - Assess newly pre-washed and centrifuged freeze dried protein isolate material for quality and attributes
  - Electrophoresis of protein powders to assess composition
  - Dry via other methods than freeze drying
  - Test for collagen in centrifuged material
  - Shelf-life studies on protein isolate and injected meat

Follow-up

- **Earlee Products Marinade**
  - Arrival- 7.2.18
- **Shipping Samples**
  - Shipping logistics to MLA
  - Sample sizes to be sent?
  - How quickly can we get samples there (do we need to freeze?)
- **Billing**
  - Last invoice
  - First downpayment + materials and analytical
- **Next Meeting**
  - Move to 9.3.18

Attachment 2: Red Meat Snack Workshop Agenda

mla DONOR COMPANY
MEAT & LIVESTOCK AUSTRALIA

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AGENDA

Red Meat Snacking Workshop

Date: 12th & 13th February 2018
Location: MLA Level 1, 40 Mount St, North Sydney

Attendees:

Xinova & Partners: Scott Needham, Yi Lan Chen, Evelyn Miles, Russel Rankin, David Ireland, Greg Caire, Nick Hazell

MLA/MDC: Christine Pitt, Allister Watson, Michael Lee, Duncan Veal, Rachel Cofrancesco, Emily Walker

Innovators: Maxime Bilet, Steve Weaver, Bradley Wardrop-Brown, Bob Hamilton, Brett McMullen, Trish Linderman

Please bring:

Innovators to bring hard concepts (samples) and soft concepts (ideas). Presentation outlining What the product is? How was it made? How it could be made commercially? Who is the target consumer group? Why is it unique and exciting? Is there a technology stretch to make it? What is the key challenge to success?

It is recommended that each concept pitch take no more than 10 minutes each

Day 1: "*Taste the Snack*" – Hard Concepts and food experience
"*Dream the Snack*" – Soft Concepts

10:00am (20 min)	**Welcome, introduction and workshop objectives**	Scott Needham/ Christine Pitt
10:20am (10 min)	**Overview of the process, roles & responsibilities**	David Ireland
10:30am (120 min)	**Presentation of Concepts - Earlee Foods** ***Taste the Snack*** (Collagen chips; Shelf ready snack sausage; Breakfast bar) ***Dream the Snack*** (Meat based cracker; Hi-protein lo-carb muesli/trail mix: Chocolate with beef fat; Hi-protein, lactose free, ice-cream; Protein enriched beverage – pre/post workout). Group fills out proforma and engage in limited discussion	Bob Hamilton/Brett McMullen/Trish Linderman

1

	on each concept + Complexity v Value maps.	
12:30pm (60 min)	**Plan for Action** Group discussion on interesting concepts, identifying challenges and how to address. Challenges to address might include commercialization partners, manufacturing difficulties, scaling, small or unidentified target market etc. This will highlight the most immediate and key areas to focus on to de-risk the concept and translate into concrete action plans for selected food concepts.	David Ireland
1:30pm (120 min)	**Presentation of Concepts - BluOak Innovations** ***Taste the Snack*** (Beef snacking chips; Enriched beef beer; Fortified pre/probiotic shelf-stable beef and lamb bars; Hydrolysed collagen peptide bars & bites; Pre/probiotic collagen recovery beverages) ***Dream the Snack*** (TBA) Group fills out proforma and engages in limited discussion on each concept + Complexity v value maps.	Bradley Wardrop-Brown
3:30pm (60 min)	**Plan for Action** Group discussion on interesting concepts, identifying challenges and how to address. Challenges to address might include commercialization partners, manufacturing difficulties, scaling, small or unidentified target market etc. This will highlight the most immediate and key areas to focus on to de-risk the concept and translate into concrete action plans for selected food concepts	David Ireland

Close Day 1

Catering time

11:00am	Morning Tea
1:00pm	Working Lunch
4:30pm	Afternoon Tea

2

Day 2: "*Share the Snack*" – Creative thinking and sharing of ideas

9:45am (15 mins)	**Review Day 1** Recap, insights, thoughts, plan for Day 2	David Ireland
10:00 am (120 min)	**Presentation of Concepts - Maxime Bilet** ***Taste the Snack*** (TBA) ***Dream the Snack*** (TBA) Group fills out proforma and engages in limited discussion on each concept + Complexity v value maps.	Maxime Bilet
12:00pm (30 mins)	**Lunch** (not working)	
12:30pm (90 min)	**Plan for Action** Group discussion on interesting concepts, identifying challenges and how to address. Challenges to address might include commercialization partners, manufacturing difficulties, scaling, small or unidentified target market etc. This will highlight the most immediate and key areas to focus on to de-risk the concept and translate into concrete action plans for selected food concepts	David Ireland
2:00 pm (120 mins)	**Share the snack Ideation** Facilitated brainstorming session to solicit comments and ideas ideas from the group. This session is designed to utilize the groups collective knowledge and experience, seeded by concepts that we have heard during the workshop. Expectations are most concepts will be very embryonic, but collective experience may quickly accelerate several concepts into action plans or 'close-out' ideas. Consider PepsiCo product offerings	David Ireland/Nick Hazell
4:00pm (60 min)	**Identification of leading opportunities** Identify the most exciting future red meat snacking concepts from both days and continue to build concrete action plans, fill any missing information and review the objectives to ensure they have been met. Detail the next steps.	David Ireland
5:00pm	**Wrap-up, Next steps & Thank you**	Scott Needham/Christine Pitt

3

Workshop close-out at 5.30

Catering time

10:45am	Morning Tea
12:30pm	Working Lunch
4:00pm	Afternoon Tea

4

Attachment 3: Workshop Outcomes

Agenda & Process



- Welcome, introduction and workshop objectives
- Overview of the process, roles & responsibilities
- Presentation of Concepts (Earlee)
  - → Taste the Snack & Dream the Snack
  - → Group discussion*
- Presentation of Concepts (BluOak)
  - → Taste the Snack & Dream the Snack
  - → Group discussion*
- Presentation of Concepts (Maxime Bilet)
  - → Taste the Snack & Dream the Snack
  - → Group discussion*
- Review
  - → Group discussion of themes & concepts
  - → Gaps, challenges & 12-month action plans

* Filling out proforma, discussion of desirability, viability and feasibility, Value vs. Complexity mapping

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Welcome and Intent

Dr Scott Needham

MDC partnership has transitioned from linear project management process to one that is design led thinking

"We are seeking iteration, optionality and agility in all of our investments."

- Dr Scott Needham

"We need to add as much value as possible to parts of the animal that may be considered waste or low value cuts."

- Dr Christine Pitt

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We also agreed on...

Pre-post and workshop output is confidential

Concepts are not to be stolen – environment of trust

Contribute to concepts in good faith (Share the Snack)

Don't be too precious with your own concepts

Utilize the collective experience to identify and improve upon the most promising concepts

Much validation work to do post meeting (e.g. consumer validation is outside this meeting)

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Outputs...

Short-list of 3 snack product concepts with a high chance of commercial success (complexity v value plot). **Specific steps** required to de-risk the short-listed concepts (desirability, feasibility, viability) with indicative time-line, $, eco-system partners identified.

A reserve list of up to 3 snack product concepts of promise. Identification of the area(s) of key concern to assist to de-risk the snack product concept (desirability, feasibility, viability)

Share the Snack – New snack concepts (synergies or extensions beyond those presented) with at least 1 being selected as the most promising.

Pepsico - Identification of at least 1 snack product concept that would be attractive

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Review of Themes and Action Plans

Fat

- Fork in road in 1950s – reimagine beef fat (tallow < $1/kg vs. butter $9/kg, 10x increase). Palm oil & margarine are out.
- Fractionate – pastries (stearate), "chocolate". Take out saturated – a monounsaturated fat (neutsloot oil). Opportunity – fractions perfect for frying, pastries, confectionery, small goods
- Supply – genetics improvement produces bigger animals, better visualization of carcass allows more efficient/targeted cuttings

- Business model – we need partners who know how to fractionate/extract at scale; we sell raw tallow to partner (expert in fractionation) to do; we need to educate market on beef fat not being bad
- Partners – Goodman Fielder, Unilever, Perus, Cargill

- Technical challenges – how do we extract value from the different fractions within fat? Clean up, take out certain elements e.g. cholesterol, make perfect for applications e.g. chips, chocolate, replace palm oil, a new margarine
- Pick up lost technology due to misunderstandings around fats

Action plans

- *Contact refiners*
- *Revisit 10yr old MLA report*
- *Trial amounts to identify near term opportunities and key technical barriers*
- *Identify changes at processors needed to supply*
- *Market survey, where are consumers?*

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Flavour

- Enables everything
- Who's interested? Food ingredient industry, consumers – drive towards natural, they won't buy powder, but they will buy products that use it – natural Oz beef flavour. Not IFF, but McCormick, PepsiCo…
- Value proposition for Aus meat – made with Australian meat; 5-8%wt of chips produces 50% by value

- Anyone who uses it has to note it is "Australian grass fed beef", like Belgian chocolate. We control this over the rights to the process, but the value goes to sector.
- Business model – devise process that meat processors can license to Teys/JBS to do. Bradley to help them set it up. Work with McCormick to develop process and then they do it. Have in contract they have to use Oz beef. Need to be careful about who gets value – they need to be part of Aus meat industry

- Devise process that works at scale
- Need info from Max and work with Bradley to scale
- Key invention is drying beef (freeze drying) before the roasting (cooking)/flavour development. And pressure rendering of fat

Action plans

- *Refine/define process, to protect it (IP)*
- *JV/construct business proposition*
- *Confirm consumer demand*

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Chips (beef)

- Meat + starch/carbohydrate (this is a meat chip that tastes like meat vs. something like popcorn. We need to understand the customer then use it to define product) – crispy texture & "high protein" (>25%) – this this is the value of the product
- Visible beef – floss, jerky fines, ...
- Sheeted dough cut, dried, fried, seasoned – finished product has high (25%) protein claim

- Understand COGS – the more meat the higher price and change in colour of meat
- If we can test the occasion (movies, work, afternoon tea...) then use it to define the design of product
- Not mass market, but niche in lots of markets

- Need to play around with meat + starch to get the right product
- Once you have the right product, you sheet then dry to pellet stage then fry for puffing (you control texture by starch)
- Alternatives to frying: IR toasting, microwave, air impingement
- We should look into visible meat bits like strands of meat floss

Action plans

- *Identify marketing and product development partners*
- *Consumer research for concept testing -> Informed prototyping development and multiple trials -> Prototype testing (can include variants for different markets) -> Refining products*

Xinova *IP for meat/starch chips?*

Bars

- Consumer engagement – listening – does that translate to sales? Eating occasions: meal replacement, fixers, gym junkies/cross fit, kids' lunchboxes, toddler, elderly, aged care (protein demand), "active ager" (+ gut health). Epic & fix – need to understand space & opportunity (case study). Formula how that hit market. Fortification, clean label, etc.
- Including personas + identify pain point (MLA data as a start). Use case understanding
- Is there a visual aspect that needs to be addressed? Enrobing with something else (fruit/meat leather). Visual appeal

- Raw material pathway – should be grass fed but need to be able to understand bioavailability (Ag research NZ)
- Partnership – how do they work. Meat Co. product or snack food Co. NZ supermarket
- Are producers the best partner rather than further down the value chain. New entrants into market, producers not existing players
- Competitive landscape: 1) red meat bars (globally), 2) other protein bars

- Regulatory constraints

- See general action plans

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Sweets
– Trend in Asia: consumer research in Asia (Singapore?), MLA AU collagen – where does this fit? Is it a "big mac + fries + diet coke" scenario? High end workers market
– Price? Portion size? Morning dose – afternoon dose? Sweet vs. savory?
– Not total compliment of amino acids etc. – will be rejected by certain groups – complementary protein source
– Protein bar space is opened up by use of collagen
– Collagen peptide synthetic production? Synthetic novel food: FDA advice?
– Supply constraints from multiple sources – understand development of collagen industry supply, availability (analogous to pectin supply issues). AU collagen industry: sausage casing – resistance
– Route to market needs to reinforce position, business model important, not for Coles
– Innovation in food science not manufacturing. How do makers work with what we have?
– BOI partner development
– See general action plans
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Chips (collagen)
– Align with pork crackle market?
– Promote protein content. Good source of protein
– Not meal replacement
– Market analysis: where to buy? Bottle shop, retail, upmarket deli, cafe, sport events, development demand, supermarket (last); where are the competitors.
– Clear occasional usage
– Satiating, portion-controlled
– Snack for office, vending machines – what is link to this chain?
– Room in current snack market
– Shape, texture, volume
– Optimise fibre/meat content. Fibre content – effect on texture. Prebiotics
– Low carb?
Action plans
– Define product: low carb, high protein, prebiotic, tasty chip alternative (room in current snack market?)
– Consumer insights
– Collagen supply chain
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Drinks

- Performance market, NOT lounge market
- Assumptions: frequency of consumption – drink daily? Degree of carbonation? More complete recovery drink
- What market research needed? Target consumers? Gym athletes, team sports, fun runs (city to surf), male, female, athlete endorsed, AIS endorse? – too specific
- Flavour development: fruit, no beef association, colour development, natural, shelf stability, chilled? Single use RTD – 500 ml size
- Customisation of recovery drink, e.g. cap release of customized drink ingredients
- Sales outlet: sports arenas, bike shops, outdoor retail
- Source of collagen: 100% Aus for MLA/MDC, Aus supply chain, links with beverage industry
- Sold ambient, consume chilled (self-chilled cans)
- Business model for customised systems. Data collection – build personal ecosystem database
- Cold fill, not fill? Must be carbonated. Sediment OK?
- Supply chain/distribution – cold chain, vending machine?

Action plans

- *Consumer insights/market assessment*
- *Collagen supply chain, limits?*

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Jerky

- Understand the opportunity space (US, Asian style…) and target customer e.g. female, kids ("jerky candy")
- How to differentiate significantly in packaging, appearance/visual, texture and flavour
- Halal product replacement
- New category? Not jerky, but aged, high value, ready to eat AU experience with red wine (share platter)
- Position as high end but accessible. Branding – Epic bar
- Partner with cattle producers
- Somewhere between jerky as we know it and charcuterie
- Iconic cuts
- Complexity is time of experimentation to proof of concept

Action plans

- *3-5 iconic prototypes read for consumer testing + design logo/brand*
- *Go to farmer's markets, The Meat Truck, local butcher, pop up tastings, for fast/real time response*
- *Meanwhile, pending proof of concept, identify partner producers who buys in and manages supply/processing chain – sell them*
- *Verify financial feasibility, it meets standards of product, and market access*
- *Build story – web presence*

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General Action Plans
3 months
Consumer insights / competitor analysis – outsource (lean start-up methodology)
Pivot point / refined point
Design sprint (9-12 weeks)
Use cases & understand size etc.
Geographic representation
Key attributes, provenance, labelling, nutrition
6 months
Detailed design (9-12 weeks): MVPs, occasions
From now – sense c partnerships + value chain design
Focused discussion from Step 1
9 months
More commercial
Value chain set
Partners identified
Manufacturing & scale up sorted
Overall aims + partners
Highest value consumers
New entrants +/or producers could be that (see Toni Kroos example)
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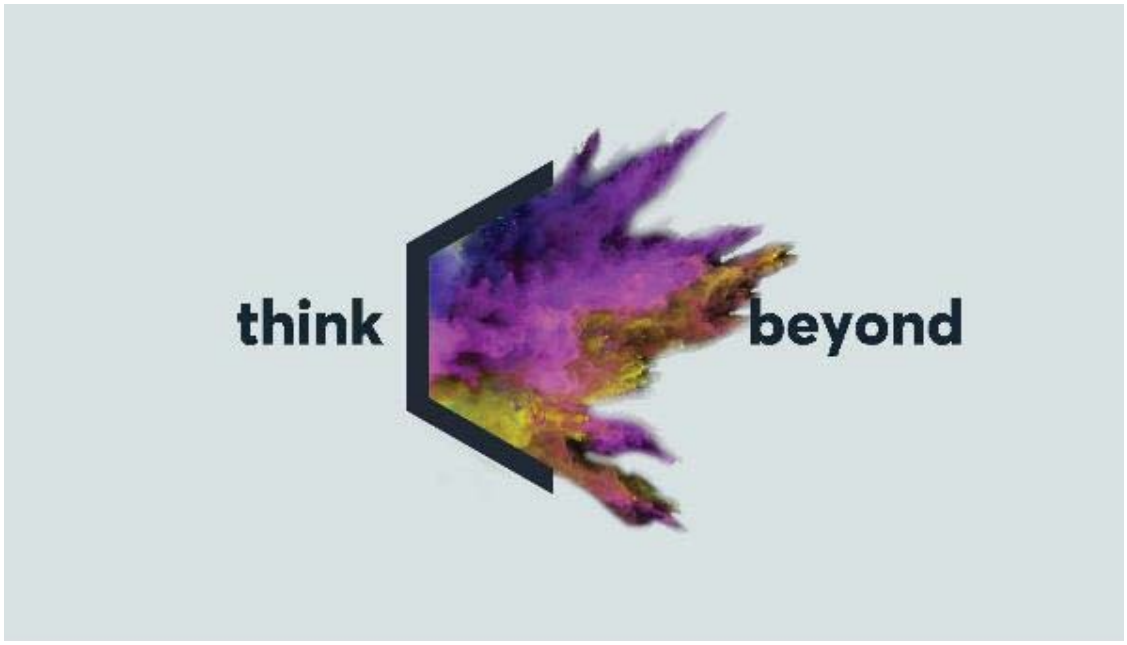
think
beyond

Attachment 4: Snack Concept Evaluation Sheet

FIRST REACTION

SCORING SHEET

INNOVATOR/PRODUCT: _____ YOUR NAME: _____

1. How much do you like or dislike this CONCEPT overall?

Dislike Extremely										Like Extremely
0	1	2	3	4	5	6	7	8	9	10

2. What do you like about it?

_____

_____

3. What do you dislike about it?

_____

_____

PLEASE NOW TASTE THE FOOD

4. Overall, how much do you like this PRODUCT ?

Dislike Extremely										Like Extremely
0	1	2	3	4	5	6	7	8	9	10

5. What are this product's TOP 3 challenges?

_____

_____