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Assessment of the mycological hazards associated with the dry ageing of red meat

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Executive summary

The dry ageing of beef and sheep meat (lamb) is increasing in popularity in Australia due to the improvement to tenderness and flavour the process affords. There are concerns, however, related to the potential risk fungal growth on meat during the dry ageing process poses to public health. More specifically, it isn't well understood what potential there may be for toxigenic fungi to grow and produce mycotoxins and whether process interventions may be necessary to reduce risk. Therefore, the objective of this project was to undertake a desktop assessment of publicly available information related to the mycological hazards associated with the dry ageing of red meat, and in particular, for beef and lamb dry-aged at temperatures ranging -0.5-3.0°C, a relative humidity of 75-85%, for 14-35 days.

This assessment has confirmed the following –

- Only anecdotal evidence exists of the moulds typically present on dry-aged beef and lamb, which includes *M. racemosus* and *P. camemberti*. No information related to the fungal species typically present on Australian dry-aged beef or lamb was found.
- In the absence of comprehensive data of the fungal species typically present on dry-aged beef and lamb, this assessment also considered fungi that had been previously isolated from cold-stored beef as potentially relevant for dry-aged beef and lamb, and included *Thamnidium elegans*, *Cladosporium cladosporoides*, *Cl. herbarum*, *Chrysosporium pannorum, Penicllium hirsutum, P. corylophylum, Aspergillus* spp. *Aureobasidium pullulans, Mucor mucedo and H. pulchrum*.
- There was no evidence to suggest that the moulds typically associated with red meat (both cold-stored or dry-aged) are capable of producing mycotoxins at between -0.5-3.0°C and 75-85% RH, as typical during the dry-ageing of red meat, and are therefore most unlikely to pose a risk to human health.
- Process steps designed to control (UV and ozone) and remove (trimming) microbial growth on or from the surface of dry-aged red meat are therefore not necessary for the purposes of preventing mould growth and mycotoxin production, but are adequate for the purposes of preparing a product that is wholesome and saleable in accordance with good manufacturing practices.

Whilst no safety risks were identified in relation to mould growth on dry-aged beef and lamb given the information presently at hand, there are considerable knowledge gaps related to the typical bacterial and mould species that develop during the dry ageing process, particularly for Australian beef and lamb. This information may be beneficial to better understand the importance of process control for optimising product quality.

The findings in this assessment were reviewed, and are supported, by Dr Ailsa Hocking and Dr John Pitt.

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1 Background

The dry ageing of beef and sheep meat (lamb) is increasing in popularity in Australia due to the improvement to tenderness and flavour the process affords. These benefits are not only advantageous for premium cuts but also for lower-grade cuts (e.g. mutton), and have the potential to attract a price premium over wet-aged products. Meat and Livestock Australia (MLA) are developing a National Standard for dry-aged red meat and would like to understand the microbiological hazards associated with the process. Specifically, dry ageing processes undertaken within, or close to, the following conditional boundaries –

1. **Temperature:** -0.5 to 3.0°C
2. **Relative humidity (RH):** 75 to 85%
3. **Time:** 14-35 days

Of particular interest, and focus for this assessment, are the fungi that may be present and grow on meat during the dry ageing process (e.g. *Thamnidium elegans*, *Rhizopus* and *Mucor* spp.), and what significance they pose to public health (i.e. potential for mycotoxin production). Further, processing steps that may have an impact on hazard reduction (if necessary), including trimming and the use of ultraviolet (UV) light and ozone, have been identified as important for consideration as part of this assessment.

Through understanding the hazards, the appropriate guidelines for processors can be developed, including the provision of appropriate mitigation strategies.

2 Project objectives

The objective of this project was to undertake a desktop assessment of publicly available information related to the mycological hazards associated with the dry ageing of red meat, and in particular, for beef and lamb dry-aged at temperatures ranging -0.5-3.0°C, a relative humidity of 75-85%, for 14-35 days.

3 Assessment of the mycological hazards associated with dry-aged red meat

3.1 Dry-aged beef: process specifications and microbiological considerations

Today's commercial dry ageing process for red meat is based on the long established tradition of drying beef to aid in preservation (Dashdorj *et al.*, 2016) and has been described as a carefully controlled decomposition designed to enhance meat tenderness and/or taste. The process consists of holding un-packaged, primal and sub-primal cuts of meat in a controlled temperature, airflow and humidity environment for at least 14 days, during which time, protein and fat break down as a result of enzymatic activity and other chemical processes (Dashdorj *et al.*, 2016). By virtue of the drying process, up to approximately 50% loss in yield occurs due to moisture loss and the required removal of the dried surface crust via a process called trimming (Galletly, 2016; U.S. Meat Export Federation). Dry-aged meat is more expensive to produce compared to the more typical, wet-aged produced meat, and is largely reserved for niche markets servicing upscale restaurants and grocery stores. That being

said, it is gaining popularity in the USA, Australia and Asia, and applications extending beyond traditional high-value beef cuts are being explored. For example, MLA (Burvill, 2016a and b) have previously commissioned studies investigating value-add for sheep meat, including mutton and hogget, processed using dry ageing techniques.

Previous reviews conducted by the CSIRO (2010) and Galletly (2016) describe process specifications for dry ageing beef, in line with good manufacturing practices (GMP), as summarised in Table 1. These are also mandated by PrimeSafe Victoria in their guidelines for ageing beef.

Table 1: Recommended process specifications for dry ageing beef

Parameter	Specification	Notes
Time (days)	≥14	Upper limit typically 35 d
Air temperature (°C)	-0.5-1.0	>1.0-3.0°C could be acceptable where the ageing process lasts 7-14 d
Relative humidity (%)	75-85	RH as low as 50% have been used
Air velocity (m/s)	0.2-0.5	

Given the outer surface of fresh red meat can be contaminated with spoilage bacteria and fungi, as well as pathogens, the conditions for dry ageing must be established rapidly and be carefully controlled to prevent any unwanted microbial growth. The reduced air temperature limits the potential for growth to psychrotrophic (cold tolerant) microbes, and by reducing RH, this further restricts growth potential to low water activity (a_w) tolerant microbes, ideally controlling the growth of bacterial pathogens and minimising the growth of spoilage microbes.

Few surveys of the microbiological diversity on the surface (i.e. crust) of dry-aged meat have been undertaken. Where undertaken internationally, the dry ageing conditions either were not consistently within the recommended process specifications outlined in Table 1 or they were not specified in their entirety. Gowda and others (2016) surveyed dry-aged beef in Belgium (conditions unspecified) and found high numbers of *Pseudomonas* (median >4.7 $\log_{10}$ colony forming units [cfu]/cm²), lactic acid bacteria (LAB; median >3.7 $\log_{10}$ cfu/cm²) and yeasts (median >4.0 $\log_{10}$ cfu/cm²) on lean and adipose surface tissues. Further, more than half the samples had detectable mould (>1 $\log_{10}$ cfu/cm^2), though their identities were not determined. Ryu *et al.* (2017) examined dry-aged beef over 60 d when stored at 1-4°C and 80-90% RH and found that total aerobic bacteria (TPC), LAB and total yeast and mould (YAM) counts increased notably within the first 25 d, each reaching ~4 $\log_{10}$ cfu/g (one exception, where the YAM count for one cut was ~6 $\log_{10}$ cfu/g), then slowly increasing by an additional 2 $\log_{10}$ cfu/g up to day sixty. Li and others (2014) assessed TPC, LAB, yeast and mould counts of surface fat and meat from beef that had been dry-aged at approximately 2.9°C for 8 and 19 d at an unknown RH. Counts were higher on the meat surfaces compared to that on the fat surfaces and all counts increased with time. For example, TPC, LAB, yeast and mould counts on meat surfaces were 8.75, 3.20, 5.68 and 0.72 $\log_{10}$ cfu/g, respectively, after 19 d. In Australia, much lower counts were reported on dry aged lamb, with counts decreasing from an average of approximately 1000, 10.9 and 0.4 cfu/cm^2 to 4.3, ≤0.25, and 0.3 cfu/cm^2 for TPC, yeast and mould, respectively, after 33 d at 0-2°C and 70-80% RH, in a UV Ozone lit environment (Burvill, 2016a). This could reflect strict processing conditions and/or better

initial product quality. Where assessed in these various studies (Burvill, 2016a and b; Gowda *et* al., 2016; Ryu *et al.*, 2017), no pathogens were detected on dry-aged beef and lamb/sheep.

3.2 Mould species associated with dry-aged red meat and the hazards associated with their growth

Historic research investigating cold-stored, red meat-associated fungi have identified many mould species, including *Thamnidium elegans*, *Cladosporium cladosporoides*, *Cl. herbarum*, *Chrysosporium pannorum, Penicillium hirsutum, P. corylophylum, Aspergillus* spp. *Aureobasidium pullulans, Mucor mucedo, M. racemosus and Helicostylum pulchrum*, manifesting as black and white spot spoilage and growing at temperatures as low as -5°C (Campano, 1985; Gill and Lowry, 1981; Gill and Lowry, 1982; Lowry and Gill, 1984). Pitt and Hocking (2009) explain that moulds only become relevant on meat when storage temperatures and a_w are lowered enough to allow them to compete with bacteria, and so dry-aged red meat provides for that scenario. However, based on the literature reviewed as part of this assessment, only two studies were found that aimed to specifically isolate and identify moulds from dry-aged meat (Tapp, 2016; Ryu *et al*., 2017).

In his dissertation, Tapp (2016) explains the importance of understanding the moulds capable of growing on red meat during the drying process and if mycotoxin production is a subsequent risk. Although over 40 mould species were isolated from beef and lamb that had been dry-aged for up to 28 d at 0.5-1.5°C with 30-80% RH, the methods used did not permit identification of the isolates. However, in a follow-up study, *Cochliobolus* sp., *Cochliobolus sativus* and *M. racemosus* were isolated from dry-aged beef (21 d at 1.7°C and <80% RH). The anamorph (asexual state) of *C. sativus* is *Bipolaris sorokiniana* and has no known association with meat, but rather, with grains and cereals. *M. racemosus* is, however, associated with the spoilage of cold-stored red meat, as previously introduced.

In a study undertaken by Ryu *et al.* (2017), a *Cladosporium* species, was initially found to be present on dry-aged beef (25 d, 1-4°C and 80-90% RH) but was not detectable after extended drying (i.e. 60 d), where, however, *Penicillium camemberti* was isolated.

As fungal identifications in the above two studies were performed using broadly based genomic techniques, 5.8S (Ryu *et al*., 2017) and 18S (Tapp, 2016) rRNA sequencing, in the absence of a complimentary assessment of colony morphology and growth rates, it is unclear if the identification to species level are accurate. In particular, *P. camemberti* is associated only with cheese manufacture, while the closely related species, *P. commune*, is a common spoilage species (Pitt and Hocking, 2009).

To understand the potential food safety risks associated with the growth of the various species of fungi historically isolated from both cold-stored and dry-aged beef and lamb, as cited above, their ability to produce mycotoxins within the environmental conditions prevalent during dry-ageing must be considered. Mycotoxins are secondary metabolites produced by moulds that can have a toxic effect on humans and animals when consumed (Hocking and Pitt, 2003; Peraica *et* al., 1999). Of the genera identified above, *Aspergillus* and *Penicillium* are the only ones known to produce mycotoxins, but are not known to be capable of producing them at temperatures between -0.5 and 3.0°C (Hocking and Pitt, 2003). For example, the minimum reported temperature for aflatoxin production by *A. flavus* is 12°C (Hocking and Pitt, 2003). *P. commune* can produce the minor mycotoxin cyclopiazonic acid (CPA), but only at temperatures above 20°C (Hocking and Pitt, 2003). Whilst *P. hirsutum* is known to produce roquefortine C, with mixed reports of CPA production, the minimum temperatures of production have

not been reported. Mycotoxins are not known to be produced by *P. corylophylum* (Pitt and Hocking, 2009).

In view of the known temperature profiles of mycotoxin production by other *Penicillium* species (Pitt and Hocking, 2009), formation of mycotoxins below 5°C is very unlikely (Hocking and Pitt, 2018). Further, with reduced RH and a subsequent reduction in the available water on the surface of dry-aged meat (i.e. a_w), this further constrains the ability of moulds to produce mycotoxins, rendering the possibility even less likely (Hocking and Pitt, 2018). The *Penicillium* and *Aspergillus* species cited here, and able to grow on red meat under chilled and/or low RH conditions, are most unlikely to produce mycotoxins under the defined conditions specified in Table 1 or pose a risk to human health (Hocking and Pitt, 2018). The other cited fungal genera and species associated with dry-aged red meat are not known to be hazardous to human health.

3.2.1 Association of *Rhizopus, Mucor, Aspergillus* and *Penicillium* spp. with dry-aged red meat and the associated food safety risks

As reviewed above, fungal species from *Mucor, Aspergillus* and *Penicillium* may be present on cold-stored red meat, including that which has been dry-aged under certain conditions, however there is no evidence that supports the possibility of mycotoxin production under the dry-ageing conditions outlined in Table 1. This review did not identify any studies that named *Rhizopus sp.* as a mould specifically isolated from cold-stored or dry-aged red meat; it is, however, mentioned anecdotally in some reviews and guidelines without substantiation (Dashdorj *et* al., 2016; Jay, 2000; PrimeSafe, 2018). Nevertheless, mycotoxin production by *Rhizopus* spp. is unknown and unlikely (Pitt and Hocking, 2009).

3.2.1 Prevalence and benefit of *Thamnidium elegans* growth on dry-aged red meat

T. elegans is a filamentous fungus with known association with aged red meat (Jensen, 1944). It has been reported that the growth of *T. elegans* complements the natural collagenolytic, enzymatic break down of the meat muscle and connective tissues, resulting in improved tenderisation and flavour development (Dashdorj *et al.*, 2016; Tapp, 2016). Kotula *et al*. (1982) explain that a process whereby *T. elegans* is deliberately introduced to the surface of ageing meat for the purposes of tenderisation was patented by Williams (1957). However, whilst *T. elegans* can grow at temperatures as low as -1°C (Jay, 2000), Kotula *et al*. (1982) were unable to show any proteolytic activity under laboratory conditions in litmus milk at 4°C, concluding that the use of *T. elegans* to improve meat texture was not likely to be feasible unless the ageing temperature was increased to 18-24°C where proteolysis was detected.

3.3 Strategies for microbiological hazard reduction

The dry ageing processing conditions outlined in Table 1 are designed to prevent the growth of bacterial pathogens and to limit the growth of spoilage bacteria and fungi, on meat, during the ageing process. Therefore, maintaining these conditions throughout, in addition to implementation of general GMP, will be a critical part of any dry ageing food safety plan. In addition to these control measures, the following aspects either need to be implemented or considered to reduce microbiological hazards and improve product quality.

3.3.1 Surface trimming

The removal of the dried surface (crust) of dry-aged red meat is required prior to sale (Galletly, 2016; PrimeSafe, 2018). This process must be undertaken carefully and hygienically to limit the transfer of microorganisms from the surface of the meat to the edible product. Concern with adequate trim removal (i.e. depth wise) relates to the potential for microbial species and any metabolites they have produced, including mycotoxins, to penetrate or permeate through the crust and into the edible meat product. Tapp (2016) comments that there are no guidelines in the USA related to the safe removal of the crust, whereas other food products, such as cheese, have guidance based on science-based knowledge, recommending cutting at least one inch around and below mould spots on hard cheeses.

In Australia, PrimeSafe Victoria (2018) guidelines for the production of dry-aged beef require that the dry-ageing process be validated via testing for mould before trimming, where any mould detected must be confirmed as *Thamnidium* spp. for process approval to be granted. A testing flow diagram directs processors to discard product if moulds other than *Thamnidium* spp. are detected.

Based on the information reviewed here related to the safety risks associated with mould growth on cold-stored and dry-aged red meat, fungi other than *Thamnidium* spp. are not indicative of a food safety risk, as mycotoxin production is not known to occur within the temperature (-0.5-3.0°C) and RH (75-85%) range suggested for the safe dry ageing of meat. Therefore, trimming, as described by Galletly (2016), is required only for the purposes of wholesomeness and saleability.

3.3.2 Effect of UV light and ozone on mould growth during the dry ageing of red meat

Galletly (2016) identified UV radiation (wavelength 200-300 nm) emitted from UV lighting (1 unit per 5 m^2) as an important dry ageing process parameter in addition to those outlined in Table 1. The UV radiation works to assist in controlling the growth of microbes by directly irradiating them on exposed surfaces of meat, or by treating re-circulated air in UV-lit chambers to minimise the contamination of meat by airborne microbes (CSIRO, 2010; Dashdorj, 2016; Savell, 2008).

The use of ozone in food processing is well reported, where the high oxidising power of ozone readily inactivates microorganisms in processing water, but can have limited effect in more complex food systems where organic constituents can interfere and reduce the effect of ozone (Kim *et al.*, 2003). Kim *et al*. (1999 and 2003) have reviewed the application of ozone for surface decontamination of meat with variable results. For fungal control, the lag phase of *Thamnidium* spp. and *Penicillium* spp. have been shown to increase with >2 µg/l gaseous ozone, whilst growth rates remained unchanged. Combining ozone (0.5 µg/l) and UV (0.2 µW/cm^2), however, worked synergistically, increasing the lag phase and decreasing growth rates (Kim *et al.*, 1999). It has also been shown that gaseous ozone (0.1 mg/l) and 60-90% RH were effective in inactivating bacteria, but higher concentrations of ozone were required to inhibit moulds (Kim *et al.*, 1999).

Whilst UV and ozone could improve the microbiological quality of dry-aged red meat, including controlling the growth of fungi, the assessment conducted here concludes that there are no known safety risks associated with their growth and, therefore, UV and ozone would not be necessary for the purposes of preventing mould growth and subsequent mycotoxin production.

4 Conclusions

This assessment has confirmed the following –

- Only anecdotal evidence exists of the moulds typically present on dry-aged beef and lamb, which includes *M. racemosus* and *P. camemberti*. No information related to the fungal species typically present on Australian dry-aged beef or lamb was found.
- In the absence of comprehensive data of the fungal species typically present on dry-aged beef and lamb, this assessment also considered fungi that had been previously isolated from cold-stored beef as potentially relevant for dry-aged beef and lamb, and included *T. elegans*, *Cl. cladosporoides*, *Cl. herbarum*, *Ch. pannorum, P. hirsutum, P. corylophylum, Aspergillus* spp. *Au. pullulans, M. mucedo, and H. pulchrum*.
- There was no evidence to suggest that the moulds typically associated with red meat (both cold-stored or dry-aged) are capable of producing mycotoxins at between -0.5-3.0°C and 75-85% RH, as typical during the dry-ageing of red meat, and are therefore most unlikely to pose a risk to human health.
- Process steps designed to control (UV and ozone) and remove (trimming) microbial growth on or from the surface of dry-aged red meat are therefore not necessary for the purposes of preventing mould growth and mycotoxin production, but are adequate for the purposes of preparing a product that is wholesome and saleable in accordance with GMP.

Whilst no safety risks were identified in relation to mould growth on dry-aged beef and lamb given the information presently at hand, there are considerable knowledge gaps related to the typical bacterial and mould species that develop during the dry ageing process, particularly for Australian beef and lamb. This information may be beneficial to better understand the importance of process control for optimising product quality.

The findings in this assessment were reviewed, and are supported, by Dr Ailsa Hocking and Dr John Pitt.

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