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Final report

FOOD SAFETY

INVESTIGATION OF MICROBIOLOGICAL GROWTH ON OFFAL DURING COOLING

Prepared by:
Neil McPhail, Gary Dykes and Ian Eustace
Food Science Australia

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EXECUTIVE SUMMARY

Australian offal products that are exported are normally packed into cartons soon after removal from the carcase. These warm products are required to be cooled at a rate that meets the refrigeration index (RI) criteria as defined in the Export Control (Meat and Meat Products) Orders. The predictive model, on which the RI is based, has been validated for hot-boned meat (bulk-packed and primal cuts) but not for offal.

The aims of this project were to:

1. undertake a microbiological validation of the predictive equation in the MLA RI calculator for beef livers, hearts and scalded tripe, and
2. generate data on lactate concentrations, pH and water activity in beef livers, hearts, scalded tripe and cheek meat.

Ten cartons each of beef hearts livers and scalded tripe were inoculated at three sites within each carton with a cocktail of five *E. coli* strains. The growth of *E. coli* was measured by testing excised samples from each site before and after freezing. The temperature at each site was logged and the RI calculated.

When the results for all three offal types are combined, agreement between the predicted and measured growth is only fair (R^2 = 0.53), although similar to that found for chilled hot-boned beef primal cuts. At the predicted RI values of interest (i.e. 1.5, 2.0 and 2.5) the model over-predicted by 0.7, 0.5 and 0.3 logs respectively.

The values for pH, lactate and water activity that are currently in the predictive equation are, respectively, pH 6.8, lactate 51 mM, and water activity 0.995. The pH results for the four offal products suggest that the pH value of 6.8 is appropriate; changing the pH value in the equation between 6.5 and 7.2 has very little effect on the RI output. The lactate results obtained in the investigation suggest that a value in the equation of 25 mM better reflects measured values. However at pH values between 6.5 and 7.2, changing the lactate value in the equation from 51 mM to 25 mM has very little effect on RI values. The value of 0.995 for water activity that is currently used in the RI calculator is very close to the values measured for hearts and cheek meat.

If the only change that is made to the constant input values in the predictive equation is to change the lactate concentration to 25 mM, the RI values for offal products will include a ‘margin of comfort’.

It is recommended that:

1. Values currently in the MLA RI calculator for pH and water activity for offal products (6.8 and 0.995 respectively) should remain;
2. Changes to the inputs to the RI calculator for offal products should be limited to amending the constant value for lactate concentration from 51 to 25 to better reflect measured values. This change will have very little effect on RI values calculated from specific cooling histories;
3. There appears to be little benefit in including separate buttons for different offal products.

INVESTIGATION OF MICROBIOLOGICAL GROWTH ON OFFAL DURING COOLING

1.0 INTRODUCTION

Offal products to be exported in the frozen form are usually packed into cartons soon after removal from the carcase. This results in warm surfaces, that may be contaminated with pathogenic and other bacteria, being located near the centre of a carton where cooling may be slow.

Cooling rates for these products should meet the Refrigeration Index (RI) criteria as defined in the Export Control (Meat and Meat Products) Orders. The RI is based on a predictive microbiology model developed by Ross *et al* (2003). The model was validated for hot-boned meat by inoculating the surfaces of beef and mutton trim with faeces or *E. coli* cultures and freezing the product in cartons. There was acceptable correlation between observed and predicted growth (Hot boning expert panel, 2004).

The model, which uses published values for pH and lactates in meat flesh and assumes a water activity of 0.995 for all offal products, has not been validated for any offal. There are no published values for lactate levels for offal apart from one report each for bovine (23 – 37 mM) and ovine (38 – 66 mM) liver shortly after slaughter. Published data for pH of heart and liver suggest values in the range 5.9 to 6.8. No data are available for water activity of offal products.

2.0 PROJECT AIM

The aims of this project were to:

1. undertake a microbiological validation of the predictive equation in the MLA RI calculator for beef livers, hearts and scalded tripe, and
2. generate data on lactate concentrations, pH and water activity in beef livers, hearts, scalded tripe and cheek meat.

3.0 METHODS

3.1 Validation of RI

Over a period of several weeks, 10 cartons each of beef livers, hearts and scalded tripe were collected from an export abattoir and transported to the Food Science Australia laboratory in insulated containers. The products were packed into 190 mm deep fibreboard cartons and three sites were selected in each carton to provide different cooling rates. For livers and hearts, the sites were approximately 50 mm, 100 mm and 150 mm from the bottom of each carton. In the case of tripe pieces which were packed in five plastic bags, the sites were at approximately half the depth at the two ends and centre of each carton.

The offal at each of the three sites in each carton was inoculated with a cocktail of *E. coli* strains. The *E .coli* inoculum was prepared from five strains of *E .coli* (Food Science Australia culture collection strains EC1604, EC1605, EC1606, EC1607 and EC1608). The strains contain no known virulence markers for pathogenic *E .coli* and between them have similar characteristics to various known isolates of *E. coli* O157:H7. Briefly, the cocktail of five *E. coli* strains grown for 14 h at 37°C was prepared in cold (0°C) Buffered Tryptone Soya Broth at a concentration of approximately one million cfu mL^{-1}. The inoculum was placed on the offals by brushing it onto the surface over a marked area of ~100 cm^2 (Figure 1). The initial count was estimated by excising two 5 cm^2 cores using a sterile cork borer. The cores were analysed as described below. The inoculated area was covered with a sterile plastic sheet and two sites marked for post-freezing sampling. Thermocouple sensors were then taped to the sheet over those sites (Figure 2). When each carton was fully packed and sealed, temperature logging at six-minute intervals commenced. Within 15 minutes of them being sealed, the cartons were then placed in an air blast freezer operating at -30°C.

After freezing for 45 to 70 hours, the cartons were removed and the inoculated sites again sampled by excising two 5 cm^2 cores through the plastic sheet using a sterile cork borer. The samples were analysed as described below.

Core samples were homogenised in buffered peptone water (BPW) and serially diluted to appropriate levels in the same diluent. *E. coli* was enumerated by plating appropriate dilutions onto Petrifilm *E. coli*/Coliform count plates according to the manufacturer's instructions. The same dilutions were also plated using the Thin Agar Layer (TAL) solid repair method to make sure that injured *E. coli* were being recovered. The TAL method was developed in line with the general TAL procedure described by Wu et al (2001) but using eosin methylene blue (EMB, Levine; Oxoid) as the selective agar. TAL plates were prepared no more than 24 hours in advance of required usage.

Agar plates were pre-poured as follows:

a. 20 mL EMB agar was poured and allowed to solidify;
b. Solidified EMB was overlain with two separate layers, each 7 mL, of tryptone soy agar (TSA; Oxoid) as a non-selective medium.

After plating appropriate dilutions, plates were incubated at 37°C for 24 h and counted. Counts were reported as numbers of *E. coli* per cm^2.

The temperature loggers were downloaded and the data used to calculate the RI values using the MLA RI calculator. For each calculation the offal button and the 'cold' starting temperature button were selected and 1.5 was subtracted from the result to allow for the lag

phase giving the ability to obtain negative RI values. If the 'hot' button had been selected, the RI Calculator would have returned 0.00 when negative growth was calculated. The average refrigeration index at each site was determined by calculating the mean of the two RI values calculated.

The counts before and after freezing were each converted to $\log_{10}$ values and the *E. coli* growth at each site calculated by subtracting the log count before freezing from the log count after freezing. The log growth was then plotted against the RI using Microsoft Excel and a linear relationship calculated.

Figure 1: Brushing the inoculum onto the surface of beef hearts

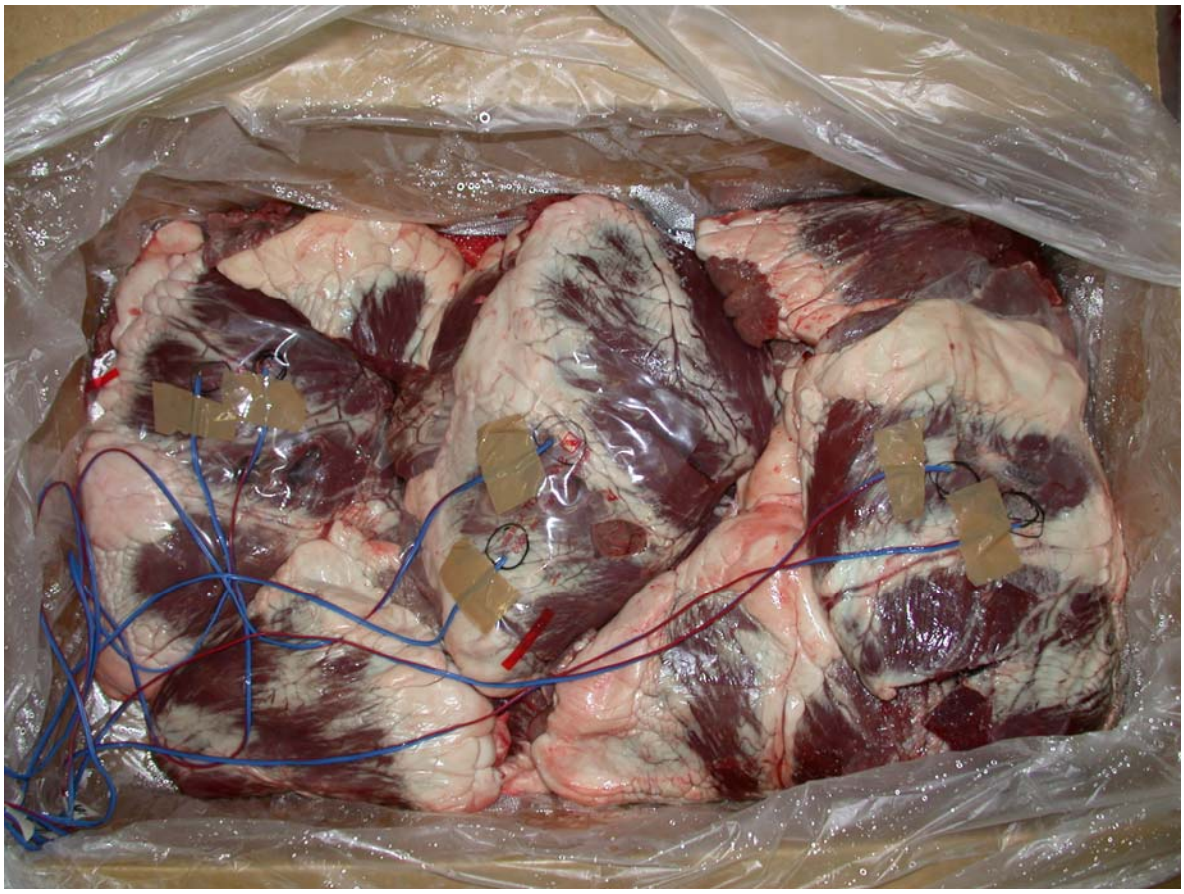

Figure 2: Thermocouples taped to the surface of beef hearts

3.2 Measurement of lactate, pH and water activity

Samples were obtained from freshly collected beef hearts, livers, scalded trip and cheek meat and immediately placed into plastic bags. A minimum of 10 different samples of each offal type were collected for determination of lactate concentration, pH and water activity. Determinations were made on the fresh sample within about 2 hours of slaughter and the next day after cooling overnight to 0 - 1°C.

3.2.1 Lactate concentration

Lactate was determined by the method of Noll (1988) using a kit supplied by Boehringer Mannheim. The principle of this determination is that L - lactic acid (lactate) is oxidized to pyruvate by nicotinamide-adenine dinucleotide (NAD) in the presence of L-lactate dehydrogenase (L-LDH).

$$\text{L-lactate} + \text{NAD} \overset{\text{L-LDH}}{\rightleftharpoons} \text{pyruvate} + \text{NADH} + \text{H}^+$$

Glutamate-pyruvate transaminase (GPT) traps pyruvate and prevents the reaction shifting to the side of lactate. By trapping pyruvate in the subsequent reaction in the presence of L-glutamate, the equilibrium can be moved to the right side of the reaction. The amount of NADH formed in the above reaction is relative to the amount of lactate. The increase in NADH is determined by spectrophotometry at 340nm. The results were expressed as mM concentration in the aqueous phase assuming all the lactate resided in the aqueous phase.

3.2.2 pH

One gram of a sample was homogenised with 10 mL of 5 mM iodoacetic acid in 0.15 M KCl, adjusted to pH 7.0, and the pH determined with a calibrated TPS Model 900P pH meter using an Ionode IJ44 electrode.

3.2.3 Water activity

Samples of surface tissue, 1 – 2 mm thick, were excised and placed in the measuring cup. Water activity was measured using an Aqualab Model CX2 water activity meter. Determinations before and after cooling were made on a minimum of 10 different samples of each type of offal.

4.0 RESULTS

4.1 Validation of RI

The growth of *E. coli* predicted using the MLA refrigeration index calculator for offal is plotted in Figures 3 and 4 against the measured growth for all three offal types.

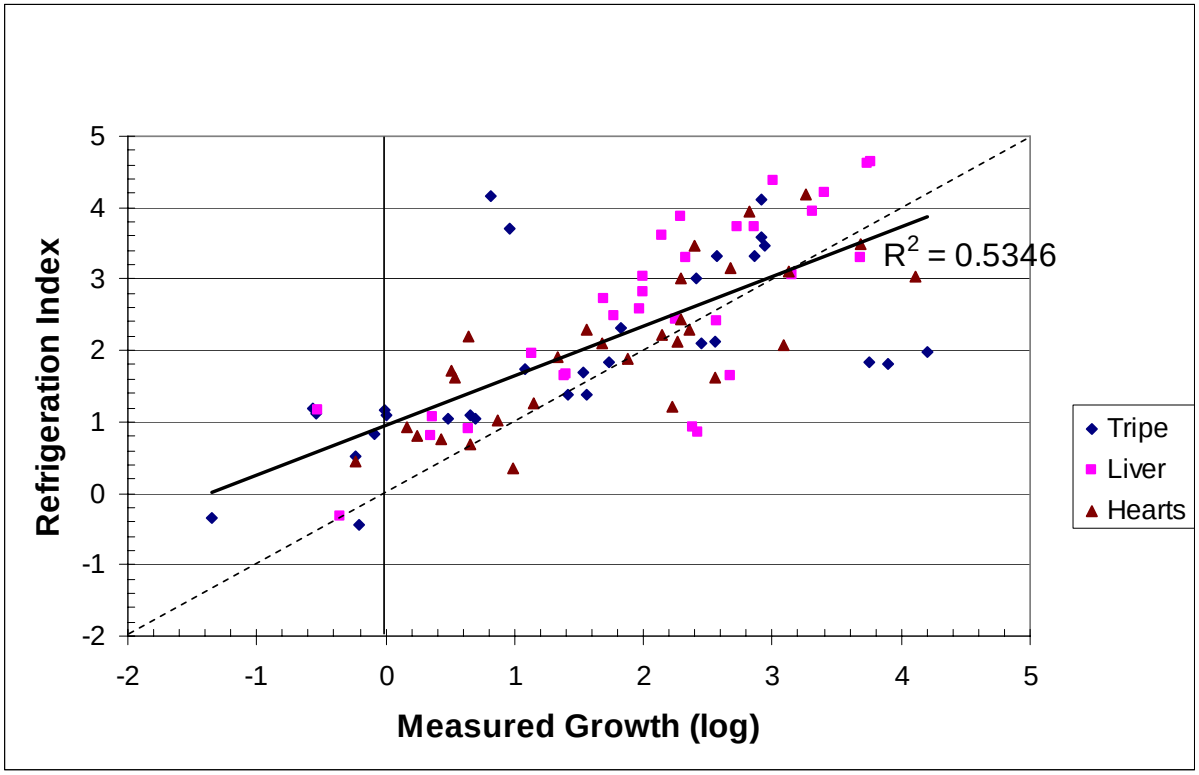

Figure 3: Measured increases of *E. coli* on frozen beef offals (on Petrifilm) compared with increases predicted using MLA RI Calculator

When the results on Petrifilm for all three offal types are combined, agreement between the predicted and measured growth is only fair (R^2 = 0.53), although similar to that found for chilled primals (Hot boning expert panel report, 2004). At the predicted values of interest (i.e. 1.5, 2.0 and 2.5), the model over-predicted by 0.7, 0.5 and 0.3 logs respectively.

A similar result was obtained when the samples were cultured on EMB (Figure 4).

In the case of individual offal products, livers and hearts provided the best agreement between predicted and measured growth (R^2 = 0.64). In the case of livers on Petrifilm, the model over-predicted by 0.55 logs at 2.0 log predicted growth. For hearts on Petrifilm, the model over-estimated by 0.3 logs at 2.0 log predicted growth.

The poorest agreement was found with scalded tripe (R^2 = 0.39). At an RI of 2.0, the model over-estimated by approximately 0.4 logs. A possible explanation for the variable results with tripe is that compared with liver and heart, the surface texture is much rougher, which may have lead to more variable application and extraction of *E. coli* from the samples. This result was also largely influenced by the results from two runs which provide the outliers. If these six points were excluded, a much better relationship was obtained (R^2 = 0.81).

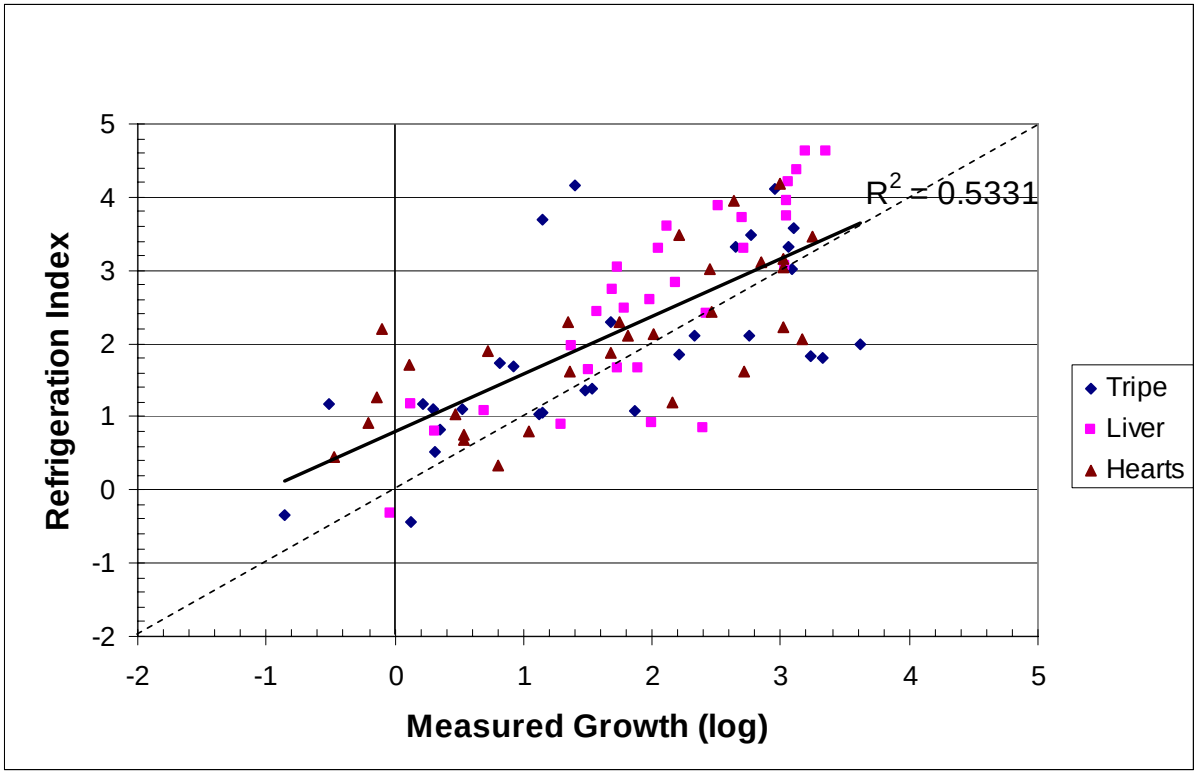

Figure 4: Measured increases of *E. coli* on frozen beef offals (on EMB) compared with predicted growth using the MLA RI Calculator

4.2 Lactate, pH and water activity

The results of lactate, pH and water activity measurements on four different beef offal products are presented in Table 1.

Table 1: Lactate concentration (in aqueous phase), pH and water activity of some beef offals ± standard deviation (range)

Offal	Lactate Concentration (mM)		pH		a_w	
	Fresh	24 h	Fresh	24 h	Fresh	24 h
Heart	41.7 ± 10.7 (27.9 – 50.7)	21.4 ± 8.7 (6.6 – 34.3)	6.24 ± 0.21 (5.93 – 6.57)	6.30 ± 0.21 (5.97 – 6.61)	0.997 ± 0.0016	0.995 ± 0.0024
Liver	12.0 ± 4.3 (7.4 – 20.5)	14.0 ± 4.7 (5.0 – 20.1)	6.50 ± 0.20 (6.35 – 6.97)	6.36 ± 0.05 (6.28 – 6.43)	0.991 ± 0.0029	0.990 ± 0.0013
Scalded tripe	8.0 ± 1.0 (6.6 – 9.6)	15.3 ± 1.7 (12.6 – 18.3)	7.25 ± 0.30 (6.48 – 7.57)	7.40 ± 0.10 (7.28 – 7.54)	1.000 ± 0.002	0.999 ± 0.0009
Cheek meat	21.8 ± 2.3 (19.0 – 25.8)	47.3 ± 6.2 (38.5 – 54.7)	6.39 ± 0.21 (6.21 – 6.93)	6.26 ± 0.30 (5.92 – 6.88)	0.994 ± 0.0016	0.995 ± 0.0013

Each result is the mean of determinations on at least 10 different samples

Heart and cheek meat had the highest lactate concentrations. Values for heart ranged up to 50.7 mM when fresh and to 34.3 mM 24 hours after slaughter. In meat flesh, lactate concentration rises with time as the pH falls. Cheek meat was the only product in this investigation in which lactate concentration increased appreciably with time – to a mean of 47.3 mM after 24 h. The lactate values for beef liver were very similar to those previously reported by Yambayamba *et al* (1996). Scalded tripe had the lowest lactate concentration (when fresh).

The pH for heart and liver was within the range previously reported and cheek meat also had a high pH. The mean pH for scalded tripe ranged from 7.25 fresh to 7.4 after 24 hours.

Scalded tripe also had a very high water activity (a_w). The tripe samples had free water visible on the surface resulting in an a_w similar to water. Liver had the lowest a_w at 0.99 whereas heart and cheek meat were about 0.995. There was negligible change in a_w after overnight cooling in sealed bags.

5.0 DISCUSSION

The investigation indicated that the current version of the RI calculator generally predicted increases in numbers of *E. coli* in offal products that were higher than measured increases.

There are some possible explanations for this. Firstly, the RI calculator assumes that the lag phase before *E. coli* begins to increase in numbers is responsible for a delay in the commencement of growth equivalent to 1.5 log (or RI) units. The lag in the test offal products may actually have been greater than 1.5 units due to the physiological state of the cells.

Secondly, the measured increases in *E. coli* were obtained from counts on samples taken from frozen product. While freezing meat doesn't always result in reductions of *E. coli* and other bacteria, reductions have been reported. The RI calculator does not take into account reductions that might occur during freezing and thawing.

Thirdly, the RI calculator has built-in constant values for pH, lactate and water activity for offal that may be too high. The values that are currently in the predictive equation are, respectively, pH 6.8, lactate 51 mM, and water activity 0.995. The pH results for the four offal products suggest that the pH value of 6.8 is appropriate; changing the pH value in the equation between 6.5 and 7.2 has very little effect on the RI output. The lactate results obtained in the investigation suggest that a value in the equation of 25 mM better reflects measured values. However at pH values between 6.5 and 7.2, changing the lactate value in the equation from 51 mM to 25 mM has very little effect. The value of 0.995 for water activity that is currently used in the RI calculator is very close to the values measured for hearts and cheek meat. The measured values for tripe must be treated with caution. They suggest a free layer of pure water at the product's surface throughout the 24 hour period of storage. This is difficult to accept. Replacement of the current water activity value in the calculation (0.995) with that for liver – 0.990 – leads to RI values for liver closer to measured values.

However there does not appear to be a compelling case for creating in the RI calculator a separate offal product category – liver. If the only change that is made to the constant input values in the predictive equation is to change the lactate concentration to 25 mM, the RI values for offal products will include a margin of comfort. If an increase is made in the correction for lag from 5 to 6 generations (i.e. to 1.9 logs) the average predicted increase would match the measured increase. Underestimation may sometimes occur, though, in situations where the measured growth is 2.5 logs or more.

From the results of the investigation, cooling histories that gave RIs of less than 1.5 gave no measured values on Petrifilm higher than 2.5 logs. Of the 30 samples with RIs between 1.5 and 2.5, two samples of hearts, two samples of livers and four samples of tripes had measured increases greater than 2.5 logs. All of these tripe samples were not hard frozen at the time of sampling.

6.0 RECOMMENDATIONS

1. Values currently in the MLA RI calculator for pH and water activity for offal products (6.8 and 0.995 respectively) should remain;
2. Changes to the inputs to the RI calculator for offal products should be limited to amending the constant value for lactate concentration from 51 to 25 to better reflect measured values. This change will have very little effect on RI values calculated from specific cooling histories;
3. There appears to be little benefit in including separate buttons for different offal products.

7.0 REFERENCES

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