Modified Atmosphere Packaging for Pipis

Prepared for Goolwa Pipi Harvesters Association

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Executive Summary

The purpose of this project was to build upon work started in 2010 to improve and validate a modified atmosphere packaging (MAP) pouch approach for extending the shelf life of live Goolwa cockles, *Donax deltoides* (Pipi), for the Goolwa Pipi Harvesters Association.

Previous work had shown that 1.5 kg trays of MAP live Pipis could have a shelf life of at least 10 days at 6ºC but only if the product was sanitized using activated Zydox (30ppm for 2 hours) prior to packing and 80% oxygen and 20% carbon dioxide MAP gas mixture was used in the tray. This assessment was made using independent sensory analysis, cockle survival and meat microbiology data.

Market feedback, however, did not favour use of the rigid 1.5 kg tray MAP presentation, so South Australian Research and Development Institute (SARDI) researchers used the key attributes of the MAP tray pack approach and adapted them to suit a 1kg plastic pouch MAP product format that was preferred by customers. The shelf life of this MAP pouch product was shown to be at least 12 days by independent sensory, cockle survival and meat microbiology data.

The SARDI MAP pouch process still used the same time-consuming and inefficient double-handling sanitation protocol as the MAP tray pack work but, because MAP product would be produced in Port Elliot instead of in Adelaide, the sanitation process would need to change to better suit the new circumstances. Hence, Logifish Consulting was commissioned to develop and validate a more efficient sanitation protocol that did not compromise on the 12 day shelf life of the product.

A series of experiments were carried out using 5 replicate 1kg pouches of live Pipis using different durations of dip in a range of concentrations of activated Zydox in either potable tapwater or seawater, and/or addition of different volumes of activated or unactivated Zydox solution to the Pipis in the pouch prior to MAP packing. All packs were held at 4oC for up to 14 days (with day of packing being day 0). The effectiveness of each treatment was assessed by sensory and meat microbiology approaches.

This approach demonstrated that Pipis that had been in the de-sanding for a minimum of 16 hours could be packed directly into a plastic pouch and 8mL of 4% unactivated Zydox (diluted in potable freshwater) could be added prior to MAP packing with 80:20 Aligal gas, and stored at 4ºC would have a shelf life of at least 12 days (with day 0 being the day of packing). Even after 12 days the freshly opened pouches would have a mild ‘seaweed’ or ‘seawater’ odour and the mean total viable count (TVC) was less than 10^4 colonies per gram. This is well below the 10^6 per gram that is usually indicative of spoiled seafood.

The successful evolution of the MAP cockles from tray packs to pouches with excellent shelf life and more efficient processing protocol has helped position the Goolwa Pipi Harvesters Association quota holders to launch a new Pipi product in the domestic and export marketplace.

Introduction

Modified Atmosphere Packaging (MAP) is now used for the vast majority of live mussel food service and retail product sold in Europe. Mussel farmers in Australia are using similar MAP approaches to enhance the shelf life of live blue mussels (*Mytilus galloprovincialis*) for supplying domestic customers that may be some distance from the growing region. Significantly longer shelf life is obtained than for mussels packed loose in air, or under vacuum, allowing product to travel to those markets by road and still have several days of shelf life remaining.

All studies have shown that MAP will not enhance the shelf life of product (live or not), unless the pre-packaging handling and sanitation of the product is also optimized and standardized. Therefore MAP trials must contain a number of post-harvest and pre-packaging treatment variations as well as differences in MAP gas composition and ratios.

In 2010 trials were carried out to explore the potential utility of the MAP approach to enhance the shelf life of live Goolwa cockles, *Donax deltoides* (henceforth called Pipis). The Pipi survival, microbiological and sensory results indicated a protocol that could deliver a MAP product that was 'fit for purpose' up to 10 days post-packaging. That process is described below:

1. Pipis were harvested from the beach, transported to the AQIS registered facility at Port Elliot, graded and put in a recirculating seawater tank to remove sand as per the standard operating procedure
2. The next day the purged Pipis were placed into aerated seawater containing 30 ppm of activated chlorine dioxide (Zydox^{TM}) at 8^{o}C. The bin was taken directly to a second AQIS registered fish processing facility in Adelaide in a refrigerated truck (allowing 2 hours).
3. The delivery temperature of water and Pipis was <10^{o}C.
4. Pipis were then dipped in chilled (6^{o}C) tapwater containing 10ppm activated chlorine dioxide. After 8 minutes they were removed and distributed into 40mm deep trays (11 x 9 inch format) to a net weight of 1.5 kg. Pipis were manipulated with gloved hands only.
5. The trays were immediately loaded into a Multivac MAP tray sealing machine and packed in 80% oxygen 20% carbon dioxide (Aligal 28; Air Liquide). The

gas fill was to 95% of the pack volume such that the lidding film held the Pipis closed within the tray.

6. MAP packs were transferred in insulated containers to a dedicated 6^{o}C refrigerator for up to 11 days.

The results showed that all packs were negative for *Listeria* and *Salmonella* in 25g of product. Almost all samples showed an *E. coli* MPN result below the limit of detection (less than 0.3 organisms per g); however on day 0 one pack had a MPN value of 0.92 organisms per g. Total viable counts (Australian Standard method 5013.11.3-2006) of Pipi meat increased from 10^5/g on day 0 to 10^8/g on day 11.

More than 99% of Pipis were alive on days 1, 5 and 9. By day 10 however, 80% of Pipis were dead, and this increased to over 90% by day 12. Sensory analysis revealed that packs were all described as having pleasant “sea, seawater, sea salt, seaweed and beach” odour on day 5. As the study progressed the intensity of the odour increased and changed such that on days 9 and 10 some panelists indicated some packs had a “slightly fishy” odour. On days 11 and 12 several of the sensory panelists reported that some of the packs had a “stale, slightly off fish/crabs” odour and were adjudged “borderline unacceptable”.

Trial shipments of the tray-packed MAP product were sent to interstate and export customers. Feedback on the quality and freshness of the Pipis was positive, but the tray pack format was seen as being too large.

During 2013 a project was undertaken to use a pouch MAP machine to overcome the product presentation issues identified in the earlier work. This project was carried out by SARDI researchers and used a similar sanitizer protocol as was used in the 2010 work (“The Pipis were sanitised at SARDI by dipping in 4°C potable water containing 10 ppm activated Zydox™ (chlorine dioxide) for at least 8 minutes. The ratio of Pipis to Zydox™ solution was 1:1 weight/volume”). The 1kg pouch MAP packed Pipis were shown to have an 11 day shelf life when stored at 4^{o}C.

The current project was carried out in mid-2014 to try to replace the lengthy and multiple handling steps for sanitizing Pipis that was effective in 2010, with a more

efficient and effective protocol that suited processing occurring in the same premises where the Pipis were de-sanded.

Methods and Results

The steps involved in this work included:

- Working with Lionel Freeman (the Australian distributor of Zydox) to determine a more effective and efficient protocol to sanitize Pipis to maximise MAP shelf-life
- Determining changes in microbiological and sensory characteristics of MAP Pipis during MAP shelf-life trials out to 14 days
- Using these data to validate a conservative 10 day use-by date for MAP Pipi product kept at 4^{o}C
- Interpreting compositional analysis data to construct a specific nutritional panel for the new MAP Pipi product

Sanitation Protocol Trials

The first experiment used 1kg MAP pouches of de-sanded Pipis exposed to five different sanitation procedures. The procedures were:

- Soak + Tapwater Dip (the 'original' 2010 protocol)
- Soak + Tapwater Dip + Shot
- Tapwater Dip
- Shot
- Tapwater Dip + Shot
- Seawater Dip + Shot

Where:

"Soak" – Pipis immersed in aerated seawater (10^{o}C) with 20ppm activated Zydox for 2 hr

"Tapwater Dip" – Pipis immersed in potable freshwater (4^{o}C) with 10ppm activated Zydox for 8 mins

"Shot" – 20mL of potable freshwater (10^{o}C) containing 0.6mL of concentrated unactivated Zydox (ie 3% by volume) was put into each pouch with the Pipis.

"Seawater Dip" – Pipis immersed in seawater (10oC) containing 3ppm activated Zydox for 3 mins

Fifteen replicate packs of each treatment were prepared and 9 were sent on ice to a NATA accredited food laboratory, with 3 retained by packing establishment and 3 taken by Logifish Consulting. All packs were held in refrigerators at 4ºC for up to 13 days.

On days 8, 11 and 13 three pouches from each treatment were opened at the food laboratory and the meat was tested for total viable count. One pack from each treatment was opened from days 8-13 by either the packing establishment or Logifish Consulting for sensory analysis.

The results are shown in Figure 1. Most Total Viable Count values were less than 20,000 colonies per g even out to Day 14 (with day of packing being Day 0). These results suggested that none of the Soak or Dip treatments (be they in potable tapwater or seawater), duration of Dip nor concentration of activated Zydox made too much difference to the levels of microbial load on the MAP Pipi product at Days 9,12 or 14 post-packing.

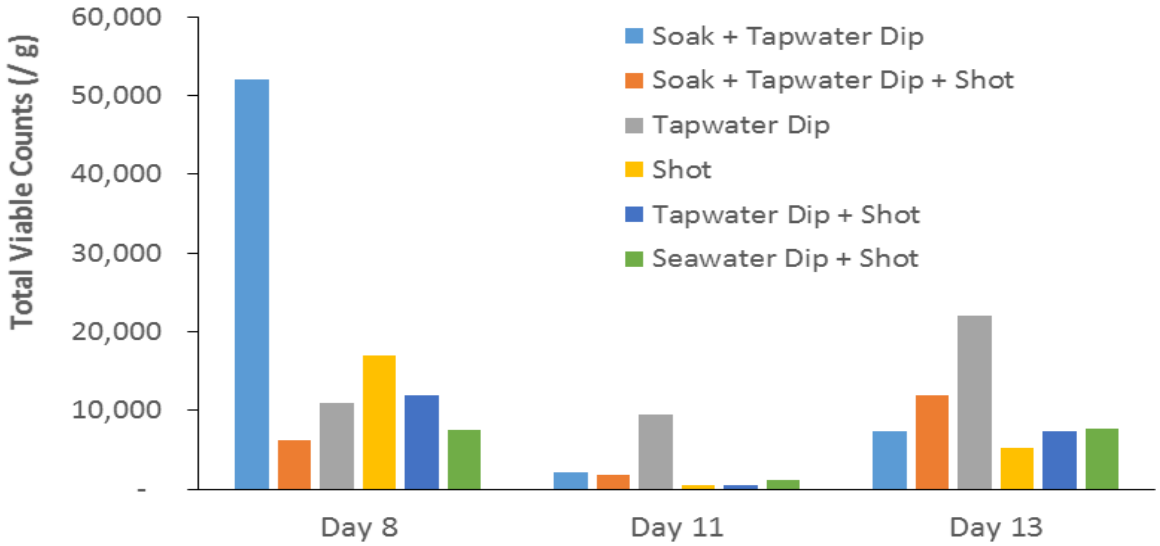

Figure 1. Total viable counts (colonies/g) at day 0 and days 8, 11 and 13 of refrigerated storage of MAP Pipis that had no unactivated Zydox or 8mL of different concentrations of unactivated Zydox (v/v). The microbiological testing was carried out by a NATA accredited laboratory, with plates incubated at 25ºC for 72 hours.

Based on these results it was considered that Soaking and Dipping were handling procedures that were unnecessary and added to the costs of MAP production. The decision was made to instead focus upon determining what concentration of

unactivated Zydox would be most effective in preventing microbial growth in the MAP pack. The 8mL volume of Shot solution was determined by the amount delivered by a hand operated pump. The following 4 treatments were tested:

- SHOT 8mL @ 7.5% Zydox
- SHOT 8mL @ 3.75% Zydox
- SHOT 8mL @ 1.87% Zydox
- NO SHOT

Five replicate packs of each treatment were prepared and 9 were sent on ice to a NATA accredited food laboratory, with 3 retained by the packing establishment and 3 taken by Logifish Consulting. All packs were held in refrigerators at 4^{o}C for up to 12 days.

On days 8, 11 and 13 one pouch from each treatment was opened at the food laboratory and the meat was tested for total viable count. One pack from each treatment was opened from days 8-13 by either the packing establishment or Logifish Consulting for sensory analysis.

The microbiological results are shown in Figure 2. Note: only one pouch of each treatment was tested on each day, so the data must be considered as indicative only. The initial (day 0) number of colonies was very low, and this level was maintained in all the treatment packs to day 11 (<4,000/g). On day 13, the colony counts increased in the “No Shot” and “1.87%” by about 4 fold however counts were still below 15,000/g, a very acceptable level. The increase in counts in the pouches treated with “3.75” and “7.5%” unactivated Zydox shots was less, with these packs only having 7-8,000 colonies per g. Sensory analysis of all the packs was excellent on all days tested.

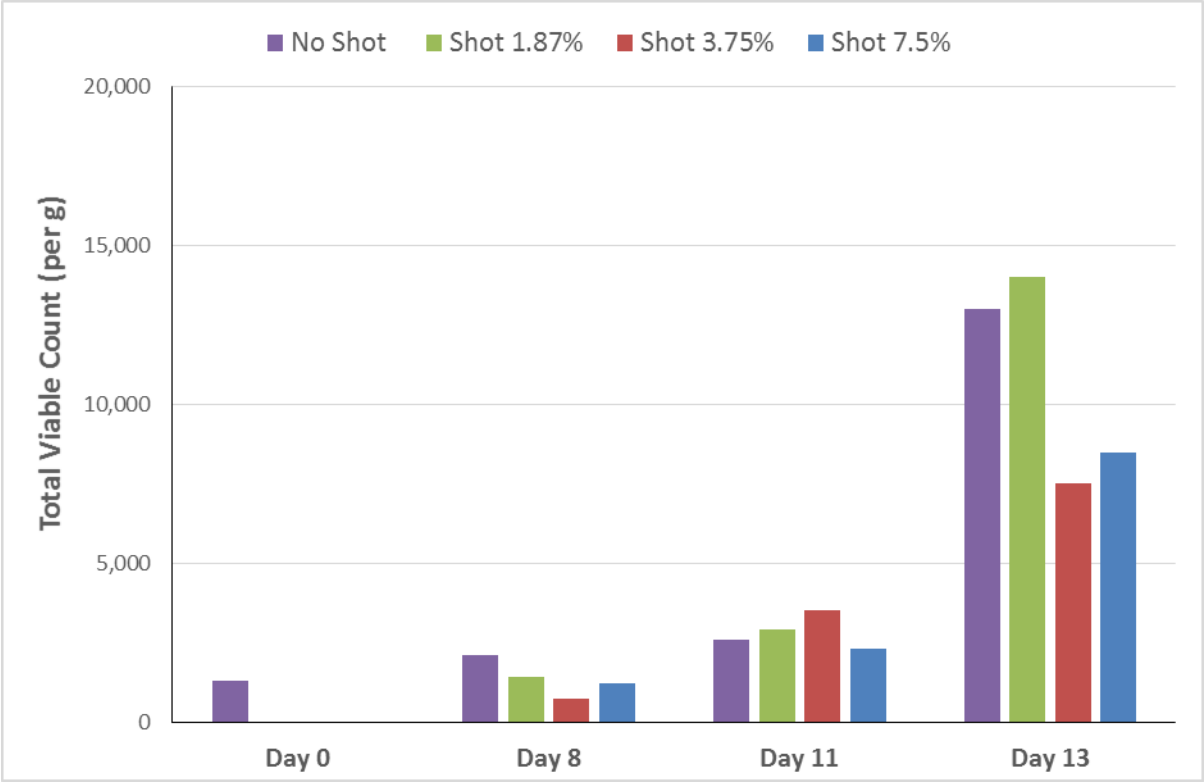

Figure 2. Total viable counts (colonies/g) at day 0 and days 8, 11 and 13 of refrigerated storage of MAP Pipis that had no unactivated Zydox or 8mL of different concentrations of unactivated Zydox (v/v). The microbiological testing was carried out by a NATA accredited laboratory, with plates incubated at 25ºC for 72 hours.

A further experiment was carried out with additional replicate packs of the 8mL of 3.75% unactivated Zydox treatment. De-sanded Pipis were weighed (1kg) into the pouches and 8mL of the freshly prepared 3.75% unactivated Zydox was added using the hand held pump device. The packs were MAP packed with the Aligal 28 gas mixture. Fifteen packs were transported on ice to the NATA accredited food laboratory, 3 packs were held at the packing establishment and 3 packs at Logifish Consulting. All packs were held in 4ºC refrigeration. Five packs at the food laboratory were opened on days 8, 11 and 13 for determination of total viable counts, and 1 pack was opened at the other sites for sensory analysis.

The results of the 5 replicate packs tested on each day are shown in Figure 3.

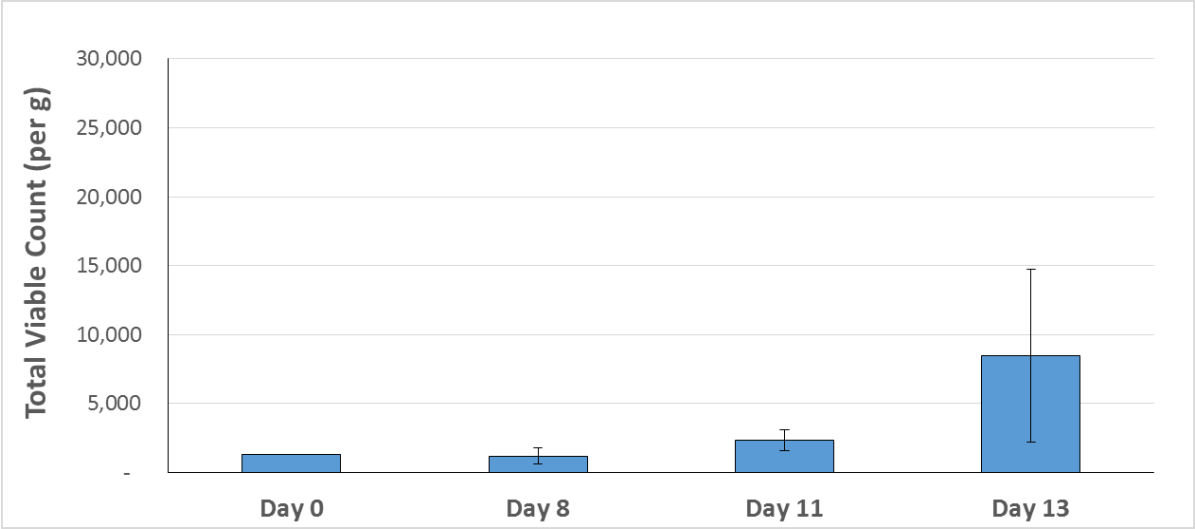

Figure 3. Total viable counts (colonies/g) of 5 replicate packs (average ± SD) of Pipis packed using the 8mL shot of 3.75% unactivated Zydox in modified atmosphere packaging protocol developed during this project. The microbiological testing was carried out by a NATA accredited laboratory, with plates incubated at 25^{o}C for 72 hours.

Again, this experiment showed that Pipis packed in the Port Elliot processing factory had extremely low total viable counts on day 0 and that this continued until at least day 13 following MAP packing with an 8mL shot of 3.75% unactivated Zydox and refrigerated storage. All packs opened on each day had a pleasant ‘seawater’ or ‘seaweed’ odour. These data indicate that MAP packed Pipis processed in this way have a refrigerated shelf life of at least 13 days post-packing.

It is notable that the total viable counts of all the Pipis tested in the current study were much lower (by 100 or 1000-fold) than Pipis tested in 2010 or 2013, despite the same analytical protocol being used. This strongly suggests that either (a) improvements to the seawater tank system for de-sanding the Pipis has substantially impacted on the level of microbial load of the Pipis, and/or (b) that transporting the Pipis by road in aerated seawater increases the microbial load in the product.

Nutritional Analysis

A sample of freshly de-sanded Pipis was sent for nutritional panel analysis by a NATA accredited laboratory. The complete analysis is below:

Australian Government
National Measurement Institute

NATA

REPORT OF ANALYSIS

Report No. RN1026026

Client	: SA PATHOLOGY	Job No.	: SAPA02/140610
	IMVS FOOD & ENVIRONMENTAL LAB	Quote No.	: QT-00972
	LEVEL 3 HANSON INSTITUTE	Order No.	:
	FROME ROAD	Date Sampled	:
	ADELAIDE SA 5000	Date Received	: 11-JUN-2014
Attention	IRENE BARKER	Sampled By	: CLIENT
Project Name :			
Your Client Services Manager	: Tim Stobaus	Phone	: (03) 9644 4849

Lab Reg No.	Sample Ref	Sample Description
V14/012753	140503	Whole Cockles

Lab Reg No.		V14/012753				
Sample Reference	Units	140503				Method
Trace Elements						
Sodium	mg/100g	870				VL247

Paul Adorno, Section Manager
Inorganics - Vic
Accreditation No. 89

25-JUN-2014

Lab Reg No.		V14/012753				
Sample Reference	Units	140503				Method
Proximates						
Fructose	g/100g	<0.2				VL295
Glucose	g/100g	<0.2				VL295
Sucrose	g/100g	<0.2				VL295
Maltose	g/100g	<0.2				VL295
Lactose	g/100g	<0.2				VL295
Total Sugars	g/100g	<1				VL295
Moisture	g/100g	88.6				VL298
Fat (Mojonnier extraction)	g/100g	0.5				VL302
Saturated Fat	g/100g	0.2				VL289
Protein (N x 6.25)	g/100g	8.1				VL299
Ash	g/100g	3.1				VL286
Carbohydrates	g/100g	<1				VL412
Energy (kj)	kJ/100g	160				VL412
Mono trans fats	g/100g	<0.1				VL289
Mono-unsaturated fat	g/100g	<0.1				VL289
Omega 3 fats	g/100g	0.2				VL289

REPORT OF ANALYSIS

Report No. RN1026026

Lab Reg No.		V14/012753				
Sample Reference	Units	140503				Method
Proximates						
Omega 6 fats	g/100g	<0.1				VL289
Poly trans fats	g/100g	<0.1				VL289
Poly-unsaturated fat	g/100g	0.3				VL289
Trans fats	g/100g	<0.1				VL289
Saturated Fatty Acids						
C4:0 Butyric	%	1.6				VL289
C6:0 Caproic	%	<0.1				VL289
C8:0 Caprylic	%	<0.1				VL289
C10:0 Capric	%	<0.1				VL289
C12:0 Lauric	%	<0.1				VL289
C14:0 Myristic	%	1.6				VL289
C15:0 Pentadecanoic	%	0.8				VL289
C16:0 Palmitic	%	16.0				VL289
C17:0 Margaric	%	0.8				VL289
C18:0 Stearic	%	10.1				VL289
C20:0 Arachidic	%	<0.1				VL289
C22:0 Behenic	%	<0.1				VL289
C24:0 Lignoceric	%	<0.1				VL289
Total Saturated	%	30.9				VL289
Mono-unsaturated Fatty Acids						
C14:1 Myristoleic	%	<0.1				VL289
C16:1 Palmitoleic	%	2.9				VL289
C17:1 Heptadecenoic	%	<0.1				VL289
C18:1 Oleic	%	9.8				VL289
C20:1 Eicosenic	%	1.8				VL289
C22:1 Docosenoic	%	<0.1				VL289
C24:1 Nervonic	%	<0.1				VL289
Total Mono-unsaturated	%	14.5				VL289
Poly-unsaturated Fatty Acids						
C18:2w6 Linoleic	%	3.3				VL289
C18:3w6 gamma-Linolenic	%	<0.1				VL289
C18:3w3 alpha-Linolenic	%	1.2				VL289
C20:2w6 Eicosadienoic	%	1.1				VL289
C20:3w6 Eicosatrienoic	%	<0.1				VL289
C20:3w3 Eicosatrienoic	%	<0.1				VL289
C20:4w6 Arachidonic	%	5.5				VL289
C20:5w3 Eicosapentaenoic	%	8.8				VL289
C22:2w6 Docosadienoic	%	<0.1				VL289
Omega 3 Fatty Acids	%	41.1				VL289
Omega 6 Fatty Acids	%	12.2				VL289
C22:4w6 Docosatetraenoic	%	2.4				VL289
C22:5w3 Docosapentaenoic	%	4.3				VL289
C22:6w3 Docosahexaenoic	%	26.9				VL289

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National Measurement Institute

REPORT OF ANALYSIS

Lab Reg No.	Units	V14/012753				Method
Sample Reference		140503				
Poly-unsaturated Fatty Acids						
Total Poly-unsaturated	%	53.4				VL289
Total Mono Trans Fatty Acids	%	<0.1				VL289
Total Poly Trans Fatty Acids	%	1.2				VL289
P:M:S Ratio		1.7:0.5:1				VL289

George Dabos, Analyst
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Accreditation No. 89

Samantha Duong, Analyst
Organics - Vic
Accreditation No. 89

25-JUN-2014

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ACCREDITED FOR TECHNICAL COMPETENCE

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Results relate only to the sample(s) tested.

This analysis was interpreted according to the FSANZ Food Standards nutritional labelling guidelines. The detailed nutritional table panel for Pipis is shown below:

NUTRITION INFORMATION Servings per package: 4 Serving size: 83 g (meat weight, excluding shell weight)		
	Average Quantity per Serving	Average Quantity per 100 g
Energy	132 kJ	160 kJ
Protein	6.7 g	8.1 g
Fat, total	0.41 g	0.5 g
– saturated	0.17 g	0.2 G
– trans	LESS THAN 0.1 g	LESS THAN 0.1 g
– polyunsaturated	0.25 g	0.3 g
– omega-3	0.17 g	0.2 g
eicosapentaenoic acid (EPA)	36 mg	44 mg
docosahexaenoic acid (DHA)	111 mg	134 mg
– monounsaturated	LESS THAN 0.1 g	LESS THAN 0.1 g
Carbohydrate	LESS THAN 1 g	LESS THAN 1 g
– sugars	LESS THAN 1 g	LESS THAN 1 g
Sodium	718 mg	870 mg

These results substantiate the following nutritional content claims being made on the pack (if desired):

- Good source of omega-3 (at least 60 mg EPA + DHA per serve; Pipis have 147 mg)
- Contributes to heart health (more than 50mg EPA + DHA per serve)

Conclusion

One of the main goals of this project was to develop and validate a more effective and efficient packaging and sanitation process for MAP pouch packed Pipis. Through a series of experiments this was achieved. The original practice of soaking Pipis in activated Zydox for 2 hours followed by batch dipping in another activated Zydox solution for 8 minutes involving multiple-handling of product has been replaced with a more continuous process using a single dose of unactivated Zydox and fewer handling steps. This will allow more Pipis to be processed and packed by MAP in a shorter amount of time and still have product with an extended shelf-life.

The microbiological and sensory characteristics of MAP packed Pipis were tested in two separate trials at days 8, 11 and 13 post-packing (with day of packing being day 0) by a NATA accredited laboratory using standard methodologies for seafood (plates incubated at 25^{o}C). Total plate counts in product on day 0 were very low (1,300/g) and remained consistently low throughout the trial being less than 4,000/g at day 11 and less than 10,000/g (or 1 x 10^4) at day 13. Even at 13 days the value is lower than the 10^5-10^6/g threshold that most authorities recognise for product that is spoiling. The microbial levels are much lower than in MAP packed Pipi products which were produced in 2010 and 2013. This could be due to improvements in the seawater system during de-sanding, and/or because the Pipis no longer have to be transported in aerated seawater to Adelaide for packing. Whatever the cause, the outcome is clearly advantageous.

The sensory analysis of the newly opened packs was consistently positive with ‘seawater’ and ‘fresh seaweed’ characteristics. None of the packs had spoiled by day 13. Together, these results suggest that MAP Pipis can conservatively be given a use-by date of 10 days (with day of pack being day 0) at 4^{o}C.

The nutritional profile of Pipis was determined by a NATA accredited food laboratory, and this information was converted into a FSANZ standard nutrition panel for a packaged food product.