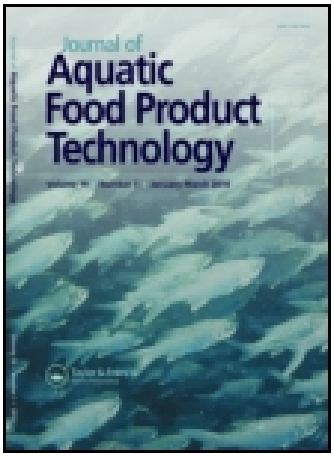

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Steven L. Slattery[a] & Paul J. Palmer[b]
[a] Innovative Food Technologies, Department of Employment, Economic Development and Innovation, Archerfield BC, Queensland, Australia
[b] Fisheries and Aquaculture, Bribie Island Research Centre, Department of Employment, Economic Development and Innovation, Woorim, Queensland, Australia
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Modified Atmosphere Packaging (MAP) for Control of Black Spot Formation in Chilled Prawns

Steven L. Slattery[1] and Paul J. Palmer[2]

[1]*Innovative Food Technologies, Department of Employment, Economic Development and Innovation, Archerfield BC, Queensland, Australia*
[2]*Fisheries and Aquaculture, Bribie Island Research Centre, Department of Employment, Economic Development and Innovation, Woorim, Queensland, Australia*

The marketing of organically labeled prawns is predominately in a cooked or raw frozen form to avoid the development of melanosis (black spot). Certification for organic status prohibits the use of any added chemicals. The application of 60% CO_2/40%N_2 modified atmosphere to chilled (raw) prawns using two species of prawn was investigated for the ability to control black spot formation. Sensory assessment and microbiological counts were used to determine the end of product shelf life. Modified atmosphere packaged (MAP) prawns exhibited no melanosis for up to 16 days. The high quality life was retained for 12 days; shelf life of 16 days, according to standard microbiological criteria, was achieved, which is more than twice previously reported for non-MAP prawns. Results suggest MAP may be an effective method for the marketing of organically grown prawns as well as those produced by conventional prawn aquaculture without application of the normal chemicals used to prevent black spot.

Keywords: MAP, black spot, shelf life, microbiology, sensory assessment, banana prawns, black tiger prawns

INTRODUCTION

Melanosis (black spot) dramatically reduces the visual acceptance of chilled (raw) prawns (Shengmin, 2009). While black spot formation is a natural process that discolors the shell after harvesting (Cobb, 1976) and is harmless for the consumer, its appearance makes marketing difficult when some consumers consider it to be related to bacterial activity (Fieger et al., 1958). A review of organically labeled prawns in the marketplace shows that chilled prawns that have never been frozen are rarely offered for sale outside of the country where they are grown (Alibaba.com, 2011; Biocentinela, 2011; Ecofish, Inc., 2004; Graig Farm Organics, 2011; Marvesta Shrimp Farms, 2011; Sureerath Co., Ltd., 2007). This may be related to black spot formation, as the only chilled prawn supplier, Marvesta Shrimp Farms, guarantees 24-h delivery.

Correspondence should be addressed to Steven L. Slattery, Senior Seafood Technologist, Innovative Food Technologies, Department of Employment, Economic Development and Innovation, PO Box 156, Archerfield BC, Qld 4108 Australia. E-mail: slattes@bigpond.com

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The presence of oxygen (O_2) is necessary in black spot formation for the enzyme driven process of pigment development (Gomez-Guillen and Montero, 2007). Nonorganic procedures rely on chemicals such as sulphite, 4-hexylresorcinol, or organic acids to inhibit polyphenol oxidase, the enzyme responsible for converting tyrosine to the black pigment melanin (Montero et al., 2001). Certification for organic status prohibits the use of any chemicals. Therefore, organic prawns are exported either frozen and/or cooked to avoid this defect.

The prime quality of fresh seafood only lasts a short time (Haard, 1992). Barbosa and Bremner (2002) stated that the high quality life of frozen seafood is lost when noticeable sensory differences develop. The shelf life of prawns stored in air has long been known to be short (Al-Dagal and Bazaraa, 1999; Green, 1949; Haard, 1992), with Cobb (1976) stating that the shelf life of uncooked head-on shrimp was only 5 days. Yamagata and Low (1995), under similar conditions to the current trials, found that control cultured banana prawns killed immediately before storage had only 4-days shelf life at 0°C due to loss of sensory quality and blackening. Previous experiments in our laboratory (Slattery et al., 1994, 1995, 2009) obtained a similarly short shelf life from control wild and farmed prawns stored under ice, with extensive black spot being exhibited within 3–4 days.

Experiments with prawns stored in carbon dioxide (CO_2) treated refrigerated seawater found no discoloration after 6 days (Barnett et al., 1978). Rutherford et al. (2007) and Shengmin (2009) found CO_2 to be more effective in inhibiting bacteria and maintaining sensory attributes of shrimp than air.

The shelf life of food can be extended by storage under vacuum packaging or modified atmosphere packaging (MAP; Robertson, 2006). Considerable research has been conducted on the use of MAP to improve the shelf life of seafood (reviewed by Statham, 1984; Phillips, 1996; Sivertsvik et al., 2002; Slattery, 2010). MAP extends the shelf life of fish as compared to air controls at the same temperature (Sen, 2005).

Lopez-Caballero et al. (2002) found that MAP delayed microbial growth on wild caught shrimp compared to ice or air storage. However, a recent study by Mastromatteo et al. (2010) using both microbial and sensory techniques failed to find any difference in shelf life between air and MAP conditions for untreated peeled shrimp. Therefore, this study using MAP to prevent black spot formation in chilled prawns was conducted.

Since MAP is an acceptable procedure for organic vegetables, fruits, meats, and dairy products, a modified atmosphere containing CO_2 and no O_2 was evaluated for use with organically and conventionally produced chilled prawns. If successful, this technique could be utilized for conventional production without the need for adding chemicals during processing and packaging, thereby fulfilling an increasing consumer need for sulphite-free prawns.

METHODS

Experimental Design

Prawns were supplied from two different aquaculture sites in southeast Queensland, Australia. The first trial was intended as a preliminary study before the main work began; however, the results are included with two subsequent packaging trials. Table 1 summarizes data of chilled prawns used in the preliminary experiments.

Two packaging trials were performed with prawns harvested from aquaculture ponds where organic principals were applied (Palmer and Slattery, 2009; Palmer et al., 2009). MAP packs of banana (*Fenneropenaeus merguensis*) prawns were tested in duplicate on each sampling day (Trial 1). The second trial involved one pack of banana prawns and two packs of black tiger (*Penaeus monodon*) prawns grown at the same site. Trial 3 was composed of black tiger prawns (triplicate packs) from a conventional prawn aquaculture farm that used Australian production-approved chemicals.

TABLE 1
Sources and species of prawns used for each trial conducted

Trial	*Farm Source*	*Pond Number*	*Grow out conditions used*	*Prawn species*	*Number of packs tested per sample day*
1	Bauple	1	Organic principles	Banana	2
2	Bauple	1	Organic principles	Banana	1
2	Bauple	2	Organic principles	Black tiger	2
3	Donnybrook	7	Conventional aquaculture	Black tiger	3

No added chemical was applied to any of the chilled prawns to prevent black spot. Based on the consistent outcomes from the authors' previous work and others (Slattery et al., 1992, 1994, 1995, 1998, 2009; Slattery, 2010; reviewed in Statham, 1984; Sivertsvik et al., 2002) on untreated chilled prawns using both sensory and microbiological assessment and similar holding conditions to what would have been used here, no control samples were used in this study.

Prawns were harvested and submerged in ice slurry (50/50 w/v) for 5 min, as per Hanpongkittikun et al. (1995). Prawns were allowed to drain before packing at the farm in a standard 22 cm × 17 cm MAP tray containing an absorbent pad (50-mL capacity). The packs contained from 180–240 g whole banana or black tiger prawns, resulting in an atmosphere to product ratio greater than 2.5:1 (Slattery, 2010). The packs were prepared using a semiautomatic tray sealing machine (Pratica Model, Technovac, Paolo, Italy) and flushed with a certified gas mixture containing 60% CO_2 and 40% nitrogen (N_2) and sealed with a film having an oxygen transmission rate (OTR) of 33 cc/m^2 day.

The packs were stored sealed at 4°C to mirror current commercial chilling conditions and to assist with the identification of any nonproteolytic *Clostridium botulinum* as per Lannelongue et al. (1982). The packs were sampled on Days 1, 5, 9, 12, and 16 (see Table 2).

Headspace Analysis

Headspace analysis was conducted on each pack. A gas chromatograph (GC; Model GC-8A, Shimadzu Corporation, Kyoto, Japan) with a CTR I stainless steel column (Part No. 8700, Alltech, Nicholasville, KY, USA) and a thermal conductivity detector were used for headspace analysis. The carrier gas was argon (Ultra High purity) operating at a primary pressure of 400 kPa. The injector/detector temperature was 60°C, and the column oven temperature was constant at 40°C. The detector current was 90 mA. A 1-mL sample of gas was withdrawn from the headspace of the packs using a precision sampling syringe (gas tight with pressure lock) for loading into the GC. Quantification of the CO_2, O_2, and N_2 was based on the peak area relative to that of a National Association of Testing Authorities' endorsed reference gas (0.79% O_2, 25% CO_2, and remainder N_2) supplied by Air Liquide Australia Limited (North Sunshine, Victoria, Australia) using the computer software System Control (V6.41, Varian Inc., Palo Alto, CA, USA).

Drip Loss Evaluation

MAP affects the amount of water lost through drip (Borderias, 1996). The weight of the empty trays, the trays after the addition of an absorbent pad used to collect drip, the trays after loading with prawns, and the finished packs with gas flush and lidding film were recorded. As microbiological sampling took priority and to avoid any contamination issues, the drip loss from the prawns could only be measured indirectly through the water gain on the pad in the tray instead of the weight of the

TABLE 2
Levels of drip loss and visual and odour demerit scores of raw prawns stored in MAP at 4°C

Parameter		*Drip (%)*	*Black spot*	*Drip*	*Odor*	*Head appearance*	*Hepatopancreas leak*	*Staining of first segment*	*Shell texture*	*Flesh appearance*	*Flesh color*	*Total demerits*
Trial	1	1.98^{b}	0	0.22^{a}	1.08	0.96	1.31^{a}	0.26^{b}	0.61^{a}	0.48	1.08	6.01
	2	5.48^{a}	0	0.13^{ab}	1.05	0.74	0.78^{b}	0.30^{b}	0.19^{b}	0.42	1.02	4.63
	3	2.97^{b}	0	0^{b}	0.91	0.83	0.72^{b}	0.69^{a}	0.14^{b}	0.26	0.76	4.26
	Probability	$p < 0.05$	ns	$p < 0.01$	ns	ns	$p < 0.05$	$p < 0.01$	$p < 0.01$	ns	ns	ns
Species	Black tiger	4.37	0	0.07	0.99	0.83	0.80	0.55^{a}	0.18	0.38	9.92	4.70
	Banana	2.81	0	0.16	1.03	0.77	0.94	0.19^{b}	0.37	0.35	0.96	4.76
	Probability	ns	ns	ns	ns	ns	ns	$p < 0.01$	ns	ns	ns	ns
Trial 1	Banana	1.98^{b}	0	0.22^{a}	1.08	0.96	1.31^{a}	0.26^{ab}	0.61^{a}	0.48	1.08	6.01
Trial 2	Banana	4.06^{ab}	0	0.09^{ab}	0.98	0.57	0.53^{b}	0.12^{b}	0.11^{b}	0.21	0.83	3.43
Trial 2	Black tiger	6.04^{a}	0	0.15^{ab}	1.09	0.83	0.90^{ab}	0.39^{ab}	0.23^{b}	0.52	1.12	5.23
Trial 3	Black tiger	2.97^{b}	0	0^{b}	0.91	0.83	0.72^{b}	0.69^{a}	0.14^{b}	0.26	0.76	4.26
	Probability	$p < 0.01$	ns	$p < 0.01$	ns	ns	$p < 0.05$	$p < 0.01$	$p < 0.01$	ns	ns	ns
Storage time (days)	1	1.85^{c}	0	0^{b}	0.05^{d}	0.13^{d}	0.02^{d}	0^{c}	0^{c}	0^{c}	0^{e}	0.21^{e}
	5	2.83^{bc}	0	0.03^{b}	0.71^{c}	0.44^{cd}	0.58^{c}	0.42^{b}	0.05^{bc}	0.03^{c}	0.38^{d}	2.55^{d}
	9	3.62^{bc}	0	0.11^{ab}	0.72^{c}	0.77^{bc}	0.72^{bc}	0.35^{b}	0.18^{bc}	0.27^{bc}	1.03^{c}	4.15^{c}
	12	5.55^{ab}	0	0.03^{b}	1.46^{b}	0.83^{b}	1.10^{b}	0.46^{b}	0.28^{b}	0.42^{b}	1.41^{b}	6^{b}
	16	7.46^{a}	0	0.25^{a}	2.2^{a}	1.65^{a}	1.91^{a}	1.09^{a}	0.61^{a}	1^{a}	1.95^{a}	10.66^{a}
	Probability	$p < 0.01$	ns	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

Means followed by a different letter are significantly different to those in the same column for individual parameters at the level stated.
The letters ns indicate that no significant differences are present.

prawns themselves. After storage at 4°C, the increase in the weight of the empty tray and absorbent pad upon removal of the prawns was calculated as the amount of drip lost from the prawns. The drip loss weight was then converted into a percentage of the original pack load as per Goncalves et al. (2008).

Demerit Scoring System

A demerit scoring system (Bremner et al., 1986), on which the Quality Index Method (QIM) is based, was used to appraise the visual and odor quality (Bremner, 2004; Hyldig et al., 2007). Demerit and QIM score sheets are modified for each type of seafood and sometimes for specific conditions. There were nine attributes appraised by three trained panelists. Black spot was rated as Absent (scoring 0), Slight (1), Moderate (2), or Extensive (3). The amount of drip apparent in the tray was scored as None (0), Slight (1), Moderate (2), or Excessive (3). The odor of the pack at opening was scored as Fresh (0), No off-odors (1), Slight (2), or Excess off-odor (3). The appearance of the head (cephalothorax) attachment to the tail (abdomen) was scored as Firmly attached (0), Slightly loose (1), Drooping (2), or Lost (3). The presence of leakage from the hepatopancreas and the changes this causes to the head appearance was scored as Unseen (0), Slight burn (1), Moderately blown (2), or Fully blown (3). The staining this leakage causes was scored as No stain (0), Slight stain (1), Moderate stain (2), or Very stained (3). The texture of the shell of the tail was scored as Smooth (0), Slightly etched (1), Course (2), or Gritty (3). The appearance of the flesh was scored as Intact (0), Slightly gaping (1), Soft (2), or Mushy (3). Finally, the color of the flesh was scored as Translucent (0), Dull (1), Slightly opaque (2), or Opaque (3).

The mean scores recorded can be seen in Table 2. A total of 27 demerit points could be scored for each sample evaluated. The scores were averaged from those recorded by three trained staff. The parameters used were not vulnerable to any visual differences because of the two species used (Erickson et al., 2007). Odor at opening was also scored before any microbiological samples were taken. The high quality life (Barbosa and Bremner, 2002) of this product was considered lost when a majority of the demerit parameters increased significantly and exhibited quite noticeable negative attributes.

Microbiological Analysis

All media and procedures were as per Kostaki et al. (2009). As the first trial was originally a preliminary evaluation and the prawns came from lined ponds with restrictions to any outside contamination, only the total aerobic mesophile and psychrotroph counts were evaluated. The second and third trials included a greater range of microbiological tests. The total number of aerobic mesophilic, psychrotrophic, dihydrogen sulphide (H_2S) producing bacteria and anaerobes were identified using the Australian Standard methods (1991a, 1991b).

To provide an element of food safety to the experiments, the methods Broda et al. (1998) developed to identify heat and ethanol resistant anaerobes, such as psychrotrophic *Clostridium* spp., were used in this investigation. Heated and ethanol treated samples were grown in Reinforced Clostridial Agar CM151 from Oxoid Ltd. (1995) under anaerobic conditions for enumeration of *C. botulinum* and *C. perfringes* spores.

Microbiological acceptability was determined using the Food Standards Code (Food Standards Australia New Zealand [FSANZ], 2007). Raw crustacean standard plate counts exceeding 5×10^6 cfu/g in one or more samples will cause the lot to be rejected. The standard considers 3 out of 5 prawn samples with counts below the microbiological level of 5×10^5 cfu/g in a sample unit as acceptable, and this interpretation has been applied here.

Statistical Analysis

Univariate one- and two-way analysis of variance (ANOVA) was used to identify significant differences between the treatments—such as trial, species, and sampling day—for all of the physical, sensory, and microbiological parameters tested. Statistix Version 1.0 from Analytical Software Inc. (Tallahassee, FL, USA) was utilized.

RESULTS AND DISCUSSION

Headspace Analysis

The headspace analysis shows that all the packs tested had remained sealed for the duration of storage at 4°C. The O_2 levels in the packs were minimal between 0 and 0.2%. After an initial drop in CO_2, the level stabilized to between 42 and 47%. This is in contrast to increases in CO_2 levels after storage reported by Layrisse and Matches (1984). Under identical conditions to the current study, Lannelongue et al. (1982) also found only a slight increase in CO_2 after storage of packed shrimp.

Because of the high water content of prawns (Borderias, 1996), CO_2 can be absorbed from a modified atmosphere. There was no distortion of the packs due to a drop in internal pressure, because there was only a small drop in CO_2 levels, unlike fish under the same type of atmosphere as reported by Tiffnety and Mills (1982/2006). Due to relatively constant CO_2 levels in the current study, this gas would have continued to inhibit much of the bacteria present in the packs for the life of the experiments (Statham, 1984).

Drip Loss Within Prawn Packs at Opening

When CO_2 is absorbed into packaged seafood, it increases cell wall permeability inducing increased drip (Borderias, 1996). Table 2 shows there were no significant differences between the two species for the amount of drip lost, measured as a percentage of the pack load. The three trials did differ significantly ($p < 0.05$), with the second trial recording a larger drip loss than the first and third. The significant interaction ($p < 0.01$) between trial and species suggests it was due to the different pond environments used for each category.

Although there was a progressive increase in drip loss over time, differences were not significant until after 9-days storage at 4°C. Excessive drip loss (7.46%) occurred by Day 16, and although this may not be commercially desirable, it is much lower than the 26% previously reported for frozen prawns by Goncalves et al. (2008). Layrisse and Matches (1984) obtained higher drip loss from *Pandalus platyceros* after using similar postharvest and MAP storage conditions as the current study.

Demerit Assessment of Prawns Within Packs at Opening

Table 2 shows the mean scores recorded for the two species of raw prawns for three trials stored in MAP at 4°C. Figure 1 shows opened packs of prawns after 16-days storage at 4°C. Denaturation of the contents of the hepatopancreas, due to its effective proteolytic digestive system (Moore and Eitenmiller, 1980), made the organ more visible. Prawns stored in air for 16 days invariably would have poorer (higher) appearance scores, as indicated by Cobb (1976). Black tiger prawns are naturally darker than banana prawns; thus, care had to be taken with the selection of the demerit parameters to be rated so that this difference did not unduly influence the scores. The lack of any significant differences between the two species for most of the parameters indicated that any bias

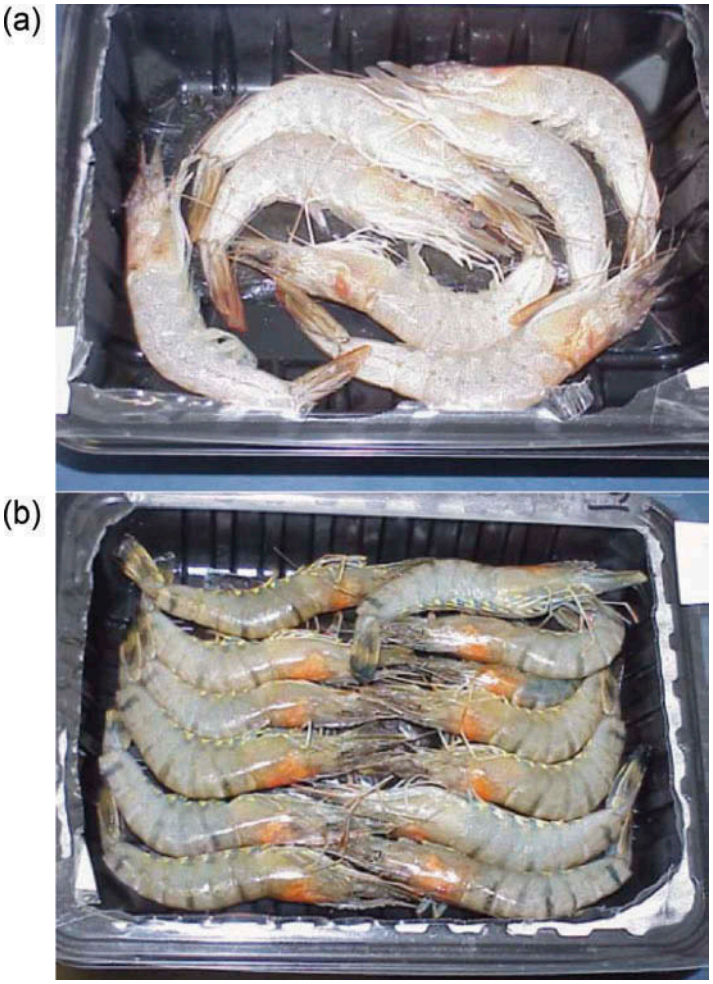

FIGURE 1 Opened packs of banana (a) and black tiger (b) prawns after 16-days storage in MAP at 4°C.

was unlikely. Small differences between the scores for the measured demerit parameters suggest that the natural degradation processes were operating at the same rate.

No black spot formation was observed on any of the prawns for up to 16-days storage in MAP at 4°C. Yamagata and Low (1995), using similar conditions to the current trials, were only able to attain 4-days shelf life before blackening caused the rejection of control prawns. Treatment with sulphite ($NaHSO_3$) was only able to limit blackening to moderate levels after 6 days, with the prawns being rejected at this time due to this as well as deterioration of other sensory attributes.

Up to 5% of fish weight can appear as unsightly drip at the base of the MAP tray (Sen, 2005). Significant differences between the trials were identified for drip score ($p < 0.01$), staining of the first abdominal segment ($p < 0.01$), and shell texture ($p < 0.01$); although there was no difference in the representative descriptors that these scores represented. Trial 3 exhibited no visible drip, while the other two trials were scored significantly higher.

The trial with the most significant hepatopancreatic leakage (Trial 1) did not lead to higher staining scores, and Trial 3 scored significantly higher than the other two for this attribute. The banana

prawns scored significantly higher for staining ($p < 0.01$), perhaps due to their more transparent nature (see Figure 1). The shell texture scores were significantly higher for Trial 1, but there was no significant difference between banana and black tiger prawns. Different water and feeding conditions for the various ponds may explain some of these trial differences, as shown by the interactions.

From the analysis of the combined trial data, the scores for each of the parameters increased significantly ($p < 0.01$) during storage at 4°C (Table 2). The totals for all demerit scores show there was a progressive loss of quality over time; however, significant drip loss ($p < 0.01$) was only observed at the end of storage at 4°C.

The fresh prawn odor at unpacking diminished as MAP storage progressed, although no off-odors had developed by Day 9 at 4°C. Some off-odor had become detectable by Day 12, but this remained slight even when the packs were opened on Day 16. The microbiological data will provide insight into this result.

Due to the action of digestive enzymes present in the hepatopancreas, this organ became more visible and the cephalothorax (head) began to loosen from the abdomen during storage. After 16 days at 4°C, the head was drooping. This may be an issue if the head is to stay intact with the abdomen when cooking the product. The breakdown of this combined digestive and storage organ progressed during storage, with moderate leakage being scored by Day 16 at 4°C. This led to only slight staining of the first abdominal segment and some softening (gaping) of the muscle tissue by the end of storage. Hanpongkittikun et al. (1995) found considerable loss of texture in black tiger prawns after 4-days storage under ice. The raw prawn tail muscle tissue did lose some transparency during storage in MAP at 4°C due to muscle enzyme activity (Cobb, 1976), but it was minor even by the end of storage. The CO_2 component of the modified atmosphere can lead to etching of the prawn shell due to the presence of minerals such as $CaCO_3$ (Moore and Eitenmiller, 1980), but in the present trials only slight changes were noted by the end of storage at 4°C.

Most sensory parameters suggested obvious degradation only after 16-days storage at 4°C. In combination with this and the increased drip loss, the authors suggest that the high quality life of the product was lost after Day 12 at 4°C. Even with well-controlled harvest conditions, Hanpongkittikun et al. (1995) only achieved less than 8-days storage under ice before rejection of black tiger prawns occurred. The defects present at rejection in the Hanpongkittikun study were greater than what was observed at the end of the current trials. The 12 days of high quality is a good outcome, as the storage temperature in MAP for these trials was 4°C, while the temperature of prawns stored under ice should be closer to 0°C. Shamshad et al. (1990) reported that banana prawns had a shelf life of 9 days when stored at 5°C and 13 days at 0°C. This indicates that any MAP prawns stored at 0°°C would have an even longer shelf life than that obtained from these trials.

Mastromatteo et al. (2010), in their study on peeled prawns, did not achieve an improvement in shelf life using MAP, as the sensory acceptable limit of ~3 days was the same as for air storage. This shorter shelf life may be explained by contamination from peeling and the fact they used a gas mixture that contained O_2. The authors, however, were able to obtain a similar shelf life to our findings by including a thymol coating for the peeled prawns. Shengmin (2009) found that overall acceptability could be extended for untreated whole prawns, although their limit was 13-days storage in MAP.

Microbiological Testing

The aerobic and anaerobic microbiological counts from the different trials and species can be seen in Figure 2. There were significant differences ($p < 0.01$) between the three trials and two species and their interactions for most microbiological counts (Table 3). The contaminating flora of aquaculture seafood is dependent on the environment in which it is grown and processed (Bhasker et al., 1995). As all prawns were packed at each farm and the packs sampled at the laboratory, the influence

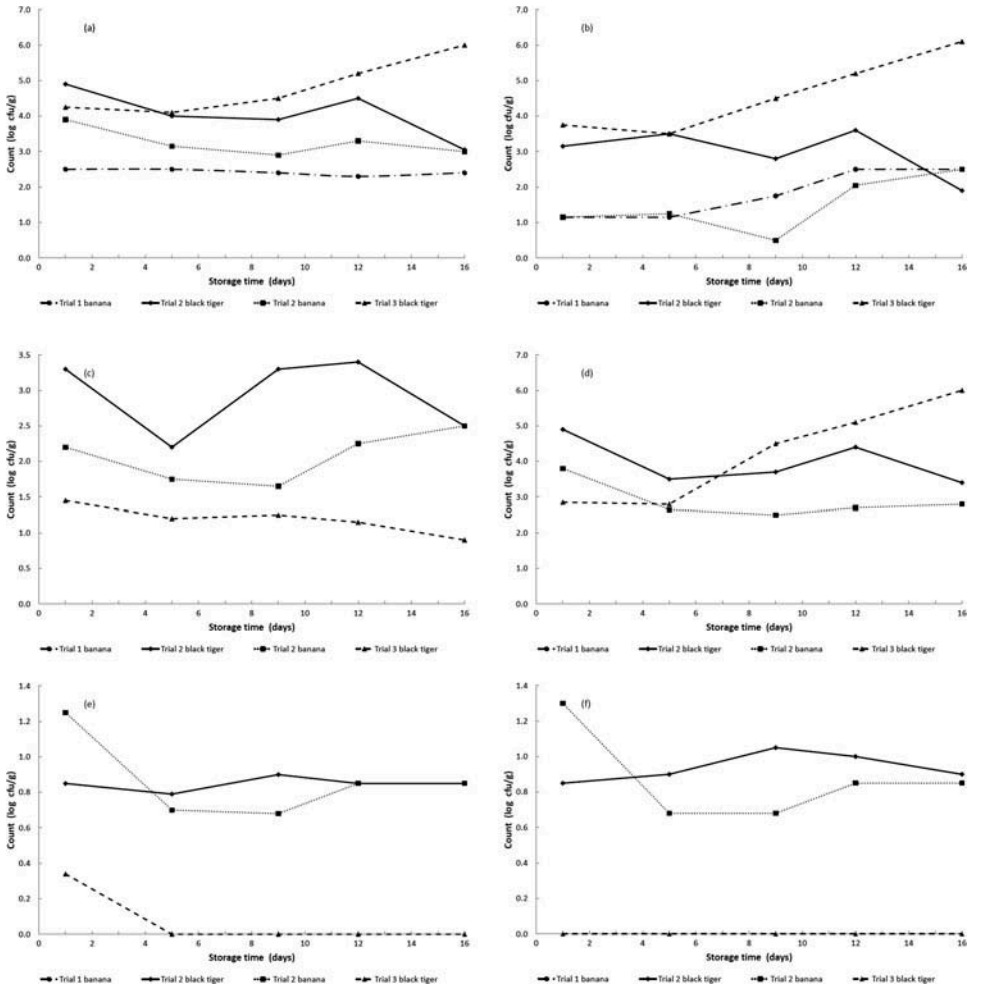

FIGURE 2 Microbial counts of two species of raw prawns for three trials stored in MAP at 4°C: total mesophile (a), psychrotroph (b), H_2S producer (c), anaerobe (d), heat-treated anaerobe (e), and ethanol-treated anaerobe (f).

of the processing environment could not be separated. There were significant differences ($p < 0.01$) between different trials for the same species and between the two species for Trial 2 because they originated from different ponds. Because different farms and even different ponds will have different bacterial profiles, growth of specific types of bacteria can vary between trials.

Trial 1 had quite low total mesophile and psychrotroph log counts. Similar flora was found on both species studied in Trial 2, but this was somewhat expected since they were both harvested from the same farm. In general, there was limited microbial growth during storage at 4°C for both these trials and it was always within the 5×10^5 cfu/g limit of acceptability set by FSANZ (2007; Figure 2).

As the prawns for Trial 3 came from a different farm, the microbial growth patterns differed during storage at 4°C. After an initial lag phase caused by changed atmosphere conditions, the initial moderate total mesophile, psychrotroph, and anaerobic counts of bacteria grew during storage until they approached the limit of acceptability by Day 12 and exceeded this by Day 16 at 4°C.

TABLE 3
Microbiological counts during storage of raw prawns in MAP at 4°C

Parameter		*Total mesophile (log cfu/g)*	*Psychrotroph (log cfu/g)*	*H_2S producer (log cfu/g)*	*Total anaerobe (log cfu/g)*	*Heat-treated Anaerobe (log cfu/g)*	*EtOH-treated anaerobe (log cfu/g)*
Trial	1	2.42^{c}	1.77^{b}	—	—	—	—
	2	3.73^{b}	2.48^{b}	2.60	3.57^{b}	0.86^{a}	0.93^{a}
	3	4.82^{a}	4.69^{a}	1.15	4.19^{a}	0.06^{b}	0^{b}
	Probability	$p < 0.01$	$p < 0.01$	ns	$p < 0.01$	$p < 0.01$	$p < 0.01$
Species	Black tiger	4.50^{a}	4.04^{a}	1.59	4.13^{a}	0.26^{b}	0.24^{b}
	Banana	2.74^{b}	1.73^{b}	2.02	2.83^{b}	0.87^{a}	0.88^{a}
	Probability	$p < 0.01$	$p < 0.01$	ns	$p < 0.01$	$p < 0.01$	$p < 0.01$
Trial 1	Banana	2.42^{d}	1.77^{c}	—	—	—	—
Trial 2	Banana	3.20^{c}	1.52^{c}	2.02^{b}	2.83^{b}	0.87^{a}	0.88^{a}
Trial 2	Black tiger	4.00^{b}	2.96^{b}	2.90^{b}	3.93^{ab}	0.85^{a}	0.95^{a}
Trial 3	Black tiger	4.82^{a}	4.69^{a}	1.15^{c}	4.19^{a}	0.06^{b}	0^{b}
	Probability	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
Storage time (days)	1	4.07^{abc}	2.91^{b}	1.95	3.45^{cd}	0.54	0.33
	5	3.60^{c}	2.95^{b}	1.47	2.93^{d}	0.25	0.28
	9	3.82^{bc}	3.32^{ab}	1.71	3.92^{bc}	0.28	0.32
	12	4.39^{ab}	4.19^{a}	1.68	4.59^{ab}	0.28	0.32
	16	4.55^{a}	4.42^{a}	1.37	5.03^{a}	0.28	0.30
	Probability	$p < 0.01$	$p < 0.01$	ns	$p < 0.01$	ns	ns

Means followed by a different letter are significantly different to those in the same column for individual parameters at the level stated.
The letters ns Indicate that no significant differences are present.

Because the anaerobic environment of MAP can cause the possible growth of *Clostridium botulinum* (Mullan and McDowell, 2003), the UK Food Standards Agency recommends a maximum 10-day shelf life (Callaghan, 2008), mainly due to repackaging issues. This product has never been frozen and is already consumer ready; it will not be repackaged, thus reducing the risk envisaged by the UK. The toxin from *Clostridium botulinum* can develop in seafood that has been stored incorrectly at an elevated temperature and even in atmospheres containing 100% O_2 (Huss et al., 1980). The majority of botulism outbreaks in the northern and temperate regions are associated with fish (Huss et al., 2003). Inactivation of *botulinum* toxin can be achieved using time/temperature combinations of 20 min at 79°C or 5 min at 85°C (Huss et al. 2003); however, due to the delicate nature of fish and prawns, they often get cooked using gentler conditions that do not reach these levels, so the presence of this contaminant in MAP products needs to be monitored. It is known that *Clostridium botulinum* spores can be either heat-resistant or relatively heat-sensitive (Broda et al., 1998; Campden and Chorleywood, 1995; Gram et al., 1987); so the three different anaerobic counts were needed to monitor the safety of the prawns in these trials.

Significant differences ($p < 0.01$) were identified between the second and third trial for the three different anaerobic counts. The heat and ethanol treatment counts, which include *Clostridial* spp., started much lower than 10^2 cfu/g and remained that way for all of storage at 4°C (Figure 2). While Trial 2 showed more anaerobic bacteria, the levels and types present were not considered a risk to consumers because at temperatures between 4 and 12°C organoleptic spoilage of fish precedes toxin production by *Clostridium botulinum* (Phillips, 1996), and the sensory evaluations from this study were favorable for at least 12 days.

Huss et al. (2003) reported that it took 28 days at 8°C for toxin to develop in chilled seafood containing 10^2 cfu/g of *Clostridium botulinum*, and hence our much shorter shelf life and lower storage temperature would have precluded this toxin production. They also stated that the natural level of *C. botulinum* in fish is much lower than levels used in most challenge studies. Heat-resistant spore-formers were more prevalent at the start of the trials; however, individual counts were all below 10^2 cfu/g. Individual counts of ethanol-resistant bacteria remained similarly low at all times.

The banana prawns had significantly lower total mesophile ($p < 0.01$), psychrotroph ($p < 0.01$), and anaerobe ($p < 0.01$) log counts, but they had higher numbers of heat ($p < 0.01$) and ethanol resistant forms ($p < 0.01$).

Only the psychrotroph and total anaerobe counts increased significantly ($p < 0.01$) during storage at 4°C. This growth should be expected from an anaerobic product stored at 4°C. The main cause of off-odor in seafood, H_2S producing bacteria (Slattery, 2010), were low for both trials, and there was no significant difference between the two trials or species of prawn. Low levels of off-odor encountered by the sensory evaluators at pack opening support these findings.

Shamshad et al. (1990) found that deheaded banana prawns which started with a bacterial load of 5×10^5 cfu/g had total aerobic counts of 6×10^7 cfu/g at the end of sensory shelf life (9-days ice storage at 5°C), although the total counts were already greater than 10^7 cfu/g by Day 4. By comparison, our trials show that the MAP was able to suppress bacterial growth for much longer for the same species and black tiger prawns. The microbial loads under MAP in the current study were also much lower at the end of shelf life than those identified by Mastromatteo et al. (2010), even though their peeled prawns had much lower initial counts. Lannelongue et al. (1982) found a similar gas mixture to the current trials prolonged the lag phase as compared to those stored in air. Layrisse and Matches (1984) obtained initial slower growth of bacteria during air storage of *Pandalus platyceros* after using similar postharvest MAP storage conditions.

The prawns from all of the trials contained counts that did not meet the definition for rejection ($> 5 \times 10^6$ SPC/g) set in Australia and New Zealand by the Food Standards Code for raw crustaceans (FSANZ, 2007). The levels of microorganisms in the raw prawns after storage in MAP at

4°C were well below the acceptable limit of 5×10^5 SPC/g (FSANZ, 2007), except for one isolated result at Day 16 for one of the replicate samples taken during Trial 3.

CONCLUSION

MAP of chilled prawn resulted in no black spot formation after 16-days storage at 4°C. This packaging method may be suitable for organic and conventional aquaculture to produce prawns without the use of black spot inhibiting chemicals. Prawns in MAP retained high quality for up to 12-days storage at 4°C. Since the visual and odor aspects of the modified atmosphere packaged prawns were acceptable for nearly all of the storage periods of these trials, the microbiological quality assessments are crucial in determining the recommended shelf life for this product.

A high quality shelf life with no melanosis of 12 days and a maximum shelf life of 16 days in MAP would allow the export of this type of fresh, never frozen product from Australia or any other country to distant markets using air freight. If the prawns were repacked from frozen stock, this should be limited to 10 days so not to exceed the UK Food Standard Agency recommendation.

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