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Effect of modified atmosphere packaging on oxidative changes in frozen stored cold water shrimp (*Pandalus borealis*)

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Abstract

Shrimps caught at sea were boiled in seawater, air blast or nitrogen frozen, glazed and then packed in plastic bags with a low oxygen transmission rate. The bags were either flushed with nitrogen (modified atmosphere packaging) or with atmospheric air before sealing. The shrimps were then stored for up to 12 months in a freezer cabinet at −17°C with fluctuating temperatures. During storage they were either exposed to fluorescent light or kept in darkness. To investigate the effect of fluctuating temperatures some of the modified atmosphere-packed shrimps were stored in darkness in a cold store at a constant temperature of −18°C. Quality changes were determined by sensory evaluation combined with chemical/physical analyses, including determination of the pigment astaxanthin and measurement of the oxidative stability by determination of thiobarbituric acid-reactive substances (TBARS). Packaging in modified atmosphere resulted in overall better quality in relation to colour fading, development of rancid flavour and toughening of the meat. Light exposure influenced both colour fading and lipid oxidation negatively. Temperature fluctuations resulted in very pronounced formation of frost in the packages. After 6 to 9 months of frozen storage, the amount of frost corresponded to the weight of the glazing layer applied before storage.

Keywords: Shrimp, *Pandulas borealis*; Frozen storage; Modified atmosphere packaging (MAP); Frost formation

1. Introduction

Boiled shell-on shrimps are regarded as a luxury product in Scandinavian countries. They are normally sold in light- and gas-permeable packages of 0.5–1 kg, and are given a shelf life of 12 months at −18°C. Prior to retail packaging the shrimps may be stored for up to 12 months in bulk packages at −25°C, but the main quality changes take place during the retail storage (DIFTA, 1993). The most important quality changes occurring during retail storage of frozen shrimps are colour fading (Ghosh & Nerkar, 1991; Chandrasekaran, 1994), lipid oxidation (Bottino, Lilly, & Finne, 1979; Reddy, Nip, & Tang, 1981; Riaz & Qadri, 1990), denaturation of protein (Bhobe & Pai, 1986), increase in volatile basic nitrogen (Riaz & Qadri, 1990; Yamagata & Low, 1995), textural changes (Gates, Eudaly, Parker, & Pittman, 1985; Bhobe & Pai, 1986; Watabe & Hashimoto, 1987; Yamagata & Low, 1985), reduced water binding capacity (Bhobe & Pai, 1986) and loss of juiciness (Bhobe & Pai, 1986). This study has mainly focused on oxidative changes during frozen storage.

Boiled shrimps are pigmented by the carotenoid astaxanthin giving the distinct red colour. During storage the red colour fades and the shrimps gradually appear more yellow. The colour changes are in part caused by photooxidation of astaxanthin (Christophersen, Jun, Jørgensen, & Skibsted, 1991), a process which is enhanced by high partial pressure of oxygen (Nielsen, Mortensen, Jørgensen, & Skibsted, 1996). The shrimps contain approximately 1.2% lipids of which the major class is highly unsaturated phospholipids (Johnston, Ghanbari, Wheeler, & Kirk, 1983). Most of the lipids are located just under the shell and the lipids may, therefore, be exposed to light and oxygen, making the unsaturated fatty acids vulnerable to oxidation. Lipid oxidation produces off-flavours that smell strongly during peeling and may be tasted even in the peeled product. To protect shrimps from dehydration during storage, they are often glazed with 5–15% of seawater (Bottino et al., 1979). The glazing water and

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the water from the shrimps may sublime from the surface of the shrimps and crystallise inside the packages, resulting in frost formation. Excessive frost formation is an indication of dehydrated shrimps. It prevents the consumers from getting a clear view of the shrimps' colour and size and, furthermore, the product appears as if it contains more water than shrimp meat.

It has been suggested that packaging in modified atmospheres depleted of oxygen may improve colour stability and prevent lipid oxidation in chill-stored shrimps (Sivertsvik, 1995). If this is combined with protection of the shrimps from light exposure and temperature fluctuations, both of which are mainly a problem during retail storage, a better overall shelf life could be expected. Technically, modified air packaging (MAP) is troubled by the shrimps horns that, sharp as a needle, may easily penetrate most plastic materials used for food packaging.

The purpose of the present study was to investigate the effect of packaging atmosphere, temperature fluctuations and light exposure on frost formation, lipid oxidation, discoloration and meat toughness of shell-on cold-water shrimps during 12 months of frozen storage.

2. Materials and methods

2.1. Product and process

Shrimps (*Pandalus borealis*) were caught in the Skagerak in March 1995. On board the trawler the shrimps were boiled and chilled in seawater and packed in polystyrene boxes with ice in plastic bags at the bottom to keep the shrimps chilled until landing. On landing the shrimps were immersed in seawater for 2 min to imitate the handling on board commercial shrimp factory trawlers. Thereafter the shrimps were frozen to a temperature of −21°C within 40 min. Some of the shrimps were blast frozen in a tunnel (−45°C, 6–7 m s^{-1}, 40 min) and some were cryogen frozen (N_2, −50 to −55°C, 5 min). As none of the chemical analyses indicated a significant difference in storage stability due to the freezing procedure, the data were pooled. The total process from landing to freezing took place within 18 h. After freezing, the shrimps were bulk-stored for 3 days at −25°C, and then glazed in fresh water, achieving a weight gain of 6.1–10.7%.

2.2. Packaging

For the storage experiment, 800 g of shrimps were packed manually in 1500 cc plastic bags composed of a three layer laminate (15 μm orientated polyamide, OPA/45 μm cast polyamide, CPA/50 μm low linear density polyethylene, LLDPE). The oxygen transmission rate (OTR) of the laminate was 26.7 cm^3 m^{-2} $(24\ h)^{-1}$ at m^{-1} (23°C, 5/95% RH). The laminate was fully transparent. The bags were flushed with 100% N_2 or with atmospheric air (see Table 1) before being sealed on a chamber vacuum packer.

2.3. Storage

The samples were either stored in a freezer cabinet or in a cold store. The freezer cabinet had an average temperature of −17°C with daily defrosting resulting in fluctuations in the air temperature from −9.6 to −32°C. Half of the samples in the freezer cabinet were stored under fluorescent light (Philips TLD 83) with a radiant flux of 340 (±160) lux. The cold store was kept dark at a temperature of -17.9 ± 3°C. All the samples were frequently redistributed within the cabinet to obtain equal temperature and light conditions. At 0, 3, 6, 9 and 12 months of frozen storage, samples were withdrawn for chemical and physical analyses.

2.4. Chemical and physical analyses

2.4.1. Gas composition

Before the packs were opened for further analyses the gas composition was analysed by a Gasspace gas analyzer (Systech Instruments Ltd) by penetrating a needle through a gas-tight membrane. Samples packed in N_2 with a residual oxygen level of >5% were discarded. The mean oxygen level in the nitrogen-packed samples was 0.84%.

2.4.2. Frost formation

For measuring frost content, a method was developed in which the frost formed in the packages was weighed directly. A package was taken from the freezer, wiped on the outside and weighed (T). It was twisted carefully to release the frost from the shrimps and placed on a freezing block of −21°C to keep the plastic cold, thereby preventing the frost from melting and recrystallizing on the cold shrimps. The package was opened and the shrimps were taken out one by one after being scraped free of frost with a −21°C cold glass spatula. The whole procedure took place within 1 min. The package with frost was left to melt for 15 min at ambient temperature with one corner placed low to collect

Table 1
Combination of packaging atmosphere and storage conditions for frozen shell-on shrimps

Code	Atmosphere in bags	Light conditions	Temperature conditions
1	Modified (N_2)	Fluorescent light	Fluctuating temperature
2	Modified (N_2)	Darkness	Fluctuating temperature
3	Modified (N_2)	Darkness	Constant temperature
4	Atmospheric air	Fluorescent light	Fluctuating temperature
5	Atmospheric air	Darkness	Fluctuating temperature

the melted water. A small hole was cut in the bottom and the water was poured out and weighed (W). As the package still contained water it was weighed (pw), dried and reweighed (pd). The left-over from the shrimps was taken out and the plastic bag was weighed (B). The frost formation of each code was calculated as the average of the frost formed in five packages.

The frost (F) was calculated as gram of frost/gram of package content:

$$F = [(W + pw - pd)/(T - B)] \times 100\%.$$

2.4.3. Sample preparation for chemical and physical analyses

After determining the gas composition, the packs were opened, visible frost was removed and the shrimps were thawed for 10–20 min in darkness at ambient temperature. Head, tail, legs and roe were removed and the residual meat and shell was cut into minor-sized pieces.

2.4.4. Level of oxidation

The level of oxidation was measured by determination of 2-thiobarbituric acid reactive substances (TBARS) by the method of Vyncke (1975).

2.4.5. Astaxanthin content

The astaxanthin content was determined spectrophotometrically after extraction by the method described by Kelley and Harmon (1971).

2.4.6. Dry matter

Shrimp (5 g) was placed on a dry aluminium tray and dried for a minimum of 16–18 h at 105°C, and then the sample was weighed to get the dry matter content.

2.5. Sensory analyses

A sensory profiling was performed by an eight-member trained sensory panel as described in Bak, Jacobsen and Jørgensen (1998).

2.6. Statistical analyses

Analysis of variance was performed for the response variables TBARS, astaxanthin content, and sensory scores for rancid flavour, toughness, and colour. The analysis of variance included main effects of storage temperature and time, packaging, light and freezing method, all two-factor interactions between the last four of these factors except the interaction between storage time and freezing method, and the three-factor interaction between packaging, light and storage time. This model was selected to match the design of the experiment, which was done to investigate the effect of storage temperature at a specific combination of light and packaging (darkness and MAP) and on the basis of prior judgement of possible importance of effects. The main effect of the freezing method and its interactions were omitted from the model after verifying their non-significance.

When significant effects of light, packaging and storage temperature were found, the results were compared. For all responses the largest deviations occurred for the treatment atmospheric packaging in combination with storage in light. The data for this treatment were then omitted and the analysis of variance was repeated to see whether this was the single cause of the significant result. Otherwise, the procedure was repeated by also omitting the data for samples packed in atmospheric air and stored in darkness.

3. Results

Packaging in atmospheric air resulted in pronounced lipid oxidation during frozen storage as compared to packaging in modified air. This can be seen from Fig. 1, where the TBARS in shrimps packed in atmospheric air were significantly higher than TBARS in samples packed in modified air. For samples packed in modified air, no significant change in TBARS was observed during the initial 9 months of frozen storage, whereas a small but significant increase was seen between 9 and 12 months of frozen storage. Storage in light resulted in significantly higher TBARS values for samples packed in atmospheric air, whereas no significant differences were observed between modified air-packed samples exposed to light or stored in darkness. As neither of the

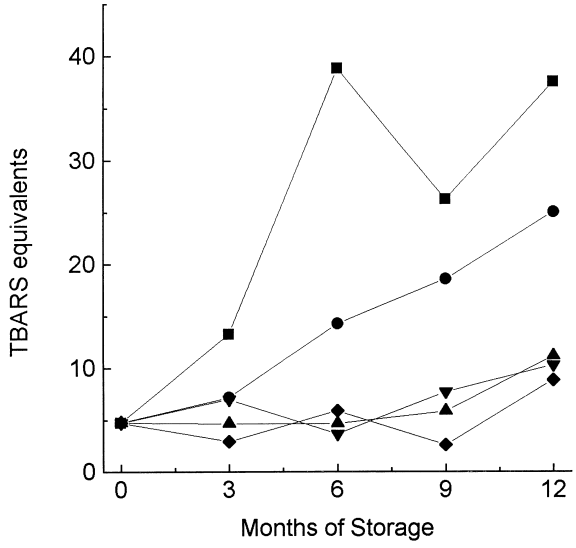

Fig. 1. Lipid oxidation measured by determination of TBARS (μmol/mg) of shrimps packed in atmospheric air and stored in a freezer cabinet in light (■) or in darkness (●), or packed in a modified atmosphere and stored in a freezer cabinet in light (▲) or in darkness (▼), or in a cold store in darkness (◆).

modified air-packed samples had any pronounced increase in TBARS, no effect of fluctuating temperatures was observed.

In accordance with the TBARS results, the sensory scores for rancid flavour were significantly higher for samples packed in atmospheric air compared to samples packed in modified air (Fig. 2). The effect of light on the rancid flavour of atmospheric air packed shrimps was significant although less pronounced than on the TBARS results. In contrast to TBARS, the sensory scores for rancid flavour for modified air-packed samples did not increase significantly between 9 and 12 months of frozen storage.

The sensory evaluation of shrimp meat toughness revealed a clear effect of the packaging method and storage conditions. As can be seen from Fig. 3, the sensory scores for toughness were significantly higher for samples packed in atmospheric air compared to samples packed in modified air. In addition, storage in light resulted in significantly tougher shrimp meat. The sensory scores for toughness were almost constant for samples packed in modified air for the first 9 months of storage, whereas a significant increase was observed between 9 and 12 months of storage, corresponding to the TBARS development.

Colour changes during frozen storage were quantified, both by determination of astaxanthin and by sensory evaluation. The most pronounced colour changes were recorded in samples packed in atmospheric air and stored in light. As can be seen from Fig. 4, the concentration of astaxanthin in light-exposed, atmospheric air-packed shrimps, decreased to less than 1/3 of the initial content during the 12 months of frozen storage. Less, but still a significant loss in astaxanthin content was also seen in atmospheric air-packed samples stored in darkness, whereas only minor changes were seen in modified air-packed samples exposed to light or stored in darkness. In addition, no significant differences were seen between modified air-packed samples stored either in the freezer cabinet at fluctuating temperatures or in the freezer store at constant temperature.

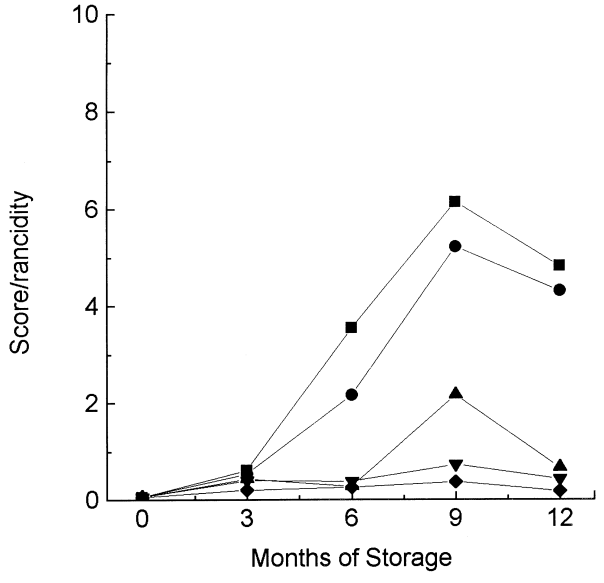

Fig. 2. Sensory scores for rancid flavour of shrimps packed in atmospheric air and stored in a freezer cabinet in light (■) or in darkness (●), or packed in a modified atmosphere and stored in a freezer cabinet in light (▲) or in darkness (▼), or in a cold store in darkness (◆).

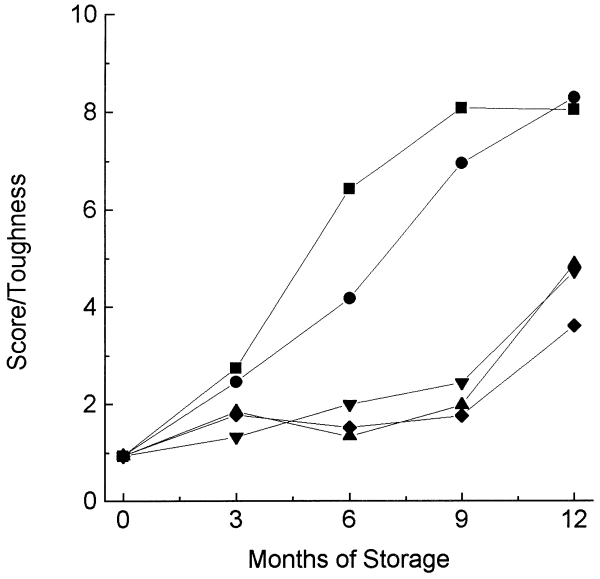

Fig. 3. Sensory scores for toughness of shrimps packed in atmospheric air and stored in a freezer cabinet in light (■) or in darkness (●), or packed in a modified atmosphere and stored in a freezer cabinet in light (▲) or in darkness (▼), or in a cold store in darkness (◆).

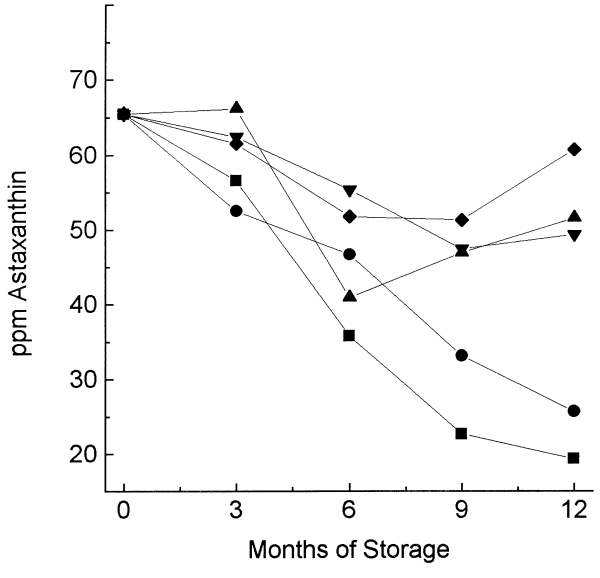

Fig. 4. Changes in concentration of astaxanthin of shrimps packed in atmospheric air and stored in a freezer cabinet in light (■) or in darkness (●), or packed in a modified atmosphere and stored in a freezer cabinet in light (▲) or in darkness (▼), or in a cold store in darkness (◆).

Results of the sensory evaluation of the colour of the shrimps were fully in agreement with the chemical analyses of astaxanthin. As can be seen from Fig. 5, significant reduction in the score was seen during storage, especially for atmospheric air-packed samples stored in light. Pronounced colour fading was also seen for atmospheric air-packed samples stored in darkness, but the colour fading seemed to be delayed for 3 months as compared to light-exposed packages.

Frost formation was determined in modified air-packed samples stored in darkness in the freezer cabinet with fluctuating temperatures or in the cold store at constant temperature. Fluctuating temperatures resulted in considerable frost formation, especially after 6 months of frozen storage, as can be seen from Fig. 6. After 12 months of frozen storage, frost formation was approximately 20% for samples stored in the freezer cabinet. Only very little frost formation was seen in samples stored at constant temperature in the freezer store.

4. Discussion

In the present study commercial handling and storage procedures have been simulated as closely as possible. Thus both light exposure and temperature fluctuations have been included. The shrimps used for the experiment had a high initial quality. After 12 months of frozen storage the overall quality of the shrimps was still good when stored in modified atmosphere at a constant temperature of −18°C in darkness. Under these storage conditions, only minor quality changes took place and the quality changes were mainly related to increased toughening of the meat and a visible colour fading through loss of astaxanthin. The minor increase of TBARS between 9 and 12 months of storage, indicating initial lipid oxidation, was below the limit for rancidity that the sensory panel was able to detect. The increased toughness seemed to be more pronounced during the last 3 months of storage, whereas the reduction in astaxanthin especially took place within the first 3 months of storage.

Textural changes of marine meat are predominantly linked to protein denaturation as a consequence of protein aggregation and loss of water-holding capacity (Shenouda, 1980). Several pathways of protein denaturation have been suggested in frozen marine meat. These include dehydration of the proteins from ice crystal formation and increasing salt concentration (Ota & Tanaka, 1978), interaction with formaldehyde from the reduction of TMAO (Matsumoto, 1979) and interaction with lipids and products from lipid oxidation and hydrolysis (Shenouda, 1980). The very strong correlation of the shrimp meat toughening with lipid oxidation observed in the present study suggests that the main pathway of meat toughening in this case is proteins interacting with lipid oxidation products. Proteins may be attacked by free radical intermediates from lipid oxidation forming protein free radicals (Karel, Schaich, & Roy, 1975), which may react with other free radicals of either lipid or protein origin forming lipid-protein aggregates (Schaich & Karel, 1975) or protein-protein aggregates (Varma, 1967). Another possibility is the secondary lipid oxidation products reacting covalently with specific functional groups on protein side

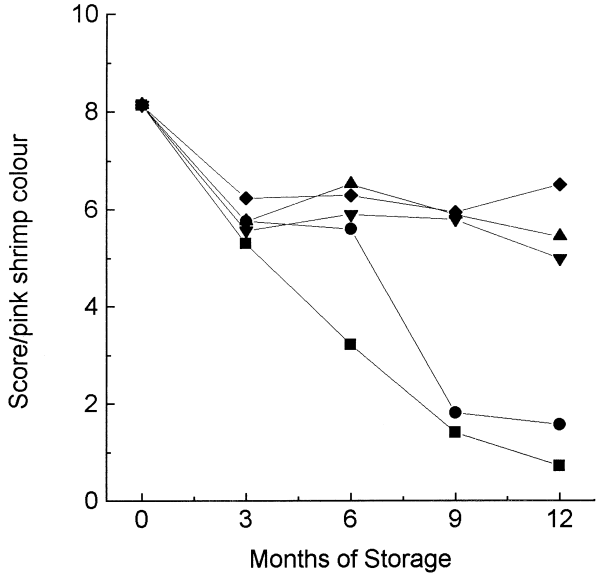

Fig. 5. Sensory scores for colour of shell-on shrimps packed in atmospheric air and stored in a freezer cabinet in light (●) or in darkness (■), or packed in a modified atmosphere and stored in a freezer cabinet in light (▲) or in darkness (▼), or in a cold store in darkness (◆).

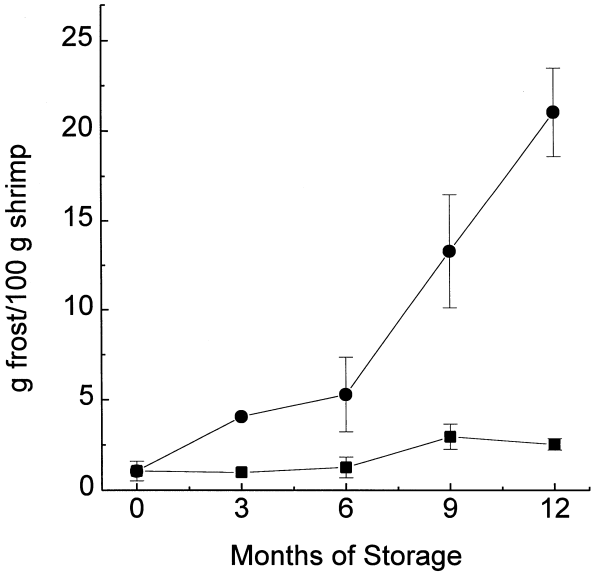

Fig. 6. Frost formation (weight %) of shrimps packed in a modified air and stored in darkness in a freezer cabinet (●) or in a cold store (■).

chains, increasing protein hydrophobicity (Takama, 1974). Although the TBARS method measures the increase in secondary lipid oxidation products and the meat toughening correlated well with TBARS, further studies are necessary to reveal which pathway is dominant.

The first observed quality change was the degradation of the pigment astaxanthin which was seen already after 3 months of frozen storage (Figs. 4 and 5), whereas lipid oxidation quantified by determination of TBARS (Fig. 1) and by sensory evaluation (Fig. 2) was pronounced only after 6 months of frozen storage. Astaxanthin has been identified as an important factor for protection of the highly unsaturated lipids against oxidation, as astaxanthin has been shown to be efficient as a scavenger of free radicals especially at low partial pressure of oxygen and as a quencher of singlet oxygen (Burton & Ingold, 1984; Di Mascio, Kaiser, & Sies, 1989). The latter may explain why astaxanthin degradation precedes lipid oxidation in the present storage experiment.

The single most important handling factor affecting shrimp quality was the exposure to oxygen during storage. All measured quality parameters were significantly affected by atmospheric air-packaging, such as lipid oxidation and rancid flavour, meat toughening, loss of astaxanthin and the red shrimp colour. Oxygen is essential for lipid autoxidation, and initiation of autoxidation might be decisive for the other quality losses of the shrimps. Unfortunately, oxygen is also the most difficult parameter to control when packing shell-on shrimps. An anoxic atmosphere may be achieved either by vacuum packaging or by packaging in modified atmosphere. In both cases it is difficult to avoid puncturing of the packages by the needle sharp horns of the shrimps and by the shrimp legs. Furthermore, during 12 months of storage, the partial pressure of oxygen will increase inside the packages as some transmission of oxygen is unavoidable through the plastic bags even at $-17^{\circ}C$. At a certain level of oxygen, autoxidation will be initiated and quality will be affected, limiting the storage life. When the shrimps are packed in atmospheric air, the exposure to light enhances the rate of quality loss in respect to lipid oxidation, colour fading and increasing toughness by at least 3 months, whereas light exposure during 12 months was insufficient to damage the shrimps packed in an anoxic atmosphere, indicating that photooxidation is less significant than autoxidation.

Temperature fluctuation is a common problem in commercial freezer gondolas, but should be minimized as it produces excessive frost in the packages, giving an overall bad appearance of the product. The effect of temperature fluctuations has in the present study been restricted to modified air packages stored in darkness, and under these conditions temperature fluctuations had only a minor influence on the quality parameters. Temperature fluctuations did not influence colour stability, whereas lipid oxidation and meat toughening increased slightly. The influence of temperature fluctuations on the oxidative stability of the lipids could be due to a general deterioration of the structure due to drying or simply a consequence of the lost glazing cover; both may expose the lipids to oxygen in the atmosphere. The increase in meat toughening may partly be due to the drying out of structural proteins in addition to lipid oxidation. Temperature fluctuations will probably enhance the overall quality loss when the shrimp packages are exposed to oxygen and light, but the extent of enhancement is yet to be resolved.

5. Conclusion

The present study clearly shows that the most common industrial practice in which the glazed shrimps are packed in plastic materials with high oxygen permeability and retail-stored at $-17^{\circ}C$ in open cabinets (gondolas) with extensive temperature fluctuations and more or less exposure to light, can be improved considerably. Atmospheric air and light exposure, in combination, significantly fade the shrimps and these parameters also increase lipid oxidation significantly. Exclusion of oxygen, especially, will give an overall better quality in relation to colour stability, lipid oxidation and shrimp meat toughening and will extend shelf life of the shrimps for at least 9 months. Excluding light influences both lipid oxidation and colour stability, giving an improved quality which will extend the shelf life for at least 3 months. For packaging in an anoxic atmosphere, it is a requirement to develop packaging materials with a sufficient strength to withstand puncturing from the shell shrimp horns.

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