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ORIGINAL ARTICLE

Stability of carotenoids recovered from shrimp waste and their use as colorant in fish sausage

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Abstract The stability of carotenoids recovered from shrimp waste using organic solvents and vegetable oils as affected by antioxidants and pigment carriers was evaluated during storage under different conditions. Solvent extracted carotenoid incorporated into alginate and starch as carriers was stored in metallised polyester and polypropylene pouches. Oil extracted carotenoids were stored in transparent and amber bottles. Also the use of recovered pigments as colorants in fish sausage was evaluated. Antioxidants, packaging material and storage period had a significant effect ($p \leq 0.001$) on the reduction of carotenoid content, while type of carrier had marginal effect ($p \geq 0.05$) on solvent extracted carotenoids during storage. Carotenoid content in pigmented oil was significantly affected by antioxidants ($p \leq 0.001$), packaging material ($p \leq 0.05$) and storage period ($p \leq 0.001$). Addition of carotenoid to the sausage enhanced the sensory colour, flavour and overall quality score of sausage and the added carotenoid was stable during processing.

Keywords Shrimp waste · Carotenoid · Stability · Colorant · Sausage

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Introduction

Shrimp waste is an important natural source for carotenoids, particularly astaxanthin and its esters (Sachindra et al. 2005, 2006a). Methods have been developed for the recovery of carotenoids from shrimp waste using organic solvents and vegetable oils (Sachindra and Mahendrakar 2005, Sachindra et al. 2006b). However, carotenoids are highly unstable compounds and need to be protected by suitable storage conditions from excessive heat, exposure to light and oxygen to prevent their breakdown. In crustacean wastes, the processing and storage also affect the carotenoids. The degradation of astaxanthin in dried crawfish meal and its prevention by use of butylated hydroxy anisole (BHA) and ethoxyquin has been reported (Meyers and Bligh 1981). Chen and Meyers (1982) have reported the stabilization of carotenoids in soy oil by addition of ethoxyquin to crawfish waste before oil extraction of pigments.

The mechanism of carotenoid degradation is hypothesized to be similar to lipid oxidation (Frankel 1985) and the antioxidants, which inhibit lipid oxidation, also decrease the degradation of carotenoid. Goldman et al. (1983) suggested that free radicals produced by lipid oxidation might interact with carotenoid to intensify their oxidation. Use of antioxidants has been attempted for prevention of carotenoid related colour degradation in fish, meat and poultry (Green et al. 1971, Chastain et al. 1982, Li et al. 1998). The major cause for carotenoid degradation in foods is oxidation with rates dependent on contact with oxygen, light, heat and presence of pro- and antioxidants (Francis 1985, Haard 1988). In animal tissues the carotenoid degradation probably involves both lipoxygenase catalysed enzymatic oxidation and non-enzymatic oxidation such as autooxidation and photosensitized oxidation (Frankel 1985, German and Kinsell 1985). In nature, the interaction of protein and carotenoids as occurring in crustaceans increases the pigment stability (Britton 1996). However, when carotenoids are recovered from crustacean wastes, they are extracted as pigments solubilized in lipid. Thus it is necessary to protect

the lipids from oxidation, which in turn can stabilize the pigments in it.

Various fish mince products such as sausage, kamaboko are very popular in urban cities as ready to eat products. Colour is one of the important sensory attributes, which determines the consumer acceptability of the products. Synthetic colouring agents or colour developers are included in the formulation of fish products to improve the colour. The main commercial colourants being used in seafood products include carmine, carmosine, caramel, paprika and annatto dye (Lee et al. 1992). Koizumi and Nonaka (1980) used ferihaemochrome forming nitrogenous bases such as imidazole and amino acid derivatives to develop pink colour in fish sausage. Use of L-xylose, 2-ketohexonic acid, along with potassium bromate, pH adjustor and surfactants for colouring the surface of fish meat products is reported (Akiji 1985). There is a need for alternate natural colouring ingredients to reduce the health risks associated with synthetic food additives. Reports on use of shrimp carotenoids as colorants in fish products are scanty.

This study was carried out to investigate the effect of antioxidants, pigment carriers and different storage conditions on the stability of carotenoids recovered from shrimp waste by solvent extraction and oil extraction methods. Further, the suitability of carotenoids recovered from shrimp waste as colouring agent in fish sausage as an alternative to the synthetic colouring agent was also evaluated.

Materials and methods

Shrimp (*Feneropenaeus indicus*) waste comprising of head and carapace was collected from a shrimp processing plant situated at Mangalore, India and transported to the laboratory under frozen (–4°C) condition and stored at –20°C till use. The material was thawed in running water before use and homogenized in a laboratory mixer. Tertiarybutyl hydroxyquinone (TBHQ) and α-tocopherol were from Loba Chemie, India. Solvents used were of AR grade.

Solvent extracted carotenoids: Carotenoids in the waste from the shrimp *Feneropenaeus indicus* was extracted using a mixture of isopropyl alcohol and hexane as described by Sachindra et al. (2006b). In brief, minced shrimp waste was homogenized with a solvent mixture (3:2) of hexane and isopropyl alcohol, filtered and extraction repeated two more times. From the pooled extract hexane layer was separated and dried with sodium sulphate. Hexane layer containing carotenoids was concentrated to 50 ml by evaporating the solvent using flash evaporator. Fat content in the hexane concentrate was determined by evaporating an aliquot of the extract. Antioxidant, TBHQ or α-tocopherol was added to the hexane extract at a level of 200 ppm of fat content. The extract without antioxidant served as control. Carrier was added to the concentrated hexane extract at a rate of 15 g/100g of waste and the solvent evaporated completely to obtain the pigmented carrier. Corn starch and sodium alginate were used as pigment carriers. The pigmented carrier was packed in metallised polyester or polypropylene pouches and stored at ambient temperature (27 ± 2°C) for 6 months.

Oil extracted carotenoids: Carotenoid in the waste was extracted using refined sunflower oil by adopting the optimized conditions for extraction (Sachindra and Mahendrakar 2005). To the pigmented oil, antioxidant TBHQ or α-tocopherol was added at a level of 200 ppm. The pigmented oil without antioxidant served as control. The pigmented oil was then stored in transparent or amber colored bottles at ambient temperature (27 ± 2°C) for 6 months.

Preparation of fish sausage with added carotenoids: Pigmented starch was prepared from carotenoid extract of shrimp waste as explained above and used for colouring. The carotenoid content in the pigmented starch ranged from 397.0 to 439.9 μg/g. Fish sausage was prepared using the minced meat from pink perch, *Nemipterus japonicus*. The formulation of fish sausage included 500 g fish meat, 14.3 g salt, 10.7 g sugar, 1.4 g sodium tripolyphosphate, 60 mg chilly oleoresin, 0.8 g pepper powder, 0.8 g garlic powder, 65 g corn starch, 35 ml refined vegetable oil and 70 ml chilled water. Sausage mix (700 g) was prepared by mixing the ingredients in sequence in a bowl chopper. The mix was stuffed into synthetic casings and cooked at 90°C for 45 min to obtain cooked sausage. For control (C) batch no carotenoid was added. To prepare fish sausage with 5 ppm carotenoid (T1), the preparation was carried out as above by replacing 8.0–8.8 g (depending on carotenoid content in the pigmented starch) of corn starch with pigmented starch. Similarly, to prepare sausage with 10 ppm carotenoid (T2), 16.0–17.6 g of corn starch was replaced with pigmented starch in the formulation. The preparation of sausage in 3 formulations (C, T1 and T2) was carried out 4 times.

Determination of carotenoid content, Hunter colour values: Carotenoid content in the pigmented carrier during storage was determined at monthly intervals by extracting the pigments in hexane and measuring the carotenoid content spectrophotometrically (Sachindra et al. 2005). Hunter L, a*, b* values were measured using Hunter Colour Instruments (LabScan XE, Virginia, USA) (port sze: 1.20", area view: 1.00", 2°, C illuminant). The pigmented oil during storage was sampled at monthly interval for analysis. The absorbance of the pigmented oil was read at 487 nm and the Hunter L, a*, b* values were measured.

Evaluation of fish sausage: Carotenoid content in fish meat, sausages mix and cooked sausage was determined similarly. Hunter L, a*, b* values were measured using Hunter LabScan XE as explained earlier. Sensory analysis of cooked sausage for colour, flavour and overall quality was carried out on a 9-point Hedonic scale (1: dislike extremely; 9: like extremely) employing 10 in-house trained panelists (ASTM 1996).

Statistical analysis: All statistical analyses were carried out using the software STATISTICA (Statsoft Inc. 1999).

The data was subjected to analysis of variance (ANOVA) and Duncan's multiple range tests. The relationship between carotenoid content and Hunter L, a* b* was determined by correlation analysis.

Results and discussion

Stability of carotenoid: Solvent extraction of carotenoids from shrimp waste yielded a product in thick paste form. It is difficult to use the paste in food applications as uniform mixing of paste with food ingredients is a problem. Thus it is necessary to prepare and store the product in an easy to use form. Starch or alginate is normally used as an ingredient in many of the comminuted meat and fish products. Thus starch or alginate is considered as a pigment carrier for solvent extracted carotenoids.

Carotenoid content in the pigmented carriers decreased during storage (Fig. 1). Presence of antioxidants, packaging material and storage period had a significant effect ($p \leq 0.001$) on the total carotenoid content, while the total carotenoid content was not affected ($p \geq 0.05$) by the carrier used. Highest reduction (from the initial carotenoid content) was observed in the absence of antioxidant and storing in polypropylene pouches in pigmented alginate (118.7 μg) and starch (125.4 μg) at the end of 6 months storage. Lowest reduction at the end of 6months storage was observed in pigmented alginate (44.4 μg) and starch (45.7 μg) containing 200 ppm TBHQ and packed in metallised polyester pouches. Similar to total carotenoid content, the percentage reduction in the carotenoid content was also significantly ($p \leq 0.001$) affected by antioxidants, packaging material and storage period.

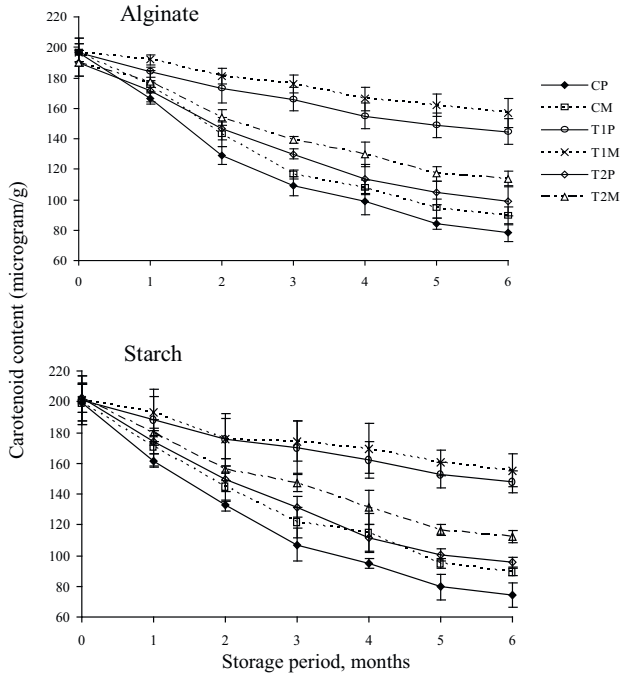

Fig. 1 Carotenoid content during storage of solvent extracted carotenoid in sodium alginate or corn starch as carrier with or without antioxidant, packed in polypropylene (P) or metallised polyester (M) pouches (n = 4). C, T1, T2: See Table 1

At the end of 6 months, the difference in reduction of carotenoid content between polypropylene packed and metallised polyester pouch packed concentrate (with same carrier and antioxidant or control) ranged from 7.4 μg (starch and TBHQ) to 17.0 μg (starch and α-tocopherol). While the differences between reduction of carotenoid content in control and antioxidant containing samples in polypropylene pouches ranged from 18.2 μg (starch and α-tocopherol) to 72.3 μg (starch and TBHQ) and in metallised polyester pouches from 20.0 μg (starch and α-tocopherol) to 62.6 μg (alginate and TBHQ). This indicated that the antioxidants have more influence on prevention of carotenoid degradation than the packaging materials used.

With decrease in carotenoid content, there was an increase in Hunter L value and decrease in a* and b* values of pigmented carriers (Fig. 2). The changes in Hunter L, a*, b* values were significantly ($p \leq 0.001$) affected by antioxidants, carrier used, packaging material and storage period. The significant difference in lightness (L) in 2 carriers was mainly due to the fact that alginate is light brown in colour while starch is white. As the whiteness increases the L value also increases. Thus the pigmented starch is lighter (higher L value) than the pigmented alginate. The correlation coefficients indicate that the reduction in carotenoid content results in reduction of colour intensity of pigmented carrier and thus the reduction in a* value ($r_{alginate} = 0.98$; $r_{starch} = 0.94$) and b* value ($r_{alginate} = 0.94$; $r_{starch} = 0.91$), and increase in lightness ($r_{alginate} = –0.85$; $r_{starch} = –0.92$).

The reduction in absorbance (at 487 nm) of pigmented oil during storage (Fig. 3) indicated the degradation of carotenoids, which was significantly affected by antioxidants ($p \leq 0.001$), packaging material ($p \leq 0.05$) and storage period ($p \leq 0.001$). Highest reduction was observed in pigmented oil without antioxidant stored in transparent bottle and lowest reduction in pigmented oil containing TBHQ and stored in amber coloured bottle. With reduction in absorbance, which is indicative of carotenoid loss, the lightness (L value) increased, redness (a*) and yellowness (b*) decreased (Fig. 4). Hunter L, a*, b* values were significantly ($p \leq 0.001$) affected by the presence of antioxidants, packaging material and period of storage. Correlation coefficients between absorbance and Hunter L (r = –0.95), a* (r = 0.98) and b* (r = 0.98) values are indicative of the positive relationship between absorbance and a*, b* value and negative relationship between absorbance and L value.

The stability of carotenoids has been studied in model systems. Scita (1992) observed that in a model system β-carotene shows faster degradation with effect of light in the presence of oxygen, the degradation rate increasing with increment in oxygen turnover and β-carotene is stabilized with antioxidants, thus concluding that the degradation is by the effect of free radicals. Synergism between carotenoid

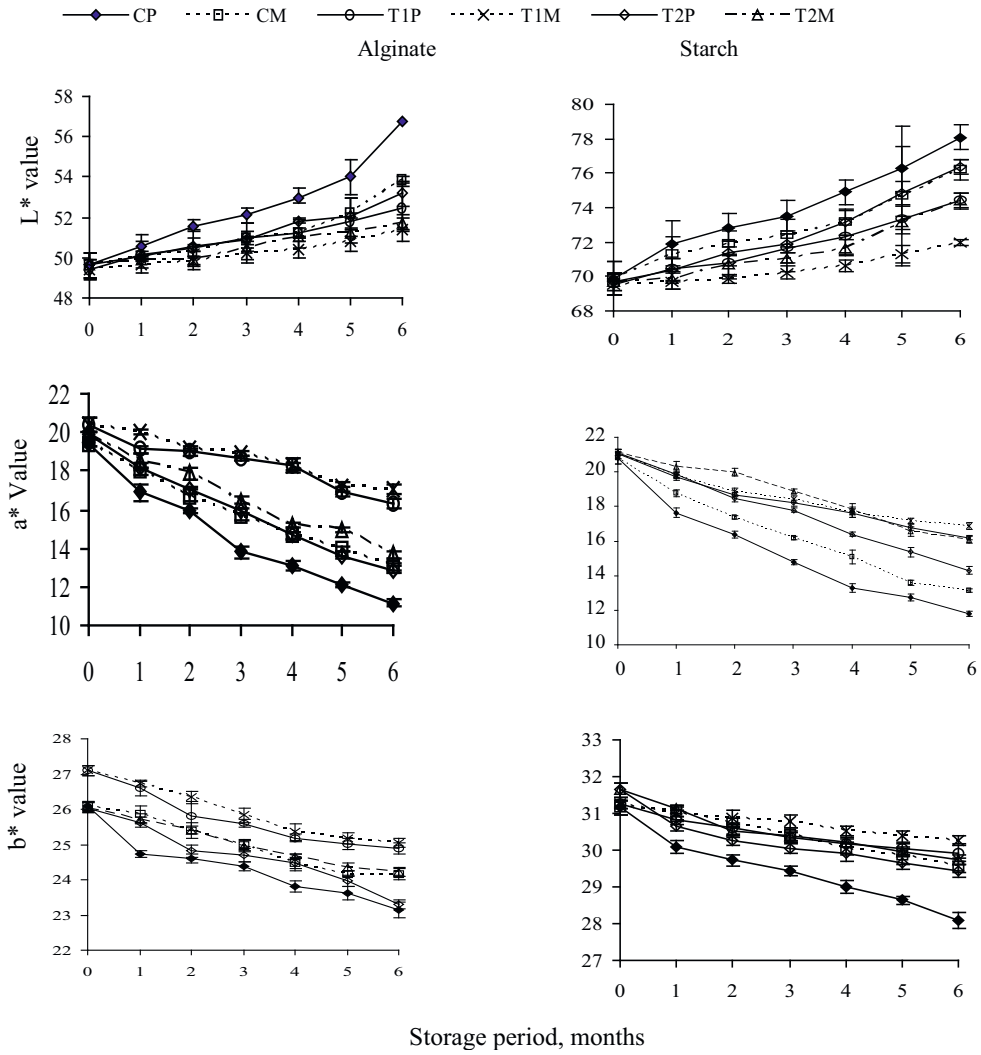

Fig. 2 Hunter L, a*, b* values during storage of solvent extracted carotenoid in sodium alginate or corn starch as carrier with or without antioxidant, packed in polypropylene (P) or metallised polyester (M) pouches (n = 4). C, T1, T2: See Table 1

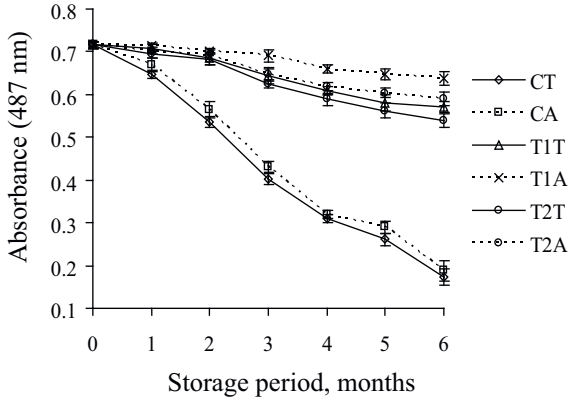

Fig. 3 Absorbance (at 487 nm) of pigmented oil, with or without antioxidants during storage in transparent (T) and amber colored (A) bottles (n=4). C, T1, T2: See Table 1

and antioxidant has also been observed in membrane model system (Haila et al. 1998). The protection of β-carotene from being degraded has been attributed to the recycling of one electron oxidized β-carotene by the antioxidant α-tocopherol (Palozza and Krinsky 1992).

Carotenoids are known to have antioxidant property (Burton 1989). Mortenssen and Skibsted (2000) indicated that carotenoids, like other antioxidants, are degraded by radicals when functioning as antioxidants and the presence of other antioxidants is thus important for the preservation of colour as they scavenge the free radicals before they react with carotenoids. Tocopherol is commonly used antioxidant to prevent oxidative degradation of colour during storage of fish and shellfish (Ingemansson et al. 1993). Li et al. (1998) used sodium erythorbate to prevent astaxanthin degradation in frozen rockfish.

It is stated that oxidation of fat in crawfish waste with formation of peroxides probably would oxidize the associated astaxanthin simultaneously and develop discoloration (Budowski and Bondi 1960). The addition of antioxidant ethoxyquin to crawfish meal stabilized the astaxanthin against

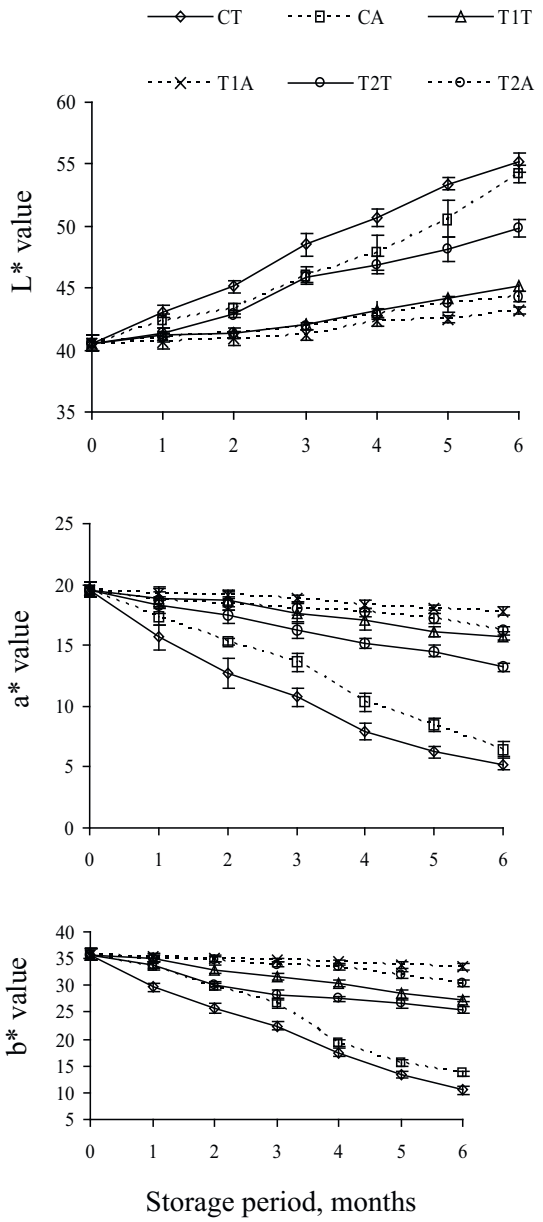

Fig. 4 Hunter L, a*, b* values of pigmented oil, with or without antioxidants during storage in transparent (T) and amber colored (A) bottles (n=4). C, T1, T2: See Table 1

degradation (Chen and Meyers 1982). Chen and Meyers (1982) also observed high pigment retention in pigmented soy oil containing ethoxyquin as antioxidant and storing in opaque bottles for 7 months. Solvent extraction adopted for recovery of carotenoids in the present study also extracts lipids. Thus addition of antioxidants is beneficial to prevent lipid oxidation and subsequent carotenoid degradation. The addition of antioxidants to the oil extracted carotenoids and storing them in amber coloured bottles showed improved stability of carotenoids during storage. TBHQ was found to be better antioxidant than α-tocopherol for stabilization of extracted carotenoids. The relative activity of antioxidants is based on combination of factors like solubility, oxygen partial pressure and reactive species with which it reacts (Di Mascio et al. 1991). The lower rate of carotenoid reduction during storage of pigmented carriers in metallised polyester pouch is due to the fact that the metallised polyester films have good oxygen and light barrier properties.

Carotenoids as colorant in fish sausage: Starch is a common ingredient in the preparation of fish sausage. Hence pigmented starch was used as a colourant in fish sausage. The carotenoid content in the fish meat used for preparation of sausage was 0.34 µg/g (Table 1). Cooking of sausage resulted in a marginal reduction ($p>0.05$) in the carotenoid content from 5.0 to 4.9 µg/g and 9.8 to 9.5 µg/g in sausages added with 5 and 10 ppm carotenoid, respectively. There was highly significant difference ($p\leq0.001$) in carotenoid content between 3 formulations of sausage mix and cooked sausage. Hunter L values decreased, a* and b* values increased with increase in carotenoid content (Table 1) and showed a significant difference ($p\leq0.001$) between 3 formulations. However, a* values were not significantly different between sausage mix and cooked sausage of same formulation.

The sensory analysis of cooked sausage (Fig. 5) indicated that, the colour, flavour and overall acceptability scores for sausage with added carotenoid were higher than that without added carotenoid. A significant difference was observed in colour ($p\leq0.001$), flavour ($p\leq0.05$) and overall acceptability ($p\leq0.05$) scores between sausages of 3 formulations. However, there was no significant difference ($p\geq0.05$) in flavour and overall acceptability scores for sausages containing two different levels of added carotenoids.

Colour is one of the important quality criteria, which determines the acceptability and marketability of many fish mince products. Hideo (1988) used immersion in onion skin

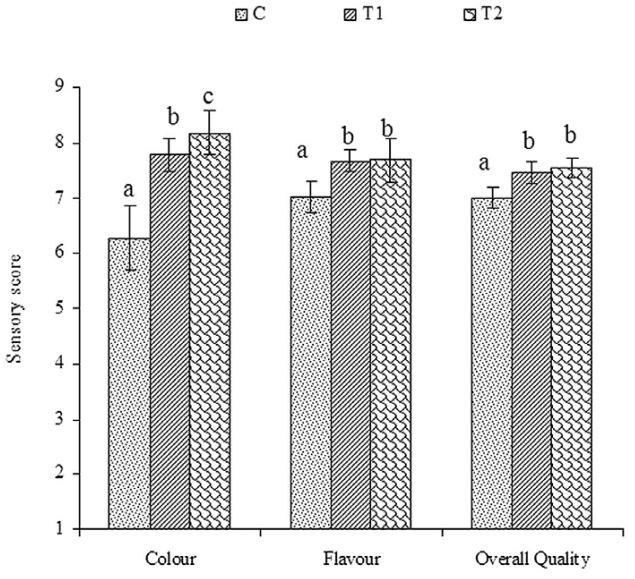

Fig. 5 Sensory scores of fish sausage prepared with or without added carotenoid (n=4) Bars with different letters differ significantly ($p<0.05$)

Table 1 Carotenoid content and Hunter colour values of fish meat, sausage mix and cooked fish sausage

Sample	Carotenoid, µg/g	Hunter colour values		
		L	a*	b*
Fish meat	0.34 ± 0.06[a]	51.1 ± 1.21[a]	–1.2 ± 0.31[a]	8.5 ± 0.42[a]
Sausage mix				
C	0.41 ± 0.11[a]	68.4 ± 0.55[b]	–0.72 ± 0.20[ab]	12.9 ± 0.50[b]
T1	5.0 ± 0.03[b]	65.1 ± 1.40[c]	5.3 ± 0.31[c]	20.6 ± 0.40[c]
T2	9.8 ± 0.13[c]	61.8 ± 0.46[de]	8.9 ± 0.42[d]	24.1 ± 0.26[d]
Cooked sausage				
C	0.36 ± 0.08[a]	66.3 ± 1.63[c]	–0.17 ± 0.33[b]	16.3 ± 0.84[e]
T1	4.9 ± 0.07[b]	62.9 ± 1.65[e]	5.5 ± 0.90[c]	21.0 ± 0.87[c]
T2	9.5 ± 0.19[c]	60.1 ± 1.67[d]	9.2 ± 0.99[d]	23.7 ± 0.70[d]

pigment extract as a technique to colour the surface of fish paste product and reported that it is difficult to achieve uniform colouration with this technique. Takahito (1993) used hydrolyzed pigments from tissue cultured cells of common madder to colour fish paste products, but suggested the use of alum, organic acids and carbonates for stabilization of colour during processing. Osterlie et al. (2001) evaluated the use of synthetic astaxanthin as colouring agent in fish pastes and reported that synthetic astaxanthin may be added during processing of pastes without negatively affecting the product flavour.

The present study indicated that the carotenoids extracted from shrimp waste could be effectively used as colouring agent in fish sausage. The advantage of the extracted carotenoids is that, it not only enhances the colour, but also improves the flavour of the product. Further, the study revealed that the carotenoids added to the sausage preparation are stable during processing and do not require any stabilizers. Synthetic colouring agents are not advised for use in food products due to safety aspects. Thus, the shrimp waste carotenoids would be a beneficial alternative to synthetic colourants in fish products.

Conclusion

As carotenoids degrade on exposure to light and oxygen, they need to be protected against oxidation during storage. Solvent extracted carotenoids can be stored by mixing the extract with carriers such as sodium alginate or corn starch. As starch or alginate is used as one of the main ingredient in minced fish products, the pigmented carrier can be used directly as a source of colouring agent in these products. Addition of antioxidants such as TBHQ or α-tocopherol and storing them in metallised polyester pouches reduced the carotenoid degradation during storage. The oil-extracted carotenoid can be protected from degradation by addition of antioxidants and storage in amber coloured bottles. The carotenoid containing starch can be used as a colouring agent in fish mince products such as sausages, which not only improves the colour, but also the flavour of the product.

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