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Microencapsulation of lobster carotenoids within poly(vinyl alcohol) and poly(D,L-lactic acid) membranes

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The use of natural pigments such as lobster carotenoids in fish feed formulations offers advantages over the use of the synthetic alternatives. Microencapsulation of the pigments, with or without the addition of antioxidants to the formulation, may be of benefit in terms of stabilizing pigment colour. In the present study, lobster carotenoids were extracted from lobster shell into petroleum ether and microencapsulated by phase separation and salt coacervation within poly(vinyl alcohol) and poly(vinyl alocohol)/poly(D,L-lactic acid) membranes. Spherical microcapsules, with smooth, thin and resilient membranes were obtained with mean diameters ranging from 50 to 150 μm, depending on the membrane material, and source of pigment. The microcapsules were pink-orange in colour, and colour stability was followed spectrophotometrically. Enhanced stability was observed in both membrane materials, in comparison to the non-encapsulated control. Rates of discoloration were determined under a variety of storage conditions, including the absence of light, reduced temperatures and under nitrogen atmosphere. The best stability of lobster carotenoids was observed under a nitrogen atmosphere within PVA/PLA membranes, representing an 11-fold enhancement of pigment stability in comparison to the controls. Under ambient conditions, the enhancement in pigment stability was approximately 6-fold. The optimum concentration of PVA during microencapsulation was 3–4%, and the microencapsulated pigments appeared most stable under acidic conditions. The rate of discoloration appeared independent of pigment concentration.

Keywords: Microencapsulation, lobster carotenoids, poly(vinyl alcohol), poly(D,L-lactic acid).

Introduction

Carotenoids are a group of aliphatic or aliphatic cyclic fat-soluble compounds widely distributed in nature (Emodi 1978). They are responsible for the brilliant red, orange and yellow colours of many fruits and vegetables, shrimp, salmon and lobsters. Synthetic carotenoids, such as β-carotene, β-apo-8 carotenal (apocarotenal) and canthaxanthin have been manufactured for a variety of applications. Commercial utilization requires that the pigments be stabilized in a convenient form, and soluble in water or fat (Dziezak 1987). Pure carotenoids are sensitive to light and air

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oxidation, resulting in colourless oxidation products unless stored under vacuum or inert gas. Insolubility of crystalline carotenoids in water and oils has been overcome by reduction in crystal size, preparation of emulsions in liquid and beadlet form, and development of colloidal preparations (Gordon and Bauernfeind 1983).

One additional obstacle to the commercial utilization of synthetic carotenoids in food applications has been caused by the adverse health effects of pigments such as canthaxanthin, due to its deposition in the human retina (Food Chemical News 1990). A convenient, stable and competitively priced natural product alternative would have a readily accessible market.

The production of Atlantic salmon and rainbow trout was 150 000 and 10 000 metric tons respectively in 1989 (Hardy *et al.* 1990). Pigmentation of the flesh was achieved by dietary supplementation of astaxanthin or canthaxanthin in the range of 35–75 mg/kg dry diet (Torrissen *et al.* 1990). Replacement of synthetic pigments by natural carotenoids may meet this potential new market. Carotenoids can be extracted from the millions of kg of lobster shell processed each year in Eastern Canada for fertilizer applications.

The introduction of crustacean processing waste directly into feed for farmed salmon, trout and lobster has certain disadvantages. The waste has a low and often variable pigment content, is prone to rapid degradation, is bulky and has a high cost associated with its handling and transportation. In addition, it has a high chitin and calcium content (Ramaswamy *et al.* 1991). Solvent extracted pigment results in high yields, providing a more concentrated form of the pigment, free of chitin and ash. Ramaswamy *et al.* (1991) described the trypsin/EDTA-aided extraction of carotenoproteins from lobster waste. The protein complex was shown to be stable to oxidation (Spinelli *et al.* 1974) and photo-oxidation was shown to be stable to oxidation (Spinelli *et al* 1974) and photo-oxidation (Cheesman *et al.* 1967).

Extraction and isolation of crustacean pigments may involve oil extraction (Chen and Meyers 1982) and acid ensilage followed by extraction into soy oil (Chen and Meyers 1983, Omara-Alwala *et al.* 1985). Carotenoproteins have been recovered from shrimp using proteolytic enzymes (Simpsons and Haard 1985) and snow crab wastes (Manu-Tawaiah and Haard 1987).

The stability of β-carotenes has been shown to be related to the water activity of the food product, and may be stabilized by the use of anti-oxidants (butylated hydroxyanisole, propyl gallate), fatty acid methyl esters (methyl stearate and oleate), salt, and the presence of vegetables oils (Arya *et al.* 1979). The loss of carotenoids during foods processing and storage was reported to be of commercial significance, resulting in loss of colour, nutritional value and flavour. Meyers and Bligh (1981) reported 45% decrease in crawfish pigment over a 3-week period. The presence of anti-oxidants and storage under nitrogen or reduced temperature reduced the rate of discoloration.

Researchers at Hoffman-LaRoche reported on an encapsulation technique for synthetic β-carotene, based on the formation of a gelatin-sugar beadlet, containing an emulsified solution of the pigment. While the technique of encapsulation was not clearly described, Bunnell *et al.* (1958) reported stable colour in the beadlet form (2·4% pigment). The beadlets were spherical (30–80 mesh) and hard, with an irregular surface. They contained a finely dispersed oil emulsion containing soluble pigment. The beads dispersed readily in water suspension, and were used as a colorant in beverages, butter and other food products. While colour stability was affected by relative humidity, oxygen, light and temperature, excellent stability was

reported due to encapsulation. The researchers co-encapsulated an anti-oxidant together with the pigment, so it is unclear from the study whether the enhanced stability was due entirely to the encapsulation technique.

The molecular weight of yellow lobster shell pigment was reported as 4400, while that of the astaxanthine-protein pigment β-crustacyanin was 21 800 (Buchwald and Jencks 1968). The higher molecular weight of the carotenoid-protein complex, coupled with the enhanced stability to oxidative degradation, would make the encapsulation of this higher molecular weight complex more attractive. In the present study, lobster carotenoids were extracted from lobster shell by solvent extraction. Microencapsulation of the extracted pigment within poly(vinyl alcohol) and poly(D,L-lactic acid) membranes was examined to stabilize the pigment in a convenient form for storage and formulation. The stability of the pigment in the encapsulated form was of particular interest.

Materials and methods

Reagents

Poly(D,L-lactic acid) (50 000 MW) was purchased from Polysciences, and Poly (vinyl alcohol) was purchased from Anachemia (78 000 MW), from Polysciences (25 000 or 133 000 MW), or from Sigma (70 000 to 100 000 MW). Chloroform, acetone and petroleum ether were of reagent grade (ASC Amer. Chem. Ltd), and β-carotene obtained from Fluka Biochemika. Sodium sulphate and sodium chloride were of reagent grade, and were obtained from Fisher.

Extraction of carotenoids

Lobster shells were frozen in sealed plastic bags and stored at −20°C. Frozen shells (500 g) were ground and extracted with acetone overnight at 4°C in the dark. The extraction mixture was filtered through glass wool and water removed with sodium sulphate. Pigment was extracted three times into petroleum ether (500, 200 and 200 mL), the phases separated in a separatory funnel and the pigment concentrated in a rotary evaporator. The pigment concentration was determined by absorbance at 472 mnm using E = 2200 (Saito and Regier 1971).

Preliminary extraction studies indicated that the choice of solvent did not influence the HPLC profile of the pigments and extraction of the pigments with acetone, isopropane or methanol showed no differences in the UV spectra or the HPLC profile in the extracts. Identification of the astaxanthin in the samples was done by spectrum comparison using the photodiode array detector. Spectrum comparison of an astaxanthin standard (Hoffman La Roche) and the extracted samples was 0·9999.

Microencapsulation of pigments

Poly(vinyl alcohol) (PVA) microcapsules were formed by dispersing 10 mL chloroform containing 5 mg β-carotene or 1–2 mL lobster carotenoids into 100 mL cold (0°C) 2% PVA aqueous solution. After 10 min of vigorous stirring (350 rpm), the dispersion was transferred into 200 ml cold 4% NaCl solution, and stirring continued for 5 min. Microcapsules were filtered, and washed with 4% NaCl solution.

Poly(vinyl alcohol)/Poly(D,L-lactic acid) (PVA/PLA) microcapsules were prepared by dissolving 5 mg β-carotene or 1–2 mL lobster carotenoids together with

50 mg PLA in 10 mL chloroform, and dispersing the solution for 10 min into 100 mL of cold (0°C), PVA aqueous solution (2%) with vigorous stirring (350 rpm). The emulsion was then transferred into 200 ml 4% NaCl solution for 5–10 min, the microcapsules were filtered and rinsed with 4% NaCl solution.

Presence of an encapsulating membrane was demonstrated by rupture of the microcapsule membranes using micromanipulators under the light microscope.

Pigment stability

Colour stability of β-carotene or lobster carotenoids was determined by absorbance at 497 and 472 respectively. Microencapsulated pigment was stored in aqueous suspension in sealed bottles, and tested for stability under the conditions described. Colour was determined by extracting 1 mL microcapsule suspension into 9 mL chloroform and measuring absorbance. Since carotenoids are insoluble in water, it was assumed that extraction into excess chloroform yielded essentially full recovery. Colour half-life ($t_{0.5}$), a measure of pigment stability, was calculated from the slope of the initial linear portion of the colour decay plot.

Size distribution

Volumetric (volume of microcapsules in each diameter class) and cumulative size distributions were determined by laser light scattering, with a Malvern Instruments 2602-LC particle analyser (Poncelet De Smet *et al.*, 1989, 1990). The mean diameter (d_{50}) and the arithmetic standard deviation ($\sigma_a = 0{\cdot}5[d_{84} - d_{16}]$) were calculated from the cumulative distribution curve.

Results and discussion

Lobster carotenoids demonstrated maximum absorbance at 470 nm in petroleum ether and 472 nm in chloroform. The yields from the solvent extraction ranged from 35·4 to 56·5 mg/kg of lobster shell. β-carotene showed maximum absorbance at 497 nm in chloroform. Subsequent experiments involved measuring pigment concentrations in chloroform at the respective wavelengths.

A typical size distribution of PVA microcapsules containing β-carotene is presented in figure 1. Distributions were symmetrical about mean diameters of 46 ± 16 and $132 \pm 54\,\mu$m for β-carotene and lobster carotenoids respectively within PVA membranes. Both PVA and PVA/PLA microcapsules were spherical with smooth, thin and resilient membranes. Previously, it has been shown that diameters and size distributions may be controlled by the formulation conditions, involving variations in mixing intensity, choice of reactor and mixer geometry, control of solution viscosity and through the addition of suitable emulsifiers (Poncelet De Smet *et al.* 1989, 1990).

The colour stability of β-carotene encapsulated within PVA/PLA and PVA membranes is illustrated in figure 2. Enhanced stability of the microencapsulated pigment was observed in comparison to the control, with PVA/PLA providing the better protection from de-coloration. A decrease in pigment colour was observed to between 10 and 15 days, after which the OD appeared to asymptotically approach a minimum value.

The initial linear decay in colour intensity observed in figure 2, was calculated as a slope, proportional to the rate of pigment discoloration. The pigment half-life ($t_{0.5}$), determined from the initial slope of the colour decay plot provides a quantitative measure of pigment stability as a basis for comparison from case to case.

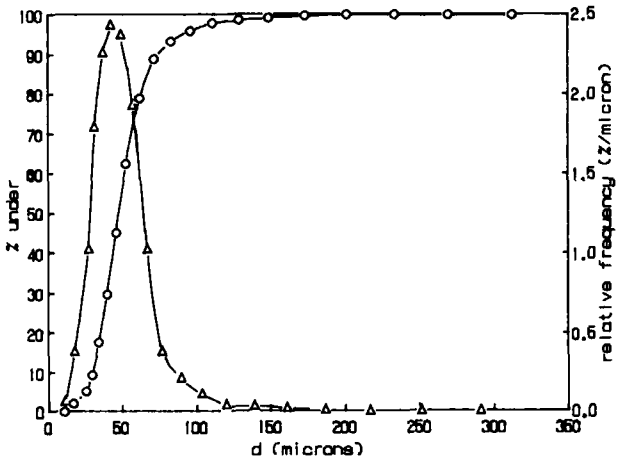

Figure 1. Volumetric (△) and cumulative (○) size distributions of β-carotene microencapsulated within PVA membranes. $d_{50} = 46 \pm 16\,\mu m$.

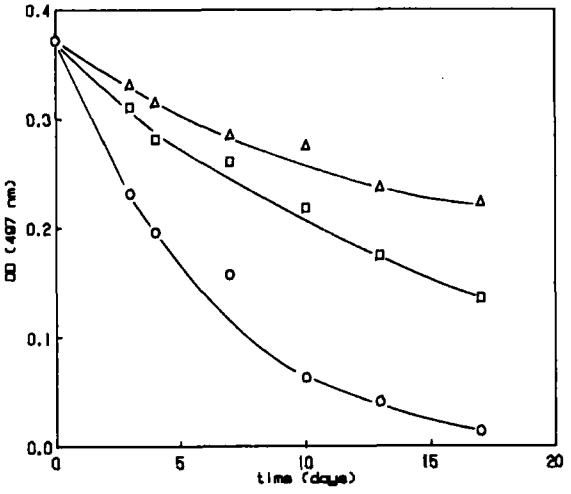

Figure 2. Colour stability (OD_{487}) of β-carotene encapsulated with PVA/PLA (△) and PVA (□) membranes. Control (○) consisted of non-encapsulated pigment.

A comparison of $t_{0.5}$ values under a variety of storage conditions, within the two membrane materials considered is provided in table 1. The relative stability value represents the stability of the microencapsulated pigment relative to the non-encapsulated control.

Table 1 demonstrates that the stability of the carotenoid pigments was enhanced overall upon microencapsulation in comparison to the controls. Enhancements in stability ranged to a factor of 11-fold for lobster carotenoid microcapsules stored under nitrogen, corresponding to a half-life of 18 days. In general, it appears that the commercial β-carotene was more stable than the lobster carotenoids, but that the enhancement in pigment stability upon microencapsulation was more significant with the lobster carotenoids. Marginal benefits were achieved by storage of the microencapsulated pigments in the absence of light, and under reduced temperature.

Table 1. Stability of microencapsulated pigments under various storage conditions. The half-life ($t_{0.5}$: days) of the pigment was determined quantitatively from the initial linear slope of the colour decay plot. The relative stability values in brackets, are the half-life values relative to that of the non-encapsulated pigment (control).

Pigment	Membrane	Non-encapsulated pigment (control)	Encapsulated pigment	Storage in dark	Storage in dark @ 4°C	Storage under nitrogen
lobster carotenoids	PVA	1·7	1·7 (1·0)	6·7 (3·9)	6·7 (3·9)	15·4 (9·1)
	PVA/PLA	1·7	9·5 (5·6)	8·3 (4·9)	—	18·2 (10·7)
β-carotene	PVA	4·2	7·7 (1·8)	8·3 (2·0)	18·2 (4·3)	2·6 (0·6)
	PVA/PLA	4·2	5·9 (1·4)	6·5 (1·5)	6·1 (1·5)	2·1 (0·5)

Microencapsulated β-carotenoids stored under nitrogen were actually less stable than the controls. Overall, PVA/PLA membrane provided a better protection for the lobster carotenoids, and the PVA membrane provided slightly better protection from discoloration for the β-carotene, in comparison to the alternate membrane material. Lobster carotenoid microencapsulated within the PVA/PLA membrane showed a 5·6-fold enhancement in stability ($t_{0.5}$ of 9·5 days) while stored under ambient conditions.

Figure 3 illustrates the effect of PVA concentration during membrane formation

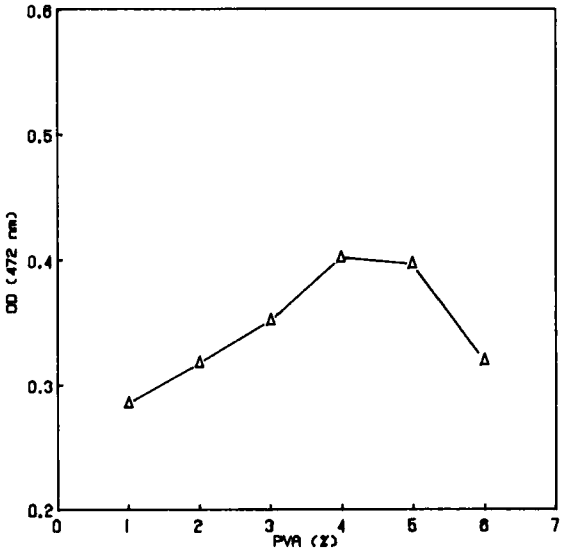

Figure 3. The effect of PVA concentration during membrane formation on the stability (OD_{472}) of microencapsulated lobster carotenoids after 5 days. The initial OD in all cases was 0·692, and the microcapsules were stored under ambient conditions.

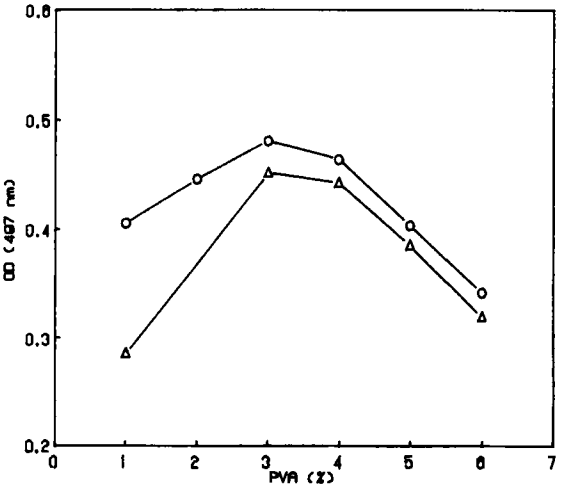

Figure 4. The effect of PVA concentration during membrane formation on the stability (OD_{497}) of microencapsulated β-carotene after 1 (○) and 5 (△) days storage under ambient conditions. The initial OD in all cases was 0·502.

on the stability (OD_{472}) of microencapsulated lobster carotenoids measured after 5 days of storage. An optimum concentration of 4–5% PVA appeared to provide for the best enhancement in stability. Higher or lower concentrations of PVA, resulted in an increasing rate of discoloration. The effect of PVA concentration during membrane formation on the stability (OD_{497}) of microencapsulated β-carotene is illustrated in figure 4. The optimum concentration ranged from 3 to 4% PVA, slightly less than that necessary to stabilize the lobster carotenoids. A lower concentration of PVA (1%) resulted in short-term colour stability, however a considerable reduction in colour was observed after 5 days of storage. Pure carotenoids are light

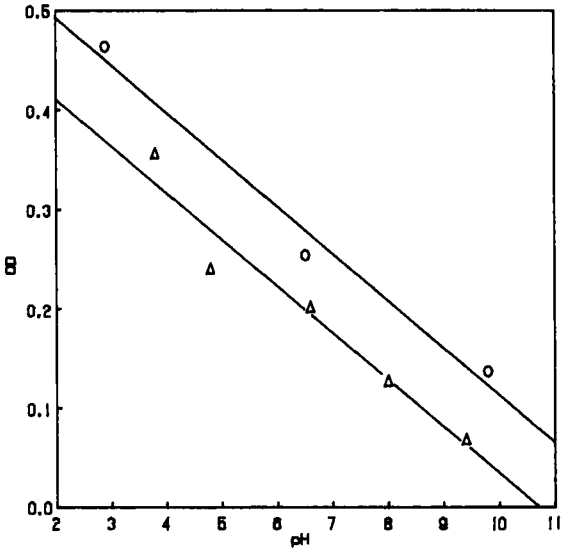

Figure 5. The effect of pH on the stability (OD) of β-carotene (○) and lobster carotenoids (△) after 12 (lobster) or 15 (β-carotene) days storage.

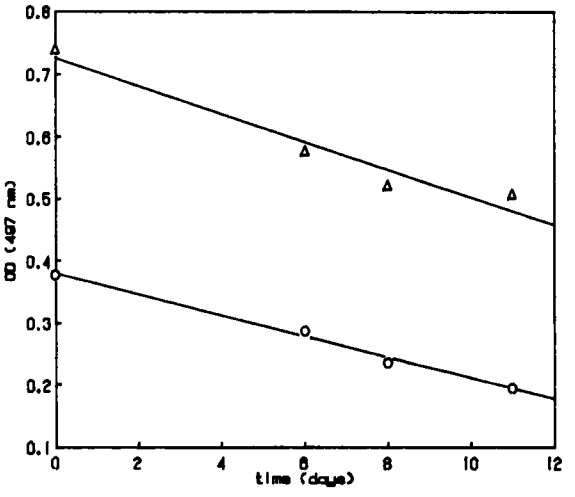

Figure 6. The stability (OD_{497}) of β-carotene with time at different initial concentrations of pigment. (○): 0·26 mg/ml; (△) 0·52 mg/ml.

and oxygen labile and are thus stabilized under vacuum or inert gas to prevent rapid degradation. With encapsulation, the physical structure of the membrane appears to be the most effective barrier to light and oxygen concentrations at 3 to 5% PVA.

The effect of pH on the colour stability of microencapsulated β-carotene and lobster carotenoids after 12–15 days of storage is illustrated in figure 5. Both pigments appeared to be more stable under acidic conditions, with a linear decrease in colour with increasing pH. Pigments appeared most stable under highly acidic conditions.

The rate of discoloration of microencapsulated β-carotene appeared to be independent of the initial pigment concentration within the microcapsules as seen by similar slopes in figure 6. Similar results were observed with the lobster carotenoids.

It appears that in general, microencapsulation does enhance pigment stability to varying degrees depending upon the storage conditions. Microscopically, the microcapsules had a pink orange colour that was salmon-like in appearance.

Pigments are generally oxygen labile, particularly in the presence of light. The presence of an encapsulating membrane barrier may provide a degree of protection from oxidation. Enhanced stability within PVA/PLA membranes may be due to some lactic acid contained within the membrane providing a degree of acidification. As noted, pigments were more stable under acidic conditions.

The application of natural pigments in food formulations for animals and humans will depend largely upon the ability to stabilize the pigment from oxidative damage and from light. Microencapsulation is one method that has proven beneficial in the past, and appears to offer promise for stabilizing lobster carotenoids and other pigments for extended periods.

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References

ARYA, S. S., NATESAN, V., PARIHAR, D. B., and VIJAGARAGHAVAN, P. K., 1979, Stability of carotenoid product in dehydrated carrots. *Journal of Food Technology*, **14**, 579–586.

BUCHWALD, M., and JENCKS, W. P., 1968, Properties of the crustacyanins and the yellow lobster shell pigment. *Biochemistry*, **7**, 844–859.

BUNNELL, R. H., DRISCOLL, W., and BAUERNFEIND, J. C., 1958, Coloring water-based foods with β-carotene. *Food Technology*, **12**, 536–541.

CHEESMAN, D. F., LEE, W. L., and ZAGALSKY, P. F., 1967, Carotenoprotein in invertebrates. *Biological Reviews*, **42**, 13–16.

CHEN, H. M., and MEYERS, S. P., 1982, Extraction of astaxanthin pigment from crayfish waste using a soy oil process. *Journal of Food Science*, **47**, 892–896.

CHEN, H. M., and MEYERS, S. P., 1983, Ensilage treatment of crayfish waste for improvement of astaxanthin pigment extraction. *Journal of Food Science*, **48**, 1516–1520.

DZIEZAK, J. D., 1987, Applications of food colorants. *Food Technology*, **41** (4), 78–88.

EMODI, A., 1978, Carotenoids. Properties and applications. *Food Technology*, **32** (5), 38–42.

FOOD CHEMICAL NEWS (1990) Canthaxanthin ADI removed by FAO/WHO because of retina effects. Vol. 32, Number 14, June 14.

GORDON, H. T., and BAUERNFEIND, J. C., 1983, Carotenoids as food colorants. In *CRC Critical Reviews in Food Science and Nutrition* Vol. 18, No. 1, edited by T. E. Furia. (CRC Press, Baton Rouge, Fla.), pp. 59.

HARDY, R. W., TORRISSEN, O. J., and SCOTT, T. M., 1990, Absorption and distribution of ^{14}C-labelled canthaxanthin in rainbow trout. *Aquaculture*, **87,** 331–340.

MANU-TAWIAH, W., and HAARD, N. F., 1987, Recovery of carotenoprotein from the exoskeleton of snow crab *Chionocetes opilio*. *Canadian Institute of Food Science and Technology Journal*, **20,** 31–33.

MEYERS, S. P., and BLIGH, D., 1981, Characterization of astaxanthin pigments from heat-processed crawfish waste. *Journal of Agricultural and Food Chemistry*, **29,** 505–508.

OMARA-ALWALA, T. R., CHEN, H. M., ITO, Y., SIMPSON, K. L., and MEYERS, S. P., 1985, Carotenoid pigment and fatty acid analyses of crayfish oil extracts. *Journal of Agricultural and Food Chemistry*, **33,** 260–263.

PONCELET, DE SMET, B., PONCELET, D., and NEUFELD, R. J., 1989, Control of mean diameter and size distribution during formulation of microcapsules with cellulose nitrate membranes. *Enzyme and Microbial Technology*, **11,** 29–37.

PONCELET, DE SMET, B., PONCELET, D., and NEUFELD, R. J., 1990, Preparation of hemolysate-filled hexamethylene sebacamide microscapsules with controlled diameter. *Canadian Journal of Chemical Engineering*, **68,** 443–448.

RAMASWAMY, H. S., SIMPSON, B. K., TU, Y., and YAYLAYAN, V., 1991, Tray-drying of carotenoproteins recovered from lobster waste. *Journal of Food Processing and Preservation*, **15,** 273–284.

SAITO, A., and REIGER, L. W., 1971, Pigmentation of brook trout (*Salvelinus fontinalis*) by feeding dried crustacean waste. *Journal of the Fisheries Research Board of Canada*, **4,** 509–512.

SIMPSON, B. K., and HAARD, N. F., 1985, The use of proteolytic enzymes to extract carotenoproteins from shrimp wastes. *Journal of Applied Biochemistry*, **7,** 212–222.

SPINELLI, J., LEHMAN, L., and WIEG, D., 1974, Composition, processing, and utilization of red crab as an aquacultural feed ingredient. *Journal of the Fisheries Research Board of Canada*, **31,** 1025–1029.

TORRISSEN, O. J., HARDY, R. W., SHEARER, K. D., SCOTT, T. M., and STONE, F. E., 1990, Effects of dietary canthaxanthin level and lipid level of apparent digestibility coefficients for canthaxanthin in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **88,** 351–362.