Effect of Solvent Polarity and Fractionation Temperature on the Physicochemical Properties of Squid Viscera Stearin

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Complete utilization of squid viscera oil was found to be feasible by solvent fractionation. The effects of solvent polarity and temperature on yield, soft melting point, fatty acid composition and solid fat content of the stearin were studied. Although yield increased with increasing solvent polarity and with decreasing fractionation temperature, the stearin obtained has a lower soft melting point. This makes it unsuitable as a table margarine. Operation at lower temperature also increased the operating cost. Solid fat content of the stearins fractionated at −1°C was 10% higher than of those at −5 or −9°C for all solvent polarities studied. Solid fat content of stearin increased with the decrease of solvent polarity at every tested temperature. The combined effects of polarity and fractionation tempeature affect the soft melting point and solid fat content, which determine the commercial application of the stearin. The percentage of saturated fatty acids of stearin decreased with increasing solvent polarity, and the percentage of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) increased with solvent polarity and fractionation temperature. The percentage of EPA + DHA in stearin decreased with temperature, except for those from 5.1 p′ solvent. The practical fractionation condition is at −1 or −5°C with a solvent polarity of 5.1 p′. The stearin fraction can be made into a series of products, such as high EPA- and DHA-containing table margarine.

KEY WORDS: Fatty acid composition, fractionation stearin, fractionation temperature, soft melting point, solid fat content, solvent polarity, squid viscera.

Squid *(Illex argentinus)* production in Taiwan's fishing industry grew from 1.3×10^4 tons in 1980 to become Taiwan's major fish industry in 1990 with a harvest of 1.8×10^5 tons (1). There have been several studies on utilization of the viscera, previously a waste product. Lai *et al.* (2) reported on extracton of oil from viscera; Lai *et al.* (3,4) and Wang and Chai (5) reported on a refining process for this oil; and Lai *et al.* (4) studied dietary effects of squid visceral oil on 30 rats. Current applications of this oil, however, remain limited. Most of the product is employed as a supplemental oil in aquaculture feed (6,7) or in capsules for human consumption.

The greatest commercial potential for the crude oil from squid viscera seems to lie in its high content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These make up about 30% of the total fatty acid composition (2). EPA has been shown to have a promising effect on the prevention of thrombosis and atherosclerosis (8). Since these results were published, efforts to increase EPA and DHA in fish oils have increased. These ω-3 fatty acids have been reported to be effective in lowering the very low density lipoproteins (VLDL) in blood, thereby lowering serum cholesterol and having a probable effect in reducing the occurrence of heart disease (9,10,11). Lawson and Hughes (12) reported that EPA and DHA in triglyceride form may be absorbed by the human body three times faster than in the ethylated form.

Fractionation of squid visceral oil results in a liquid fraction (olein) and a solid fraction (stearin). Current interest in utilization of squid viscera oil is focused on increasing the EPA and DHA content in the olein fraction. The solid stearin fraction then becomes a low-value by-product. The enrichment process is mostly done by fractionating olein from whole viscera oil. Studies have been conducted to increase the percentage of EPA and DHA, thereby increasing the value of squid viscera oil products. When Lee and Wang (13) fractionated squid viscera oil with acetone or *n*-hexane (ratio 1:1-3) at −20°C, the percentage of EPA and DHA in the olein fraction increased to about 40%. Perng (14) obtained 69% methylated EPA and DHA through short-path distillation. After hydrolyzation and ethylation, Liang *et al.* (15) obtained about 75% ethylated EPA and DHA with supercritical CO_2 extraction. However, the products obtained were not absorbed as well as in the triglyceride form (12).

We propose a different approach to enrich the EPA and DHA in the final product. The enrichment starts from olein, which is generally considered to be richer in EPA and DHA than the whole viscera oil. Whereas the by-product stearin is usually used for lower-priced industrial applications, it can be turned into a high-value, cost-effective edible product high in both EPA and DHA.

We suggest a stearin fractionation scheme (Fig. 1) that has not been previously applied. Crude viscera oil is first fractionated into stearin and olein instead of using whole crude visceral oil for enrichment; then, the enrichment of EPA and DHA can start from olein obtained either from dry fractionation of the crude viscera oil or from the by-product of solvent fractionation of refined oil. The major product, stearin, can be used to produce EPA + DHA-containing plastic fats, to fulfill the complete utilization of viscera oil.

The objectives of this research were to study the feasibility of the complete utilization of squid visceral oil and to study the effect of solvent polarity and fractionation temperature on the physicochemical properties of the stearin.

MATERIALS AND METHODS

Refined oil. A crude oil was prepared by autolysis of squid viscera. The crude oil was held at 4°C to separate the stearin and olein through dry fractionation. The stearin obtained in this way was designated as unrefined oil. The unrefined oil was neutralized with caustic soda to an acid value of less than 0.1. The oil was decolorized by mixing

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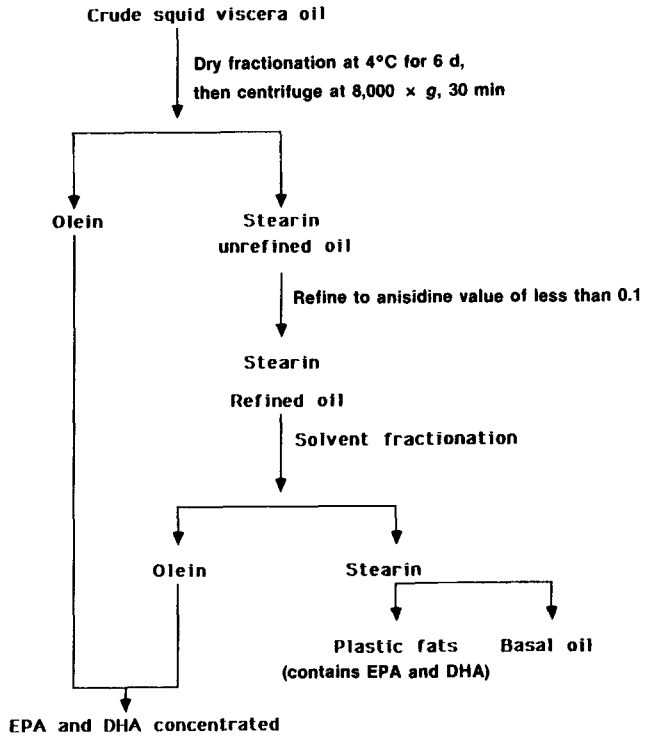

FIG. 1. A scheme for the complete utilization of squid visceral oil.

neutral active white clay (Wako, Osaka, Japan)/silica gel in a 1:1 ratio with oil and *n*-hexane at 0.3:1.0:1.0 for 2 h. The mixture was then centrifuged at 8000 × *g* for 30 min; afterward the precipitates were discarded. *n*-Hexane was recovered by refluxing the clarified supernatant under vacuum. The obtained oil after removal of *n*-hexane was designated as the refined visceral oil.

Solvent fractionation. Twelve fractionation conditions were tested with four solvent mixtures prepared from mixtures of pure acetone and *n*-hexane. Polarity ranged from 5.1 to 0.1 p′. The mixtures were: pure acetone (5.1 p′); acetone and *n*-hexane in a 7:3 ratio (3.6 p′); acetone and *n*-hexane in a 3:7 ratio (1.6 p′); and pure *n*-hexane (0.1 p′). These solvents were mixed with an equal amount of refined oil. After standing for 36 h at −1, −5 or −9°C, samples were then centrifuged at these temperatures at 15000 × *g* for 30 min. The supernatant was used as a feedstock for EPA and DHA enrichment (Fig. 1). The precipiates were washed several times with solvent of the same temperature. Solvents were recovered by reflux under vacuum at 40–50°C, and the residue was the fractionated stearin.

The properties of fractionated stearin. The following properties of each resulting stearin sample were assessed for yield, soft melting point, fatty acid composition and solid fat content. The yield of obtained stearin was calculated as a weight percentage of the refined oil.

For the soft melting point, a modified capillary tube method was used (16). About 10-mm length of melted fat was drawn into a capillary tube (1-mm i.d., 2 mm o.d., 80 mm length). Tubes containing melted fat were placed on an ice block for one hour to ensure solidification. They were then suspended 3 cm above a beaker filled with water. Water temperature in the beaker was increased about 2°C/min to about 10°C below the melting point of the sample. Heating rate was then slowed to about 0.5°C/min, and water in the beaker was stirred to ensure uniform temperature. The melting point was taken when the fat in the capillary rose 2 mm below the tip.

Fatty acids were determined by gas chromatography (17) with a Model 3700 Varian gas chromatograph (Varian, Palo Alto, CA). A glass column, 2 mm in i.d. × 3 m in length was packed with 10%, 80–100 mesh chromosorb. An FID (flame ionization detector) was used. The following conditions were used. Injection and detector temperatures were 230°C. Initial column temperature was held at 160°C for 10 min, then raised quickly to 180°C and held for 62 min. Air and hydrogen gas flow rates were 350 and 30 mL/min, respectively. Flow rate of make-up gas (N_2) was 30 mL/min, and the carrier gas (N_2) was held at 10 psi.

Solid fat content. Pulse NMR (nuclear magnetic resonance; Praxis NMR SFC 900) (Praxis Corp., San Antonio, TX) (18) was used to measure free induction decay/m s at 60.0, 10.0, 21.1, 26.7, 33.3 and 37.8°C. Solid fat content (SFC) was calculated by the following equations.

$$\text{SFC} = \text{percentage of solid fat} = 100\% - \text{oil \%} \qquad [1]$$

where

$$\text{oil \%} = \frac{\text{FID (t°C)}}{\text{FID (60°C)}_{\text{unknown}}} \times \frac{\text{FID (60°C)}}{\text{FID (t°C)}_{\text{standard oil}}} \qquad [2]$$

Yields and soft melting points of fractionated stearins were analyzed by one-way analysis of variance (ANOVA) at $P > 0.05$. Duncan's multiple range test was used to resolve the difference among the means.

RESULTS

Yield of stearin. Solvent polarity, not fractionation temperature, is the prominent factor affecting yield (Table 1). Yield increased with increasing polarity. With a polarity of 5.1 p′, the yield of stearin was about 36%. Yields of stearin obtained between −1 and −5°C at polarities between 0.1–3.6 p′ were not different. However, as the polarity increased to 5.1 p′, the yield increased significantly. For

TABLE 1

Effect of Temperature and Polarity on Yield of Fractionated Stearin from Refined Squid Viscera[a]

Solvent polarity	Fractionation temperature		
	−1°C	−5°C	−9°C
5.1 p′	36.5 ± 2.3%a	36.0 ± 1.5%a	36.6 ± 2.7%a
3.6 p′	26.8 ± 3.6%b,c	26.3 ± 2.9%b,c	29.8 ± 0.7%b
1.6 p′	21.3 ± 2.6%b,c	20.8 ± 2.4%b,c	23.5 ± 2.5%b,c
0.1 p′	19.3 ± %c	20.0 ± 2.5%c	20.9 ± 1.3%c

[a]Oil/solvent mixture, 1:1 (vol/vol); fractionation condition: 36 h at designated temperature after rapid cooling from ambient temperature; values followed by the same letter in each column are not significantly different ($P < 0.05$).

those fractionated at −9°C at a polarity of 3.6 p′, the yield was significantly higher than those from 0.1 p′ solvent. The yield was significantly higher as polarity increased further to 5.1 p′.

Solft melting point. Solvent polarity, not fractionation temperature, is the predominent factor affecting the soft melting point (Table 2). Soft melting points of stearins fractionated at the same polarities and temperatures between −1 and −9°C were nearly equal. The effect of polarity not only occurred between 5.1 p′ and 0.1 p′ at a fractionation temperature of −1 or −5°C, but also occurred between 5.1 p′ and 1.6 p′ at a fractionation temperature of −9°C. There is no significant difference when comparing either one of the two sets of data of 3.6 and 1.6 p′ at fractionation temperatures of −1 or −5°C to the data of either higher or lower polarity. At −9°C, as polarity decreased to 1.6 p′, the soft melting point was significantly higher than at 5.1 p′. These results show that the combined effect of −9°C and solvent polarities was different from that of −1 and −5°C and solvent polarities.

Fatty acid composition. As the solvent polarity increased, the percentage of saturated fatty acids in the resulting fractionated stearin decreased (Table 3), and percentage of EPA + DHA increased. At −1°C and 5.1 p′ polarity, the content of EPA + DHA was 17.4%. The ratio of saturated to unsaturated fatty acids was close to 1 for those samples fractionated with 5.1 p′ or 3.6 p′ solvent mixtures at all three fractionation temperatures. The percentage of EPA + DHA in stearin decreased with decreasing fractionation temperature under all solvent polarities, except 5.1 p′.

Solid fat content. As fractionation temperature was raised above −5°C, the solid fat content increased with temperature under all tested polarities (Figs. 2–5). Solid fat content of stearin fractionated at −1°C was 10% higher than from −5 or −9°C under all solvent polarities. Stearin solid fat content from −5 or −9°C was not significantly different at any polarity employed.

Figures 6–8 show that solid fat content increased with lower polarity at every temperature. The increase was less pronounced at 1.6 p′ and below. Solid fat content of stearin from 0.1 p′ and 1.6 p′ were close. Figure 6 reveals that the solid fat content of stearin fractionated at −1°C was about 49% at 10°C and that the content decreased with increasing testing temperature to about 21% at 37.8°C at 0.1 or 1.6 p′. When polarity increased to above 3.6 p′, solid fat amounted to 36.7% at 10°C and decreased further with increasing temperature. Figures 7 and 8 show the effect of solvent polarity on the solid fat content of stearin fractionated at −5 and −9°C, respectively. The trends are

TABLE 2

Effect of Temperature and Polarity on the Soft Melting Point (°C) of Stearin Fractionated from Refined Squid Visceral Oil[a]

Solvent polarity	Fractionation temperature		
	−1°C	−5°C	−9°C
5.1 p′	37.0 ± 1.5a	36.7 ± 1.2a	37.0 ± 1.0a
3.6 p′	38.4 ± 1.3a,b	38.4 ± 0.9a,b	38.8 ± 1.2a,b
1.6 p′	40.0 ± 1.3a,b	39.9 ± 1.4a,b	40.8 ± 0.9b
0.1 p′	40.7 ± 1.8b	41.0 ± 0.3b	41.0 ± 1.1b

[a]Footnotes as in Table 1.

TABLE 3

Effect of Temperature and Solvent Polarity on the Percentage of Fatty Acid of Stearin Fractionated from Refined Squid Viscera Oil[a]

	C14:0	C15:0	C15:1	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:1	C20:4	C22:0	C22:1	C20:5	C22:4	C22:6	S^b	E + D[c]
Raw material	4.7	0.2	0.6	16.2	5.0	1.7	1.1	1.8	15.8	2.4	2.4	4.7	3.7	1.8	3.7	14.8	1.7	17.7	31.1	32.5
In searin	5.9	0.2	0.7	19.9	4.9	1.7	1.0	2.2	15.5	2.5	2.7	5.0	3.6	1.8	3.7	12.8	1.2	14.7	35.0	27.5
In olein	4.8	0.2	0.6	17.5	4.9	1.5	0.9	1.7	16.7	2.4	2.4	4.8	3.8	1.8	3.5	14.9	0.5	17.1	29.4	32.0
At −9°C																				
5.1 p′	9.3	0.5	1.2	31.1	4.5	1.8	0.9	3.2	13.8	1.6	1.8	5.1	2.9	1.4	2.9	9.0	2.0	7.0	51.6	16.0
3.6 p′	10.5	0.3	1.2	33.1	4.2	1.7	0.7	3.5	12.9	1.4	1.6	4.9	2.7	1.3	5.2	8.1	0.1	6.6	52.8	14.7
1.6 p′	12.5	0.7	1.3	38.4	3.7	1.5	0.9	4.1	10.3	0.9	1.6	4.5	2.2	1.1	5.0	6.2	—	5.1	60.7	11.3
0.1 p′	12.3	0.7	1.3	39.1	3.9	1.5	0.7	3.8	9.9	0.9	1.6	4.4	2.3	1.1	4.4	6.1	1.5	4.5	62.4	10.6
At −5°C																				
5.1 p′	9.7	0.7	1.8	31.6	4.8	1.9	1.0	3.1	13.5	1.3	1.5	5.1	2.8	1.2	4.4	9.1	—	6.5	51.1	15.6
3.6 p′	10.4	0.7	1.5	34.1	4.9	1.9	0.9	3.2	13.4	1.2	1.5	5.1	2.8	—	3.4	8.9	—	6.1	52.3	15.0
1.6 p′	12.1	0.7	1.5	35.2	3.9	1.9	1.0	3.6	12.6	1.2	1.5	5.4	2.9	—	3.6	7.6	—	5.3	56.2	12.9
0.1 p′	12.3	0.8	1.6	36.6	3.3	1.4	1.0	3.8	11.3	1.8	1.8	5.3	4.0	—	3.6	6.5	—	4.9	58.4	11.4
At −1°C																				
5.1 p′	10.0	0.7	1.3	30.1	4.1	1.6	1.1	3.3	11.7	1.8	2.1	4.2	2.5	1.2	4.7	8.0	2.2	9.4	51.3	17.4
3.6 p′	10.2	0.5	1.1	32.2	3.8	1.4	0.9	3.5	12.0	1.4	1.5	4.5	2.5	1.2	5.1	7.9	2.1	8.2	53.3	16.1
1.6 p′	11.5	0.7	1.3	33.1	4.0	1.5	0.8	3.5	11.0	1.3	1.4	4.0	2.5	1.2	4.6	7.4	2.1	8.1	55.9	15.5
0.1 p′	12.9	0.6	1.4	38.2	3.3	1.3	0.8	4.1	9.2	1.1	1.4	3.6	2.0	1.0	4.5	5.8	2.2	6.6	62.7	12.4

[a]Oil/solvent mixture = 1/1 (vol/vol). Fractionation condition: 36 h at designated temperature after rapid cooling from ambient temperature. Footnotes as in Table 1. [b]S, percentage of saturated fatty acid. [c]E + D, percentage of eicosapentaenoic acid + docosahexanoic acid.

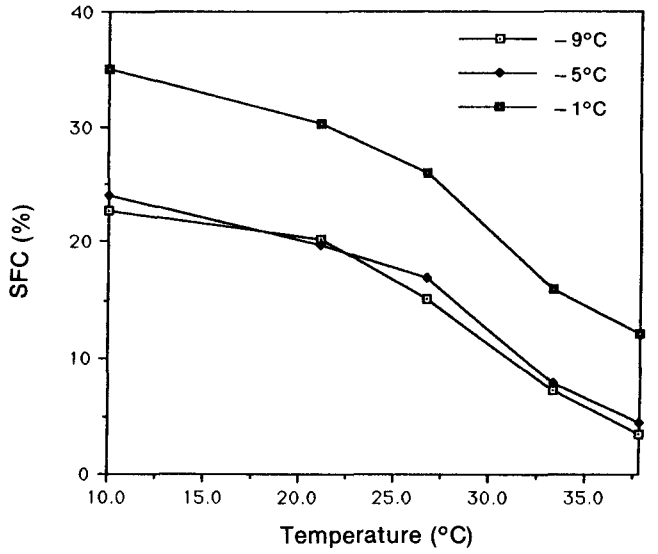

FIG. 2. Effect of fractionation temperature on solid fat content (SFC) of stearin from refined squid visceral oil with solvent fractionation at 5.1 p′. Fractionation conditions: oil/solvent mixture (1:1, vol/vol), rapid cool to designated temperature from ambient, set for 36 h.

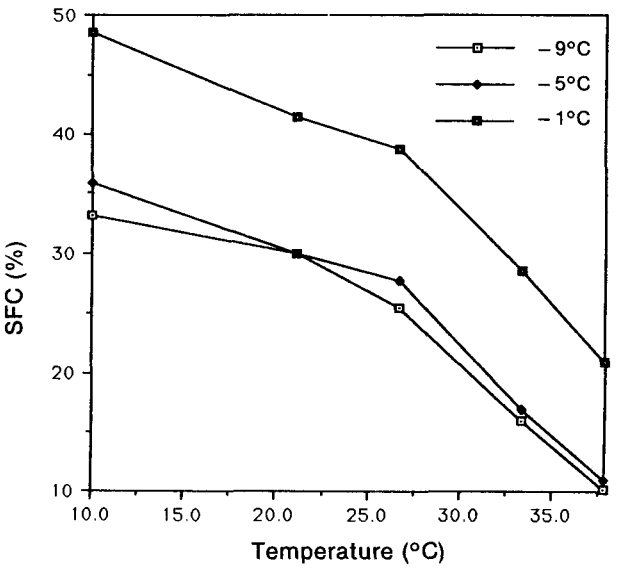

FIG. 4. Effect of fractionation temperature on solid fat content (SFC) of stearin from refined squid visceral oil with solvent fractionation at 1.6 p′. Fractionation conditions as in Figure 2.

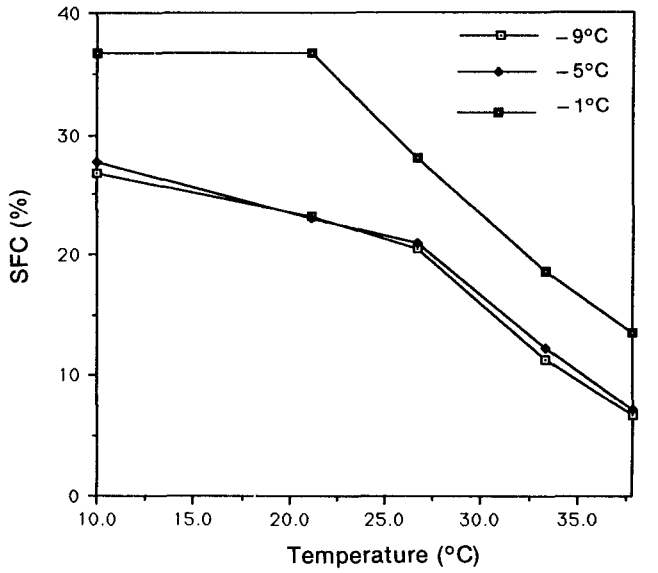

FIG. 3. Effect of fractionation temperature on solid fat content (SFC) of stearin from refined squid visceral oil with solvent fractionation at 3.6 p′. Fractionation conditions as in Figure 2.

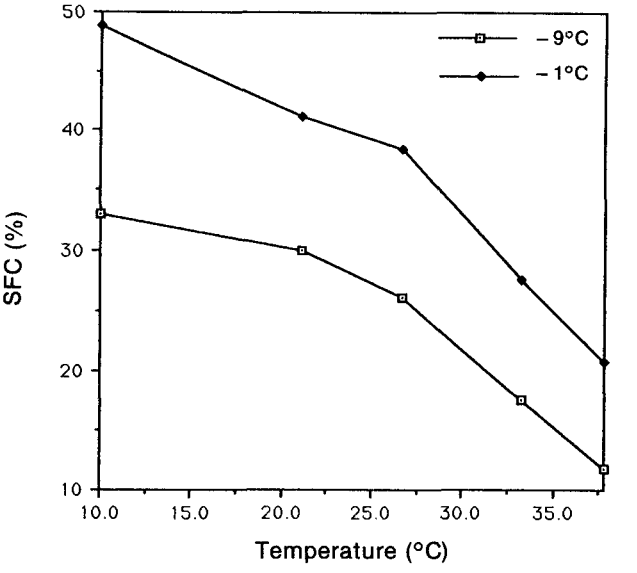

FIG. 5. Effect of fractionation temperature on solid fat content (SFC) of stearin from refined squid visceral oil with solvent fractionation at 0.1 p′. Fractionation conditions as in Figure 2.

similar to those in Figure 6, but a difference in percentage of solid fat is observed. Comparing those fractionated at −5° and −9°C to −1°C, the solid fat contents of stearins fractionated at −5 or −9°C were both lower than at −1°C.

DISCUSSION

Effect of solvent polarity on yield and soft melting point. Table 1 shows that yield of fractionated stearin increased with increasing solvent polarity. This may be attributed to squid refined viscera oil containing about 70% unsaturated fatty triglycerides (Table 3). It is well known that saturated fatty acids are less polar than unsaturated ones. Therefore, polar solvent fractionated more glycerides from squid refined oil than a nonpolar one, so the yield increases with solvent polarity. As solvent polarity was raised above 3.6 p′, the miscibility of the visceral oil in solvent increased as did the yield, regardless of the fractionation temperature.

Because squid refined viscera oil contains a high proportion of unsaturated fatty glycerides, fractionation at identical fractionation temperatures while increasing the solvent polarity will result in stearin with more unsaturated

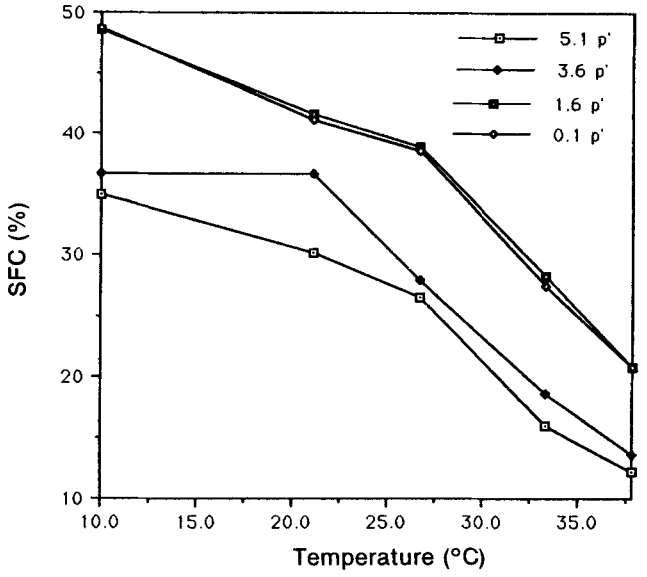

FIG. 6. Effect of solvent polarity on solid fat content (SFC) of stearin from refined visceral oil with solvent fractionation at −1°C. Fractionation conditions as in Figure 2.

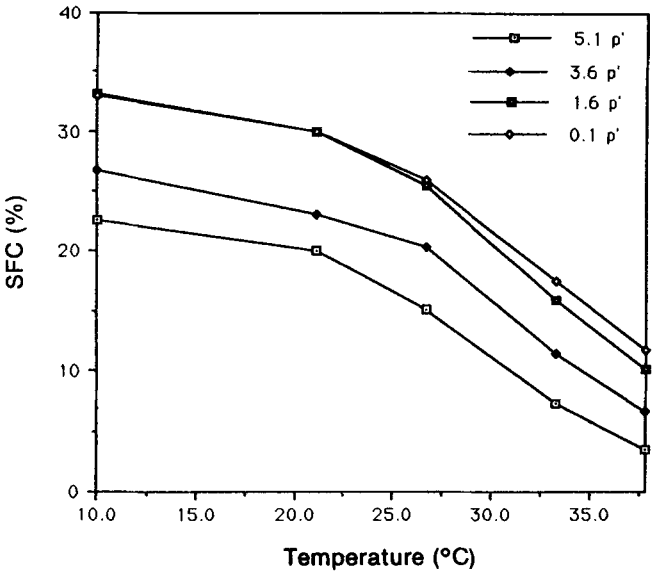

FIG. 8. Effect of solvent polarity on solid fat content (SFC) of stearin from refined visceral oil with solvent fractionation at −9°C. Fractionation conditions as in Figure 2.

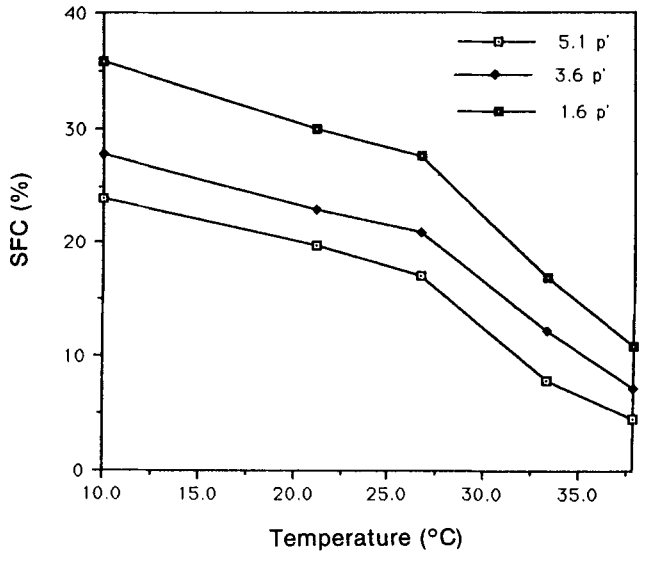

FIG. 7. Effect of solvent polarity on solid fat content (SFC) of stearin from refined visceral oil with solvent fractionation at −5°C. Fractionation conditions as in Figure 2.

fatty glycerides dissolving more in the polar solvent systems. As the solvent polarity exceeded 3.6 p′, the proportion of unsaturated fatty glycerides increased enormously and affects the state of the crystal lattice of the triglyceride. This may explain the reduction in the soft melting point. The soft melting point of stearin fractionated with 5.1 p′ solvent was significantly lower than that of 0.1 p′ for three fractionation temperatures studied.

The combined effect of fractionation temperature and solvent polarity. Yield of stearin fractionated from −9°C at 3.6 p′ was significantly higher than from 0.1 p′, as shown in Table 1, but not at the other fractionation temperatures of −1 or −5°C. The difference may result from the combined effects of fractionation temperature and solvent polarity. As fractionation temperature was lowered to −9°C, which is near or below the crystal temperature of most glycerides, more stearin will be fractionated from higher polarity solvent than from lower polar solvent because squid refined viscera oil contains about 70% unsaturated fatty triglycerides (Table 3). The yield from 3.6 p′ solvent is thus higher than from 0.1 p′.

The soft melting point of stearin obtained from −9°C at 5.1 p′ was significantly lower than from 1.6 p′ (Table 2), but not at the other fractionation temperatures of −1 or −5°C. The discrepancy may result from the same reason as discussed above. At −9°C, although more saturated glycerides fractionated from the less polar solvent, more unsaturated glyceride fractionated from the higher polar solvent. Thus the soft melting point of stearin was significantly higher from 1.6 p′ than from 5.1 p′.

Effect of uneven distribution of various types of triglycerides. Boucher and Skau (19) reported that as the fractionation temperature is lowered, yields of stearin increase. Wong (20) supported this observation with fractionations of rice oil at different temperatures. However, Table 1 shows no significant difference between different fractionation temperatures at a solvent polarity of 5.1 p′.

The yields of stearin mainly depended upon the fatty acid composition of crude viscera oil triglycerides, polarity of the solvent and the temperature of fractionation. If the distribution of various types of triglycerides in the crude viscera oil is constant, yield of stearin will increase with decreasing fractionation temperature, regardless of the effect of solvent polarity, as long as temperatures near or below the crystal temperatures of the stearins are employed. Results shown in Table 1 may therefore be attributed to uneven triglycerides distribution.

Soft melting points did not differ significantly among stearins fractionated with solvent polarities higher than 1.6 p′ at −1 or −5°C or higher than 3.6 p′ at −9°C

(Table 2). The melting point of saturated fatty acid is higher than that of unsaturated acid. The order of triglyceride melting points is $GS_3 > GS_2U > GSU_2 > GU_3$. If the fractionation temperature is near or below the crystal temperature of glycerides, the stearin crystallizes out. Therefore, the melting point of stearin reflects the triglyceride's composition, as well as the state of the crystal lattice of the triglyceride. The soft melting point of lipids dissolved in nonpolar solvent will be higher than that dissolved in polar solvent (20,21). If the distribution of various types of triglycerides is constant in stearin, the soft melting point of stearin fractionated at 5.1 p′ and −9°C will be the lowest and the one from 0.1 p′ and −1°C will be the highest. However, the results shown in Table 2 disagree with this conclusion, which may be attributed to uneven distribution of various types of triglycerides. Table 3 shows that the percentages of fatty acids of stearin fractionated with like solvent mixture at different temperatures were similar. Results in Tables 1 and 2 supported the speculation of the uneven distribution of various types of triglyceride in refined viscera oil.

Commercial applications. As shown at lower solvent polarities in Figures 6–8, the solid fat content of stearin increased. Although solid fat content is higher, the plastic range was narrow. Such characteristics in an oil do not favor use in a table spread, but it could be used for shortening or other base oils after blending. Both stearins fractionated from solvents with polarities higher than 3.6 p′ had the widest plastic range. Stearin from 5.1 p′ had the highest yield and the highest percentage of EPA and DHA (15–17%), and the soft melting point was close to the human body temperature (37°C). Therefore, it could be used as a topping, shortening or table margarine. Stearin fractionated from −1°C with solvent of 5.1 p′ showed a plastic range of 35–21°C (Fig. 6). Plastic ranges were 28–10°C and 27–10°C for fractionations at −5°C (Fig. 7) and −9°C (Fig. 8), respectively. The product from −1°C would be useful in summer and that from −5°C and −9°C in other, colder seasons. Because those products did not undergo any high-temperature processing, they need only to be packaged and stored to preserve their quality.

Triglycerides of stearins fractionated from 5.1 p′ solvent mixture contain about 15–17% of EPA and DHA. The percentage of EPA and DHA in the fractionated stearin decreased with lower fractionation temperatures in every solvent system except 5.1 p′ (Table 3). This may be due to the fact that triglycerides containing fatty acids other than EPA + DHA crystallized out more easily as fractionation temperature decreases, therefore decreasing the ratio of EPA + DHA in the stearin.

Lowering the fractionation temperature to below −9°C may increase the yield. For example, if 5.1 p′ solvent at temperature below −9°C is used, more triglyceride with more unsaturated fatty acid will crystallize out, thus increasing the yield. However, the soft melting point will be lower than 37°C and the product may lose the characteristics of table margarine or shortening oil. If polarities of 3.6 p′ or lower are employed at temperatures below −9°C, the stearin thus obtained may resemble that fractionated at 5.1 p′ and −5°C. Therefore, the practical fractionation conditions are at −1°C or −5°C with a solvent polarity of 5.1 p′.

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