Hensley, D. L.; Beuerman, D. S. N.; Carpenter, P. L. "The Inactivation of Glyphosate by Various Soils and Metal Salts". *Weed Res.* **1978**, *18*, 287–291.
Hoagland, R. E.; Duke, S. O. "Biochemical Effects of Glyphosate [N-(Phosphonomethyl)glycine]". In *Biochemical Responses Induced by Herbicides*; Moreland, D. E., St. John, J. B., Hess, F. D., Eds.; ACS Symposium Series 181; American Chemical Society: Washington, DC, 1982; pp 175–205.
Karadakov, B. P.; Ivanova, H. R. "Spectrophotometric Study of Copper(II) and Lead(II) Complex-Formation with Glycinebismethylphosphonic Acid". *Dokl. Bolg. Akad. Nauk* **1979**, *32*, 325–328.
Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 3rd ed.; Wiley: New York, 1978.
Pearson, R. G. "Hard and Soft Acids and Bases". *J. Am. Chem. Soc.* **1963**, *85*, 3533–3539.
Rueppel, M. L.; Brightwell, B. B.; Schaefer, J.; Marvel, J. T. "Metabolism and Degradation of Glyphosate in Soil and Water". *J. Agric. Food Chem.* **1977**, *25*, 517–528.
Smith, J. V., Ed. *Inorganic X-Ray Powder Data File*; American Society for Testing and Materials: Philadelphia, 1967; File 5-0661.
Sprankle, P.; Meggitt, W. F.; Penner, D. "Adsorption, Mobility, and Microbial Degradation of Glyphosate in the Soil". *Weed Sci.* **1975**, *23*, 229–234.
Tsuboi, M. "Vibrational Spectra of Phosphite and Hypophosphite Anions, and the Characteristic Frequencies of PO_3^{2-} and PO_2^- Groups". *J. Am. Chem. Soc.* **1957**, *79*, 1351–1354.

Received for review September 14, 1987. Accepted March 21, 1988.

Processing and Quality Assessment of Solubles Prepared from Dogfish Processing Wastes

John C. Mowbray, Humberto A. Rossi, and Tuu-jyi Chai*

Solubles were produced through the digestion and concentration of ground raw dogfish processing wastes under acidic and heated conditions. The optimum conditions for the digestion were pH 3.5 and 50 °C. Proximate analysis of the soluble product indicated, on a dry-weight basis, 67.4% crude protein, 26.9% lipid, and 7.6% ash. The nonprotein nitrogen constituents contained free amino acids, urea, trimethylamine, volatile base nitrogen, and ammonia in the amounts 41.3, 3.2, 1.5, 1.0, and 1.3%, respectively. Free amino acids ranged between 65 and 85% of the protein nitrogen present. Fatty acid analysis indicated the predominance of unsaturated fatty acids.

Spiny dogfish (*Squalus acanthias*) is an abundant yet underutilized resource available to the seafood industry on the eastern coast of the United States (Seymour, 1982). Obstacles to the development of food processing for this species include high proportion of processing waste, rapid deterioration of flesh quality (Southcott et al., 1960; Boyd et al., 1967; Bilinski et al., 1985), and the unpleasant name. Although there is no market in the United States at present, dogfish in Europe has long been consumed both fresh and smoked (Kizevetter, 1971). Dogfish flesh has a high lipid content (Boyd et al., 1967), which makes it suitable for smoking (Shiau and Chai, 1985). Oily fish has also been receiving positive publicity due to recent research findings of the possible health benefits of diets rich in ω^3 fatty acids (Kromhout et al., 1985; Phillipson et al., 1985).

The greatest obstacle to the development of dogfish processing is the problem of waste disposal (Jackson, 1987). Dogfish processing wastes consist of the head, viscera, skin, and small fins. Due to their large proportion, high water content, and high degree of perishability which readily produces a large quantity of ammonia, it is not practical to heat-dry these processing wastes to meal as is done with more conventional species. These wastes are also extremely difficult to process due to the abrasive nature of the skin and the tendency of the wastes to clog grinding equipment. Therefore, the wastes must be disposed of, and this process can be expensive. Current methods of disposal include landfill burial and dumping at sea, both of which have been associated with pollution problems (Jackson, 1987). Since dogfish processing produces such a great amount of waste, the development of an economical and useful waste process would benefit all other aspects of seafood industry.

The objective of this study was to develop an alternative economical process and quality evaluation of a useful, soluble product prepared from dogfish processing wastes.

MATERIALS AND METHODS

Dogfish Processing. Iced, freshly harvested female dogfish were transported to the laboratory where they were promptly decapitated and dressed. Male dogfish, which are small in size, were not harvested and hence not included in this study. Each sample consisted of 250–550 lb of female dogfish. The skinned carcasses were set aside for human consumption, the livers were allocated for oil production, and the skin and fins were frozen for other uses such as a possible replacement for traditional blue crab baits. The remaining head, viscera, embryos and pups, blood, and all debris were collected for soluble production.

Solubles Production. The dogfish processing wastes were ground in a Hobart food mixer with a meat grinding attachment with a $^1/_8$-in. pore size head. The ground wastes were transferred to a steam-jacketed kettle outfitted with a variable speed agitator. The pH and temperature were adjusted, and the mixture was agitated for 4 h under these conditions. After the digestion was complete, the temperature was increased to 80 °C to drive off moisture, concentrating the product to about 70% total solids. The solubles were packed in sanitized 5-gal plastic buckets, sealed, and stored at 23 °C for further use and testing.

Optimum Process Determination. The primary environmental factors influencing the rate of digestion were pH and temperature. The optimum pH was determined by adjusting four equal portions of the ground processing wastes to pH 3.5, 4.0, 4.5, and 5.0 with concentrated hy-

Seafood Science and Technology Division, Horn Point Environmental Laboratories, University of Maryland, Cambridge, Maryland 21613.

0021-8561/88/1436-1329$01.50/0

Table I. Body Composition of Female Dogfish

batch	date; location landed	body composition, %						
		head	viscera[a]	skin[b]	fin	fillet	cartilage	liver
A	3/10/85; Ocean City, MD	15.7	20.1	13.9	5.9	26.6	7.4	10.4
B	3/30/85; Gloucester, MA	16.9	23.2	13.6	5.5	23.2	4.8	12.9
C	4/29/85; Ocean City, MD	15.9	25.6	13.2	5.5	24.4	4.4	11.0
F	7/8/85; Gloucester, MA	15.8	18.9	16.7	6.7	26.7	5.3	10.0
I	3/26/86; Ocean City, MD	19.6	19.6	13.9	7.0	25.2	5.7	9.1
av		16.8	21.5	14.3	6.1	25.2	5.5	10.7

[a] Viscera, including embryos, pups, intestines, and all other internal organs except livers. [b] With belly meat intact.

drochloric acid. The pH-adjusted wastes were then incubated at room temperature (23 °C), and the digest was periodically sampled and analyzed for free amino nitrogen via the ninhydrin reaction (Harding and McLean, 1916).

The optimum temperature was determined by adjusting a portion of ground wastes to the optimum pH and then dividing it into four equal parts that were incubated at 40, 50, 60, and 70 °C. The digest was then periodically sampled and subjected to free amino acid determination.

Chemical Analysis. Proximate analysis of the final product was performed by AOAC methods (AOAC, 1980). Amino acid analysis were achieved through the acid hydrolysis of the soluble (Moore and Stein, 1963) and subsequent ion-exchange chromatography, via the automatic amino acid analyzer. Fatty acids present were determined by gas chromatography of the methyl esters of a soluble extract. Free amino acid content was determined with the ninhydrin reaction described by Harding and McLean (1916). The presence of trimethylamine was detected by the picrate salt method of Dyer (1945). Urea and ammonia were determined by the AOAC distillation method (AOAC, 1980). Volatile base nitrogen content was determined by Conway's method of microdiffusion analysis (Su, 1977). Thiobarbituric acid number was determined by the 2-thiobarbituric acid method of Tarladgis et al. (1960).

Microbiological Analysis. All microbiological analyses were performed according to FDA methods (FDA, 1984). Aerobic plate counts were completed with plate count agar with incubating for 48 h at 35 °C. Anaerobic plate counts were determined with trypticase peptone glucose yeast extract agar and incubating for 4 days at 28 °C in a gas pak (BBL). Mold and yeast counts were detected with acidified malt extract agar at pH 5.5 and incubating for 4 days at 20 °C.

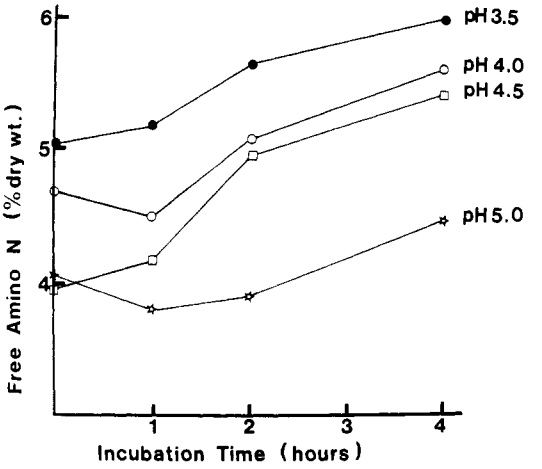

Figure 1. Effect of pH on digestion of raw processing wastes at room temperature (23 °C).

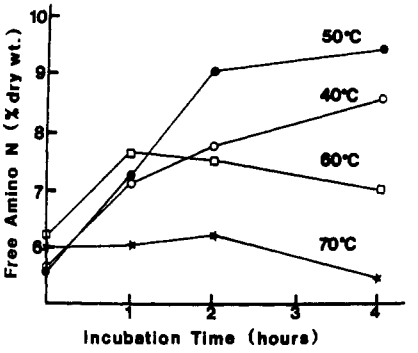

Figure 2. Effect of temperature on digestion of raw processing wastes at pH 4.0.

RESULTS AND DISCUSSION

In the eastern coast of the United States, female dogfish weighed from 10 to 14 lb and male fish from 4 to 7 lb. Therefore, in this region only female dogfish were commercially fished because of their larger size. A yield study indicated that over 60% of the processed female dogfish was waste material (Shiau and Chai, 1985). Body composition is shown in Table I. These wastes included the head, viscera, skin, and fins, with the head and viscera as the major portions of the wastes. Seasonal changes from spring to summer did not cause fluctuation of body composition. Spiny dogfish caught in New England and the mid-Atlantic region of the eastern coast of the United States had similar body composition. In commercial processing situations, the cartilage is usually kept intact with the fillets. The livers might be recovered for use in oil production.

Figures 1 and 2 indicate the optimum processing conditions that appeared to be pH 3.5 and a temperature of 50 °C. For practical processing, pH 4.0 was used in this study. pH 4.0 was low enough for digestion, able to retard bacterial spoilage during the process, did not require as much acid, and took less neutralization required later when the soluble was incorporated in a feed system. Nine batches of dogfish solubles made by pilot-scale processing were performed, and results indicated that all samples were reduced to a very small particle size in 1.5 h at pH 4.0 and 50 °C. Digestion would be continued and accelerated during the concentration process at 80 °C and hence would be completed prior to the end of the concentration process. Therefore, pH 4.0 and 50 °C were suggested to be the optimum conditions for digestion in the large-scale production of the industry.

The optimum digestion conditions in this study were comparable to the work of Strasdine and Jones (1983). They reported that in processing a stable silage the addition of 1.5% formic acid into ground dogfish and incubating it at 45 °C would result in complete digestion in 1–2 days. The digestion in our process was much faster because of the application of heat during digestion and following concentration. In our process, heating at 80 °C would not only concentrate the solubles but also completely digest any remaining wastes.

As shown in Table II, the proximate analysis on a dry-weight basis indicates that the soluble product contained

Table II. Proximate Composition of Dogfish Solubles[a]

soluble sample	date	% composition of solubles: TKN[b]	crude protein, % N × 6.25	TCA-sol N	true protein (TKN - TCA N) × 6.25	lipid	ash	moisture
B	3/30/85	7.50	46.86	6.50	5.96	18.72	4.80	31.34
E	6/2/85	7.09	44.32	5.65	8.96	20.40	5.35	30.02
F	7/8/85	7.81	48.82	6.17	10.24	22.67	5.31	28.15
G	8/7/85	7.25	45.32	5.69	9.76	18.81	5.53	32.11
H	2/19/86	7.39	46.20	6.30	6.85	21.72	5.10	30.20
I	3/26/86	7.89	49.33	6.41	9.30	20.33	5.25	29.84
av		7.50	46.85	6.13	8.52	20.46	5.23	30.23
		10.78[c]	67.36[c]	8.63[c]	13.40[c]	26.86[c]	7.58[c]	
ground raw wastes		2.73	17.07	1.28	9.09	5.64	1.94	78.99

[a] Dogfish soluble is a soluble paste prepared from the degradation (pH 4.0, 50 °C) and concentration (80 °C) of ground dogfish processing wastes. For details see Results and Discussion. [b] TKN, total Kjeldahl nitrogen. [c] Percent dry weight basis.

Table III. Amino Acids Composition of Dogfish Soluble

amino acid	% amino acid in dogfish soluble protein: sample E	sample I	av	catfish requirement,[a] % protein
cysteine	1.06	1.02	1.04	
aspartic acid	9.50	9.53	9.52	
threonine[b]	4.67	4.76	4.72	2.0
serine	5.37	5.56	5.47	
glutamic acid	13.58	13.23	13.40	
proline	5.55	5.57	5.56	
glycine	8.24	8.47	8.37	
alanine	5.87	5.87	5.87	
valine[b]	5.65	5.20	5.41	3.0
methionine[b]	2.66	2.65	2.66	2.3
isoleucine[b]	5.14	5.09	5.11	2.6
leucine[b]	8.70	8.39	8.54	3.5
tyrosine	2.52	2.75	2.64	
phenylalanine[b]	3.79	3.78	3.79	5.0
histidine[b]	2.53	2.72	2.63	1.5
lysine[b]	6.95	7.02	6.99	5.0
arginine[b]	8.20	8.38	8.30	4.3
tryptophan[b]				0.5
total	99.98	99.99	100.02	

[a] Data derived from *Nutrient Requirements of Warmwater Fishes and Shellfishes* (National Research Council, 1983). [b] Essential amino acids. Tryptophan was not determined.

crude protein between 63 and 70%, lipids between 27% and 31%, and ash ranging from 7 to 8%. The average moisture content of various batches of soluble was approximately 30%. Indications were that the proximate chemical composition was relatively consistent and did not vary with season and fishing locations between the New England and mid-Atlantic regions. A trichloroacetic acid (5%) extract of the soluble yielded between 80 and 90% of the total Kjeldahl nitrogen present. This indicates that the final true protein present in the soluble was between 10 and 20% of the total nitrogen, depending on the processing conditions and the degree of digestion. The TCA extract of raw ground processing wastes when subjected to nitrogen analysis indicated that half of the nitrogen was nonprotein and about one-third of the nitrogen in the finished soluble was nonprotein nitrogen (Table II). This was confirmed when the total amino acids were analyzed, showing that the total true protein was 45%, accounting for about two-thirds of the crude protein (Table III). The proximate composition of dogfish soluble was similar to the results of Strasdine and Jones (1983) who reported that the digested dogfish silage consisted of 73% crude protein, 22% fat, and 13% ash. Minor differences exist that might be due to their inclusion of cartilage and the variation in fish used. Past studies performed by Satia et al. (1975) reported that dogfish and dogfish wastes were poorly assimilated by salmon when used as feed. The dogfish soluble produced in this study may be more biologically available for feed uses due to the high free amino acid content. Other studies using wastes similar to these had good results in a comparative feeding study using salmon (Asgard and Austreng, 1985).

Table IV. Nonprotein Nitrogen Constituents and TBA Values of Solubles Made from Fresh and Spoiled Dogfish

sample	nonprotein nitrogen constituent, %: free amino acid (FAA - N × 6.25)	TMA	urea	NH_3	VBN	malonaldehyde, mg/kg
E[a]	41.25	1.53	3.22	1.31	1.04	5.0
G[b]	48.75	4.68	0.19	2.30	1.70	14.2

[a] Soluble was made from the fresh dogfish processing wastes. [b] Soluble was made from the spoiled dogfish processing wastes.

The amino acid assay indicated the presence of the full spectrum of amino acids in the soluble. All of the essential amino acids were present, but methionine, phenylalanine, and lysine were present in levels lower than the requirements for many animals. For example, to apply this soluble for catfish and other fish feeding, an adequate supplement with other feed ingredients was necessary to reach the efficient growth rates.

Ethoxyquin was added into the soluble product to prevent the oxidation reaction. The TBA value ranged from 2.8 to 5.2 mg/kg malonaldehyde at initial preparation, and it increased up to 30.5 mg/kg during storage when the antioxidant was not added (data not shown). With the addition of 0.006% v/v ethoxyquin to the soluble, the TBA value remained in the range 3.5–6.8 mg/kg malonaldehyde through the entire course of 1-year storage. The high lipid content of the soluble could cause it to be susceptible to oxidative changes, and the use of antioxidants would be necessary. Lipid oxidation products have been shown to be toxic when fed to carp (Watanabe and Hashimoto, 1968) and channel catfish (Murai and Andrews, 1974). When salmon were fed with rancid sprat silage, some vigorous feeding response and normal growth rate were observed although slight changes in the morphological appearance and distribution of the eosinophilic granule cells occurred (Jackson et al., 1984).

To observe the effect of raw material freshness on soluble quality, one shipment of dogfish was divided into two equal parts, half being processed immediately and half being held at 2 °C for 14 days until intermediate spoilage was evident. To compare the solubles produced using these two conditions, the nonprotein nitrogen constituents were analyzed (Table IV). The levels of free amino nitrogen were found to be slightly higher in the soluble made

Table V. Fatty Acid Composition of Dogfish Soluble[a]

fatty acid	from fresh wastes	from state wastes
14:0	2.7	5.0
15:0	0.2	0.6
16:0	14.2	14.7
$16:1\omega^7$	4.2	14.7
18:0	3.2	4.1
$18:1\omega^9$	15.7	13.4
$18:1\omega^7$	4.8	6.2
$18:1\omega^5$	1.0	0.9
$18:2\omega^6$	1.4	1.8
$18:3\omega^3$	0.17	1.5
$18:4\omega^3$	0.6	1.2
$20:1\omega^9$	12.7	11.2
$20:1\omega^7$	1.0	0.9
$20:3\omega^6$	0.3	0.3
$20:3\omega^3$	1.3	1.5
$20:5\omega^3$	0.9	0.8
$22:1\omega^{11}$	5.3	6.3
$22:1\omega^9$	11.2	9.4
$21:5\omega^3$	0.3	0.3
$22:5\omega^6$	0.3	0.3
$22:5\omega^3$	2.2	1.3
$22:6\omega^3$	9.5	9.4
unknown	5.7	3.8

[a] Fatty acids are presented by percentage.

from the spoiled fish. This could be due to proteolysis that may have occurred during the 14-day storage period. The presence of trimethylamine, as expected, was greater in the spoiled samples when compared to the soluble previously made from fresh dogfish wastes. These results were similar to those reported by Southcott et al. (1960). Urea was found at lower levels in the soluble made from the spoiled fish, probably due to the conversion to ammonia by urease-producing organisms during storage. It is well-known that urease-producing bacteria are widely present in fresh fish (Chai, 1970). The ammonia level in the same sample was corresponding higher due to the same urease reaction. The levels of volatile base nitrogen were found to be slightly higher in the spoiled sample, probably due to the greater amount of ammonia present. Similar findings were reported by Moyer et al. (1959). The soluble made from the wastes of the spoiled fish contained more TBA-reactive substances than the fresh samples. This was likely due to the oxidation of lipids that might have occurred during storage, prior to processing.

Fatty acid composition of dogfish soluble is shown in Table V. Unsaturated acids accounted for about four-fifths of the total fatty acids. The predominant fatty acids were $18:1\omega^9$, 16:0, $20:1\omega^9$, $22:1^9$, $22:6\omega^3$, $22:1\omega^{11}$, $18:1\omega^7$, and $16:1\omega^7$. ω^3 fatty acids accounted for about 15% of the total acids. Despite the relatively low level of ω^3 fatty acids in dogfish as compared with many fatty fish, dogfish soluble does supply these acids that are critical in nutrient requirements for the feeding application particularly when used as aquaculture feed. When dogfish soluble was prepared from the deteriorated processing wastes, the fatty acid profile was similar to that of soluble prepared from fresh wastes except that some acids were quantitatively changed. In the soluble prepared from stale wastes, the acids with less numbers of carbon such as 14:0, 15:0; $16:1\omega^7$, $18:3\omega^3$, and $18:4\omega^3$ were increased in their levels while some other acids with higher numbers of carbon were slightly decreased accordingly.

The results of the microbiological tests indicate that the product is relatively free of microorganisms (Table VI). The low number of organisms present was likely due to contamination after the process. Due to the low pH and low moisture content, proliferation of microorganisms would be unlikely to occur in the soluble product.

Table VI. Microbiological Analysis of Dogfish Solubles

sample	microbiological count, CFU/g: aerobic	anaerobic	mold and yeast
A	$<3.0 \times 10$	$<3.0 \times 10$	$<3.0 \times 10$
B	1.1×10^2	1.8×10^2	7.5×10
C	6.0×10	$<3.0 \times 10$	$<3.0 \times 10$

Several advantages of this dogfish soluble can be seen as compared with the raw processing wastes and other waste products. The acidification of soluble neutralized the ammonia and other alkaline deteriorative products so that the final product possessed an agreement odor and long shelf life. Unlike the liquefied silage, which required enormous containers, storage space, and specific transportation that would add immense cost and inconvenience, concentrated solubles took up less volume. When the soluble was subject to more concentration, products varying in moisture including a powder form could be produced for many uses.

Dogfish soluble could be used in many diversified ways. Agricultural applications for dogfish soluble include poultry or swine feeds. Jeng and Hwang (1985) have demonstrated that the supplement of small sharks waste soluble to the diet was effective in promoting the growth of fresh water prawn. Previous studies have indicated that cod viscera incorporation into autolysate was suitable for use in bacterial media (Clausen et al., 1985). Aquacultural application may be one of the best uses for this product. A feeding study of catfish incorporating a diet of dogfish soluble with other ingredients has been carried out in this laboratory with promising results (Mowbray, Harrell and Chai, manuscript in preparation).

If this product is suitable for feed applications, we may have a solution to a major obstacle to developing a dogfish processing industry: the problem of the disposal of the wastes generated during processing. Dogfish presents the greatest challenge to the seafood processing industry due to the large portion of wastes and the difficulty in handling these processing wastes because of their high moisture and high perishability with great production of ammonia. The ability to completely process and utilize this species would benefit the entire seafood processing industry as these methods may easily be adapted to other species that are much easier to handle.

This study provides a practical and economic process to convert these processing wastes into useful products rather than retaining them as conventional requiring costly disposal. Many seasonal seafood industries recognize that an underutilized species such as dogfish would be good to process during the off season; however, they are reluctant to process dogfish because of the large amount of waste produced. The solution of processing wastes would facilitate the development of processing of underutilized species for human food that could become major seafood resources. Furthermore, the maximum utilization of seafood processing wastes can help the progress of innovative "complete utilization" of seafood processing resulting in no wastes and little pollution.

ACKNOWLEDGMENT

Contribution No. 1959 from the Center for Environmental and Estuarine Studies, University of Maryland, Cambridge, MD. This study was supported by the Mid-Atlantic Fishery Foundation with funding from the Saltonstall-Kennedy Program (Grant No. 85-23-58017M).

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.

Asgard, T.; Austreng, E. Dogfish offal, ensiled or frozen as feed for Salmonids. *Aquaculture* **1985**, *49*, 289–303.
Bilinski, E.; Gildberg, A.; Raa, J. Factors controlling the deterioration of the spiny dogfish (*Squalus canthias*) during iced storage. *J. Food Sci.* **1985**, *48*, 808–812.
Boyd, J. W.; Southcott, B. A.; Schmidt, P. J. Quality of Pacific coast dogfish during frozen storage. *J. Fish. Res. Board Can.* **1967**, *24*, 527–535.
Chai, T. Studies on the bacterial flora of fish pen slime. M. S. Thesis, University of Massachusetts, 1970; p 154.
Clausen, E.; Gildberg, A.; Raa, J. Preparation and testing of an autolysate of fish viscera as growth substrate for bacteria. *Appl. Environ. Microbiol.* **1985**, *50*, 1556–1557.
Dyer, W. J. Amines in fish muscle, I. Colorimetric determination of triethylamine as the picrate sale. *J. Fish. Res. Board Can.* **1945**, *6*, 351–356.
FDA. *Bacteriological Analytical Manual*, 6th ed.; Food and Drug Administration: Washington, DC, 1984.
Harding, V. J.; MacLean, R. M. A colorimetric method for the estimation of amino acid alpha nitrogen, II. Application to the hydrolysis of proteins by pancreatic enzymes. *J. Biol. Chem.* **1916**, *24*, 503–515.
Jackson, W., North Atlantic Products, Inc., Rockland, ME, personal communication, 1987.
Jackson, A. J.; Kerr, A. K.; Bullock, A. M. Fish silage as a dietary ingredient for salmon, II. Preliminary growth findings and nutritional pathology. *Aquaculture* **1984**, *40*, 283–291.
Jeng, S.-S.; Hwang, D.-F. Effect of feeding fish soluble and fish meal produced from small sharks wastes on the growth of fresh water prawn. *J. Fish. Soc. Taiwan* **1985**, *12*, 64–73.
Kizevetter, I. V. V.S. Gordievskaya Shark Flesh in the Food Industry. (Translated from Russian into English by H. Mills, U.S. Department of Commerce), 1971.
Kromhout, D.; Bosschreter, E. B.; Coulander, C. L. The inverse relation between fish consumption and 20 years mortality from coronary heart disease. *N. Engl. J. Med.* **1985**, *312*, 1205–1209.
Moore, S.; Stein, W. H. *Methods Enzymol.* **1963**, *6*.
Moyer, R. H.; Southcott, B. A.; Baker, E. G.; Tarr, H. C. A. Keeping quality of pacific coast dogfish. *J. Fish Res. Board Can.* **1959**, *16*, 791–794.
Murai, T.; Andrews, J. W. Interaction of dietary α-tocopherol, oxidized menhaden oil and ethoxyquin on channel catfish. *J. Nutr.* **1974**, *104*, 1416–1431.
Phillipson, B. E.; Rothrock, D. W.; Connor, W. E.; Harris, W. S.; Illingsworth, D. R. Reduction of plasma lipids lipoproteins and apoproteins by dietary fish oils in patients with hypertriglyceridemia. *N. Engl. J. Med.* **1985**, *312*, 1210–1216.
Satia, B. P.; Brammon, E. L. The value of certain fish processing wastes and dogfish (*Squalus suckley*) as food for Coho Salmon (*Oncorhynchus* kisutch) fry. *Prog. Fish. Cult.* **1975**, *37*, 76–80.
Seymour, H. H. Shark processing demonstration project—A final report to the Mid-Atlantic Fisheries Development Foundation. University of Delaware, 1982.
Shiau, C. Y.; Chai, T. Smoked dogfish processing and its refrigerated storage stability. *J. Food Sci.* **1985**, *50*, 1348–1350.
Southcott, B. A.; Moyer, R. H.; Baker, E. G.; Tarr, H. C. A. Keeping Quality of Pacific Coast Dogfish II. *J. Fish Res. Board Can.* **1960**, *17*, 811–814.
Strasdine, G. A.; Jones, Y. Ensiling dogfish (*Squalus acanthias*) processing wastes for animal feed. B.C. Research: Vancouver, B.C., Canada, 1983.
Su, H. J. *Seafood Chemistry Laboratory*; Wensheng: Taipei, Taiwan, 1977; pp 81–94.
Tarladgis, B. G.; Watts, B. M.; Younathan, M. T.; Dugan, L. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.* **1960**, *37*, 44–48.
Watanabe, T.; Hashimoto, Y. Toxic components of oxidized saury oil inducing muscular dystrophy in carp. *Bull. Jpn. Soc. Sci. Fish.* **1968**, *34*, 1131.

Received for review May 7, 1987. Revised manuscript received April 22, 1988. Accepted May 19, 1988.

Preparation of Esters Resembling Natural Waxes by Lipase-Catalyzed Reactions

Kumar D. Mukherjee* and Irmgard Kiewitt

Esters composed of long chain (C_{16}, C_{18}) and/or very long chain ($\geq C_{20}$) acyl and alkyl moieties, which resemble some naturally occurring waxes of commercial importance, are prepared conveniently in high yield by alcoholysis or esterification reactions catalyzed by lipases. Triacylglycerols of seed oils containing large proportions of very long chain (*n*–9)-(*Z*)-monounsaturated acyl moieties (20:1, 22:1, 24:1) are subjected to lipase-catalyzed alcoholysis with suitable mixtures of long chain and very long chain (*n*–9)-(*Z*)-monounsaturated alcohols (18:1–24:1) to yield esters resembling the waxes from jojoba (*Simmondsia chinensis*) or orange roughy (*Hoplostethus atlanticus*), for example. Similar products are also obtained by lipase-catalyzed esterification of suitable mixtures of (*n*–9)-(*Z*)-monounsaturated fatty acids (18:1–24:1) with those of the corresponding alcohols.

Lipase-catalyzed reactions such as hydrolysis, esterification, and interesterification of lipids have received considerable attention during the past few years in view of their potential biotechnological applications in oils, fats, and oleochemicals industries (Macrae, 1984). In a recent study from this laboratory, it was shown that lipase-catalyzed alcoholysis of triacylglycerols with long chain alcohols yielding wax esters is by far the fastest of the interesterification reactions such as those with fatty acids, methyl esters, triacylglycerols, or glycerol (Schuch and Mukherjee, 1987a,b).

In the present paper we report applications of lipase-catalyzed alcoholysis and esterification reactions in the preparation of esters resembling naturally occurring waxes of commercial interest. The starting materials used in this study are triacylglycerols from seed oils of white mustard, *Sinapis alba* (Mukherjee and Kiewitt, 1984), and honesty, *Lunaria annua* (Mukherjee and Kiewitt, 1986), which are rich in very long chain (*n*–9)-(*Z*)-monounsaturated acyl moieties (gadoleoyl, 20:1; erucoyl, 22:1; nervonoyl, 24:1).

Federal Center for Lipid Research, Institute for Biochemistry and Technology, H. P. Kaufmann-Institute, Piusallee 68, D-4400 Münster, Federal Republic of Germany.

0021-8561/88/1436-1333$01.50/0