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Low Temperature Alcohol Treatment of Oily Fish (Sardine) in View to Produce Fish Protein Concentrate (FPC) like Product

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Abstract

FPC like product from sardine (*Sardinops melanosticta*) was prepared using ethanol and n-hexane by low temperature treatment. Results showed nice protein content (85%), low water content (4%), and 1.18% of residual lipids in prepared FPC. Amino acid profile was similar to casein and egg. Protein digestibility and amino acid index were higher than casein. Protein efficiency ratio was 2.83.

No content of lysinoalanine was detected in prepared FPC. FPC samples showed very nice functional properties, high emulsifying and hydration capacities. Equilibrium moisture content ranged from 1.25% at 5% RH (relative humidity) to about 19% at 85% RH. After 10 months of storage at room temperature (vacuum packing with nitrogen) only a slight change in specific activity of actomyosin Ca^{+2}-ATPase was detected and total activity expresed as actomysin as total Ca^{+2}-ATPase activity was preserved in 26.25%. Thiobarbituric acid number (TBA N) was increased during storage.

Fish protein concentrate developed so far by not solvent extraction has no functional properties, affinity with water or emulsifying capacity, that are essential to further processing.

It appears that FPC without functional properties is mainly relegated to the role of food suplement.[1)]

Suzuki et al.[2)] reported that moisture content of dried fish muscle was critical to avoid protein denaturation during storage. Fukushima[3)] found that ethanol concentration far from 50% was the most favorable condition for preserving the native state of soy bean protein.

The purpose of this report, was to produce from sardine, a fish protein concentrate like product, with functional (emulsifying and hydration capacities) and nutritional properties. Without fishy odor, defated, and that preserve as much as possible the native state of the protein.

Materials and Methods

Fresh sardine (*Sardinops melanosticta*) was obtained from commercial source and

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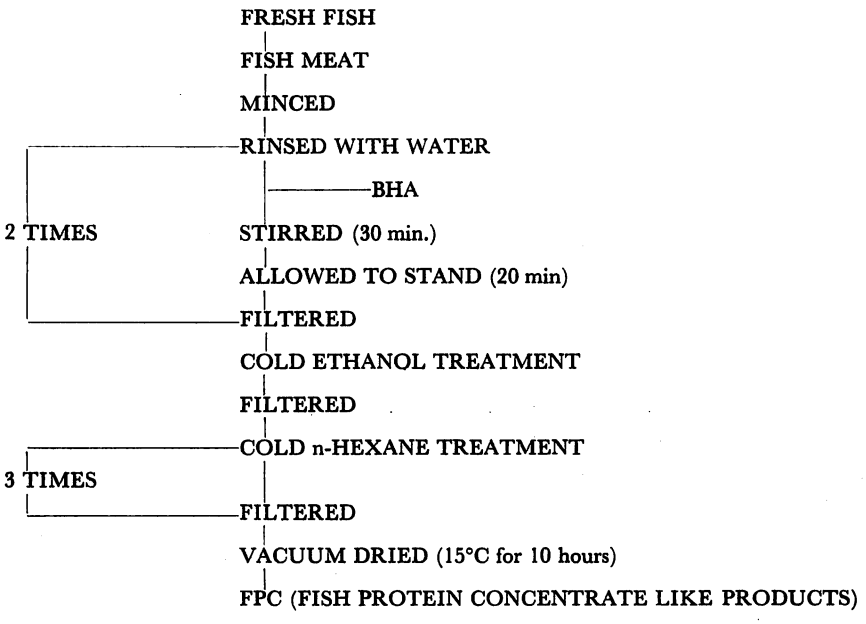

Fig. 1. Flow diagram for the preparation of FPC from sardine (*Sardinops melanosticta*) by ethanol and n-hexane at low temperature treatment.

immediatly processed according to Fig. 1.

Fish meat was obtained from sardine, by removing head, tail, viscera and scales. Then the fish meat was minced by a chopper, rinsed with 10 volumes of cold water with stirring for 30 min. The obtained supernatant was removed and the precipitate fraction was pressed with a filter cloth. This process was carried out at 2°C. The rinsed process was repeated 3 times.

The rinsed and pressed material was treated with previously precooled at −20°C ethanol, which last concentration was 80 or 90%, in a cold room for 1 hour, and then pressed with a filter cloth. The resultant material was treated with 5 volumes of previlusly precooled at −20°C n-hexane, in a cold room with stirring for 1 hour. Then the defated material was pressed with a filter cloth. This process was repeated 2 times.

The ethanol and n-hexane treated material was vaccum dried at 15°C for 10 hours in view to remove the organic solvent.

Analytical Determinations.

Total lipid content was determined as decribed by BLIGH et al.[4]. Moisture content was determined using Kett apparatus type F-1A (Kett Electric Laboratory, Japan).

Specific activity and actomyosin as Ca^{+2}-ATPase activity was determined as described by KATO et al.[5].

Total protein content was determined using solid sample Biuret method as described by OOSHIRO[6].

For water soluble protein determinations (WSP), samples were suspended in phosphate buffer solution, pH 7.5, IS 0.05, then homogenized with ultraturrax homogenizer for 3 min, and centrifuged for 30 min at 5000 rpm. Protein content was determined in supernatant and precipitate fraction as described by OOSHIRO[6,7].

Salt soluble protein (SSP) was determined same as was described for water soluble protein but using 0.6 M of KCl.

Emulsifying capacity of FPC was determined as described by WEBB et al.[8], and CARPENTER et al[9]. In a 500 ml pyrex glass was added 0 g to 0.5 g of FPC, 20 g of 1 M NaCl solution and 10 g of soy bean oil the mixture was then stirred with a mechanical stirrer and soy bean oil was added (1 ml/sec) until end point, showed by infinite resistance in the resistance sensing unity. The blanks were made by the same proceeding but without FPC.

Hydration capacity was measured as described by BRYANT et al.[10] 0.2 g of FPC was suspended in solutions at various pH and/or ionic, allowed to stand for 1 hour, then centrifuged at 2400 G for 30 min, and the remainder water in the sample was calculated.

Rancidity was determined using the 2-Thiobarbituric acid method, as described by SHIBATA et al.[11]. Results were expresed in thiobarbituric acid number (TBA N) according to SINNHUBER et al.[12].

In vitro protein digestibility was determined using a multienzyme system as described by HSU et el.[13], recording the pH drop after 10 min. Also was utilized the utilized the method described by OSHIMA[14] using pepsin.

Moisture adsorption of FPC at various relative humidities at 35°C, was determined as described by RASEKH et al.[15]. Saturated salt solutions were prepared as described by ROCKLAND[16].

Amino acids were analyzed with a Hitachi automatic amino acid analyzer, model 034, as described by MOORE et al.[17], after hydrolysis of the samples at 110°C at vacuum for 24 hours.

Tryptophan was determined by alkaline hydrolysis procedure as described by NOLTMAN et al.[18]. Half-cystine was determined as S-carboxymethylated cysteine, as described by CRESTFIEL et al[19].

Protein efficiency ratio was determined using C-PER (computed protein efficiency ratio) based on essential amino profile, as described by HSU et al.[20].

Results and Discussion

Table 1 shows, partial chemical composition of FPC prepared from sardine by ethanol and n-hexane at low temperature treatment. Protein could be concentrated from 16.4% to 85%, moisture content was 4% and water activity (expressed as equilibrium relative humidity) was 0.38 (Fig. 5). Total recovery expressed as protein recovery was 55.8%.

Residual lipid content was 1.18%. Table 2 shows that after FPC preparation

Table 1. Partial chemical composition of FPC prepared from sardine, by ethanol and n-hexane at low temperature treatment and fresh fish.

Material	Moisture content (%)	Total protein (%)	Total lipids (%)	Recovery (%)
Fresh fish (fillets)	73	16.4	11.80	—
FPC	4	85.0	1.18	55.8

Table 2. Content of water soluble protein (WSP) and salt soluble protein (SSP) during processing and after 10 months of storage.

Material	Dry matter (%)	SSP WM	SSP DM	Recovery (%)	SSP after 10 months (%) (DM)	WSP WM	WSP DM	Recovery (%)
Fresh fish	27	10.3	38.1	100	—	8.2	30.4	100
Rinsed	26	6.9	26.5	69.55	—	2.6	10.0	32.8
FPC	96	13.5	14.1	37.0	6.25	3.7	3.9	12.6

SSP (salt soluble protein) could be recovered in 37%. And during 10 months SSP content decreased from14.1% to 6.25%. These results can be interpreted as protein denaturation during storage.

Water soluble protein could be removed in about 88% by ethanol and n-hexane at low temperature treatment. This was important in view to ensure the stability of FPC during the storage.[21] Results show that the increase in TBA number after 10 months of storage, in vacuum, with nitrogen gas, was not so high. TBA number increased from 11.27 to 30.9 (Table 3).

Table 3. Thiobarbituric acid number (TBA N)* of FPC from sardine prepared by ethanol and n-hexane at low temperature treatment.

Material	TBA N just after preparation	TBA N after 6 months of storage	TBA N after 10 months of storage
FPC	11.27	29.49	30.9

* mg of malonadehyde/1000 g of sample

SINNHUBER et al.[12] reported that freshly prepared fish meal showed a TBA number of 21 and badly oxided samples near 300.

Results suggest that the low content of water soluble protein, 3.7% (total protein content was 85%) was favourable in the low rate of rancidity, expressed as TBA number, observed in FPC prepared from sardine by ethanol and n-hexane at low temperature treatment. Specific activity and actomyosin as total Ca^{+2}-ATPase activity were measured in fresh fish and dried FPC.

Table 4. Specific activity and actomyosin as total Ca^{+2}-ATPase activity of fresh fish and FPC.

Material	*Specific activity	*Specific activity after 10 months of storage	**Total activity	**Total activity after 10 months of storage
Fresh fish	0.11	—	71.6	—
FPA	0.42	0.404	48.0 (100%)	12.6 (26.25%)

* Specific activity: mole Pi/min/mg protein

** Total activity: (actomyosin as total Ca^{+2}-ATPase activity) mole Pi/min/10 g muscle

Table 4 shows that in 10 months storage of FPC, at vacuum with nitrogen, at room temperature (20°C), only a slight change in specific activity was detected and total activity was preserved in 26.5%. These results are in part in agreement with those reported by SUZUKI et al.[2] which reported that fish muscle with low moisture content (4.8%), was very stable to denaturation during storage at room temperature. Is possible to suggest same as SUZUKI et al.[2], that this condition will be much welcome as a new food material as it is ideal for storage and transportation.

Table 5, shows actomyosin as total Ca^{+2}-ATPase activity, specific activity and remainder activity of FPC prepared using 80% and 90% of ethanol concentration.

Table 5. Actomyosin as total Ca^{+2}-ATPase activity, specific activity and remainder activity in FPC prepared using 80% and 90% of ethanol concentration.

Material	*Specific activity	**Total activity	Remainder total activity (%)
Fresh fish	0.074	23.50	
FPC (80% ethanol)	0.16	12.72	54
Fresh fish	0.11	71.6	
FPC (90% ethanol)	0.42	48.0	67

* and ** see table 4.

Remainder total activity of FPC prepared using 90% of ethanol concentration was higher than FPC prepared using 80% of ethanol concentration. This result is in agreement with those reported by FUKUSHIMA[22] which found that ethanol concentration near to 50% produce higher denaturation of soy bean protein than ethanol concentration far from this value.

BRYANT et al.[10] have developed a FPC with high rehydration and emulsifying capacity. Fig. 2, shows emulsifying capacities of FPC prepared by ethanol and n-hexane at low temperature treatment and BRYANT et al.[10] prepared FPC.

Results show that Emulsifying capacity of FPC prepared from sardine by ethanol and n-hexane at low temperature treatment was higher than FPC prepared by BRYANT et al.[10] at high temperature treatment. This result is in agreement with SPINELLI et al.[21] which reported that treatment with solvent at high temperature reduce

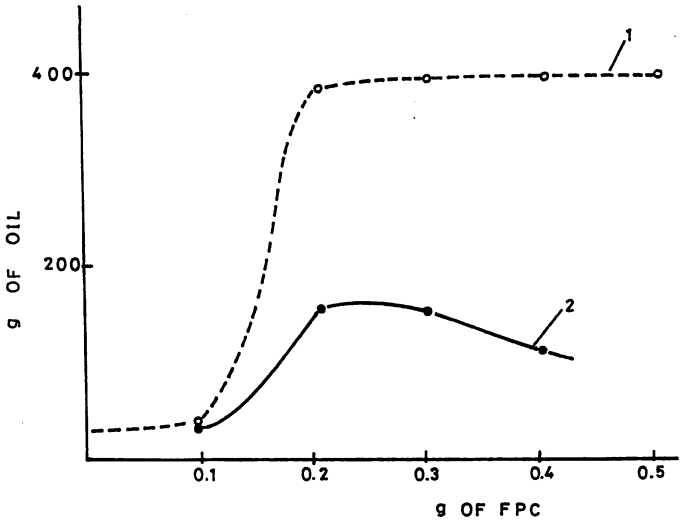

Fig. 2. Emulsifying capacity of different concentrations of FPC (1 g FPC=0.85 g protein) at pH 2.

1. FPC prepared by ethanol and n-hexane at low temperature treatment.
2. FPC developed by BRYANT et al.[10], with high emulsifying capacity.

drastically the functional properties of myofibrillar protein, specially emulsifying capacity which sometimes is completely destroyed. Fig. 3, shows moisture adsorption curve at 35°C of FPC prepared in this report.

The type of isotherm shows a minimum "moisture: equilibrium relative humidity range stability of 4.25. This result is in agreement with ROCKLAND[23], which reported

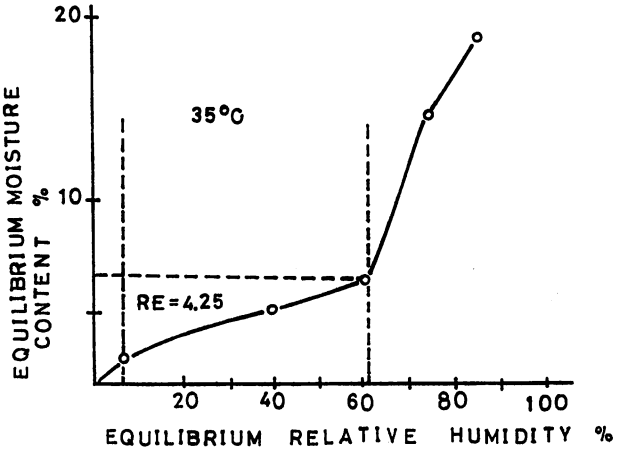

Fig. 3. Moisture adsorption at 35°C of FPC prepared by ethanol and n-hexane at low temperature treatment.

RE: Range stability[22]

similar curve for products with high protein content and small content of starch or cellulose.

Moisture content ranged from 1.25% at 5% RH (relative humidity) to about 19% at 85% RH. The water content of FPC was 4%, and fall in the isotherm zone of minimal chemical changes, ensuring the stability during storage. Rasekh et al[15] reported that 16% of moisture content in FPC although avoid mold growing, is necessary to storage the FPC below this condition, ought to 16% of moisture content appears to represent that portion of the isotherm where capillary condensation of water occurrs.

Fig. 4, shows hydration capacities of FPC prepared by ethanol (90%) and n-hexane at low temperature treatment, and FPC prepared by Bryant et al.[10], at various pH.

Hydration capacity of FPC prepared by ethanol and n-hexane at low temperature treatment, from pH 2 to 9 was higher than Bryant et al.[10] developed FPC. However at alkaline pH, from pH 10 to 12, both FPC showed similar values. After 10 months of storage hydration capacity of FPC prepared in this work was increased

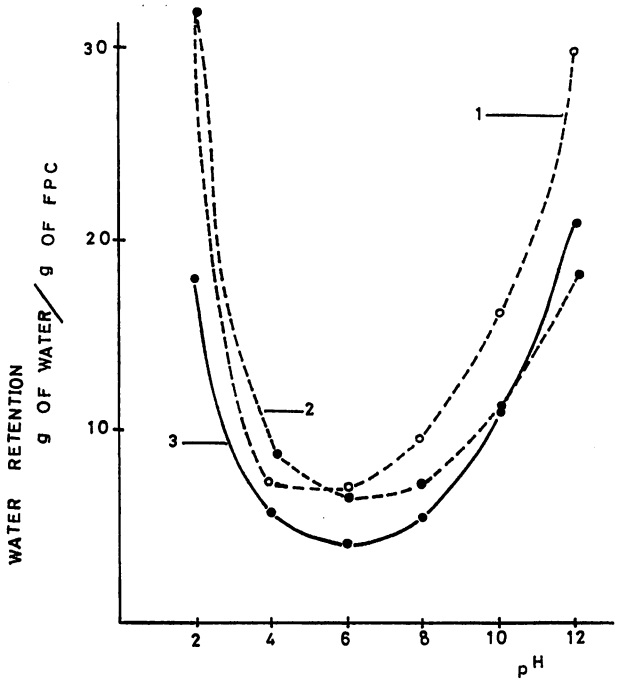

Fig. 4. Hydration capacity of FPC at various pH.

1. Hydration capacity of FPC prepared by ethanol and n-hexane at low temperature treatment.
2. Hydration capacity of FPC prepared by ethanol and n-hexane at low temperature treatment, just after preparation.
3. Hydration capacity of Bryant et al.[10] developed FPC.

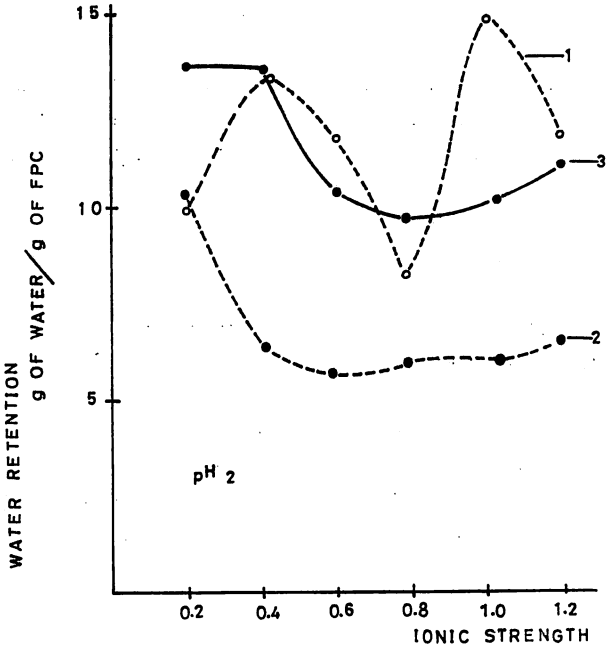

Fig. 5. Hydration capacity of FPC at various ionic strength and pH 2.
1, 2, 3, same as Fig. 3.

between pH 8 to 12. Fig. 5, shows the influence of ionic strength in rehydration capacity of FPC at pH 2, prepared by BRYANT et al.[10] and FPC prepared in this report.

Ionic strength showed to have strong influence in rehydration capacity of FPC. Hydration capacity of FPC prepared by ethanol and n-hexane at low temperature treatment was reduced in 60% at IS 0.2 and from IS 0.4 to 1.2, the reduction was 80%.

After 10 months of storage hydration capacity of FPC prepared in this report was increased between IS 0.4 to 1.2.

Amino acid composition of FPC from sardine prepared in this report was similar to FAO/WHO provisional pattern[24], egg and casein. The first limiting amino acids were sulfur amino acids (methionine, cystine). SIDWELL et al.[25], and SIKKA et al.[26] have also reported similar results using isopropanol and ethanol treatment respectively. Formation of lysinoalanine, in alkali and/or heat treated proteins is of interest to the food industry, due to reports concerning its toxic effects.[27,28] No content of lysinoalanine was detected in FPC from sardine prepared in this work. Table 6.

Table 7, shows E:N (ratio of essential amino acids to nonessential amino acids), E:P (ratio of essential amino acids to protein, 100 g), E:T (ratio of essential amino acids to total amino acids), chemical score (is the percentage of the most deficient essential amino acid in the protein, compared with the requirement pattern) and

Table 6. Amino Acid Composition of FPC from sardine, egg, FAO/WHO Provisional Pattern (1973) g aa/100 g protein.[27]

amino acid	egg	casein	sardine (FPC)	FAO/WHO
Essential aa				
Isoleucine	5.8	4.56	4.63	4.0
Leucine	8.9	7.31	7.06	7.0
Lysine	6.7	6.86	8.90	5.4
Total aromatic aa	10.3	9.77	8.44	6.1
Phenylalanine	6.7	4.41	4.09	3.05
Tyrosine	3.6	5.36	4.35	3.05
Total sulfur aa	5.3	2.73	3.33	3.5
1/2 Cystine	3.0	0.35	0.37	
Methionine	2.3	2.38	2.96	
Threonine	5.1	4.30	5.04	4.0
Tryptophan	1.5	1.24	1.58	1.0
Valine	7.5	5.26	4.69	5.0
Nonessential aa*				
Arginine	6.7	3.28	6.28	5.2
Glycine	3.6	1.56	3.70	2.2
Aspartic acid	10.40	6.04	9.32	7.7
Serine	6.0	3.87	3.84	7.7
Histidine	3.5	1.76	3.06	2.5
Alanine	3.5	2.16	4.86	6.1
Glutamic acid	25.2	20.45	14.89	14.7
Proline		9.98	3.30	10.7
Ammonia		9.17	7.36	
Lysinoalanine**			—	

* A mixture of nonessential amino acids in FAO/WHO provisional pattern based on the proportion of these amino acids as found in skim milk protein[24]

** Lysinoalanine was determined using MOORE et al. method[14] for usual amino acids, but citrate elution buffer concentration was changed to 0.2 N.

*Table 7. E: N, E: P, E: T, ratios, chemical score and EAAI of FPC made from sardine by ethanol and n-hexane at low temperature treatment and casein.

	E: N	E: P	E: T	EAAI (%)	Chemical score (egg) (%)	Chemical score (FAO/WHO) (%)
FPC	0.77	0.44	0.43	85.46	62.8	95.1
Egg	—	—	—	100	100	—
FAO/WHO[27]	—	—	—	—	—	100
Casein (Merk)	0.72	0.42	0.419	82.25	51.51	78

* Nomenclature in the text.

EAAI (essential amino acid index: is based on the ratios of the amounts of essential amino acids in a protein relative to this amount in whole egg protein).[24]

E:N, E:P, E:T, chemical score and EAAI of FPC prepared by ethanol and n-hexane at low temperature treatment were higher than Merk casein.

Table 8, shows in vitro protein digestibility and computed protein efficiency ratio (C-PER) of FPC prepared from sardine by ethanol and n-hexane at low temperature treatment and standard Merk casein. Protein digestibility of FPC was higher than standard casein, 94.62%, and 88.28% respectively.

Table 8. In vitro protein digestibility and computed protein efficiency ratio (C-PER) of FPC prepared by ethanol and n-hexane at low temperature treatment and casein.

Material	Digestibility (%)	C-PER
FPC	94.62	*2.83
Casein	88.28	*3.0

* Value reported by SIKKA et al.[26] and SIDWELL et al.[25]

C-PER of was 2.83. This result was similar to those reported by SIDWELL et al.[25] and SIKKA et al.[26] using rats-feeding. According to the results is possible suggest that FPC prepared from sardine by ethanol and n-hexane at low temperature treatment has a nutritive value similar to casein.

Conclusions

The results show that FPC prepared by ethanol (90%), and n-hexane at low temperature treatment have nice functional and nutritional properties. Represented by the results reported in emulsifying and hydration capacities, and protein content, protein digestibility, C-PER, and amino acids profile.

After 10 month of storage at room temperature with nitrogen gas, only a slight changed in odor was detected. The principal problem was related with the stability of FPC during the storage, although TBA number increased after 10 months of storage, the rate of oxidation was low compared with oxidized samples[12].

Using low temperature alcohol treatment in FPC preparation, was possible to avoid in part the protein denaturation during preparation and storage.

The use of antioxidants in view to ensure the complete stability of FPC during preparation and storage must be investigated. In this report was used BHA (butylated hydroxyanisole) 0.05%, solution which was added at the step of rinsing with water. Is possible that using a mix of BHA and BHT (butylated hydroxytoluene)[29] was more effective on preventing oxidation of residual lipids during processing and storage.

Also is necessary to study the compatibility of FPC in food formulation.

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