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A NOVEL METHOD FOR THE PRESERVATION OF DEODOURIZED FISH PROTEIN AS POWDER BY THE USE OF EDIBLE SURFACTANTS

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A stable, wholesome, non toxic, nonhygroscopic and deodourized fish protein powder has been developed by an economically feasible process aimed at eliminating solvent extraction of oils and fats. The product also facilitates the fighting protein malnutrition because transportation of fresh or frozen protein to distant places often is not possible. Bio-technical problem has totally been abrogated by controlling the biochemical problem. It has been possible to protect lipid in fish protein, so as to stop its deterioration inspite of having lipid content as high as 20%. The protein powder is devoid of any hazardous elements. Innovation of the process is to preserve the most perishable valuable part of the fish at room temperature.

Key words: Fish protein, Protein powder, Edible surfactants.

Introduction

The term fish protein concentrate (FPC) is understood [1] to include any form of dried fish including fish meal intended for human consumption. Storage requirements are taken to involve satisfactory behaviour for six months at a temperature upto 37.8°. There is a technological problem of preventing the rancidity: Exclusion of oxygen is the basic approach but fundamental studies are needed. There is a major technological problem of getting the antioxidants into the product so that every lipid particle or droplet is protected [1].

Most methods were either be set with processing problems or resulted in products that were not approved by nutritionists, pediatricians, FAO and others. FDA informally ruled out that FPC could not be made from fish containing viscera, skeleton, tails and heads [2].

The authors for the University of Chile in Santiago described a method of treating FPC with detergent sodium dodecylsulphate (SDS) in order to reduce the lipid content.They succeeded in significantly lowering the lipid content from that of the starting material, but it raised serious objections because SDS converts protein into foam [3]. The product was not edible.

Major attention was given to develop a solvent process using isopropyl alcohol, to extract red hake. Later efforts were directed towards the use of other species of fish for production of FPC [4].

Nabisco Astra Nutrition Development Corporation produced fish protein concentrate from eviscerated fish rather than whole fish [5]: but the protein could not be preserved to the extent of even packing it into polythene bags.

Commercial production and acceptance of FPC has been slower than at first expected. Apparent reasons may be as follows:-

(i) Technological production problems were greater than expected.

(ii) Better quality standards and products were needed to satisfy food regulatory agencies.

(iii) Considerable capital investment and sophisticated technology was needed.

(iv) It was difficult to broaden the consumer acceptance [6].

FPC prepared by hot solvent extraction of whole fish is a protein source of high nutritive value. However, it is a denatured product with poor functional properties. When tasted after rehydrating, it has a very gritty mouth feel. In general, incorporation of FPC into food products with desirable characteristics has met with little success [7].

Kastro *et al.* [8] extracted lipid from fish protein isolate using binary system of organic solvents (Hexane: isopropanol) containing non-ionogenic surface active substances (NSAS). The protein isolates, freed of lipids by the described treatment, has no fishy odour during storage for 1 year. Nothing has been mentioned about the biotechnical problem of FPC.

With the aid of biochemical and biotechnical innovations it has been possible to develop a stable marine protein isolate. It is composed of loosening of skin, visceral organs and skeleton by the action of chemicals. This is followed by the elimination of all these undesired elements with a view to preserving most perishable valuable food with the help of edible surfactants. The most important achievement is that the product has been obtained in powder form by processing it in water. The product is deodourised non-hygroscopic and has a shelf life of more than 36 months without deterioration of fish protein.

Experimental

The process begins with mechanical removing of heads and tails from market fresh fish*. It is followed by a chemical

bath [9] where skins and guts are loosened for 12 hr. by maintaining a temperature of 55° with mechanical shaking. The chemical bath is composed of 3-6% [9] chemicals and 200-500% water on the weight of raw fish. Skins and guts are removed under 1 atmosphere pressure of water. Corium, fat and skeleton are further loosened in a fresh chemical [9] for another 12 hr. under the same conditions.

The flesh and bones are separated through a mechanical device and shower of 1 atmosphere pressure of water is again operated so as to remove chemicals.

The pulp is hydrolysed in 5% hydrochloric acid solution for 6 hr. with constant stirring and heating upto 55°. It is further neutralized with 33% ammonia solution for 3 - 4 hr. till the pH of the hydrolysed protein is near neutral.

It is further treated with 2.5 % solution of edible surfactants [10] on the weight of fish till all of oil is emulsified at 55°. The amount of surfactant may vary from 1-10 % depending on the lean or fat fish. The pH of the emulsified protein slurry is again adjusted to pH 5.2 with hydrochloric acid.

The protein cake is obtained from buchner funnel/muslin cloth and vacuum.

The cake is dried at 40-50° with hot air blowing and is milled to obtained powder.

The yield is 18 % of the fresh fish. It may vary from 8 - 20 % depending on the kind of fish.

The species of the fish utilized in processing were: (i) *Diagramma* species, (ii) *Sardinella* species, (iii) *Otlithus* species.

Results and Discussion

Statistical analysis. Standard deviation for the levels of protein deterioration was used to compare the storage treatment of products (Table 1). Standard deviation of the three samples A, B, and C has been found 0.4, 0.37 and 1 and relative deviation 0.4953, 0.4995 and 0.4993 respectively.

Standard deviation for the level of trimethylamine production (Table 2) showed s.d. 1.1, 0 and 1.4 for the samples A, B and C and relative deviation of 0.4995, 0.4861 and 0.4 9984 respectively. All these fall within the practical useful statistical limits.

Table 3 shows the microbiological analysis of the fish protein powder in which the micro-organisms have been reduced from 3 - 4.5x 10^3/g against the FDA regulation of edible limit of 500 -10,000 CFU/gm. Table 4 shows the miscellaneous parameters tested of the preserved fish protein powder.

In this paper emphatic point was to creat an economically feasible method for the production of fish protein powder. The other parameters about amino acid contents and admitted biological values have not been taken into consideration in this research.

Efforts were made during the past by many scientists of the world to resolve this problem but none of them has met with little success.

The protein preserved in these laboratories showed neither any liquefaction of protein powder nor any significant change in protein level; change in total volatile levels varied for 15 -110 mg/100gm of the sample during 36 months of storage.

TABLE 1. LEVELS OF PROTEIN (Nx6.25 gm %) IN DIFFERENT LABORATORY PREPARED SAMPLES DURING STORAGE AT ROOM TEMPERATURE (22-38°) [11].

Storage time (months)	Sample A	Sample B	Sample C
6	35	39	42
12	35	39	38
18	35	39	40
24	36	41	39
30	33	40	39
36	34	39	34

± S.D/A = 0.4; ± S.D/B = 0.37; ± S. D/C. = 1
Relative Deviate of Standard Curve = 0.4953; R.D.S.C. = 0.4953; R.D.S.C. = 0.4993
A = *Diagramma* species, B = *Sardinella* species, C = *Otolithus* species. Three replicate were performed.

TABLE 2. LEVELS OF TMA-N (mg/100gm) IN DIFFERENT LABORATORY PREPARED SAMPLES DURING STORAGE AT ROOM TEMPERATURE (22 - 38°). [12].

Storage time (months)	Sample A	Sample B	Sample C
6	8	4	2
12	9	4	5
18	11	4	8
24	12	4	11
30	14	4	11
36	16	4	11

± S.D/ A = 1.1 ± S.D / B = ZERO ± S.D / C = 1.4
*R.d.S.C. = 0.4995 R.D.S.C=0.4861 R.D.S.C. = 0.49984
*Relative deviate experesses the distance of a value from the centre of the curve in units of Standard Deviation. Three replicates were performed.

TABLE 3. MICROBIOLOGICAL ANALYSIS OF FISH PROTEIN POWDER: [13a, b, c].

Test	Results
1. CFU/g	4.5x10^3
2. MPN *Coliforms*/g	3
3. MPN *Faecal coliforms* s/g	3
4. Yeast count /g	Nil
5. Mold count/g	20
6. *Salmonella*/25g	Absent

Three replicates were performed.

Since all the processing has been performed in water, thereby it has reduced the microbial flora to a considerable low level of 3 - 4500 (CFU/gm) of powder and is devoid of hazardous elements. Miscellaneous tests performed have shown that fish protein powder can take moisture upto 22% when the artificial humidity is maintained at 76% with sodium chloride solution.

The increase in melting point of the preserved oil from 33- 35° depicts that the oxygen on the unsaturated bond of oil has not been allowed to initiate the process of oxidation which in turn is responsible for deterioration of fish protein.

The product was subjected for acute (72 hr.); and chronic (28 days) toxicity tests on normal adult healthy mice. No animal showed any untowards effect during the said observation period (Table 4).

TABLE 4. MISCELLANEOUS PARAMETERS TESTED FOR THE PRODUCT.

1. *100 gm of the *Diagramma* species protein powder contains: (i) Protein 35%; (ii) Moisture = 19% at 50° for 48 hr.; (iii) *Oils/fats =20% (iv) Carbohydrate = 26%; (v) Ash = Nil (vi) Melting point = 14-16° of the oil; (vii) Melting point of the preserved oil = 33-35°; (viii) Moisture percent determined at76% humidity (NaCl). = 22%
2. Toxicology: The product did not show any untoward effect on normal adult healthy mice when, subjected, for acute (72 hr.) and chronic (28 days) toxicity tests.
3. Pink colour was observed for three samples of proteins in Biuret reaction which depicts the presence of dipeptide.
4. Presence of blue colour with ninhydrin indicates the free carboxyl and amino group in all the three samples of the proteins.
5. Appearance of voilet ring shows the presence of carbohydrates in fish proteins in Molisch reaction.

* Solvent system Ethanal: ether : 3:1

Tests performed such as Biuret, Ninhydrin and Molisch showed that the preserved protein is having the dipeptide with intact carboxylic and amino groups and is not a denatured protein and also contains carbohydrates.

Conclusion

Fundamental research reveals that fish protein can be preserved as powder for more than 3 years without deterioration at ambient temperatures (22-37°). The term biotechnical problem is misnomer provided the mechanism of biochemical problem is completely understood. There is no technological problem of getting the antioxidants into the product. It is feasible to protect every lipid particle intact in fish protein powder inspite of having lipid content as high as 20% or more.

The main hypothesis behind this research was exclusion of oxygen from the fish protein lipid concentrate by most of the workers, which has been achieved successfully.

Extraction of oil/fat with solvent system was a wastage. The dipeptide product bears a direct suitability for incorporation into veaning foods with few exceptions where the solubilization is the primary requirement.

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