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CHANGES IN THE PROTEIN FRACTION OF *MERLUCCIUS BILINEARIS* MUSCLE UNDER LACTIC ACID BACTERIAL FERMENTATION USING A *LACTOBACILLUS ACIDOPHILUS* STARTER CULTURE

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RESUMEN

Se evaluó el efecto de fermentación láctica sobre la fracción proteica del músculo de Merluccius bilinearis. *En nitrógeno no proteico aumentó con el tiempo de fermentación indicando acción bacteriana proteolítica. Se observaron aumentos significativos en los porcentajes de los amino ácidos cistina, isoleucina, fenilalanina y tirosina después de dos meses de fermentación. Los porcentajes de arginina disminuyeron significativamente después de una semana, y posteriormente, a los dos meses del tratamiento.*

ABSTRACT

The effect of lactic acid bacterial fermentation on the protein fraction of Merluccius bilinearis *muscle was evaluated. The non-protein fraction increased progressively with corresponding decreases in the percentage protein (dry weight) indicating proteolytic activity during fermentation. Significant increases in the percentages of the amino acids cystine, isoleucine, phenylalanine and tyrosine were observed after two months of fermentation. Percentages of arginine decreased significantly after one week and again after two months of fermentation.*

INTRODUCTION

Lactic acid bacteria are Gram Positive asporogenous prokaryotes typified by their production of lactic acid as an end product. In the food industry lactic acid bacteria are commonly used for the production of various fermented milk products such as cheese and yogurt (Batt, 1986). Meat and vegetable fermentations are less developed in the food industry. In oriental countries fermentation techniques have been used in the development of various fish products, mainly fish pastes and sauces (Burkholder, 1968; Steinkraus, 1983). However, research in the area of microbial genetics, starter cultures and process technologies is still needed (Wasserman, B. *et al.*, 1988).

The ability of lactic acid bacteria to utilize protein is weak as compared with other groups of bacteria, however, they present a complex proteolytic system and have absolute requirements of preformed amino acids for their growth. The proteolytic systems of lactic acid bacteria are important from the rheological point of view for the curing or maturation processes of fermented products (Law and Kolstad, 1983).

The purpose of this investigation is to study

the changes in the protein fraction of fish muscle preparation during fermentation with a *Lactobacillus acidophilus* starter culture.

MATERIAL AND METHODS

Preparation of the fish muscle

Whiting *(Merluccius bilinearis)* was purchased at local seafood markets in Point Judith, Rhode Island, USA. The flesh was separated from the skin manually using a sharp knife and further strained using a mincing attachment (Hole diameter of 5 mm) of a kitchen Aid Mixer (Model DLC-7, Hobart Corp., Troy, Ohio).

Microorganisms

Lactobacillus acidophilus ATCC 9224 strain culture was purchased from the American Type Culture Collection (Rockville, Maryland). The culture was propagated in skim milk medium (Difco 0-032-01, Difco Laboratories, Detroit, Mich.) and incubated at 100 rpms for 24 hours at 37°C. One milliliter of the revived culture was placed in 2 ml vials and stored at −70°C until further use.

Sample analysis

Total solids and ash content were determined following the AOAC (1980) procedures. Lipid content was determined using the method of Bligh and Dyer (1959). Total nitrogen was determined by digesting the sample using the micro-Kjeldahl procedure (AOAC, 1980) and measuring nitrogen with an ammonia electrode attached to an Orion microprocessor ionanalyzer model 901 (Orion research Corp., Cambridge, Mass.). The non-protein fraction (NPN) was determined by precipitating the protein with 17 °/o trichloroacetic acid solution by the same method. Total protein nitrogen was calculated by substracting the NPN from the total nitrogen. Carbohydrates were calculated by difference.

Amino acid analysis

a. *Sample preparation:*

Frozen samples of the fermented fish muscle were evacuated by submitting the samples to three freeze-thaw cycles under vacuum using liquid nitrogen. Evacuated samples were hydrolyzed in 5 ml 6N HCl at 110°C for 24 hours. Hydrolyzates were filtered through sintered glass and then evaporated to dryness. Dried samples were washed two times with 1 ml deionized water (DI), evaporated to dryness and redissolved in 2 ml of 0.1 N HCl, filtered through 0.45 μm membrane filters (Gelman Sciences, Inc.) and stored at −30°C until further use.

b. *HPLC analysis:*

The amino acids were analyzed using a Perkin Elmer High Performance Liquid Chromatograph (HPLC) (Norwalk, Conn.) for post column amino acid analysis. This consisted of a Series 4 Solvent Delivery System two post column reagent pumps, an RTC-1 reaction temperature control, L-C 10 fluorescence detector and a Model 3600 Chrom-2 data station. Separations were achieved on a AA 911 amino acid cation ion-exchange column (0.46 x 25 cm) of sulfonated polystyrene-divinylbenzene (Interaction Chemicals, Mountain View, Ca.) equilibrated at 52°C with a flow rate of 0.7 ml/min as described in the Perkin Elmer amino acid Analysis System Manual (1983). The isoindole derivatives were detected with excitation at 350 nm and emission at 418-700 nm. Buffers of pH 3.15 and 7.4 and o-phthalaldehyde diluent were purchased from Pickering Laboratories (Mountain View, Ca.). Sodium hypochlorite (NaOCl) (4-6 °/o) was purchased from Fisher Scientific Corp. (Fair Lawn, New Jersey). The amino acid standard was purchased from Pierce Chemical Corp. (Rockville, Illinois).

Whiting mince fermentation

A pure culture of *Lactobacillus acidophilus* was grown as described before. The culture was allowed to grow until a cell density in the range of 2.0 - 8.0 x 10^6 cells/ml was reached. The fish muscle was mixed with a 2.5 °/o NaCl solution at a 2:1 ratio (mince:solution) at 35°C for 15 minutes after which 10 °/o (g/100g fish) molasses (Grandma's Molasses, Duffy Mott Co., Inc., Stanford, Conn.) and 15 °/o (ml/100g fish) inoculant were added. The mixture was placed in 500 ml nalgene polypropylene bottles (Thomas Scientific, Balance, Phil.) and stored at 25°C.

Samples were taken at 0, 1, 6 and 8 weeks of fermentation. Samples of the drained muscle mince and the liquid drained were analyzed for protein content as described before at 0, 1, 2 and 6 weeks of fermentation. The moisture content of the samples was determined and the protein calculated as percentage of the dry weight. Changes in the amino acid composition of the whiting mince were monitored for a period of 7 weeks. Samples

TABLE 1. **Protein changes of the fish muscle-molasses mixture during fermentation with *Lactobacillus* (ATCC 9224)[a].**

Time (week)	*°/o Moisture (g/100g)*	*Total N (g/100g)*	*Non-Protein N (g/100g)*	*Protein[b] N (g/100g)*	*°/o Protein[c]*
Fish Mince:					
0	87.7+–0.1	1.37+–0.21	0.009+–0.002	1.36	69.3
1	84.6+–0.4	1.55+–0.09	0.019+–0.001	1.53	62.2
2	84.6+–0.2	1.55+–0.09	0.026+–0.001	1.52	59.6
7	83.5+–0.1	1.49+–0.08	0.037+–0.003	1.45	54.8
Drained Liquid:					
0	93.4+–0.1	0.20+–0.01	0.008+–0.001	1.19	17.9
1	91.9+–0.1	0.36+–0.02	0.025+–0.003	2.06	25.4
2	90.0+–0.6	0.45+–0.00	0.028+–0.001	2.63	28.9
7	92.8+–0.3	0.48+–0.05	0.042+–0.005	2.75	38.7

a. Values represent the mean +– std. of single determinations of duplicate samples.

b. Protein N = Total N — Non-protein N

c. °/o Protein (dry weight) = ((Protein N x 6.25)/(100 — °/o H_2O)) x 100

were taken at day 0 and then after 1, 2 and 8 weeks. Amino acids were determined as described before.

RESULTS AND DISCUSSION

As can be seen in Table 1, a progressive reduction in the percentage protein (dry weight) of the fish mince with a corresponding increase in the non-protein nitrogen is observed throughout 7 weeks of fermentation.

The percentage protein in the fish muscle mince was reduced from 69.3 to 54.8 °/o (dry weight) in this period of time. In the drained liquid, the percentage protein increased from 17.9 to 38.7 °/o (dry weight) in 6 weeks indicating liquefaction of the fish muscle protein. Raa (1981) suggests that the liquefaction observed during fermentation is mainly due to the presence of proteolytic enzymes originally present in the fish. The degree of proteolysis must therefore be taken into consideration in the development of fish products using bacterial fermentation. Also, a pH of 4.2 was obtained after 2-3 days of fermentation. At this low pH, the growth of most pathogenic and spoilage flora will be inhibited (Banwart, 1979). This inhibition will eliminate microbial competition and the growth of lactic acid bacteria will be favored.

Changes in the amino acid composition of the fish muscle-molasses mixture at different stages of the fermentation are shown in Table 2.

Significant increases ($P < 0.05$) in the amino acid percentages of cystine, isoleucine, tyrosine and phenylalanine were observed after two weeks of fermentation. The percentages of arginine were reduced from 6.9 to 6.5 in one week and then to 5.2 after 8 weeks of fermentation. As reported by Jonsson (1979), arginine can be readily decomposed by lactic acid bacteria in the absence of glucose. Cheeseman and Fuller (1966) reported that arginine can be metabolized by some strains of lactic acid bacteria by a dehydrolase system in which arginine is metabolized into citrulline and ornithine. However, these authors report that *Lactobacillus acidophilus* do not produce arginine dehydrolase required to achieve this conversion. The reduction of arginine in the system may be an indication of the presence of other species of acid tolerable bacteria present in the whiting mince before inoculation.

TABLE 2. **Changes in the amino acid composition of the fish muscle-molasses mixture during fermentation with *Lactobacillus acidophilus* (amino acid (a.a) composition expressed as grams a.a/100 grams of total a.a.).**

Amino acid[a]	*Time (weeks)*			
	0	*1*	*2*	*8*
Aspartate	10.5+–0.4	10.9+–0.2	11.0+–0.2	11.4+–0.2
Threonine	4.2+–0.1	4.1+–0.1	4.3+–0.1	4.7+–0.2
Serine	3.3+–0.2	3.4+–0.0	3.4+–0.0	3.4+–0.1
Glutamate	17.1+–1.0	17.7+–0.1	17.7+–0.3	17.5+–0.3
Proline	3.2+–0.2	3.0+–0.2	3.1+–0.1	3.2+–0.0
Glysine	3.6+–0.2	3.8+–0.0	4.0+–0.2	3.8+–0.0
Alanine	5.6+–0.3	6.0+–0.0	6.1+–0.0	6.0+–0.1
Cystine[b]	0.4+–0.1	0.6+–0.0	0.7+–0.0	0.8+–0.0
Valine	5.5+–0.1	5.5+–0.2	5.4+–0.2	5.6+–0.1
Methionine	2.8+–0.2	3.0+–0.1	2.9+–0.1	2.9+–0.4
Isoleuc[b]	5.1+–0.1	5.1+–0.1	5.3+–0.1	5.8+–0.0
Leucine	8.6+–0.4	9.1+–0.0	9.0+–0.0	9.1+–0.1
Tyrosine[b]	3.1+–0.1	3.7+–0.0	3.7+–0.0	3.8+–0.1
Phenylal[b]	4.4+–0.1	4.4+–0.1	4.5+–0.0	4.8+–0.0
Lysine	10.1+–0.9	9.5+–0.3	9.3+–0.4	8.3+–0.7
Histidine	2.6+–0.2	1.6+–0.5	1.7+–0.3	2.1+–0.0
Arginine[b]	6.9+–0.0	6.5+–0.0	6.4+–0.1	5.2+–0.0

a. Values represent the means +– std. dev. of one determination on duplicate samples.

b. A significant change was observed ($P < 0.05$). Underlined amino acid means are not significantly different.

CONCLUSION

Losses in the protein content of the fish mince are evidenced by a progressive reduction in the percentage protein (dry weight) with corresponding increases in the non-protein fraction in the fish mince. A 10 °/o reduction of the percentage protein (dry weight) observed in the solid fraction after one week of fermentation indicates that if this fermentation technique is to be applied in the formulation of fish products, these should be made soon after the desired pH for antimicrobial growth (4.2) is reached (2-3 days). Maximum utilization of the fish protein can only be accomplished if both the protein in the liquid and solid fractions of the fermented mixture are used in product formulations. Further research can be focussed in this area. The increases observed in the amino acid percentages for cystine, isoleucine, tyrosine and phenylalanine, all essential amino acids, indicate an improvement in the nutritional value of the fish mince after the fermentation process.

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