Food Chemistry 31 (1989) 41–50

Changes in Some Chemical Characteristics and Lipid Composition of Salted Fermented Bouri Fish Muscle (*Mugil cephalus*)

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(Received 2 December 1987; revised version received and accepted 4 March 1988)

ABSTRACT

*'Feseekh' is the traditional name in Egypt of salted-fermented Bouri Fish (*Mugil cephalus*). The effect of the salting-fermenting process on gross chemical composition was studied. The lipid content decreased from 12·6 to 11·2% in small fish and from 7·5 to 6·3% in large fish accompanied by an increase in peroxide value and production of free fatty acids. TLC plates were used to study the lipid classes and individual phospholipids of fresh and salted fish. Phospholipids (PS), monoglycerides (MG) and triglycerides and hydrocarbons (TG & HC) showed a decrease while diglycerides (DG) and free fatty acids (FFA) showed a significant increase. The process resulted in a depletion in phosphatidyl inositol (PI), phosphatidyl choline (PC), sphingomyelin (SL) and phosphatidyl ethanolamine (PE). At the same time phosphatidic acid (PA) and lysophosphatidyl choline (LPC) showed significant increases. GLC analysis was used to study the fatty acid composition of fresh and salted fish. The salting and fermenting process of fish resulted in a significant increase in C 16:0, while C 20:4, C 22:5 and C 22:6 showed a great decrease. The ratio of unsaturates/saturates (U/S) decreased after the salting and fermenting process.*

INTRODUCTION

Salting of food and particularly salting of fish is an ancient treatment still in use nowadays even in developed countries, either for economic reasons,

Food Chemistry 0308-8146/89/$03·50

owing to its low production costs, or in order to satisfy consumer habits. In Egypt 'Feseekh' is the Arabic name for a salted fermented Bouri fish. Whole noneviscerated fish are washed with tap water, and left to decompose for a day before salting. The salting process involves stuffing of the gills and covering of the entire fish with approximately 15–25% salt by weight. Historically, fish fermentation has been associated with the treatment of fish with salt. Normally, fermentation is taken to mean the transformation of organic substances into simpler compounds by the action of enzymes or microorganisms. This definition covers the traditional methods of fermentation as applied to fish products reasonably well (Mackie *et al.*, 1971). Cole (1963) reported that fermented products are autolyzates, that is, they are products in which the protein of the fish is largely or partly broken down to its constituent amino acids in the absence of air. He also added that, if air is admitted during processing, the proteins putrefy instead of fermenting.

Amano (1961) studied the influence of fermentation on the nutritive value of fish. He reported a nitrogen loss and developing rancidity.

Shahine (1956), studied the chemical composition of salted-fermented fish 'Feseekh' at various stages of fermentation. His work was focused on protein, nitrogenous compounds and volatile fatty acids. Little information is available regarding the lipid composition of salted fermented Bouri fish.

This investigation was undertaken mainly to study the lipid composition of Bouri fish obtained from El-Zawia Fish Farm at Kafr El-Sheikh in either small or large sizes. 'Feseekh' was prepared and the effect of this salting-fermenting process on some chemical characteristics, lipid classes and fatty acid composition was determined.

MATERIALS AND METHODS

Materials

Fresh Bouri fish were obtained from El-Zawia fresh water fish farm at Kafr El-Sheikh. The average length and weight of the small and large fish sizes used are shown in Table 1.

Preparation of 'Feseekh'

The salting of fish was conducted inside three perforated plastic boxes of about 3 kg each. The fish was kept immersed in the drip by covering the mouth of the container by a plank weighted with stone. The container was kept under non-aerobic conditions for approximately one month at room

TABLE 1
Average Size and Weights of Small and Large Bouri Fish (*Mugil cephalus*)

Average lengths and weights of one fish	*Small size*	*Large size*
Total length (cm)	22·4	26·5
Length of head (cm)	3·6	4·2
Weight of whole fish (g)	107·0	220·8
Head weight (g)	20·0	51·5
Weight of viscera (g)	6·3	13·6

temperature. The product thus prepared had a yellowish colour, and a characteristic flavour. The yield was approximately 80%. Six salted fish were filleted, minced and mixed well to form a representative sample. Portions were taken immediately for each of the following analyses.

Methods

Moisture, sodium chloride content and ash content were determined according to the method of the AOAC (1975).

The lipid content was determined by extraction with $CHCl_3$:MeOH (2:1) using the procedure described by Kates (1972) which is a modification of Bligh & Dyer (1959). Aliquots were taken for the determination of total lipds by the procedure of dry weight determination (Kates, 1972).

Free fatty acids and peroxide values were determined on aliquots of total lipid extract using the methods of the AOCS (1957). A mean equivalent of 300 has been assumed for the FFA.

Total phospholipid phosphorus was determined on aliquots of the total lipid extract by the method of Rouser *et al.* (1970).

Separation and quantification of lipid classes

Lipid classes were separated on TLC chromatographic plates coated with Silica Gel G (0·5 mm thickness) and a solvent system composed of petroleum ether:diethylether:acetic acid (70:30:2) using the method of Rouser *et al.* (1970). The separated visualized and identified lipid classes were quantified by scanning at 700 nm ('Shimadzu CS-190').

Separation of phospholipid class

The phospholipid class was separated from the total lipid extract by the same method as for separating lipid classes. The phospholipid class

remained at the origin of the plates and was scrapped and eluted by the method of Braddock & Dugan (1972) modified by El-Sebaiy *et al.* (1980). Total phospholipid phosphorus was determined in this class by the method of Rouser *et al.* (1970).

Separation and quantification of individual phospholipids

Individual phospholipids were fractionated and quantified using the method described by Bunn *et al.* (1969). Borated silica gel plates and a solvent system composed of chloroform:methanol:water (65:25:1) were used. Identifications of individual phospholipids were made by specific spray reagents and a lipid extract prepared from albino rat which contained the known fractions of phospholipids.

Fatty acids analysis

The methyl esters of the fatty acids were prepared by the method of Hilditch & Williams (1964), and analyzed by GLC using a GCV chromatograph (PYE Unicam) equipped with flame ionization detector using the following conditions: PEGA, 10% column, initial temperature, 60°C, rate, 8°C/min, final temperature, 190°C, detector temperature, 300°C, injection temperature, 220°C, chart speed, 2 min/cm; gas flow rate, 30 ml/min for nitrogen and 33 ml/min for hydrogen and 330 ml/min for air. The identification of the esters was carried out using authentic samples. The quantitative evaluation of the chromatograms was based on the area of each peak calculated using a Zero Setting Compensating Planimeter. The results of fatty acids analysis are expressed as the percentages occupied by each component methyl ester peak relative to total peak area. Response factors for all components were not determined, but the peak areas obtained when measured amounts of pure individual fatty acid methyl esters and standard mixtures (imported from Applied Science laboratories, Inc. State College, Pennsylvania) were applied to the chromatograph at intervals, served as a guide to response.

RESULTS AND DISCUSSION

Some chemical characteristics of fresh and salted fermented Bouri fish

After the end of the salting and fermenting process, the yield of Feseekh obtained was about 80% of the original weight. Table 2 shows some chemical characteristics of fresh and salted fermented Bouri fish, in small and large sizes; namely, moisture content, sodium chloride, crude protein,

TABLE 2
Some Chemical Characteristics of Fresh and Salted Fermented Bouri Fish Small and Large Size (Feseekh)

Analysis	*Bouri fish treatments*			
	Small size		*Large size*	
	Fresh	*Salted fermented*	*Fresh*	*Salted fermented*
Moisture content %	77·30	64·10	76·10	65·5
Sodium chloride %	0·00	12·30	0·00	15·70
Ash content %	4·85	17·18	4·75	20·42
Crude protein %	82·50	71·10	87·40	73·20
Lipid content %	12·60	11·20	7·50	6·30
Free fatty acids (as g/100 g lipid)	3·80	24·85	4·20	15·71
Peroxide value	4·31	26·86	4·88	14·62
Phospholipid phosphorus (% in dry muscles)	0·13	0·039	0·265	0·037
Phospholipid phosphorus (% in lipid)	1·04	0·35	3·56	0·59

lipid content, free fatty acids as g/100 g lipid, peroxide value, phospholipid phosphorus in dry muscle and phospholipid phosphorus in lipid.

The moisture content decreased after the salting and fermenting process from 77·3 to 64·1% in the small and from 76·1 to 65·5% in the large fish. The loss in weight of fish and the loss in moisture were accompanied by an increase in the salt and ash contents. The salt content reached 12·3% and 15·7% for small and large sizes, respectively. Ash content increased from 4·85 to 17·18% in small fish and from 4·75 to 20·42% in large fish as a result of increasing salt content. These results are in agreement with those mentioned by Shahine (1956) who studied the composition of salted fermented fish, 'Feseekh', at various stages during a period of 58 days. He noticed a gradual decrease in moisture from 72% to 48·41% and an increase in salt content to reach 17·8%. The loss in moisture and the increase of salt observed by Shahine (1956) was much greater because the period of salting was much longer than that used in our studies. Levanidov (1958) reported the mechanical losses taking place during salting, e.g. dropping of scales, tearing of internal organs, and the losses which are the differences between water and organic substances.

Several factors affect the rate of salt penetration in the muscle. Cruess (1951) reported that the rate of salt penetration varied with the thickness of flesh, temperature, and surface/volume ratio of the flesh. Narayanaswarny *et*

al. (1980) found that temperature also exerted a definite influence on rates of NaCl penetration and moisture loss; these were maximum at 40°C and minimum at 10°C.

Crude protein decreased from 82·5 to 71·1% in small fish and from 87·4 to 73·2% in large fish. Shahine (1956) found a decrease in crude protein from 71·6 to 51·5%. The decrease in the crude protein content is attributed to the breakdown of proteinaceous material by bacteria and enzymes and leaching out in the brine.

Lipid content of fresh Bouri fish was much greater in small size than in large size. The lipid content decreased after the process of 'Feseekh' preparation from 12·6 to 11·2% in small fish and from 7·5 to 6·3% in large fish, accompanied by free fatty acid production. The production of free fatty acids was greater in the small size bouri; this may be attributed to the higher activity of lipolytic enzymes in small fish. Phospholipid phosphorus decreased from 1·04 to 0·35% in small fish and from 3·56 to 0·59% in large fish. The results show that salt does not inhibit those enzymes responsible for the liberation of free fatty acids from the greater part of the phospholipid group. Such loss in lipids and liberation of free fatty acids has been previously mentioned by Cardin & Bordelean (1957).

The peroxide value of the starting fresh bouri was about the same in the small and large fish. Equivalents of thiosulfate/kg of fat increased about sixfold in the small fish and threefold in the large fish after the preparation of Feseekh. This may indicate that greater proportions of unsaturated fatty acids were liberated and were liable to oxidative decomposition at the double bonds. The resulting substances, mostly ketones and aldehydes, appear to be largely responsible for flavour and taste of the products. These results have been previously reported by Cardin *et al.* (1958) for salted cod. They added that about 90% of the specific flavour is contained in the volatile reducing substances.

Lipid classes of fresh and salted fermented Bouri fish

The composition of total lipids studied by thin-layer chromatography is shown in Table 3. Seven lipid classes were identified and quantified; namely, phospholipids, monoglycerides, diglycerides, free fatty acids, triglycerides and hydrocarbons which contain cholesteryl esters. Phospholipids, monoglycerides and triglycerides and hydrocarbons decreased in both small and large sizes of fish after preparing 'Feseekh'. The decrease was much higher in large size fish. Diglycerides and free fatty acids showed a significant increase. The increase in diglycerides reflects the hydrolysis of triglycerides while the increase of the free fatty acids reflects the hydrolysis of both phospholipids and triglycerides.

TABLE 3
Lipid Classes of Fresh and Salted Fermented Bouri Fish (Small and Large Sizes) (Feseekh)[a]

Lipid classes[b]	*Bouri fish treatments*			
	Small size		*Large size*	
	Fresh	*Salted fermented*	*Fresh*	*Salted fermented*
Phospholipids (PS)	11·4	10·1	10·1	7·23
Monoglycerides (MG)	13·2	5·56	3·67	1·81
Diglycerides (DG)	9·65	15·7	15·6	16·6
Free fatty acids (FFA)	19·3	26·0	23·9	34·3
Triglycerides and hydrocarbons (TG & HC)	46·5	42·7	46·8	40·1

[a] The solvent system used was petroleum ether:diethyl ether:acetic acid (70:30:2).
[b] Average of four determinations.

TABLE 4
Individual Phospholipids of Fresh and Salted Fermented Bouri Fish (Small and Large Sizes) (Feseekh)

Treatments of fish		*Phospholipid classes*[a]							*Total recovered*
		PS	*LPC*	*PI*	*SL*	*PC*	*PE*	*PA*	
Small size									
Fresh	*b*	4·24	3·53	3·53	3·12	9·65	2·18	0·94	27·2
	c	15·6	13·0	13·0	11·5	35·5	8·02	3·46	
Salted-fermented	*b*	5·04	2·94	1·35	1·70	3·53	1·29	3·47	19·3
	c	26·1	15·2	6·99	8·79	18·3	6·68	18·0	
Large size									
Fresh	*b*	4·59	2·00	2·29	3·18	3·18	1·00	0·94	17·2
	c	26·7	11·6	13·3	18·5	18·5	5·82	5·47	
Salted-fermented	*b*	6·06	3·00	0·40	0·53	0·88	2·47	3·53	16·9
	c	35·9	17·8	2·37	3·14	5·22	14·6	20·9	

[a] The sequence of the classes in the Tables as separated on the chromatographic plates.
[b] Phospholipid phosphorus in μg P:20 μl containing 28·24, 21·0, 18·83 and 18·22 μg were applied on the TLC plates. Total recoveries were 96·3, 92·0, 91·2 and 92·6%, respectively.
[c] Phospholipid as percentage of total recovered.

Individual phospholipids of fresh and salted fermented Bouri fish

As shown in Table 4, seven phospholipid classes were identified and quantified in the muscles of small and large Bouri fish; namely, phosphatidyl serine (PS), lysophophatidyl choline (LPC), phosphatidyl inositol (PI), sphingolipid (SL), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) and phosphatidic acid (PA). Trace amounts of phosphatidyl glycerol (PG) were found in all examined sample. The salting-fermenting fish process in both small and large sizes resulted in a depletion in PL, SL, PC and PE. The depletion was much greater in large size fish than in small. At the same time LPC and PA showed a significant increase which indicates higher rates of enzymic hydrolysis of phospholipids.

TABLE 5
Fatty Acid Composition of Fresh and Salted-Fermented Bouri Fish, Small and Large Sizes (Feseekh)

Fatty acids g/100 g fish lipids	*Treatments of Bouri Fish*			
	Small size		*Large size*	
	Fresh	*Salted-fermented*	*Fresh*	*Salted-fermented*
C 14:0	4·37	5·99	6·10	8·27
C 15:0	2·00	2·10	3·33	3·04
C 16:0	9·35	14·5	12·2	16·9
C 17:0	3·27	3·93	5·88	6·00
C 18:0	4·45	5·66	4·64	5·78
$\sum$ Saturates	23·4	32·2	31·1	40·0
C 16:1	19·2	19·4	16·6	16·4
C 18:1	20·1	19·9	17·6	17·6
C 20:1	00·34	trace	0·85	trace
$\sum$ Monoenes	39·7	39·3	35·1	34·0
C 18:2	5·73	5·70	6·44	6·11
C 18:3	3·67	3·82	3·26	3·30
C 18:4	4·55	4·98	3·95	4·27
C 20:4	2·10	0·66	1·44	0·54
C 20:5	6·77	5·88	5·87	5·10
C 22:5	5·10	3·25	4·69	3·12
C 22:6	8·98	4·22	8·12	3·54
$\sum$ Polyenes	36·9	28·5	33·8	26·0
U/S ratio	3·27	2·10	2·21	1·50

Fatty acid composition of fresh and salted fermented Bouri fish

Table 5 shows the fatty acid composition of fresh and salted fermented Bouri fish in small and large sizes. It was observed that both sizes had the same profile of fatty acid structure although the small sizes contain higher percentages of unsaturated fatty acids. The unsaturates/saturates ratio (U/S ratio) was 3·27 in fresh Bouri, small size, and 2·21 in the large size fish. The major saturated fatty acid was C 16:0 and the major unsaturated fatty acids were C16:1 and C 18:1. Appreciable amounts of polyunsaturated fatty acids C18:2, C18:3, C18:4, C20:5, C22:5 and C22:6 were also present. As shown in Table 5, the odd chain 15:0 and 17:0 fatty acids were found, which is considered a unique characteristic of the Bouri fish oil. Such a result was mentioned by Lu *et al.* (1979). However, the reports of the previous studies of Gruger *et al.* (1964), Sen & Schlenk (1964) and Orthoefer *et al.* (1976) indicated that the fatty acid composition of Bouri (mullet) varies somewhat depending upon location of catch, season, sex and age of the fish.

The percent U/S decreased, after the salting–fermenting process, from 3·27 to 2·1 in small size Bouri and from 2·21 to 1·5 in the large sizes. All the saturated fatty acids, especially C 16:0, increased in both sizes. Higher decreases in polyenes were observed in C 20:4, C 22:5 and C 22:6 fatty acids.

REFERENCES

Amano, K. (1961). The influence of fermentation on the nutritive value of fish. In *Fish and Nutrition*. Ed E. Heen & R. Kreuzer, Fishing News Books Ltd, pp. 180–200.

AOAC (1975). *Methods of Analysis of the Association of Official Analytical Chemists.* (12th edn, Washington, DC, USA).

AOCS (1957). *Official and Tentative Methods.* The American Oil Chemists Society. 35 East Wacker Drive, Chicago I, Illinois.

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian J. Biochem. Physiol.*, 1959, **37**, 911.

Braddock, R. J. & Dugan, L. R. (1972). Phospholipid changes in muscle from frozen stored Lake Michigan Coho Salmon. *J. Food Sci.*, **37**(3), 426–9.

Bunn, C. R., Keere, B. B. & Elkan, J. G. H. (1969). A technique for improved thin layer chromatography of phospholipids. *J. Chromatog.*, **45**, 326–8.

Cardin, A. & Bordelean, M. A. (1957). Changes occurring in the fat during processing of salted fish. Fisheries Research Board Can. Progr. Repts. Atlantic Coast Stas., **66**, 16–17.

Cardin, A., Bordelean, M. A. & Laframboise, A. (1958). The composition of the fatty acids liberated from the phospholipids in the preparation of salted Cod fish. *J. Fish Res. Bd Can.*, **15**(4), 555.

Cole, R. C. (1963). Some methods of fish preservation. *Fishing News International*, **2**, 385–90.

Cruess, W. V. (1951). Factors in the salting and smoking of fish. *Canner*, **113**(6), 12–14, (7), 14, 16–18.

El-Sebaiy, L. A., El-Mahdy, A. R., Mostafa, E. I. & Mohamed, M. S. (1980). A modified method for the isolation and quantification of oil seed phospholipids. *Food Chemistry*, **5**, 217–29.

Gruger, E. H., Nelson, R. W. & Stansby, M. E. (1964). Fatty acid composition of oils from 21 species of marine fresh water and shell fish. *J. Am. Oil. Chem. Soc.*, **41**, 662.

Hilditch, T. P. & Williams, P. N. (1964). *The Chemical Constitution of Natural Fats* (4th edn). Chapman and Hall, London, p. 614.

Kates, M. (1972). *Techniques of Lipidology: Isolation, Analysis and Identification of Lipids*. North Holland Publishing Company, Amsterdam, London, American Elsevier Publishing Co. Inc., New York.

Levanidov, L. P. (1958). Quantitative characteristics of fish after salting. Israel Program of Scientific Translation (PST) Catalogue, No. 109, 39–45.

Lu, J. Y., Ma, Y. M., Williams, C. & Chung, R. A. (1979). Fatty and amino acid composition of salted mullet Roe. *J. Food Sci.*, **44**, 676–77.

Mackie, I. M., Hardy, R. & Hobbs, G. (1971). Fermented fish products. F.A.O. Fisheries Reports No. 100.

Narayanaswarny, D., Narasimha Rao, C. V. & Govindan, T. K. (1980). Penetration of sodium chloride during prolonged salting of fish. *Fisheries Technology*, **17**(1), 63–64.

Orthoefer, F. T., Deng, T. C. & Demnison, R. A. (1976). Mullet lipids and fatty acids. Presentation at 73rd SAAS Meeting, Mobile, A1, Feb. 1–4.

Rouser, G., Fieskher, S. & Yamamato, A. (1970). Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids*, **5**(5), 494–6.

Sen, N. & Schlenk, H. (1964). The structure of polyenoic odd and even numbered fatty acids of millet. *J. Am. Oil Chem. Soc.*, **41**, 421.

Shahine, A. B. (1956). Chemical Composition of Salted Fermented Fish 'Feseekh' at Various Stages of Fermentation. MSc thesis. Cairo University.