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Changes in amino acid and fatty acid composition during shelf-life of marinated fish

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Abstract: Quality aspects of marinated fish were examined over a 120 day period. Nutritional quality parameters (proximate composition, fatty acid profile and amino acid profile) were determined. Changes in amino acids and fatty acids were examined in the muscle of marinated fish during storage. Aspartic acid, threonine, proline, glycine, tyrosine and lysine concentrations in marinated anchovy and rainbow trout greatly influenced their quality. Polyunsaturated fatty acid concentrations decreased significantly ($p < 0.05$) in marinated anchovy but not ($p > 0.05$) in marinated rainbow trout, while total saturated fatty acid concentrations increased significantly ($p < 0.05$) in both marinated fish during storage. These changes in fatty acid and amino acid concentrations were found to be useful as an index of freshness and decomposition of marinated fish in storage.

Keywords: marination; rainbow trout; shelf-life; anchovy; amino acids; fatty acids

INTRODUCTION

Soaking foods such as meat, fish and vegetables in a seasoned liquid mixture is called marination. The purpose of marination is for the food to absorb the flavours of the marinade or, as in the case of a tough cut of meat, to tenderise it. Because most marinades contain acid ingredients, the marination should be done in a glass, ceramic or stainless steel container, never in an aluminium one. The food should be covered and refrigerated throughout the process. Marinades are fish products consisting of fresh, frozen or salted fish or portions of fish processed by treatment with edible acids and salt and put in brines, sauces, creams or oils. Marination preserves fish by means of sodium chloride and acetic acid solutions. Today, herring is the basic raw material for marinades, although they are also made from sprat, sardine and gadoids.[1]

A review of the scientific and technical literature revealed some information about the shelf-life of marinated fish. Varlık *et al*[2] studied the effect of temperature on the penetration of vinegar/salt during the marination process. Gün *et al*[3] determined the maturation time for rainbow trout marination. Fuselli *et al*[4] studied the microbiology of the marination process of anchovy. Aksu *et al*[5] investigated changes in marinated anchovy during production in different acid/salt concentrations and determined the shelf-life. Dokuzlu[6] studied the effect of the acid/salt ratio used in the marination process of anchovy on the microbiological load, sensory quality and shelf-life of the product. Varlık *et al*[7] determined the shelf-life of marinated fish meatballs. Erkan *et al*[8] examined the effect of modified atmosphere packaging on the shelf-life of marinated and breaded rainbow trout. Metin *et al*[9] studied the effect of modified atmosphere packaging on some quality criteria of the shelf-life of marinated and breaded rainbow trout. Poligne and Collignan[10] investigated the quality and stability of the end product of anchovy marinated using acetic and gluconic acids. Arık *et al*[11] studied the proximate composition of marinated fish and its shelf-life. Özden and Baygar[12] examined the effect of different packaging methods on some quality criteria of marinated fish.

The aim of this study was to determine the lipid, protein, moisture, ash, salt and acid composition of marinated fish and changes in amino acid and fatty acid composition during its shelf-life and to investigate the possibility of using these changes as quality indices.

MATERIALS AND METHODS

Samples

Fresh anchovies (*Engraulis encrasicholus*) (mean weight 14 ± 0.8 g, mean length 12 ± 2.5 cm), a Marmara Sea pelagic species, were obtained from a fishing port. Fresh fish packed in ice in wooden boxes were transported to the laboratory within 3 h. Fish samples amounting to 10 kg were used for the experiment. Fresh rainbow trout (*Salmo gairdneri*) (mean weight 280.9($\pm$11.08) g, mean length 26($\pm$4.1) cm) were

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obtained from a fish market in Istanbul. A total of 16 kg of commercial size fish (260–295 g) were packed in ice in polystyrene boxes containing holes for drainage and delivered to the laboratory within 30 min after harvest. Fish were individually beheaded, gutted, filleted and washed.

Marinating process and storage

Fillets were dipped in 3% acetic acid/10% salt marinating solution at a ratio of 1:1.5 (w/w) for 48 h (rainbow trout) or 24 h (anchovy) at 4 ± 1 °C. After marination the fish were drained at ambient temperature (18 ± 1 °C) for 15 min, placed in high-barrier plastic film bags (O_2 transmission rate 6.89 ml m^{-2}, CO_2, 5.42 ml m^{-2}, N_2 2.48 ml m^{-2} at 4 °C; vapour permeability 7.86 g m^{-2} day^{-1} atm^{-2} at 37.8 ± 1 °C, 90 ± 2% RH) and packaged under vacuum for 20 s at 760 mmHg. Plastic film bags were obtained from UPM-Kymmene, Walki-Pack, Plastic Films Factory (Valkeakoski, Finland). A Röschermatic vacuum-packaging machine (Röschermatic, Röscher AG, Barsenbrück, Germany) was used. Sampels were stored at 4 ± 1 °C and analysed every 30 days to determine the shelf-life (up to 120 days).

Proximate analyses

Moisture content was determined by drying an accurately weighted sample of minced fish in an oven at 103 ± 2 °C for 3 h.[13] Ash content was determined by heating the residue for 3 h at 550 °C (AOAC[14] method 938.08). Protein content was determined by AOAC[14] method 940.25. Total lipid content was determined in a 5 g sample of minced fish using the acid hydrolysis extraction procedure (AOAC[14] method 948.15). Total salt and acetic acid concentrations were determined according to the method described by Karl.[15]

Total amino acid composition

Total amino acid composition was determined using an amino acid analyser (Eppendorf LC 3000, Hamburg, Germany). Samples were hydrolysed in 6 M HCl in vacuum-sealed tubes at 110 °C for 24 h. The amino acid analyser conditions were as follows: precolumn, PEEK-Pre Column 'LUFA' VL 00 286 998 07; analytic column, PEEK-Separation Column 'LUFA' L 00 282 98 07; injection volume, 20 µl; flow rate, 0.2 ml min^{-1}; reactor temperature, 125 °C; excitation, 440 nm; emission, 570 nm; programme time, 83.50 min. Amino acids were identified with Hydrolysate Benson Calibration Standard H (Eppendorf-Biotronik, Hamburg, Germany).

Lipid extraction and fatty acid composition

Total lipids were extracted according to AOAC[14] method 948.15. Fatty acid composition was determined after methylation (AOAC[14] method 991.39) by gas chromatography (Perkin-Elmer Autosystem XL, FID, GC Software Turbochrom 4.1, Awenud-Shelton, DE, USA) using a Supelco SP-2330 column (30 m × 0.25 µm × 0.25 mm). The chromatographic conditions were as follows: injection volume, 0.5 µl; injection temperature, 230 °C; carrier gas, helium, 10 psi; detector temperature, 240 °C; column temperature, 120 °C for 2 min, programmed at 5 °C min^{-1} up to 220 °C; programme time, 31.00 min. Fatty acids were identified by comparison of their retention times with those of authentic standards (Supelco 37 Components FAME Mixture, Kat Nr 4-7885) and their contents were calculated on a weight percentage basis.

Statistical analysis

Data were subjected to analysis of variance followed by Duncan's multiple range test ($p < 0.05$).[16]

RESULTS AND DISCUSSION

The moisture contents in fresh and marinated anchovy and trout are shown in Table 1. As expected, moisture contents (fresh anchovy, 69.76%; fresh trout, 76.23%) were reduced in marinated fish (anchovy, 66.75%; trout, 74.02%). Ash (1.92/1.76%), protein (19.10/19.43%) and fat (11.51/4.11%) contents in marinated anchovy/trout were higher than those in fresh fish. The relative amounts of fat, protein, ash and other components increased as a result of the water loss caused by the penetration of salt into the meat. A similar proximate composition (72.10% moisture, 19.87% protein, 4.84% fat, 1.54% ash) has been reported by Arık *et al*[11] for marinated fish. The salt and acetic acid concentrations in marinated anchovy/trout were 5.3/5.1 and 2.2/2.1% respectively. Similar salt (5.9%) and acetic acid (1.8%) contents have been reported by Gün *et al*[9] in marinated rainbow trout.

The total amino acid content in marinated anchovy was 12 101 mg per 100 g, which is 98.6% of that in fresh anchovy muscle (12 264 mg per 100 g), while the total amino acid content in marinated trout was 14 951.5 mg per 100 g, which is 99.9% of that in fresh trout muscle (14 954.5 mg per 100 g). Both marinated and fresh muscle of trout showed higher contents of amino acids than found in anchovy, so fresh and marinated trout would serve as a better protein source.

The major amino acids in marinated and fresh muscle of anchovy and trout were aspartic acid, glutamic

Table 1. Proximate composition (%) of fresh and marinated anchovy and rainbow trout

	Moisture	Ash	Protein	Fat
Fresh anchovy	69.76 ± 2.86	1.62 ± 0.15	18.02 ± 0.92	10.32 ± 0.75
Marinated anchovy	66.75 ± 3.16	1.92 ± 0.12	19.10 ± 1.04	11.51 ± 0.98
Fresh trout	76.23 ± 2.02	1.47 ± 0.09	18.57 ± 0.57	3.71 ± 0.85
Marinated trout	74.02 ± 2.96	1.76 ± 0.18	19.43 ± 0.96	4.11 ± 0.71

acid and lysine, which constituted approximately 31% of total amino acids. Marinated anchovy and trout had high contents of lysine (1184 and 1470 mg per 100 g respectively); hence they may serve as a good additive in the diet of Koreans and other Asians whose food is mainly grain-based and low in lysine.

Tables 2 and 3 show the essential and non-essential amino acids determined in fresh and marinated anchovy and rainbow trout. The most abundant of the non-essential amino acids were aspartic acid, glutamic acid, alanine, serine and glycine.

Changes in amino acids in relation to freshness were examined in the muscle of marinated anchovy and trout during cold storage. In this study, amino acid increases were found to be useful as an index of freshness and decomposition of marinated fish in storage. In the course of marinated anchovy storage the increases in concentration of aspartic acid, threonine, serine and glycine were significant ($p < 0.05$) at day 120 and the increases in concentration of phenylalanine, lysine and arginine were significant ($p < 0.05$) at day 90. Significant ($p < 0.05$) increases in the concentration of all amino acids except arginine in marinated rainbow trout samples were found on the final day of storage (day 120). Aspartic acid, threonine, proline, glycine, tyrosine and lysine concentrations in

Table 2. Amino acid contents (mg per 100 g (%)) in anchovy marinade

Amino acid	Fresh fish	Fish marinade	Storage time (days)			
			30	60	90	120
Aspartic acid	1337.5 (10.90)	1245 (10.28)	1164 (9.89)	1144 (10.20)	1276.5 (8.91)	1684.5 (12.06)
Threonine	578 (4.71)	555.5 (4.59)	485 (4.12)	483 (4.30)	640.5 (4.47)	755 (5.40)
Serine	717 (5.84)	660.5 (5.45)	620.5 (5.27)	633 (5.64)	781.5 (5.45)	959 (6.87)
Glutamic acid	1213.5 (9.89)	1194.5 (9.87)	988 (8.39)	973 (8.67)	1333.5 (9.31)	1194 (8.55)
Proline	292.5 (2.38)	439.5 (3.63)	545 (4.63)	454 (4.04)	238.5 (1.66)	554 (3.96)
Glycine	587.5 (4.79)	556.5 (4.59)	536.5 (4.55)	569.5 (5.07)	728.5 (5.08)	870 (6.23)
Alanine	750.5 (6.11)	784.5 (6.48)	685 (5.82)	645 (5.75)	626 (4.37)	671 (4.80)
Valine	690 (5.62)	742 (6.13)	748.5 (6.36)	708.5 (6.31)	899.5 (6.28)	829 (5.93)
Methionine	361 (2.94)	394.5 (3.26)	342 (2.90)	303 (2.70)	454 (3.17)	450.5 (3.22)
Isoleucine	641.5 (5.23)	648 (5.35)	606.5 (5.15)	563 (5.02)	801.5 (5.59)	770.5 (5.52)
Leucine	948.5 (7.73)	971.5 (8.02)	931 (7.91)	866.5 (7.72)	1175.5 (8.21)	1177 (8.43)
Tyrosine	295 (2.40)	311.5 (2.57)	334.5 (2.84)	293.5 (2.61)	430 (3.00)	434 (3.10)
Phenylalanine	455 (3.70)	573 (4.73)	648.5 (5.51)	589.5 (5.25)	758 (5.29)	659.5 (4.72)
Histidine	621 (5.06)	398.5 (3.29)	405 (3.44)	406.5 (3.62)	474 (3.31)	400.5 (2.86)
Lysine	1144 (9.32)	1184 (9.78)	1260 (10.70)	1182 (10.53)	1532 (10.70)	1351 (9.67)
Arginine	771.5 (6.29)	753 (6.22)	832.5 (7.07)	801 (7.14)	1050 (7.33)	652.5 (4.67)
Ammonia (decomposition product)	860.5 (7.01)	689 (5.69)	634 (5.38)	600 (5.34)	1117.5 (7.80)	546 (3.91)
Total	12 264.5 (99.9)	12 101 (99.9)	11 766.5 (99.9)	11 215 (99.9)	14 317 (99.9)	13 958 (99.9)

Table 3. Amino acid contents (mg per 100 g (%)) in rainbow trout marinade

Amino acid	Fresh fish	Fish marinade	Storage time (days)			
			30	60	90	120
Aspartic acid	1600.5 (10.70)	1860 (12.44)	1752.5 (9.14)	1453.5 (8.05)	1500 (9.43)	2764.5 (11.87)
Threonine	718.5 (4.80)	858 (5.73)	869 (4.53)	736.5 (4.08)	725 (4.55)	1157.5 (4.96)
Serine	833 (5.57)	926 (6.19)	1011 (5.27)	860.5 (4.76)	892.5 (5.61)	1589.5 (6.82)
Glutamic acid	1510.5 (10.10)	1597 (10.68)	1788 (9.33)	1563 (8.66)	1506.5 (9.47)	2410 (10.35)
Proline	429 (2.86)	527.5 (3.52)	235 (1.22)	386 (2.13)	165 (1.03)	820.5 (3.52)
Glycine	758 (5.06)	703 (4.70)	1052.5 (5.49)	814.5 (4.51)	824.5 (5.18)	1447 (6.21)
Alanine	962.5 (6.43)	846 (5.65)	859.5 (4.48)	1681.5 (9.32)	682.5 (4.29)	1122.5 (4.82)
Valine	916 (6.12)	769 (5.40)	1267 (6.61)	1120 (6.20)	1014.5 (6.37)	1355 (5.81)
Methionine	481 (3.21)	487.5 (3.26)	672.5 (3.50)	616.5 (3.41)	522.5 (3.28)	824 (3.53)
Isoleucine	789.5 (5.27)	774 (5.17)	1116.5 (5.82)	975.5 (5.40)	892 (5.60)	1280.5 (5.49)
Leucine	1199 (8.01)	1172.5 (7.84)	1756.5 (9.16)	1563.5 (8.66)	1345.5 (8.46)	2056 (8.82)
Tyrosine	371.5 (2.48)	419 (2.80)	540.5 (2.82)	469.5 (2.60)	439 (2.76)	723 (3.10)
Phenylalanine	710.5 (4.75)	699.5 (4.67)	956 (4.58)	853.5 (4.73)	738.5 (4.64)	1077.5 (4.62)
Histidine	461 (3.08)	354.5 (2.37)	642.5 (3.35)	579.5 (3.21)	535 (3.36)	688.5 (2.95)
Lysine	1510 (10.09)	1470 (9.83)	2166 (11.30)	1873 (10.38)	1744 (10.96)	2445 (10.50)
Arginine	922 (6.16)	888.5 (5.94)	1054.5 (5.50)	1172 (6.49)	977 (6.14)	745 (3.19)
Ammonia (decomposition product)	782 (5.22)	599.5 (4.00)	1421.5 (7.41)	1322 (7.32)	1399.5 (8.79)	779 (3.45)
Total	14 954.5 (99.9)	14 951.5 (100.2)	19 161 (99.9)	18 040.5 (99.9)	15 903.5 (99.9)	23 285 (100)

marinated anchovy and trout would greatly influence their quality. Ammoniac resulting from decomposition showed an increase in level during storage of marinated anchovy and trout.

In fish, amino acids are the main components of non-protein nitrogen, accounting for 50–85%, and the most important amino acids from a quantitative point of view are proline, arginine, glycine, alanine, histidine, glutamic acid and taurine. In cooked fish, amino acids are directly responsible for flavour and taste and can be precursors of aromatic components. During storage of chilled fish the first changes in amino acids are caused by muscle autolysis, and later changes by the growth of micro-organisms.[17–21] Amino acids have also been used as quality indices for various fish and crustacean species. The amino acids tyrosine, arginine and lysine are very important during fish spoilage, since these amino acids can produce biogenic amines by decarboxylation (tyramine, agmatine and cadaverine respectively), which are very important from the toxicity point of view and as quality control indices for fish spoilage.[22]

Yıldiz[23] reported similar amino acid composition and composition changes for cold-stored rainbow trout. Oladapa *et al*[24] studied changes in amino acid composition during traditional processing of (smoked, solar-dried) freshwater fish species. Aspartic acid, glutamic acid and lysine were found as the major amino acids in these products. Changes in these amino acids were significant. Lysine in particular affected product quality.[24] Rosa and Nunes[25] stated that arginine, lysine and leucine are the major essential amino acids in aquatic organisms, and aquacultural products are therefore known as high-quality protein sources.

Many amino acids, such as glutamic acid, aspartic acid, alanine and glycine, are responsible for flavour and taste. These amino acids are important because they give marinated fish their characteristic taste and flavour. Amino acids have also been used as quality indices for various fish and crustacean species.[26] Ruiz-Capillas and Moral[21] reported that the free amino acids β-alanine and 1-methylhistidine could be used in combination with the dipeptide anserine and tryptophan as quality indices for both ice- and atmosphere-stored hake. In the case of molluscs and crustaceans, most authors recommend ornithine as a freshness index.[21] Ruiz-Capillas and Moral[27] also observed a decrease in arginine content in snow crab muscle during iced storage.

The fatty acid contents in fresh and marinated muscle of anchovy and rainbow trout are shown in Tables 4 and 5. The contents of polyenes in the muscle of fresh anchovy and rainbow trout were 36.23 and 33.88% respectively, somewhat lower than in marinated fish (anchovy, 34.14%; trout, 33.27%). There was no significant difference in the content of saturated fatty acids between fresh (anchovy, 31.20%; trout, 24.29%) and marinated (anchovy, 30.44%; trout, 24.92%) fish. Monoenes in marinated fish were present at 20.67% (anchovy) and 31.35% (trout), while those in fresh muscle were present at 20.98% (anchovy) and 30.87% (trout).

Table 4. Fatty acid composition (%) of total lipid in anchovy marinade

Fatty acid	Fresh fish	Fish marinade	Storage time (days) 30	60	90	120
$C_{12:0}$	0.24	0.22	0.22	0.22	0.27	—
$C_{13:0}$	0.06	0.06	0.07	0.07	0.07	—
$C_{14:0}$	7.53	7.5	7.34	7.07	7.48	8.09
$C_{15:0}$	0.80	0.82	0.86	0.80	0.84	0.88
$C_{16:0}$	15.93	16.37	16.71	16.45	16.46	17.05
$C_{17:0}$	1.14	1.14	1.1	1.12	1.06	1.11
$C_{18:0}$	3.14	3.26	3.33	3.48	3.16	3.39
$C_{20:0}$	1.27	—	—	0.08	1.19	1.22
$C_{21:0}$	0.1	0.13	0.1	0.15	1.78	1.87
$C_{22:0}$	0.78	0.74	—	0.75	—	0.75
$C_{23:0}$	—	—	—	—	0.46	0.25
$C_{24:0}$	0.21	0.2	0.12	—	0.09	0.16
Total saturated	31.2	30.44	29.85	30.19	32.86	34.77
$C_{14:1}$	0.2	0.2	0.2	0.2	0.21	0.26
$C_{16:1}$	6.22	6.03	5.96	6.00	5.96	6.41
$C_{17:1}$	—	—	0.91	0.99	0.63	1.04
$C_{18:1n-9}$	11.17	11.17	11.10	11.61	11.18	11.72
$C_{20:1n-9}$	2.73	2.8	1.24	2.78	3.37	3.09
$C_{22:1n-9}$	0.29	0.27	0.26	0.28	0.42	0.29
$C_{24:1n-9}$	0.37	0.2	0.24	0.22	0.76	1.11
MUFA	20.98	20.67	19.91	22.08	22.53	23.92
$C_{18:2n-6}$	2.09	1.68	1.75	1.58	2.08	1.99
$C_{18:3n-6}$	0.88	0.53	0.81	1.34	0.15	0.14
$C_{18:3n-3}$	0.78	0.80	0.83	0.79	0.81	0.81
$C_{20:2}$	0.16	0.22	0.23	0.24	0.23	0.26
$C_{20:3n-6}$	—	—	0.09	0.07	0.07	—
$C_{20:3n-3}$	0.14	0.18	0.25	0.17	0.15	0.18
$C_{20:4}$	3.69	2.50	4.45	4.02	0.71	3.26
$C_{22:2}$	—	—	—	—	—	—
$C_{20:5}$	9.97	9.75	9.84	9.51	9.89	9.69
$C_{22:6}$	18.52	18.48	19.26	18.67	19.31	17.41
PUFA	36.23	34.14	37.51	36.39	33.4	33.74
Not detected	11.59	14.74	12.99	11.35	11.21	9.46

In both fresh and marinated fish the predominant saturated fatty acid (SFA) was palmitic acid (16:0), the most abundant monounsaturated fatty acid (MUFA) was 18:1n-9 and the predominant polyunsaturated fatty acids (PUFA) were 20:5n-3 and 22:6n-3.

Thus both fresh and marinated fish contained high amounts of n-3 long-chain polyunsaturated fatty acids such as eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) and hence may contribute to the prevention of diseases related to geriatric and cardiovascular disorders and certain forms of cancer, among others.[28] Sigurgisladóttir and Pálmadóttir[29] reported the following fatty acid levels for herring, salmon and cod respectively: $C_{16:0}$, 16.2, 13.6 and 18.2%; $C_{18:0}$, 1.4, 2.4 and 3.2%; $C_{20:5}$, 5.6, 3.8 and 15.1%; $C_{22:6}$, 6.8, 5.1 and 0.8%. High levels of n-3 fatty acids (above 30%) in *Engraulis encrasiotolus* and *Sardina pilchardus* and medium levels of n-3 fatty acids in *Scomber scombrus* have also been

Table 5. Fatty acid composition (%) of total lipid in rainbow trout marinade

Fatty acid	Fresh fish	Fish marinade	Storage time (days) 30	60	90	120
$C_{12:0}$	0.06	0.04	0.05	0.06	0.05	—
$C_{13:0}$	—	0.03	—	—	—	—
$C_{14:0}$	3.87	3.61	3.69	3.76	3.71	4.18
$C_{15:0}$	0.51	0.49	0.51	0.53	0.51	0.56
$C_{16:0}$	15.36	15.35	16.67	16.79	16.75	17.68
$C_{17:0}$	0.68	0.66	0.67	0.67	0.66	0.66
$C_{18:0}$	3.42	3.58	3.86	3.85	3.98	3.98
$C_{20:0}$	0.3	—	0.12	0.14	0.31	1.33
$C_{21:0}$	0.09	0.1	0.32	0.33	0.62	0.34
$C_{22:0}$	—	1.01	1.14	1.14	—	0.86
$C_{23:0}$	—	0.05	—	—	—	0.50
$C_{24:0}$	—	—	—	—	—	—
Total saturated	24.29	24.92	27.03	27.27	26.59	30.09
$C_{14:1}$	0.1	0.09	0.09	0.1	0.09	0.21
$C_{16:1}$	5.4	5.36	5.17	5.28	5.30	5.62
$C_{17:1}$	—	—	0.68	0.7	0.69	0.67
$C_{18:1n-9}$	23.56	24.15	23.21	23.39	23.79	23.19
$C_{20:1n-9}$	1.46	1.49	1.52	1.36	1.51	0.20
$C_{22:1n-9}$	0.07	0.04	0.06	0.04	0.11	0.16
$C_{24:1n-9}$	0.28	0.22	0.23	0.21	0.32	0.53
MUFA	30.87	31.35	30.96	31.08	31.81	30.58
$C_{18:2n-6}$	12.71	12.33	12.20	12.17	12.28	12.32
$C_{18:3n-6}$	0.17	0.23	0.31	0.27	0.21	0.30
$C_{18:3n-3}$	1.4	1.33	1.31	1.34	1.36	1.50
$C_{20:2}$	0.83	0.8	0.86	0.81	0.84	0.86
$C_{20:3n-6}$	—	—	—	—	—	—
$C_{20:3n-3}$	0.43	0.37	0.44	0.23	0.32	0.40
$C_{20:4}$	1.08	0.16	0.17	0.16	0.53	0.27
$C_{22:2}$	0.07	—	—	—	0.56	—
$C_{20:5}$	3.89	3.96	3.73	3.96	4.00	3.88
$C_{22:6}$	13.3	14.09	13.73	13.75	13.85	13.01
PUFA	33.88	33.27	32.75	32.69	34.05	32.54
Not detected	10.9	10.91	8.9	8.99	7.54	6.80

reported by Zlatanos and Sagredos.[30] The total lipids and lipid composition in fish may vary with season, species, sex and/or food intake.[31] Rosa and Nunes[25] stated that 16:0, 18:1n-9, 20:5n-3 and 22:6n-3 are the main fatty acids in aquatic organisms that are responsible for the peculiar taste and odour of marinated products.

Changes in fatty acids in relation to freshness were examined in the muscle of marinated anchovy and trout during cold storage. PUFA concentrations decreased significantly ($p < 0.05$) in marinated anchovy but not ($p > 0.05$) in marinated trout, while total SFA concentrations increased significantly ($p < 0.05$) in both marinated fish during storage. In this study, fatty acid increases and decreases were found to be useful as an index of freshness and decomposition of marinated fish in storage.

Ovayolu[32] studied fatty acid composition changes in marinated anchovy during cold storage. Voldrich *et al*[33] examined the effect of smoking and marination on fatty acids in mackerel meat. Yang *et al*[34] investigated fatty acid changes caused by salting in greyfish. Oladapa *et al*[24] studied changes in the fatty acid composition of traditionally processed (smoked, solar-dried) freshwater fish species.

The changes in predominant fatty acids that these authors have reported are similar to those found in our own study. During storage there were increases in 16:0, 18:1n-9, 20:5n-3 and 22:6n-3 concentrations. Oxidation during storage affects product quality, mainly because of the increase in the total amount of saturated fatty acids and the decrease in the total amount of unsaturated fatty acids.

CONCLUSIONS

Changes in amino acids and fatty acids were examined in marinated fish during cold storage. The changes in concentration of aspartic acid and lysine during storage of marinated fish are significant and can be utilised as a freshness index. The ammonia which is a by-product of decomposition can be utilised as an indicator of decreasing quality during storage. Increasing SFA and decreasing PUFA concentrations indicate that oxidation is in progress, and this has an important effect on quality. In marinated fish products of high or average fat content, autoxidation triggered by enzymatic reactions causes loss of quality during storage, even when preventive measures are taken before and during the marination process.

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