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Effect of frozen storage and cooking method on amino acid composition of mullet fish (*Mugil cephalus*)

Abstract

The current study was carried out to investigate the effect of frying and grilling cooking methods on amino acid composition of Mullet fish (*Mugil cephalus*) obtained from Wadi El-Ryan Lake, at Fayoum, Egypt. The study also included the effects of frozen storage of raw Mullet fish steaks for 6 months on amino acid profile. Fresh Mullet had good protein quality as indicated by high of total amino acids (TAA), total essential amino acids (EAA) and Amino acid score (AAS). These quality indicators were slightly decreased after 180 days of frozen storage. Fried and grilled products have a high nutritional quality of protein and high nutritional value as indicated by high values of (TEAA), Amino acid score (AAS) and percentage satisfaction (P.S%/150) of essential amino acids upon consuming 150 g of the products and the grams consumed to cover the daily requirements of essential amino acids for adult man were lower.

Keywords: mullet fish, amino acid, frozen storage, frying, grilling

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El Lahamy AA,[1] Khalil KI,[2] El Sherif SA,[1] Abdelazim SAA,[3] Mahmud AA[2]

[1]National Institute of Oceanography and Fisheries, Fish Processing Technology Laboratory, Egypt

[2]Food Science and Technology Department, Egypt

[3]Food Technology Research Institute, Agricultural Research Center, Egypt

Correspondence: El Lahamy AA, National Institute of Oceanography and Fisheries, Fish Processing Technology Laboratory, Cairo, Egypt, Email adelammar11@yahoo.com

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Introduction

Fish and fishery products have long been recognized as healthy foods with excellent nutritional value, providing high-quality protein, minerals, vitamins, essential fatty acids and trace elements. Fish is widely consumed in many parts of the world by humans due to its high content of good protein that characterizes by an excellent amino acid composition and easily digestibility.[1] Fish proteins are easily digestible and contain significant amounts of all the essential amino-acids, principally lysine and the sulphur-containing amino-acids (methionine and cysteine) that are often present in low quantities in vegetables, cereals and legumes. Fish protein can therefore be used to complement the amino acid pattern and the overall protein quality in human diet.[2,3]

Fish is usually cooked in different methods such as boiling, smoking, roasting, frying and grilling. These processing and cooking methods improve the hygienic quality by inactivation of pathogenic microorganisms and enhance the digestibility and bio-availability of nutrient in the digestive tract.[4] Freezing is a much preferred technique to preserve fish and fish products for long period of time. It permits to preserve the flavor and the nutritional properties of foods better than storage above the initial freezing temperature. It also has the advantage of minimizing microbial or enzymatic activity.[5] Freezing requires lowering the temperature to –18°C or lower and is a popular method of preserving fish.[6] Frozen storage has been widely employed to retain fish properties before it is consumed or employed in other technological processes. Thermal processing techniques are widely used to improve eating quality and safety of food products and to extend the shelf life of the products. Fish and fishery products are cooked in different ways to improve its hygienic quality by inactivation of pathogenic microorganisms and to enhance its flavor and taste.[7] During cooking, some chemical and physical reactions take place which either improve or impair the food nutritional value. Therefore, digestibility is increased because of protein denaturation in food while, the content of thermo labile compounds, fat-soluble vitamins or polyunsaturated fatty acids is often reduced.[8]

Material and methods

Mullet fish (*Mugil cephalus*)

Fresh Mullet fish (*Mugil cephalous*) was obtained from Wadi El-Rayan Lake during February 2016. Averages of weight and length ranged between 1.3-1.5kg and 46-52cm, respectively. Mullet fish samples were kept in an

ice-box and transported to the laboratory of Fish Processing Technology, Shakshouk Station for Fish Research, National Institute of Oceanography and Fisheries, Fayoum Governorate, Egypt. The fish samples were beheaded, gutted and cut into steaks then washed gently with tap water. The prepared raw fish steaks samples were divided into two groups and packed in polyethylene bags then stored at -18°C for 6.0 months. At intervals of 6.0 months, samples of frozen fish fillets were withdrew, thawed and cooked with two methods; frying and grilling.

Cooking methods

Frying and grilling cooking methods were carried out on the pre-frozen Mullet fish steaks at zero time of storage and at 6.0 months.

Frying

Frozen Mullet steaks were thawed, soaked in saturated salt solution for two minutes, and then spices mixture was put in the steaks cavity and rubbed with flour. The steaks were fried in sunflower oil heated at 180°C for 5 minutes for each side of the steaks using electrical fryer pan Moulinex and then the fried steaks were drained in basket to remove excess oil.

Grilling

Frozen Mullet steaks were thawed, rubbed with bran and grilled using electrical grill machine at 260°C for 15 minutes for each side of the steaks. The grilled fish samples were spiced for 1 minute using a special spiced solution containing black pepper, cumin, red pepper and garlic.

Analytical method

Amino acids analysis

Amino acids determination: Amino acid contents were determined by using HPLC and Amino acid analyzer LC3000 Eppendr of Germany at National Research Center according to the method described by Andrew.[9] Tryptophan was not determined.

Amino acid score (A.A.S.): Amino acid score was calculated for the essential amino acids (g/100g protein) using Standard[10] according to Bhanu[11] using the following equation:

$$\text{A.A.S.} = \frac{\text{E. A. A. in tested sample (g / 100 g protein)}}{\text{E. A. A. of reference protein(g / 100 g protein)}} \times 100$$

A.A.S. value less than 100 indicated the deficiency in the considered amino acid. The acid which showed highest deficiency (lowest A.A.S.) was called the limited amino acid (L.A.A.).

Daily Requirements (G.D.R.) and the Percentage Satisfaction (P.S.%/150g): Gram daily requirements (g) of products were illustrated by essential amino acids composition (g/100g sample) which should be consumed to cover the daily requirements for adult man (recommended daily dietary allowances).

Daily requirements (G.D.R.) were calculated using the following equation:

$$\text{G.D.R.} = \frac{\text{Reference pattern}\left(\text{USRDA},1989\right)\left(100\text{g sample}\right)}{\text{gAA / 100g product}}$$

Percentages of satisfaction (P.S. %) of the daily requirements for adult man in the essential amino acid when consuming 150g of mullet fish products (P.S.%/150g) were calculated using the recommended daily dietary allowances given by USRDA.[12]

The percentages of satisfaction (P.S.%/150g) were calculated using the following equation:

$$\text{P.S.\% / 150g} = \frac{150\text{g} \times 100}{\text{G.D.R., g}}$$

Effect of frozen storage and cooking methods on amino acid of mullet fish

Amino acid composition of mullet

Effects of frozen storage at -18°C of raw Mullet fish and cooking method on the nutritional value of raw and cooked products were investigated based on their contents of amino acids.

Amino acid profiles of the raw Mullet fish sample were determined at zero time and after 180 days of frozen storage and data obtained are presented in Table 1. The results showed the high quality of Mullet fish protein as it was indicated from its high contents (fresh sample) of the different amino acids including the essential and non-essential amino acids. Expect the amino acid tryptophan, all the essential amino acids were found in Mullet fish. The essential amino acids amounted by 41.54g/100g protein which represent more than 47.0% of the total amino acids found in Mullet fish. Glutamic acid was found to be the predominate amino acid with concentration of 11.07g/100g protein followed by aspartic acid with concentration of 9.02g/100g protein. Leucine, Lysine, arginine, isoleucine, valine, phenylalanine, threonine and serine were determined by 6.98, 6.62, 5.15, 4.83, 4.48, 4.35, 4.53 and 4.17g/100g protein, respectively. The other amino acids were also found with considerable concentrations. Similar results were found by Shady.[13] who mentioned that glutamic acid was the abundant amino acid followed by aspartic acid of the total amino acids determined in several species of fish.

Table 1 Effect of frozen storage on amino acid composition of Mullet fish steaks

Amino acid (AA)	g AA /100g protein in Mullet fish steaks	
	Fresh(Un frozen)	Frozen stored(6.0 months)
Essential Amino Acids (EAA)*		
Isoleucine	4.83	4.76
Leucine	6.98	6.7
Threonine	4.53	4.34
Valine	4.48	4.3
Phenylalanine	4.35	4.1
Tyrosine	2.95	3.05
Methionine	2.85	2.8
Cystine	1.1	0.95
Tryptophan	ND	ND
Lysine	6.62	6.6
Histidine**	2.85	2.75
Non Essential Amino Acids (NEAA)		
Serine	4.17	4.52
Glycine	5.02	4.6
Alanine	5.85	5.61
Proline	4.9	4.55
Aspartic	9.02	8.58
Glutamic	11.07	10.75
Argnine	5.15	5.2
TEAA	41.54	40.35
TNEAA	45.18	43.81
TAA	86.72	84.16

Effect of frozen storage on amino acid composition of mullet fish steaks

The results presented in Table 1 show the effect of frozen storage for 6.0 months at –18°C on amino acid composition of Mullet fish. Frozen storage showed no considerable changes on amino acids contents of Mullet fish. Cystine showed the highest loss that determined by about 13.0%, while tyrosine, serine and argnine slightly increased. Data showed that after 6.0 months of frozen storage, the essential, non-essential and total amino acids were determined by 40.35, 43.81 and 84.16 g/100g protein, respectively which represented more than 97.0 % of their concentrations in the raw samples before storage. Similar results were found by Wesselinova.[14] who showed that frozen storage at –35ºC did not influence dramatically on the amino acid values of the examined fish species. He reported that the highest loss of amino acids during frozen storage was determined by 13 % for cysteine. Also, Castrillon[15] reported that the amino acid that was most damaged during the process of freezing storage and defrosting was cysteine. The increases observed in some amino acids during frozen storage could be due to transition of one kind of amino acid to another through oxidation, deamination, etc.[14]

Effect of cooking methods on amino acid composition of mullet fish steaks

The effect of cooking methods (frying and grilling) on amino acids compositions of the Mullet fish steaks was determined. Amino acids were determined in fresh Mullet fish and its fried and grilled products before storage. After 6.0 months of frozen storage of Mullet fish, samples of Mullet fish steaks were fried and grilled and amino acids were determined in the frozen stored fish sample and the fried and grilled samples prepared from the pre frozen stored fish steaks. Data obtained are presented in Table 2 and Table 3. It was found that several amino acids showed slight decreases due to frying and grilling processes while some others slightly increased either in the cooked samples from fresh fish steaks (Table 2) or from the frozen stored (for 6.0 months) steaks (Table 3). The results showed that in the fried steaks made from fresh Mullet fish sample (Table 2), the sulphur containing amino acids Cystine showed the highest loss which was accounted by 51.81% and methionine decreased by 20.35 %. Also, the amino acids; serine, threonine, lysine, phenylalanine and tyrosine decreased by 22.54, 17.21, 11.63%, 9.19 and 8.81 % respectively. On the other hand, the results recorded in Table 2 showed slight increases in fried Mullet fish contents of some amino acid such as histidine, glycine and alanine. The same trends were also found in the amino acid composition of the grilled Mullet sample. The results (Table 2) showed that the loss was particularly more observed in the sulphur containing amino acids cystine and methionine that determined by 23.63 and 22.45 %, respectively.

Table 2 Effect of cooking methods on amino acid composition of fresh Mullet fish steaks

Amino acid (AA)	g AA/100g protein in Mullet fish steaks		
	Fresh steaks	Fried steaks	Grilled steaks
Essential Amino Acids (EAA)*			
Isoleucine	4.83	4.1	4.33
Leucine	6.98	6.65	7.35
Threonine	4.53	3.75	4.18
Valine	4.48	3.9	4.25
Phenylalanine	4.35	3.95	4.25
Tyrosine	2.95	2.69	3.49
Methionine	2.85	2.27	2.21
Cystine	1.1	0.53	0.84
Tryptophan	ND	ND	ND
Lysine	6.62	5.85	6.22
Histidine**	2.85	3.05	2.5
Non Essential Amino Acids (NEAA)			
Serine	4.17	3.23	3.98
Glycine	5.02	5.29	5.12
Alanine	5.85	5.97	6.17
Proline	4.9	4.48	4.72
Aspartic	9.02	8.8	9.05
Glutamic	11.07	10.9	11.02
Argnine	5.15	4.87	5.19
EAA	41.54	36.74	39.62
NEAA	45.18	43.54	45.25
TAA	86.72	80.28	84.87

*Essential amino acids according to FAO.[21]

**Indispensable amino acid in human adult according to FAO.[22]

ND, not determined

Table 3 Effect of cooking methods on amino acid composition of pre frozen Mullet fish steaks (stored for 6.0 months

Amino acid (AA)	g AA/100g protein in Mullet fish steaks		
	Frozen stored (6.0 months)	Fried steaks from frozen stored (6.0 months)	Grilled steaks from frozen stored (6.0 months)
Essential Amino Acids (EAA)*			
Isoleucine	4.76	3.95	4.3
Leucine	6.7	6.45	7.15
Threonine	4.34	3.6	3.9
Valine	4.3	3.72	4
Phenylalanine	4.1	3.75	4.12
Tyrosine	3.05	2.5	3.25
Methionine	2.8	2.15	2.05
Cystine	0.95	0.5	0.65
Tryptophan	ND	ND	ND
Lysine	6.6	5.6	6
Histidine**	2.75	2.94	2.35
Non Essential Amino Acids (NEAA)			
Serine	4.52	3.1	3.75
Glycine	4.6	5.05	5.1
Alanine	5.61	5.8	6
Proline	4.55	4.32	4.45
Aspartic	8.58	8.48	8.7

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Table Continued

Amino acid (AA)	g AA/100g protein in Mullet fish steaks		
	Frozen stored (6.0 months)	Fried steaks from frozen stored (6.0 months)	Grilled steaks from frozen stored (6.0 months)
Glutamic	10.75	10.5	10.65
Argnine	5.2	4.68	5.1
EAA	40.35	35.16	37.77
TNEAA	43.81	41.93	43.75
TAA	84.16	77.09	81.52

*Essential amino acids according to FAO.[21]

**Indispensable amino acid in human adult according to FAO.[22]

ND, not determined.

The results shown in Table 2 indicated that the essential amino acids contents of fresh, fried and grilled Mullet fish steaks were 41.54, 36.74 and 39.62g/100g protein, respectively while their contents of the non- essential amino acids were 45.18, 43.53 and 45.25g/100g protein, respectively and the total amino acids contents were 86.72, 80.28 and 84.87g/100g protein, respectively. These data show that frying process resulted more loss in amino acids in comparison to the effect of grilling process.

Data given in Table 3 show amino acids contents of the pre frozen and stored (6.0 months) Mullet fish steaks and the fried and grilled samples prepared from the frozen stored samples. The trends found in amino acid compositions of the fried and grilled samples obtained from the fresh Mullet fish steaks were also observed in the fried and grilled products obtained from the frozen stored for 6.0 months sample. In the fried samples, the loss was particularly more observed in serine and threonine which decreased by 31.41% and 17.05%, respectively. Isoleucine lysine, and valine also showed a noticed decreases estimated by 17.01%, 15.15% and 13.48%, respectively. Sulphur containing amino acid; Cystine and methionine showed a reduction accounted by 47.36% and 23.21%, respectively while the losses observed in tyrosine and phenylalanine were determined by 18.03 % and 8.53% respectively. On the other hand, results recorded in Table 3 showed slight increases in fried Mullet contents of some amino acids such as histidine, glycine and alanine. Tryptophan was not determined in all Mullet fish samples.

In the grilled Mullet sample, the loss was particularly more observed in sulphur containing amino acid; cystine and methionine that showed a reductions accounted by 26.78% and 31.57%, respectively while the decreasing observed in serine, histidine and threonine were determined by 17.03%, 14.54 and 10.13%, respectively. On the other hand results recorded in Table 3 showed slight increases in grilled Mullet fish sample contents of some amino acid such as tyrosine, leucine, phenylalanine, aspartic, glycine and alanine.

Data presented in Table 3 showed that the essential amino acids contents of the 6.0 months frozen stored Mullet fish steaks and their fried and grilled products were 40.35, 35.16 and 37.77g/100g protein, respectively while their contents of the non essential amino acids were 43.81, 41.93 and 43.75 g/100g protein, respectively and the total amino acids were 84.16, 77.09 and 81.52g/100g protein, respectively. The general trend that could be observed was frying process resulted in more losses in amino acids comparing with the grilling process.

Similar results were reported by Oluwaniyi[16] who found that frying fish with palm oil appeared to have the worst effect on the amino acid composition resulting in the loss of essential amino acids of Catfish (*Clariasgariepinus*) and Tilapia (*Oreochromisniloticus*). Previous workers have reported varying effects of processing on the protein and amino acid contents of fish. Some investigators reported no significant effect on protein and amino acid contents following boiling and frying of fish,[17] while others reported that heat processing resulted in reduction in protein content by destroying some amino acids or making them unavailable.[16,18,19]

The changes found in amino acids of the fried and grilled Mullet fish samples may be attributed to the influence of heat during cooking operations. These observations could be due to some reactions such as deamination, decarboxylation and oxidation of amino acids which are known to be occurred during heat processing of protein containing food products.[20]

Amino Acid Score (AAS)

To assess the quality of protein contents of the fried and grilled Mullet fish steaks, amino acid scores (AAS) of the essential amino acid were calculated and data obtained are presented in Table 4 and Table 5.

Table 4 Amino acid scores (AAS) of fried and grilled Mullet fish steaks made from fresh sample

Essential Amino acid	Standard FAO/WHO (1991) (g/100 g protein)	Amino acid scores (%)		
		Fresh steaks	Fried steaks	Grilled steaks
Ile	2.8	173	146	155
Leu	6.6	105	101	111
Lys	5.8	114	101	107
Met+Cys	2.5	158	112	122
Phe+Tyr	6.3	116	105	123
Thr	3.4	142	110	123
Try	1.1	ND	ND	ND
Val	3.5	128	111	121
His	1.9	150	161	132

Table 5 Amino acid scores (AAS) of fried and grilled Mullet fish steaks made from frozen stored sample

Essential Amino acid	Standard FAO/ WHO (1991) (g/100 g protein)	Amino acid scores (%)		
		Frozen steaks	Fried steaks	Grilled steaks
Ile	2.8	170	141	153
Leu	6.6	101	97	108
Lys	5.8	113	96	103
Met+Cys	2.5	150	106	108
Phe+Tyr	6.3	113	99	116
Thr	3.4	127	105	114
Try	1.1	ND	ND	ND
Val	3.5	122	106	114
His	1.9	144	154	123

The results indicated the high nutritive value of Mullet protein which is confirmed from the higher values of amino acid scores for the different essential amino acid in relation to the standard protein

reference.[10] Amino acid scores for the different essential amino acid available in raw (uncooked) Mullet fish steaks ranged between 105 for leucine to 173 for isoleucine.

Moreover, changes occurred in amino acid composition due to cooking process showed no considerable changes on the levels of the different essential amino acids. Amino acid scores for the essential amino acids of grilled Mullet steaks made from fresh steaks were found to be higher than the fried samples. The results (Table 4) indicated that amino acid scores for the essential amino acids ranged between the lowest value of 101% for leucine in fried sample to the highest value of 155% for isoleucine in the grilled sample. The high values of amino acid scores for the essential amino acids available in the grilled and fried Mullet samples indicated the high quality of Mullet protein of the cooked Mullet samples.

The results (Table 5) indicated that amino acid scores for the essential amino acids in Mullet fish sample stored for 6.0 months at –18°C showed high values in the range of 101% for leucine to 170% for isoleucine. In the same trend, amino acid scores for the essential amino acids in the cooked Mullet fish steaks made from pre frozen stored steaks (6.0 months) showed values ranged between the lowest value of 96% for lysine in fried sample to the highest value of 153% for isoleucine in grilled sample. Amino acid score (AS) for the essential amino acids (EAA) of fried and grilled Mullet fish sample made from steaks before storage were found to be higher than 100 indicating no deficiency in any essential amino acids due to cooking method as compared with FAO references protein. while, (AS) of the same product after 6.0 months storage were less than 100 for leucine (97%), lysine (96%) and Phenylalanine+Tyrosine (99%) in fried Mullet samples.[21,22]

Estimating the Gram Daily Requirements (GDR) and the Percentage of Satisfaction (PS %/150): The quantities consumed of the fried and grilled Mullet fish steaks to cover the daily requirements of adult (GDR,g) and the percentages of satisfaction of these requirements by consuming 150g (PS%150%) in relation to USRDA[12] were calculated and data obtained are presented in Table 6 and Table 7.

Table 6 Daily requirements (GDR) and the percentages of satisfaction (PS %/150) of fried Mullet steaks made from fresh and frozen stored samples

Essential Amino Acid	USRDA (1989	Fried steaks from fresh sample			Fried steaks from frozen stored sample		
		g/100gsample	G.D.R (g)	P.S %/150 g	g/100g sample	G.D.R (g)	PS %/150 g
Isoleucine	0.819	0.975	84	179	0.905	90	165
Leucine	1.971	1.58	125	120	1.48	133	112
Lysine	1.008	1.39	73	207	1.28	79	190
Threonine	0.567	0.891	64	235	0.824	69	217
Tryptophan	0.315	ND	ND	ND	ND	ND	ND
Valine	0.819	0.927	88	170	0.851	96	155
Histidine	1.008	0.725	139	108	0.673	150	100
Meth+Cys	1.071	0.665	161	93	0.606	177	84
Phenyl.+Tyr	1.197	1.58	76	198	1.43	84	179

Table 7 Daily requirements (GDR) and the percentages of satisfaction (PS %/150) of grilled Mullet steaks made from fresh and frozen stored samples

Essential Amino Acid	USRDA (1989)	Grilled steaks from fresh sample			Grilled steaks from frozen stored sample		
		g/100g sample	G.D.R (g)	P.S %/150 g	g/100g sample	G.D.R (g)	PS %/150 g
Isoleucine	0.819	0.981	83	180	0.937	87	171
Leucine	1.971	1.66	119	126	1.55	127	117
Lysine	1.008	1.41	71	210	1.3	78	193
Threonine	0.567	0.947	60	251	0.85	67	224
Tryptophan	0.315	ND	ND	ND	ND	ND	ND
Valine	0.819	0.962	85	176	0.872	94	159
Histidine	1.008	0.566	178	84	0.512	196	76
Meth +Cys	1.071	0.691	155	97	0.588	182	82
Phenyl. +Tyr	1.197	1.75	68	219	1.6	75	200

The results presented in Table 6 showed that Meth+Cys amino acids in the fried Mullet steaks made from fresh Mullet fish showed the highest GDR and the lowest PS/150% that were calculated by 161g and 93%, respectively. The same findings were also found for the fried sample made from Mullet steaks stored for 6.0 months. Data indicated that Meth+Cys amino acids showed the highest (GDR 177g) and lowest PS/150% (84 %). Based on these data it could be concluded that the consumption of 150g of fried Mullet steaks made from fresh or frozen stored Mullet fish steaks covered more than the daily requirements of all the essential amino acid except for Meth + Cys that covered about 84-93 and % of their daily requirements.

The results presented in Table 7 showed that histidine amino acid in the grilled Mullet steaks made from fresh Mullet fish showed the highest GDR and the lowest PS/150% which were calculated by 178g and 84%, respectively. Also, the grilled sample made from Mullet steaks stored 6.0 months at –18°C showed the same trend. Data indicated that histidine amino acid showed the highest GDR (196g) and lowest PS/150% (76%). Based on these data, the consumption of 150g of grilled Mullet steaks covered more than the daily requirements of all the essential amino acids except for Meth+Cys and histidine which covered 97% and 84%, respectively for grilled sample made from fresh Mullet fish and 76% and 82 %, respectively for the grilled sample made from frozen stored Mullet fish.

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Conflict of interest

The authors have no competing interests.

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