Plant Archives Vol. 19 No. 2, 2019 pp. 3043-3049 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF ADDING TABLE SALT AND CITRIC ACID ON SOME PROPERTIES OF IRAQI FISH (*LIZA ABO MUGILIDAE*) AFTER COOKING IN A MICROWAVE OVEN

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Abstract

Fish is considered an essential food source that should be entitled to be part of the human choice due to its richness in proteins, vitamins, minerals, and essential fatty acids. However, there is rising concern about the correct labelling of fish products where it is vulnerable to contamination and corruption. This current study aimed to assess moisture levels, protein content, peroxide value (PV) in fish. Local Iraqi fresh fish *(Liza abo mugilidae*) purchased from Baghdad market (from Tigris River) were examined for its moisture and crude protein contents by reference methods. Significant differences in proximate composition were determined after cooking the fish in the microwave for 8 min for three treatments: table salt, acid and salt and acid mixture in different concentration. In general, local fish showed the highest moisture content (80.1 g/100 g). Significantly, lower moisture and lower crude protein/nitrogen and higher PV contents were observed after cooking the fish in microwave oven treated with various NaCl salt concentrations and citric acid treatment and a mixture of both NaCl salt and citric acid. The results indicate that the best conditions to cook Iraqi local fish is without adding salt or citric acid before cooking in microwave oven for safe fish quality, and therefore, they can be added after microwave cooking.

Key words **:** Fish, microwave oven; protein; peroxide value (PV); moisture, citric acid, table salt.

Introduction

The microwave oven is a standard method in food processing as a unit operation such as blanching, drying, thawing and cooking, hence, time of the process can be reduced, and the loss of nutritional quality of products can be prevented (Agren and Hinninen 1993). Electromagnetic waves with a frequency of 300 MHZ to 300 GHz was shown have a deadly effect on a some of pathogenic microorganisms, both those occurring only in the vegetative phase such as (*Listeria* sp. *Salmonella* sp., *Campylobacter* sp.) or those capable of forming high-resistance spores for unfavorable external factors such as (*Bacillus* spp., *Clostridium* spp.) (AOAC, 1990).

Fish is worldwide known to be an important source of human nutrients, which contains valuable lipids and proteins (Bligh and Dyer, 1959). Western food regulation encouraged consumers to include fish in their food purchases (Breck, 2014). The chemical content of seafood is usually classified as water, protein, fat and ash with the possibility of low carbohydrate levels. However, the concentrations are considered negligible. Also, the composition of the fish body differs and is related to the fish's ability to feed, growth, and the quality of feed provided to fish (Castro *et al.,* 2007). The main component is water in terms of size and weight, in all seafood products and has a key role as a determinant of product value, shelf life and sensory attributes. Over time, commercial and marketing processing methods have evolved to maintain the moisture of processed food such as adding it to seafood during collection, manufacturing and storage to compensate low humidity or loss of punctuation during frozen storage and/or during thawing. Nevertheless, it is necessary to distinguish between the addition of water to compensate for the shortage of moisture and the addition of water for commercial purposes and illegal gain.

Protein is the second major seafood component. One of the methods used to measure the protein content in

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foods is the Kjeldahl method, based on the measurement of nitrogen content. (Colwell *et al.,* 2011). Nitrogen is found in fish in two forms, the first is protein nitrogen, and the other is non-protein nitrogen (NPN) which are consisting mainly of amino acids, peptide, amines, amine oxides, guanidine compounds, quaternary ammonium compounds, boron and Urea. In addition to the above, hundreds of muscle tissues in fish include a type of protein called contractile proteins such as myofibrillar, as well as sarcoplasmic proteins present in the fluid in the muscles, which are mainly enzymes, and these proteins are the only ones that are soluble in water. Another type of protein is the meat proteins that are represented in the connective tissues, and its function is to bond the muscle with the skeletal bundles. Moreover, these compounds originated mostly from the sarcoplasm.

Fish are different in their nitrogen content, but generally nitrogen content in the muscle tissue of finfish ranges from 18-22 grams of crude protein per 100 grams, equivalent to 2.9-3.5 grams of nitrogen per 100 grams (Darvishi *et al.,* 2013). There is a strong relationship between moisture and protein levels in meat (De Greef *et al.,* 1992) and seafood (Castro *et al.,* 2007; Dong *et al.,* 2011) due to physiological reasons, but generally, the content of water and protein in muscle meat is approximately 77% and 23% respectively, Thus, the ratio of the protein-water is about 3.35 grams. These data can be used to estimate the amount of additional water that can be added. Most pre-packaged of the fish products are supposed to carry a declaration of the amount of fish present as a percent of the final weight of the commercial product, as required by the quantitative ingredients declaration (QID) which was included in the European Food Labelling Directive. On the other hand, there is a growing concern about the actual ratios of ingredients as well as correct labelling on seafood products being vulnerable to mislabeling. For example, 75 fraud cases have reported in the Food Fraud Database of the US Pharmacy Convention (http://www.foodfraud.org) so far (Bligh and Dyer 1959). Most of the time, these concerns include the replacement of wild species with cultured species, the geographically distorted geographical origin, as well as the addition of excessive water. In the European Rapidly Alert System for Food and Feed (Breck 2014), fish and fishery products were listed eighty-six times in conjunction with fraud and/or adulteration where this system is concerned with fraudulent health certificates and illegal imports (Breck, 2014). Food fraud appears when several criteria are met. This requires an opportunity as an appropriate target, a motive (which may be moral, economic and/or social), appropriate interpretation (justification by the involved parties) and lack of supervision. The nature of food fraud is that the legal representative manipulates the actual composition of the food product in order to avoid quality assurance and quality assurance schemes implemented by consumers, manufacturers, distributors and needs a different way to ensure food safety (Bligh and Dyer, 1959). In order to ensure rapid and low-cost production, food supply networks were developed, resulting in the emergence of non-transparent, non-protected, fraudulent systems. These systems are highly applicable to seafood products, which are supplied from different parts of the world and are often delivered through large-scale connectivity networks. Extraneous water addition to poultry meat fixing the water/protein ratio to 3.40 for chicken breasts (non-preparations) w regulated by the Poultry Meat Marketing Regulation Standards (EC) 543/2008 (Fogaça *et al.,* 2011). Likewise, parameters for water to protein ratios on a fat-free basis for pork and beef was specified to (3.40–3.50) and (3.60–3.70) respectively by the Food Industries Manual (Guler *et al.,* 2008). There may also be a relationship between moisture content and protein as well as its relationship with fat. One of the issues is the lack of sufficient reports to measure the actual moisture/protein content of seafood products in the market because they (*i.e.* seafood) are highly susceptible to commercial fraud and neglect of correct measurements.

This study aims for understanding water management practice for some seafood species sold in Iraqi markets, as an important and common food product consumed in Iraq. Official reference methods were applied to determine the moisture and crude protein contents in seafood obtained from local supermarkets and retailers. In contrast to saltwater fish, the results of the study showed that lower levels of n-3 EPA and DHA with higher levels of C18 PUFA in freshwater fish contain. On the other hand, other results suggest that freshwater fish can stretch and desaturate shorter fatty acids, converting them into EPA and DHA. Thus, by altering foods with low nutritional value, foods with high nutritional value can be obtained (Henderson and Tocher 1987; Honikel 2009). Despite its high nutritional value, fish is considered as a high, delicate food due to the fish components, pH close to neutral, water activity is high and presence of a natural microflora with deterioration potential (Honikel, 2009). As a result, awareness about the proximate structure and physiochemical properties of fish species are essential parameters for defining the processing conditions and product preservation.

Materials and Methods

Iraqi local fish (*Liza abo mugilidae*) samples were purchased from local supermarkets, (specialized fish shops and open-air markets) in Baghdad. All samples were marked then stored at "18°C till arrival to the laboratory. Samples ground before moisture and crude

Fig. 1: Iraqi local fish (*Liza abo mugilidae*) experimental samples.

protein analysis, Samples (150.0 g ±1.0 g) of each weight treatment were transferred to 14 cm diameter and 2 cm high Pyrex Petri dishes covered with an "l ml thickness" PVC. The microwave oven (Electrolux, model EMM2005, 2450 MHz) operated at 400 W. The samples were heated in this microwave oven at the centre of the 27cm diameter of rotary plate for 8 minutes with various salt concentrations (0.5, 1,1. 5 g), treated with citric acid (0.5,1,1.5 g) and a mixture of salt and citric acid (salt + acid) compared with control.

Proximate composition

Moisture Analysis

Moisture determination was executed by the referenced method (ISO 937:1978). This method takes into account the weight loss acquired after fully drying the test subject until reaching a mass of 105 ± 2°C, divided by the weight of the test part. Moisture determination has been done on each sample.

Crude Protein Analysis

The crude protein content was determined by the referenced method (ISO 937:1978) (Jensen *et al.,* 2013). Protein analyses were carried out on each sample (2x).

Ash Determination

Ash contents determination achieved by the reference method (AOAC 1996). This method considering the weight losses obtained after moisture and then burn the material in a muffle furnace for 4 hrs. At 550ºC or until it is free from all carbonaceous materials and white or greyish white ash remains.

Total lipid determination

Total lipids in fish tissue determination were achieved according to the standard AOAC method (AOAC 1996) which was altered by Lee *et al*., (1996); solvents used to extract lipid included chloroform-methanol, n-hexane-isopropyl alcohol, and methylene chloride-methanol. After mixing dried fish tissue with solvent, the mixture was filtered. The solvent was evaporated by using a rotary evaporator at 35ºC. Finally, the lipid's content of the samples was determined gravimetrically.

Fatty acid analysis

Fatty acids were extracted from the zone (belly of the fish) by using the method mentioned (Karl *et al.,* 2010). Ventrecha zone was subject to the test because the major lipid fraction of the muscler is concentrated in it. Derivatives were isolated by esterification and saponification with 1.2 MHCI and 0.1 M KOH both in methanol. Hexan used to extract the fatty acid derivatives and passed through Gas Chromatography GC (USA), using CP-Sil 88 capillary column (60 m × 0.25 mm), a helium and flame ionization detector (FID) as the carrier gas. The fatty acids were determined by comparison with their peaks obtained using fatty acids methyl esters standard solutions under the same operating conditions (Krzynowek and Murphy 1987).

Peroxide value methods

The following analytical standard methods of IUPAC (Lee *et al.,* 1996); the peroxide value (Method 2.501) was determined iodometrically; the results were expressed in meq/kg.

Statistical Analysis

The SAS (2012), the statistical analysis system program was used to determine the effect of different treatments within experiment parameters. The least significant difference (LSD) test was used to the significant comparison between the treatment means.

Results and Discussion

Analyzed parameters in the different muscle zones appeared that the chemical composition was significantly different for; the major constituent in all analyzed fish muscle zones was protein table 1. In fish total lipid content, muscle distribution was considered inhomogeneous, with a higher lipid content observed in the named ventrecha zone, which indicate that this region is the fish's fat deposit area. Accordingly, ventrecha zone was the zone with the smaller moisture content. Results show values of moisture, protein, total lipids and ash contents were (80.2%, 19.6 %, 1.4 %, and 1.1%) sequentially table 1.

Table 1: Proximate consistent of fish fresh muscles*.

Parameter	Composition (%)
Moisture	80.2
Protein	19.6
Total lipid	1.4
Ash	1.1

* Each sample in duplicate.

In previous research on the proximate composition of muscles, moisture values observed were 79.5 and 77.9%, protein 17.6 and 16.1%, total lipids 0.6 and 2.5%, and ash 0.9 and 0.8% in pirarucu fish muscle zones (Martins *et al.*, 2015). (Moreira *et al.*, 2001; Nederlands and Visbureau, 2014) observed moisture values of 75.0 and 75.5%, protein 20.1 and 21.4%, total lipids 0.6 and 2.6%, and ash 2.5% and 0.9% in dorsal zone of the fish. The recorded variations may be due to aspects as sex, age of animal, species, diet, seasonality and capture source, as well as the analyzed muscle portion (Honikel 2009). Mohamed *et al*., 2010 found a different range of fish content in Sudan according to their species, as well as lipid content was 1.8 to 17.3%, and moisture was 73 to 80%, Protein content was 59.8% in *S. schall* and 77 to 79.1% in the remaining species. Generally, the proximate composition of the pirarucu fish muscle was similar to that observed by (AOAC 1990) in the farmed code muscle where moisture, protein, total lipids and ash results were (78.0%, 18.6%, 1.0% and 1.3%) respectively.

For the determining levels of moisture and protein of five different treatments with salt and acid compared with fresh sample ISO reference methods. There are several techniques for determining moisture content, for example, near-infrared, nuclear magnetic resonance and guided microwave spectroscopy, however, in the current study, moisture content measurement was based on oven drying (Olagunju *et al.*, 2012; Oliveira *et al.*, 2014). The protein (nitrogen) levels were identified by the widely used Kjeldahl method while Dumas combustion method was used to determine alternatives for nitrogen content, as well as other methods such as electrophoretic, chromatography, colorimetric, immunology-based mass and spectrometry methods (Osman *et al.*, 2007). (Tables 2, 3) shows the fish moisture and protein contents of the treatment's samples investigated in this study.

Table 2 indicates that the adding of salt, acid and their mixture was significantly decrease the moisture and protein contents levels in the fish samples with increasing the concentration of salt and acid with the maximum decreasing in their mixture with different mixing percent, the moisture and protein content was 54%, 15.40% for salt 0.5g moisture , protein followed by (48 %, 11.12) for salt treatment 1g, 34 %, 9.23% for salt treatment 1.5 g and mixed samples 29%, 7. 03% for mixed salt + acid treatment and compared with control was 81%, 20.11% and different moisture and protein levels, the one with 57%, 14.31% for acid treatment 0.5 g, 44 %, 11.18 for acid treatment 1g, 31% and 8.53 for acid treatment 1.5g, 25%, 5.05 for salt + acid mixed treatment compared with control was 79% and 19.12%. Fish with 29% of moisture had a rubbery texture with the difficulty of biting and mastication whereas fish with 54% moisture became soft and flabby. It was suggested that the reduction of moisture was due to the heat drying which caused dehydration and denaturation. Results from (Paquot and Hautfenne 1987) are compatible with the current study results which exhibited moisture content of 82.1% and 17.4% of protein content for fresh fillets. Similarly, proximate composition of salmon fish is compatible with previous studies, e.g. moisture content ranged between 60%–75% and the protein content ranged between 17%–25% which was reported in this study where many numbers of salmon samples were investigated (Ranken *et al.*, 1997). On the other hand, noted by Oliveira, (2014) study evaluating 178 salmon samples from Ireland, Norway and Scotland showed that average moisture content of 69.1% which is fairly close to the data obtained in the present study. A recent study by (Rathod and Pagarkar 2013) reported Pangasius fish moisture 76.6% and protein contents 14.4%. On the other hand, a study by (Regulation (EC) No. 178/2002 2002) reported that pangasius fish moisture and protein contents of 82.7% and 14.2%, respectively. For black tiger shrimp, the moisture content of 80.5% and protein content of 17.1% have been reported while for white shrimp 77.2% moisture and 18.8% protein were reported (Bligh and Dyer 1959). Furthermore, for pink shrimp, the moisture content of 80.1% and protein content of 18.1% were reported (Paquot and Hautfenne 1987). Finally, for tilapia, the moisture content of 75.8% and protein content of 18.8% were reported (Regulation (EC)

Table 2: Effect of adding the different percentage of salt, citric acid and a mixture of both on fish's Moisture and protein % after cooking in the microwave oven*.

Treatment	Moisture %	Protein %	Treatment	Moisture %	Protein %
Control	81 ± 2.94 a	18.11 ± 1.03 a	Control	79 ± 2.63 a	19.12 ± 1.17 a
Salted (0.5) g	54 ± 2.06 b	15.40 ± 0.77 b	Acid (0.5) g	57 ± 2.44 b	14.31 ± 0.74 b
Salted (1) g	48 ± 1.59 b	11.12 ± 0.49 c	Acid (1) g	44 ± 2.05 c	11.81 ± 0.44 bc
Salted (1.5) g	34 ± 1.08 c	9.23 ± 0.52 cd	Acid (1.5) g	31 ± 1.76 d	8.53 ± 0.37 c
Mixed Salt 1 g + Acid 0.5g	29 ± 1.44 c	7.03 ± 0.27 d	Mixed Salt 0.5g + Acid 1g	25 ± 0.97 d	5.05 ± 0.16 d
LSD value	7.024 *	3.418 *	.....	6.849 *	3.291 *
*(P<0.05). Means having with the different letters in same column differed significantly.					

*Each sample is duplicated.

Table 3: Effect of a microwave oven cooking on fish's protein % using different percentages of table salt, citric acid and their mixture*.

Treatment	Protein	Treatment	Protein
Control	20.11	Control	19.12
Salted (0.5) g	15.40	Acid (0.5) g	14.31
Salted (1) g	11.12	Acid (1) g	11.81
Salted (1.5) g	9.23	Acid (1.5) g	8.53
Mixed salt 1 g + Acid 0.5g	7.03	Mixed salt 0.5g + Acid 1 g	5.05

*Every single sample is duplicated.

No. 543/2008, as well as the moisture range of 74.4%–77.8%, have been published (Rodrigues *et al.*, 2010).

In this study, the effect of a microwave oven cooking on the quality of fish fat was observed. Also, the effect of adding different proportions of table salt and citric acid and their mixture on the peroxide value of the oil extracted from the microchip fish for the mentioned treatments. The results showed in table 4 indicated that the heating of fresh fish for 8 minutes caused significantly increase in the peroxide value of 3.63 for control and the highest PV of (15.32) in mixed salt 0.5g + acid 1g treatment. The total amount of other products did not increase because of heating. The reason behind these results is probably to the oxygen low amount present during boiling for the intensive oxidation process to take place, especially concerning that the internal temperature measured for the fillets, regardless of the technique used or cooking method, was never higher than 90°C, due to a water large amount which founded in the tested product. Furthermore, the decomposition of hydroperoxides were occurred because of high temperatures.

Lipid fraction of the fat deposit region (ventrecha) in pirarucu showed 42.7% of saturated fatty acids (SFAs) and 8.4% of polyunsaturated fatty acids (PUFAs), 48.9% of monounsaturated fatty acids (MUFAs), Table 5. The similar fatty acid profiles (MUFAs > SFAs > PUFAs) were noted in three fresh water fish species (Matrinchã, Piraputanga and Piracanjuba) by (Honikel, 2009), (Sargent *et al*., 1995) and (Stancheva and Merdzhanova, 2011) in farmed common carp, a teleost fish as pirarucu. On the other hand, (Sriket *et al*., 2007) and (Jensen *et al*., 2013) reported a higher SFAs content than MUFAs in seawater fish and farmed cod, respectively. Freshwater fish have lower levels of PUFAs than marine fish, especially EPA and DHA. The differences in fatty acid profiles of marine and freshwater fishes are related to their natural diet and the habitat of the species such as herbivorous, omnivorous or carnivorous species (Stancheva and Merdzhanova 2011; Tocher 2003). The C18:1 was the major fatty acid in the fatty acid profile of pirarucu, followed by C16:0 and C18:0. A study by Castro *et al*., (2007) indicated a similar result in three kinds of freshwater fish. Also,

Table 4: Effect of cooking in the microwave oven with different percentage of table salt, citric acid and a mixture of them on fish's lipid PV*.

Treatment	PV (meq/ Kg)
Control	3. 03 ± 0.07 e
Salted (0.5) g	4.88 ± 0.11 de
Salted (1)g	5. 35 ± 0.11 d
Salted (1.5) g	8.57 ± 0.25 bc
Mixed salt 1 g + acid 0.5g	9.61 ± 0.39 b
acid (0.5) g	5. 21 ± 0.18 de
acid (1) g	6.23 ± 0.29 cd
acid (1.5) g	10. 03 ± 0.42 b
Mixed salt 0.5g + acid 1 g.	15.32 ± 0.74 a
LSD value	2.066 *
* (P<0.05). Means having with the different letters in same column differed significantly	

* each sample in duplicate.

Jensen *et al*., 2013 founded that C22:6(EPA) is the main fatty acids which followed by C16:0 and C20:5(DHA) in the fatty acid content of farmed cod. Previous literature reported that the majority of the fatty acids were C18:0 and C16:0 in fish muscle (Welt *et al.*, 1994 and Sargent *et al*., 1995). In generally, the C18:1 is the main fatty acids present in the animal origin products in contract with the vegetative ones (FAO, 2009). Pirarucu have a considerable quantity of fatty acids n-3(1372.4 mg/100 g of fresh muscle), as C18:3(ALA), C20:5(EPA) and C22:6(DHA), which has importance for human health. The results showed that fish contains higher n-3 levels than n-6PUFAs table 5. However, freshwater fish have shown an ability for conversion C18 fatty acids, such as C18:2 and C18:3, into EPA, DHA, and C20:4 (Welt *et*

Table 5: Fatty acids composition in fresh fish muscle.

Fatty acids	Composition (mg/100 g fresh muscle)
C 14:0	631.7 ± 30.3
C 14:1	123.9 ± 2.1
C 16:0	4780.8 ± 49.2
C 16:1	139.6 ± 5.9
C 18:0	1590.2 ± 55
C 18:1	7787.8 ± 63
C 20:0	99.2 ± 2.2
C 20:1	138.7 ± 1.6
C 18:3	459.8 ± 23.6
C 20:3	67.3 ± 1.8
C 22:0	173.2 ± 1.4
C 20:5	336.6 ± 29.5
C 24:1	41.2 ± 12.0
C 22:6	571.6 ± 18.0
Σn-3	1372.4 ± 72.6
Σn-6	65.3 ± 1.6

al., 1994). Because of the high content of unsaturated fatty acids, a high peroxide numbers after cooking in the microwave oven is noted beside the addition of salt and citric acid in all concentrations. Therefore, the addition of table salt, acid and the mixture of them leads to the decline of the qualitative characteristics of the fish under study when cooking with a microwave oven, and therefore, they can be added after cooking.

Conclusion

The Iraqi local fish showed the highest moisture content (80.1 g/100 g), also the protein, total lipids and ash contents were 19.6%, 1.4 %, and 1.1% respectively. The fish samples were treated with various NaCl salt concentrations and citric acid treatment and a mixture of both NaCl salt and citric acid, Significantly, lower moisture and lower crude protein content with increasing the concentration of salt and acid with the maximum decreasing in their mixture according to different mixing percent. The heating of fresh fish for 8 minutes in microwave caused an increase in the peroxide value of (3.63) for control in contract with the highest PV of (15.32) with minimum protein content in both mixed salt plus acid treatments were observed after the fish cooking in microwave oven. We conclude that the best conditions to cook Iraqi local fish (*Liza abo mugilidae*) by microwave oven, is cooking it as it is without adding salt or citric acid before, for safe and high fish quality, and therefore, they can be added after microwave cooking.

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