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Effect of cooking methods and refrigeration conditions on quality and safety of the crayfish (*Procambarus clarkii*)

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ABSTRACT

This work aimed to investigate the nutritive value, quality, and safety of raw and cooked crayfish (*Procambarus clarkii*) samples collected from several locations of the River Nile. The extracted meat of the crayfish tails was sampled to be examined. They were divided into two batches; the control samples (without any treatment), and the other batch in which samples were brined, rinsed with tap water, drained, spiced (with garlic, salt, lemon, and cumin), cooked, and stored in the refrigerator at 4±1°C for 9 days. Based on their cooking methods (frying& microwave cooking), the refrigerated boiled tails were divided into two groups and compared to the control. The chemical composition, physicochemical, and microbiological quality of the raw samples were adjusted. The effect of cold storage on the safety and quality of the cooked samples was determined. The effect of the two cooking methods on the samples was also recorded. The recorded nutritive values of raw crayfish tails were addressed as follows: moisture = 78.61%, crude protein = 18.46%, fat = 1.58%, ash = 1.33%, carbohydrates = 0.02% and 65.08 mg\16 g N for total EAAs. Additionally, the values of quality and safety indices were recorded, including: pH (6.02), TVB-N (11.20 mg/100g), TMA (0.123 mg/100g), TBA (0.113mg MDA/kg) and TPC (185 cfu/g sample) on wet wt.. An apparent increase was detected in the total protein, fat, and ash with respect to both types of cooking, whereas water content recorded a progressive decrease, particularly in the fried samples. However, a little change was found throughout refrigeration storage. Remarkably, crayfish proved to possess a high quality and safety. Thus, the current study recommended using crayfish to fill the gap in the Egyptian fish market. In addition, the frying method was determined to be better than the microwave cooking method.

INTRODUCTION

Although crayfish or crawfish is considered an excellent source of high biological proteins, minerals, vitamins and polyunsaturated fatty acids but its high water content, the lack in amino acids content and microorganisms lead to more rapid spoilage **(Mahmoud, 2010)**. Crawfish can be eaten either alive or boiled, and the meat of its tail forms a high quality product, which is sold world-wide. The hepatopancreas is often left in crawfish transferred to local markets because of its desired flavor **(Romaire *et al.*, 2005)**. Several investigations have been conducted to determine the chemical composition, quality attributes and safety of raw crayfish and to study the effect of different cooking methods on the nutritive value and safety of some new crayfish products; fried, canned and burger **(Ibrahim *et al.*, 1996; Mona *et al.*, 2000; Nyström, 2002; Baheyeldine, 2007; Zaglol & Eltadawy, 2009; Elsherif & Abd Ek-Ghafour, 2015; Abdelhady, 2017; Abraha *et al.*, 2018)**.

Therefore, the present work was performed to determine the chemical composition, physicochemical, microbiological quality of raw crayfish, and simultaneously study the effect of cold storage (4±1°C) on quality and safety of cooked (fried and microwave) crayfish samples.

MATERIALS AND METHODS

1. Crayfish samples

About 25 kg of crayfish, *Procambarus clarkii*, (33.50±0.25 g, 11±0.11 cm) were collected from several locations of the River Nile and transferred in an icebox to Fish Processing and Technology Laboratory, El-Kanater El-Khairia Branch, National Institute of Oceanography and Fisheries (NIOF).

2. Ingredients

Sodium chloride, spices, wheat flour and sunflower oil were purchased from local market.

3. Technological processes

Crayfish samples were carefully washed with cold water, and head and carapace were manually removed. Crayfish meat was then manually peeled and the tail meat was removed from the shells. The meat of crayfish tails was divided into (a) control samples (without any treatment), and (b) samples soaked in 10% sodium chloride solution for 5 min. at an ambient temperature. The latter samples were then rinsed with tap water, drained and spiced (with garlic, salt, lemon and cumin). All samples of raw and boiled tails were packed in polyethylene bags and stored in refrigerator at 4±1^{o}C for 9 days. Refrigerated boiled tails' samples were divided into two groups based on their cooking methods (frying and microwave cooking) and compared to the control.

3.1. Cooking methods

The samples of the treated meat of crayfish tails were cooked with different methods as follows:

3.1.1. Frying

Prepared crayfish tails meat were coated with thin layer of wheat flour (72% extraction) and left for 4 min and then deep-fried in sunflower oil preheated at 160^{o}C for 5-6 min.

3.1.2. Microwave cooking

The prepared meat of crayfish tails was cooked in a microwave oven (Samsung model M 1932) at 600 watt for 6-8 min. All fried and microwave cooked samples were left at an ambient temperature, packed in polyethylene bags, tightly closed, and stored in refrigerator at 4±1^{o}C for 9 days.

3.2. Analytical methods

All samples of crayfish tails meat (raw and cooked) were subjected to analysis at intervals 0, 3, 6, and 9 days. Chemical composition; (moisture, protein, fat and ash content) and trimethylamine nitrogen (TMA-N) **(AOAC, 2002)**. Quality attributes, total volatile basic nitrogen (TVB-N), thiobarbituic acid (TBA) and the pH value **(Pearson, 1991)** were analyzed. In addition, amino acid composition **(Anderson *et al.*, 1977)** was also determined. Microbial load was considered, and the total plate count (TPC) using plate count agar **(FAO, 1992)** was examined. Sensory tests **(Fey & Regenstein, 1982)** were evaluated. The results obtained (Microsoft Office Excel, 2010) were statistically analyzed and they (n=3) were expressed as Mean±SD.

RESULTS AND DISCUSSION

1. Chemical composition

The results of chemical composition of raw and cooked crayfish products are shown in Table (1). Raw crayfish (*P. clarkii*) meat contained 78.97% moisture, 18.45% crude protein, 1.19% fat, 1.34% ash and 0.05% carbohydrates content.

After cooking, these values of moisture, crude protein, fat, ash and carbohydrates content changed markedly to record 38.18%, 35.83%, 18.88%, 6.35%, and 0.53% in fried samples and 58.20%, 35.22%, 2.73%, 3.25% and 0.15% in microwave-cooked samples, respectively. Both frying and microwave processes led to an increase in the values of total protein, fat and ash content whereas, a progressively decrease was found in water content, in particular the fried samples.

Table 1. Chemical composition (ww) of raw and cooked crayfish products

Constituent (%)	Raw	Cooked crayfish products	
		Fried	Microwaved
Moisture	78.97±0.24	38.18±0.12	58.20±0.48
Protein	18.45±0.15	35.83±0.17	35.22±0.05
Fat	1.19±0.23	18.88±0.44	2.73±0.15
Ash	1.34±0.98	6.35±0.38	3.25±1.05
Carbohydrates	0.05±0.03	0.53±0.12	0.15±0.03

Values are means ± standard deviation of three samples

Although crayfish meat is a cheap source of animal protein for the Egyptian citizens, but it is similar to the high expensive shrimp and lobster **(Baheyeldine, 2007; El-Kholie *et al.*, 2012)**. The current results presented in Table (1) coincide with those of other studies **(Holand *et al.,* 1993; Elmossalami & Emara, 1999; Zaglol & Eltadawy, 2009; Sobeia, 2010; El-Kholie *et al.,* 2012; Elsherif & Abdelgafour, 2015; ´Smietana *et al.,* 2021)**. In their analysis of the chemical composition of crayfish meat, they stated a ranged value from 76.60-82.15% for moisture, 13.88- 19.77% for crude protein, 0.26-1.99% for fat, 1.18-1.59% for ash and 0.07-0.16% for carbohydrates content. Variation in chemical composition of crayfish meat is due to several parameters; season, sex, species, age, feeding, spawning and catch location.

These results are in consistence with the results of several studies; although cooking methods enhance nutritional quality, digestibility and bio-availability of nutrients in the digestive tract and sensorial quality, they reduce contaminants and extend shelf life of the cooked fish samples. Nevertheless,the cooking methods cause several changes in major and minor constituents of cooked fishr composition. Additionally, any change in the loss of water content, denaturation of protein, loss or increase in fat, and ash content depends mainly on heating and the time consumed in each used cooking method **(El & Kavas, 1996; Bognar, 1998; García–Arias *et al.,* 2003; Akinneye *et al.,* 2010; Alipour *et al.,* 2010; Kocatepe *et al.,* 2011; Pourshamsian *et al.,* 2012; Talab, 2014; Abd-Allah**; **Ibrahim, 2020)**. On the other hand, **Koubaa *et al.* (2012)** and **Bainy *et al.* (2015)** decided that though the cooking methods may have a little effecton the chemical composition or may have no recorded impact, yet they apparently affect both the texture and the color of products.

2. Quality criteria

Table (2) shows some physico-chemical and microbiological parameters of raw and cooked crayfish products. Cooking loss recorded 31.17 and 18.33% of fried and microwave cooked samples, respectively. Quality indices of raw crayfish meat were 6.02 pH, 0.11mg MDA\kg as TBA, 11.20 mg/100g TVN, and 0.12 mg/100g TMA content. The values of TBA, TVN, and TMA increased in the cooked samples while those of pH declined. Furthermore, all values, except for TBA, were higher in microwave cooked samples compared to fried samples. With regard to TPC, it was found that raw sample recorded 185×10^3 cfu\g sample, sharply depressed to be 4.60×10^3 and 4.71×10^3 cfu\g sample of fried and microwave cooked samples, respectively.

Results of pH, TBA, TMA and TPC (Table, 2) are lower while TVN content is higher than the results obtained in the study of **Elsherif and Abdelgafour (2015)** who reported that, crayfish meat had 6.98 pH, 8.21 mg TVN\100 g, 0.72 mg TMA\100 g sample, 0.27 mg MDA/kg and TPC 2.15×10^3 cell/g sample.

The effect of cooking methods decreased the TBA value in cooked samples; this may be due to MDA as secondary oxidation product reacts easily with protein molecules, loss in the frying oil, Maillard reaction, or breakdown of MDA into volatile compounds **(Maqsood & Benjakul, 2010; Narciso-Gaytán *et al.,* 2011; Tavares *et al.,* 2018)**. However, the present results are lower than the recommended limits of TBA value and TVN content. Therefore, crayfish samples in this study had a high quality based on fish, shell fish and fish products of good quality having a TBA value less than 2 mg Malonaldehyde/kg sample; while poor quality fish would have 3–27 mg/kg and TVB-N 30–40 mg/100 g for freshwater fish and 60 mg/100 g for marine fish **(Connell, 1990; Bonnell, 1994)**. These results agree with the same trends of other studies **(Talab, 2014; Bastías *et al*., 2017; Hernández-Sánchez *et al*. 2020)**.

Table 2. Physico-chemical and microbiological parameters of raw and cooked crayfish products

Item	Raw	Cooked crayfish products	
		Fried	Microwaved
Cooking loss (%)	-	31.17	18.33
pH	6.02±0.30	5.75±0.04	5.80±0.63
TBA (mg MDA/kg)	0.11±0.45	0.41±0.19	0.15±0.18
TVN (mg/100g)	11.20±0.16	8.54±0.01	11.20±0.16
TMA (mg/100g)	0.12±0.01	0.80±0.10	0.94±0.11
TBC (cfu/g)	185±0.96	4.60±0.45	4.71±0.17
Psychrophilic (cfu/g)	*Nd	3.30±0.15	3.47±0.13

*Nd: not determined. Values are means±standard deviation of three samples

3. Amino acids composition

Results in Table (3) exhibit the essential amino acids (EAAs) composition (mg\16 g N) of raw and cooked crayfish products. Raw crayfish meat contained 8 EAAs. EAAs (mg\16 g N) of raw crayfish meat took the following order; valine > leucine > phenylalanine > lysine > tyrosine > methionine > histidine > isoleucine while cyctine did not detect. All values of EAAs decreased sharply in cooked samples, especially valine and leucine content in fried samples.

Table 3. Essential amino acids (EAAs) composition (mg\16 g N) of raw and cooked crayfish products

EAAs	Raw	Cooked crayfish products	
		Fried	Microwaved
Valine	15.51	2.70	3.85
Methionine+cystine	(5.41+UD)= 5.41	(0.66+UD)=0.66	(1.68+UD)= 1.68
Isoleucine	1.75	1.08	1.55
Leucine	11.49	5.94	7.15
Phenylalanine+tyrosine	(10.80+6.37)=17.17	(7.22+3.30)=10.52	8.59+4.04
Histidine	5.16	2.93	4.02
Lysine	8.59	5.45	5.97

UD: undetectable.

The nonessential amino acids (NEAAs) composition (mg\100gm protein = mg\16 g N) of raw and cooked crayfish products are presented in Table (4). Raw crayfish meat contained 8 NEAAs too. Raw meat of crayfish has a higher content of glutamic (96.82 mg\100 gm protein) and lower content of proline (3.21mg\100gm) than the other ones. Besides, it contained NEAAs in the following order: arginine > aspartic > serine > alanine > therionine > glycine.

Table 4. Nonessential amino acids (NEAAs) composition (mg\16 g N) of raw and cooked crayfish products

NEAAs	Raw	Cooked crayfish products	
		Fried	Microwaved
Aspartic	10.11	4.80	5.98
Theronine	4.32	1.45	2.18
Serine	9.46	3.03	4.09
Glutamic acid	96.82	37.88	52.85
Glycine	3.36	1.51	1.99
Alanine	8.93	5.78	7.58
Arginine	20.24	9.45	10.69
Proline	3.21	1.36	1.20

There is no doubt that any change that occurres in the chemical composition is notably reflected on other components; this fact is confirmed in many studies. **Zaglol and Eltadawy (2009)** found 9 EAAS of raw crayfish meat; cysteine (0.339), valine (6.707), methionine (5.928), therionine (1.49), isoleucine (6.699), leucine (9.831), tyrosine (2.297), phenylalanine (4.104), histidine (3.090) and lysine (6.462, g\ 16g N). They added that the NEAAs were aspartic (9.480), serine (2891), glutamic (11.207), proline (4.006), glycine (5.815), alanine (5.590) and arginine (11.384 mg\ 16 g N). In addition**,** **El-Kholi *et al.* (2012)** reported that EAAs of crayfish flesh were cysteine (1.44), valine (5.71), methionine (2.96), isoleucine (4.41), leucine (8.70), tyrosine (4.77), phenylalanine (4.41), histidine (1.98) and lysine (7.60, g\ 16g N). They also showed that NEAAs were aspartic (11.43), therionine (1.75), serine (5.12), glutamic (15.33), proline (3.09), glycine (4.47), alanine (5.02) and arginine (7.90 g\16 g N). **Smietana *et al.* (2021)** showed that spiny-cheek crayfish (*Faxonius limosus*) abdomen muscles contained total EAAs (g/16g N) were 3.78 histidine, 19.42 arginine, 7.42 isoleucine, 13.12 leucine, 14.10 lysine, 4.54 methionine, 5.71 tryptophane, 5.96 tyrosine, 6.81 phenylalanine, 7.28 valine and 6.63 threonine. They added that the NEAAs (mg\g meat) were 9.38 alanine, 17.70 aspartic, 2.08 cysteine, 29.55 glutamic, 12.02 glycine, 5.22 proline and 6.88 serine. Therefore, it could be observed that AAs composition varied compared to the pesent results (Tables 3 & 4). This variation in AAs composition is due to the same factors as pre- mentioned in the chemical composition and the cooking methods used.

4. Effect of refrigerated storage periods

The fefect of refrigerated storage periods on the chemical composition of cooked crayfish products is shown in Table (5). A slight increase was found in values of moisture and ash while crude protein, ash and carbohydrate content decreased in all cooked crayfish samples compared to zero time. In addition, chemical composition has the same trend of fried and microwave-cooked samples throughout refrigeration periods. At the end of storage, values of chemical composition were 38.82 and 58.57% moisture content, 35.38 and 34.89% crude protein, 18.53 and 2.52% fat, 6.79 and 3.64% ash and 0.48 and 0.10% carbohydrate content of fried and microwave-cooked samples, respectively. Therefore, the effect of refrigeration storage conditions have a negligible change on chemical composition of cooked crayfish products. Hence, any occurring change in the chemical composition would eminently affect all quality indices of the cooked crayfish meat samples.

Table 5. Effect of refrigerated storage periods on chemical composition of cooked crayfish products

Constituent (%)	Refrigerated storage periods (day)							
	0		3		6		9	
	Fried	Microwaved	Fried	Microwaved	Fried	Microwaved	Fried	Microwaved
Moisture	38.18±0.12	58.20±0.48	38.39±0.86	58.63±0.28	38.53±0.15	58.57±0.90	38.82±0.01	58.57±0.90
Protein	36.06±0.30	35.67±0.20	35.83±0.17	35.22±0.05	35.63±0.37	35.21±0.32	35.38±0.25	34.89±0.10
Fat	18.88±0.44	2.73±0.15	18.74±0.46	2.61±0.14	18.66±0.15	2.57±0.22	18.53±0.12	2.52±0.34
Ash	6.35±0.38	3.25±1.05	6.52±0.65	3.40±0.05	6.68±0.01	3.53±0.31	6.79±0.23	3.64±0.15
Carbohydrates	0.53±0.12	0.15±0.03	0.52±0.03	0.14±0.01	0.50±0.01	0.12±0.19	0.48±0.50	0.10±0.01

Values are means±standard deviation of three samples

Table 6. Effect of refrigerated storage periods on Physico-chemical and microbiological parameters of cooked crayfish products

Item	Refrigerated storage periods (day)							
	0		3		6		9	
	Fried	Microwaved	Fried	Microwaved	Fried	Microwaved	Fried	Microwaved
TBA (mg MDA/kg)	0.41±0.19	0.15±0.18	0.62±0.25	0.38±0.03	1.73±0.08	0.81±0.05	3.52±0.45	1.67±0.19
TVN (mg/100g)	8.54±0.01	11.20±0.16	11.48±0.16	16.94±0.09	16.80±0.10	18.34±0.02	30.74±0.11	39.54±0.32
TMA (mg/100g)	0.80±0.10	0.94±0.11	1.05±0.04	1.65±0.07	1.51±0.22	2.19±0.3	2.07±0.16	3.62±0.18
TPC (cfu/g)	4.60±0.45	4.71±0.17	4.88±0.04	4.95±0.10	5.25±0.30	5.43±0.23	5.39±0.10	5.55±0.16
Psychrophilic	3.30±0.15	3.47±0.13	3.47±0.22	3.84±0.20	3.77±0.09	4.11±0.04	4.06±0.22	4.20±0.06

Values are means ± standard deviation of three samples

Results in Table (6) demonstrate the effect of refrigerated storage periods on physico-chemical and microbiological parameters of cooked crayfish products. Moreover, values of pH, TBA, TVN, TMA increased sharply in all cooked samples, especially in microwave-cooked samples compared to the fried ones during refrigeration storage conditions compared to zero time. At the end of storage, values recorded 3.52 and 1.67mg MDA\kg sample as TBA, 30.74 and 39.54 mg TVN\ 100gm s, 2.07 and 3.62 mg TMA\100gm of fried and microwave-cooked samples, respectively. With regard the effect of refrigerated storage periods up to 9 days on microbial load, counts of TPC and psychrophilic bacteria increased markedly in both cooked crayfish samples compared to zero time. Furthermore, the TPC recorded values were 5.39 and 5.55, while those of the spychrophilic were 4.06 and 4.20 cfu/g sample with respect to fried and microwave-cooked samples, respectively.

Results of sensory quality of cooked crayfish products as affected by refrigerated storage periods are tabulated in Table (7). Score of appearance of fried samples was excellent up to 3 days of storage, good after 6 days and accepted at the end of storage. While, in case of microwave cooked samples, it was very good at zero time, good at 3 and 6 days, and rejected at the end of storage. Texture property was very good up to 3 days and good at 6 days for all the cooked crayfish samples. At 9 days of storage, the texture of fried samples was accepted, while that of the microwave cooked was rejected. Flavor score was very good for the fried samples and good for the microwave cooked samples up to 3 days. Fried samples were accepted at 9 days, while microwave cooked samples were rejected. Score of over acceptability of fried crayfish samples was more accepted than other one.

Table 7. Effect of refrigerated storage periods on sensory quality of cooked crayfish products

Property	Refrigerated storage periods (day)							
	0		3		6		9	
	Fried	Microwaved	Fried	Microwaved	Fried	Microwaved	Fried	Microwaved
Appearance	9.3	8.5	8.7	7.3	7.5	6.8	6.3	5.4
Texture	8.2	8.3	7.9	7.7	7.4	7.2	6.3	5.4
Flavor	8.5	7.4	7.9	6.9	6.3	5.6	5.6	5.0
Overall acceptability	8.7	8.1	8.2	7.3	7.1	6.5	5.9	4.8

Scores: Excellent: 8.6-10; very good: 7.6-8.5; good: 6.6-7.5; accepted: 5.6-6.5.

This variation of sensory tests of cooked samples may be due to palatability by panelists where fried samples were more accepted than microwave cooked samples. Additionally, the effect of refrigeration storage up to 9 days led to a marked decrease in all sensorial properties, especially in microwave cooked samples compared to fried samples. This is due to heat transferring and time of each cooking method **(Fabre *et al.*, 2018)** with respect to the values of glutamic acid, aspartic acid, alanine, and glycine that are responsible for flavor and taste. Amino acids were also used as the quality indices for different species of fish and crustaceans **(Ruiz-Capillas & Moral, 2004)**. It was noticed that the frying method improved flavor, eating quality, and fat nutritional values **(Li *et al.*, 2016; Abd-Allah & Ibrahim, 2020)** compared to microwave cooking.

CONCLUSION

Based on the results obtained, crayfish meat has a high quality and has been proved safe for consumers. Frying method is better than microwave cooking. cCrayfish meat is recommended to be used to fill the gap in the Egyptian fish market. In addition, the heads and shells of crayfish are considered a good source to produce some bioactive compounds.

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