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RESEARCH PAPER

Comparision of cooking processes on nutritional value of fresh and cooked-blast chilled crayfish (*Astacus leptodactylus* Eschscholtz, 1823)

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Nutritional value
Proximate composition

Abstract

In this study, the effects of three different cooking methods (boiling, sous-vide cooking, microwave cooking) were evaluated on the nutritional content value of blast chilled crayfish. Proximate, fatty acid and amino acid composition, vitamin and mineral contents of crayfish were investigated before and after the cooking processes.

It was determined that the cooking methods with the least loss of vitamins and minerals were sous vide and microwave cooking. On the other hand, it was determined that there was no loss in EPA fatty acid in the boiling (BC) group samples, but there was a significant loss in the sous vide cooking (SVC) and microwave cooking (MC) group samples. In addition, the loss in DHA fatty acid in all groups cooked in DHA fatty acid was found to be statistically significant ($p<0.05$). While sous vide cooked crayfish had the highest protein content, microwave cooking was determined as the most appropriate cooking method in terms of EAA (Essential Amino Acids) ratio.

Consequently, it had been suggested that the sous vide and microwave cooking methods were the best methods preserving the nutritional value compared to the boiling method

Introduction

Preservation of the quality and nutritional content of foods is a process that starts from raw materials and continues in controlled processing steps. The rapid cooling process applied to the cooked foods in the cook-chill food service is also important for the preservation of the nutritional value of the cooked product. In the 1960s, the increasing demand for ready-made fast meals prepared by the food catering industries emphasized the value of cooked-chilled and cooked-frozen foods. Great interest in the fast food in 1960s and 1970s resulted in the use of

technology in commercial or industrial scale. Cook-chill systems are used mainly in foods from the catering service industry as well as chilled ready meals from supermarkets. The cook-chill technique is simple and provides flexibility in foodservice. The methodology involves the full cooking of food, followed by fast chilling and storage at cool temperatures. A safe product can be obtained if recommended temperature/time controls are followed. The preparation steps (i.e. including heating and cooling) are crucial factors for retaining nutritional value and sensory quality. Blast chiller, blast freezers or combination systems are capable of rapid cooling of the temperature of hot foods (+70 ^{o}C) to low safe storage temperatures (+3 ^{o}C or -18 ^{o}C). Refrigerated cook-chill foods deliver convenience and safety with the retention of nutritional content (El-Ansari & Bekhit, 2014). The interest in ready-to-eat and cooked-chilled foods continues to increase with the more conscious food choice of consumers (Chandrasekara & Shahidi, 2015). Especially time and temperature are critical parameters that must be strictly controlled for all heat treatments carried out in cook-chill systems (Copur & Tamer, 2003).

Meat, fish and their products are foods that provide important nutritional benefits because they contain all the amino acids necessary during digestion and high-quality proteins consisting of micronutrients necessary for human beings such as zinc and vitamin B_{12} (Shahidi et al., 1986; Venugopal, 2018). In recent years, more attention has been paid to aquaculture in order to meet the increase in global demand for shellfish (Chen & Xiong, 2008). Crayfish has become a cheap and popular source of aquatic food with a good source of minerals, amino acid content and flavorful nutritive content (El-Kholie et al., 2012; El-Sherif & El-Ghafour, 2015).

According to FAO (2019) data, crayfish production based on hunting in the world is 11654 tons. *Astacus leptodactylus* is the crayfish species that are caught and traded from inland waters in Turkey. Turkey's crayfish production is 1233 tons and a significant part of it is exported (Russia, European countries, etc.) as live or frozen (TUIK, 2020).

Micro and macronutrients are essential for the proper functioning of metabolic activities in the human body and for the proper functioning of all cells. Fish and shellfish are rich in essential amino acids, unsaturated fatty acids, micronutrients (vitamins, minerals) and macronutrients. Moreover, long-chain fatty acids from seafood oils can reduce the risk of chronic diseases such as diabetes, cardiovascular, inflammatory diseases and neurological disorders (El-Sherif & El-Ghafour, 2015; Sarojnalini & Hei, 2019). What matters is how much our bodies can benefit biologically from the foods we consume. Today's conscious consumers tend to consume foods with cooking methods that preserve both nutritional value and flavor. Crustaceans such as lobster, shrimp, crayfish are generally cooked by boiling (FAO, 1978; Wojtowicz, 1974). But boiling may cause the water-soluble materials to leach into water and reduce the nutritional value. For this reason, innovative cooking techniques are needed to preserve nutritional value. Sous vide cooking allows slow cooking in a short time in the package which maintains the flavor of the food and preserves its nutritional values (Creed, 1998). Similarly, it is stated that foods cooked in the microwave preserve their nutritional values, especially vitamins, compared to other traditional cooking methods (Bordoloi & Ganguly, 2014).

There is limited information on the nutritional differences of crayfish due to the different cooking processes applied. This study aims to compare the nutritional values of fresh and cooked crayfish with different cooking methods (BC: boiled, SVC: sous vide, MC: microwaved) to be used for researchers, industrial producers, and consumers.

Materials and Methods

Preparation and cooking of raw materials

Crayfish (*Astacus leptodactylus*) caught alive from Lake Eğirdir in Isparta province were brought to Istanbul University, Faculty of Aquatic Sciences, Seafood Processing Technologies laboratory in a perforated styrofoam box within 12 hours (Figure 1). Crayfish were supplied in 2 styrofoam boxes of 6 kg each (12 kg crayfish in total). After washing and straining, height and weight measurements were made. The average length of the crayfish used in the analyses was 13.04 ± 1.01 cm and their average weight was 41.20 ± 6.43 g.

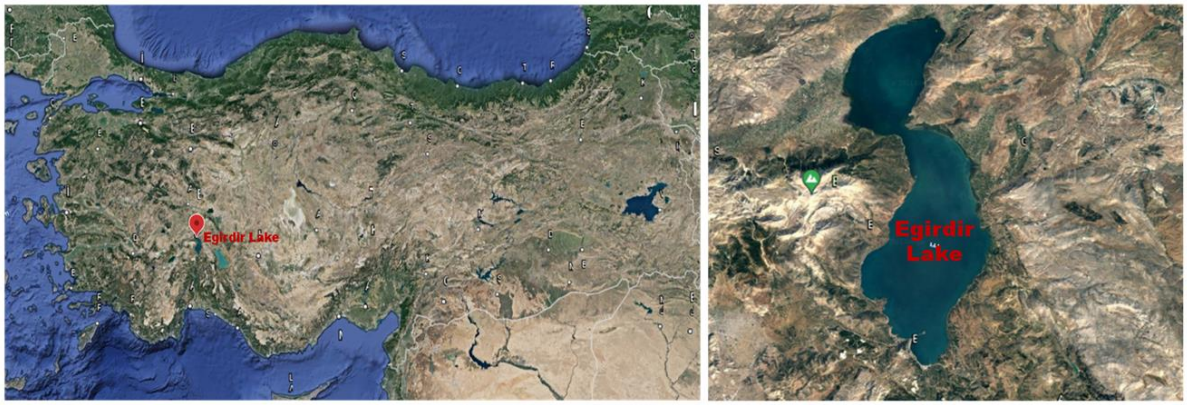

Figure 1. Satellite image of Lake Egirdir-Isparta/Turkey, from which we supplied the crayfish (Google Earth, 2022).

Live crayfish were stored in a deep freezer for 5 hours at -26°C to obtain crayfish with the same conditions before cooking. The frozen crayfish were thawed in the refrigerator. The raw crayfish (FC: control) were divided into three groups (SVC: Sous vide cooking, BC: Boiling, MC: Microwave cooking) for the cooking process (Figure 2). Cooked crayfish were immediately cooled in blast-chiller after the cooking processes. Vacuum packages were used for the raw crayfish, cooked in sous vide, and boiled crayfish. For microwave cooked crayfish microwave cooking packaging was used.

The heads were separated from their bodies and only the tails were used to ensure homogeneous heat transfer in sous vide cooking. The crayfish tails were vacuum-packed (Polinas Polibarr Y10C1B, 90 µ, 160 cm^3/m^2/day oxygen permeability, moisture permeability 8.50 g/m^2/day; Manisa, Turkey) and cooked sous vide at 65ºC for 15 minutes (Sous-videTM, Professional PolyScience Chef Series, USA). During the cooking process, the internal temperature was measured by placing thermocouples in the middle of the three packages. In boiling, crayfish were cooked at 100ºC for 10 minutes. After the cooking process it was vacuum packed (Polinas Polibarr Y10C1B, 90 µ, 160 cm3/m^2/day oxygen permeability, moisture permeability 8.50 g/m^2/day; Manisa, Turkey) by Henkovac model (Holland) vacuum machine (Cycle time 40/60 s, Voltage 230V/1/50/60 Hz, Power 0.4 kW, Volume pump capacity 8 m^3). The remaining crayfish were placed in 12/40 PET/CPP laminate microwave packs (Excelsior Technologies LTD, UK) and cooked in the microwave (LG, 1200W, South Korea) for 4 minutes.

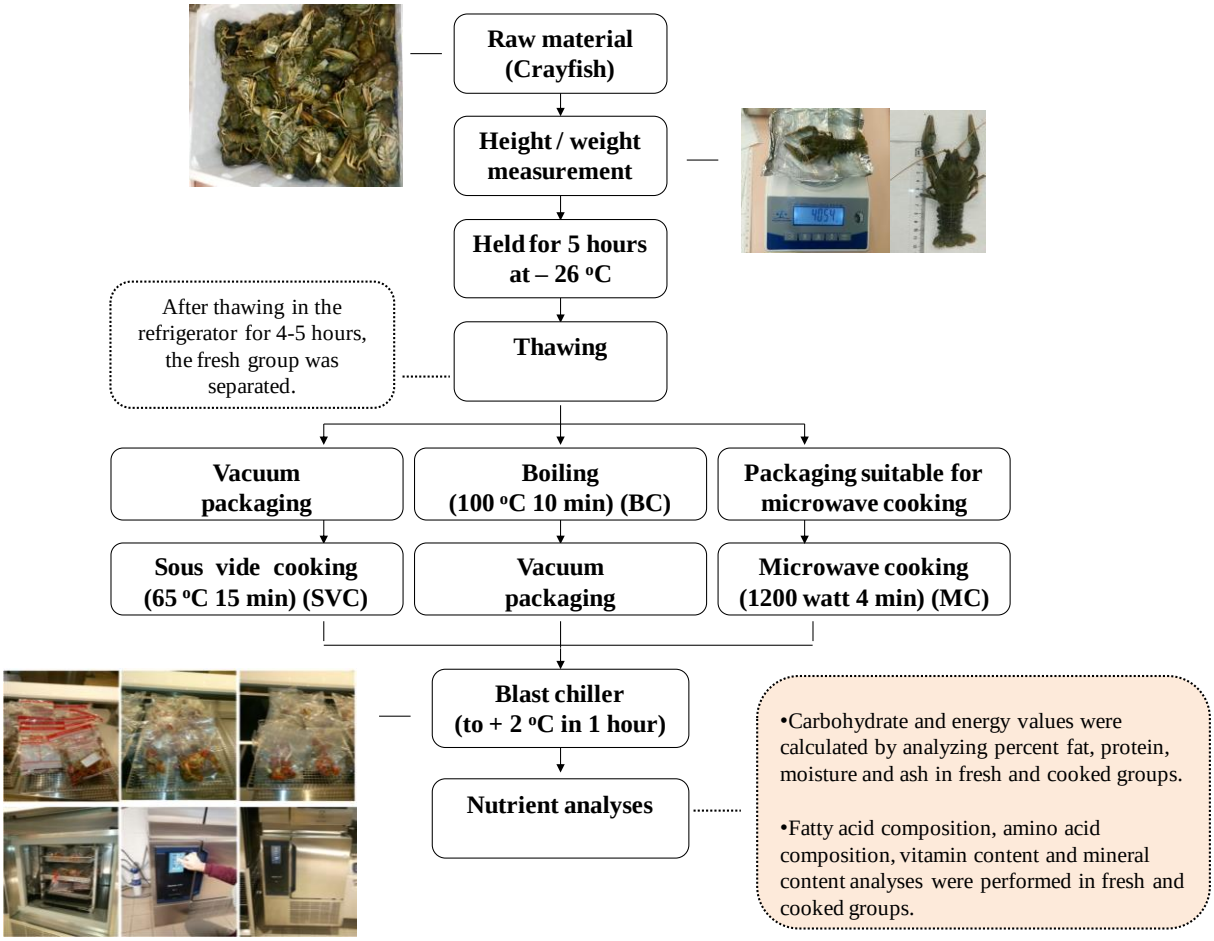

Figure 2. Cooked crayfish flow chart

Chemical analyses

Proximate composition and energy value: Crude protein analysis was performed according to AOAC (1998a). The main purpose of the protein analysis method is the conversion of free nitrogen in foods into ammonium ions. For this, the sample was first decomposed with concentrated sulfuric acid at high temperatures. The resulting solution was distilled with 32% sodium hydroxide solution. The weak base formed by binding the liberated ammonia to the solution with acid was titrated with 0.2 N HCl. The result was subtracted from the blind sample and multiplied by a factor of 6.25. In this way, the amount of crude protein (%) in the sample was calculated. Ash content was performed according to AOAC (1998b). Ash content was measured by weighing 1 g of sample into porcelain crucibles previously kept at 550ºC. These crucibles were kept in a muffle furnace set at 550ºC (Protherm Furnace, PLF 110/6, Turkey) for 7 hours. After weighing the cooled crucibles, the ash content was calculated as a percent value. Moisture content was determined by the standard method of AOAC (2002). Moisture was measured by weighing 10 g of sample in a petri dish and kept in an oven drying at 105°C for 3 hours to constant weight. Crude fat was determined according to Bligh & Dyer (1959) method. 10 g of sample was shaken in 100 ml of methanol + chloroform (1:2) solution. The mixture was filtered into a separatory funnel. 20 ml of 0.04% $CaCl_2$ was added to it and the separatory funnel was shaken and left overnight. The lower phase of the prepared solution was transferred to the balloon. The chloroform was evaporated in the rotary evaporator (Buschi, R 3000, Switzerland). After waiting for 1-2 hours in the oven set at 100ºC, the balloons were weighed and the weighing results were calculated as % fat.

Carbohydrates content was calculated by subtracting the sum of the protein, fat, moisture, and ash amounts determined by analysis from 100.The Atwater method was used for the calculation of total calories. The energy value expressed as kcal 100 g^{-1} edible part was estimated using factor 4-4-9 calories per gram of protein, carbohydrate and fat, respectively (Merill & Watt, 1973).

The study was carried out in two replicates. All analyzes were performed in three parallel samples.

Instrumental analyses

Fatty acid composition (%): Fatty acid profile determination was done according to the procedure described by Demirtas et al. (2013). Fatty methyl esters (FAMEs) were determined according to the method of ISO 12966-2 (2011). 0.1 g of oil was weighed into a screw cap test tube, 0.5 ml of 2.0 N KOH and 5 ml of heptane were added and vortexed. Anhydrous sodium sulfate was then added for drying. After standing for 1 min, the solution was injected directly into gas chromatography (GC, Perkin Elmer, Auto system GLX, Shelton, USA). Chromatographic separation was performed using a Supelco SP™ -2380 (30 m x 0.25 mm i.d., 0.25 µm film thickness) column equipped with a flame ionization detector (FID). Working conditions were as follows: carrier gas, helium; flow rate was 0.5 mL/min; injector temperature, 280 ^{o}C; detector temperature, 260 ^{o}C; oven temperature program, initial temperature was 120 ^{o}C for 2 min, increased at 5 ^{o}C/min to 220 ^{o}C, held for 10 min. Data collected and quantitied with a TotalChrom Navigator (Perkin Elmer Inc, Wellesley, MA, USA) and the results were expressed as percent concentration.

Total amino acids: Amino acid analysis was performed according to Dimova (2003). This analysis was carried out using HPLC system (Shimadzu LC-20 AT, Tokyo, Japan), equipped with a UV detector. It was the measurement of the peak area given by the hydrolysis of the proteins in the sample to the amino acid components, derivatized with phenyl isothiocyanate, in the HPLC UV detector. Tryptophan content was determined according to the procedure of Çevikkalp et al. (2016). While determining tryptophan, it was liberated by using a basic solution to the proteins in the sample and the peak area was measured with the HPLC FL (fluorescence) detector.

The quantification was performed by correlating peak areas of the samples with the concentrations according to the calibration curve.

Vitamins: Vitamins were determined using an HPLC (Shimadzu 20 series, Japan). The following methods were used for vitamin analyses of this study: vitamin E Manz & Philipp (1981); vitamins B_1 and B_2 Reyes et al. (1989); vitamin B_3 Ndaw et al. (2002); vitamin B_6 Kall (2003) and vitamin B_{12} were made according to Guggisberg et al (2012).

The basic principle of the vitamin B_1, B_2, and B_6 method is the measurement of the peak area given in the HPLC FL detector by enzymatic incubation at 45°C, after keeping 1 g of sample in 0.1 N hydrochloric acids in an autoclave at 121°C for 30 minutes. Vitamin B_6 value represents the sum of pyridoxine, pyridoxal, and pyridoxamine forms. The principle of the Vitamin B_{12} method is the measurement of the peak area given by the HPLC UV detector after the sample extracted in acetate buffer is subjected to enzymatic incubation at 37°C and separated in the immunoaffinity column. The principle of the vitamin B_3 method, for example, is the measurement of the peak area it gives in the HPLC FL detector after acid/base treatments (1 hour in an autoclave at 121°C).

Minerals: Preparation of samples for mineral analysis was done according to AOAC (1990). 8 ml of HNO_3 and 2 ml of hydrogen peroxide H_2O_2 were added to 500 mg lyophilized sample and left for 20 minutes. The heating program was carried out in 2 steps using the Berghof Microwave System. (1) 600 W 55 min at 200 C; (2) 250 W at 100 C was applied for 10 minutes. Then, it was made up to 50 mL with ultrapure water

containing 0.5% nitric acid in 50 mL flasks. Then, the samples were filtered with a 0.45 µm filter and stored in the refrigerator at +4°C until analysis.

Analyzes were made using the Perkin Elmer brand Optima 7000DV model ICP-OES device at 27.12 MHz. All glassware and equipment were cleaned with 10% HNO_3 prior to sample analysis. In the preparation of calibration solutions, Single Element Aqueous Certified Reference Materials (Ca, Zn, Mg, Se, K, P, Na, Mn, Li, Fe, Cu, Cr, Mo, Ba) aliquots were used. Certified reference materials (VHG-V21+K-100-400G, VHG laboratories, USA) used in the study were prepared by diluting stock standard solutions in 1% HNO_3 to the desired concentrations (5 ppb – 50 ppm).

The blind test was performed by repeating the sample preparation procedure without the sample. The composition of the blank solution was compared with the sample solution to determine the elemental composition in the crayfish samples.

Statistical analyses

Data on nutritional value analysis results were subjected to one-way analysis of variance using SPSS (IBM SPSS Statistics 22). Tukey's multiple comparison test was used for statistical significance difference ($p<0.05$) between groups (Sumbuloglu & Sumbuloglu, 1990).

Results and Discussion

Proximate composition and energy value

The results of proximate composition and energy value of fresh and cooked crayfish are shown in Table 1.

Table 1. Changes in proximate composition and energy value of fresh and cooked crayfish

Sample	Crude fat (%)	Moisture (%)	Ash (%)	Crude protein (%)	Carbohydrate (%)	Energy value (kcal 100 g^{-1})
FC	1.95±0.05[a]	76.37±0.24[a]	1.52±0.32[a]	17.47±0.46[a]	2.69±0.27[a]	98.21±2.51[a]
BC	3.00±0.37[b]	75.74±1.07[a]	1.38±0.08[a]	18.94±0.06[b]	0.94±0.02[b]	106.56±3.35[b]
SVC	2.94±0.07[b]	75.51±0.57[a]	1.43±0.04[a]	19.02±0.03[b]	1.01±0.03[b]	106.58±0.72[b]
MC	3.01±0.47[b]	75.22±0.79[a]	1.30±0.22[a]	18.89±0.11[b]	1.58±0.90[c]	109.00±6.17[c]

Results are the mean ± standard deviation. [(a,c)]Different letters on the same column between groups show statistical significance ($p<0.05$). FC, fresh crayfish; BC, boiled crayfish; SVC, sous vide cooked crayfish; MC, microwaved crayfish

Most researchers stated that the moisture content of raw fish, bivalve and crustaceans decreased after heat treatment. Whereas their protein and fat values generally increased after the cooking process (El-Kholie et al., 2012; Garcia-Linares et al., 2004; Gonzalez-Fandos et al., 2004; Unusan, 2007; Mohan et al., 2017; Kocatepe et al., 2011; Hosseini et al. 2014; Nieva-Echevarría et al., 2017; Bongiorno et al., 2018; Cropotova et al., 2018; Humaid et al., 2020). In this study, a decrease was observed in the moisture, ash, and carbohydrate percentage of the crayfish after cooking while an increase was observed in the crude fat and protein percentages seeing all previous researchers seemed to agree on this determination. Our findings were similar to this observation of previous studies mentioned above. Boiling (BC) and microwave (MC) cooked of crayfish provided a significantly higher percentage fat ($p<0.05$) which resulted in higher energy. It was determined that there was no statistically significant difference in the proximate composition of sous vide cooked crayfish than fresh crayfish. Scientific studies (Steppeler et al.,2016; Yin et al.,2020; Bhat et al.,2021; Liu et al.,2021) show that sous vide cooking increases the protein digestibility of the food compared to boiled cooking and the result is a more juicy and homogeneous cooked food which is also suitable for gastrointestinal health.

Therefore, sous vide cooking can be recommended as the best cooking method for human nutrition.

Carbohydrates and fats are energy source nutrients. However, they cannot replace proteins in the construction of new tissues and the repair of old cells in the living body (Keskin, 1959). Therefore, a protein-free diet is unthinkable. Protein denaturation occurs after heat treatment in foods. This causes food to lose water and changes the structure of proteins (Dutson and Orcutt, 1984; Bhat et al.,2021). Rostini & Pratama (2018) stated that myosin, actin and sarcoplasmic proteins found in shrimp-type seafood are denatured during heat treatment. They also reported that this situation causes water loss and, consequently an increase in the amount of protein and fat. In this study, the highest protein and fat increase was observed as 19.02 % in sous vide cooking and as 3.01 % in microwave cooking respectively.

Fatty acid composition

Seafood oils are rich in ω-3 PUFAs and have high nutritional value. Since they are not synthesized in the animal or human body, it is beneficial for health to be taken into the body through diets (Steffens,1997; Gladyshev et al.,2007; Sengor et al.,2013). Therefore, the PUFA composition of seafood is important in human nutrition (Gall et al.,1983; Kalogeropoulos et al.,2004; Moradi et al.,2011; Candela et al.,2011). However, it has been suggested that various processing techniques such as smoking, marinating, etc., applied to seafood cause changes in the nutritional value (fatty acid, amino acids compositions, vitamins and minerals) and proximate composition of seafood products (Mai et al.,1978; Medina et al., 1994; Gokoglu et al.,2004; Otles & Sengor, 2005; Kaya et al., 2008, Su & Babb, 2007; Weber et al., 2008; Sengor et al., 2013, Turan & Kocatepe, 2014; Momenzadeh et al., 2017). Essential fatty acids, which are important for human health are not synthesized in the body and must be taken from outside, have many benefits from cardiovascular diseases to neurological disorders (FAO, 2010). It has been proven by many studies that there are ω-3 PUFAs such as EPA, DHA, and C18:3n3 and MUFA fatty acids such as C16:1n7, C18:1n9c, C20:1n9, among these important fatty acids in seafood (Cengiz et al., 2010; Aziz et al.,2013; Durmus, 2019; Prato et al.,2019). Pigott (1989) reported that terrestrial plants were high in omega-6 (ω-6) PUFA and had limited omega-3 (ω-3) PUFA, aquatic plants contain significant amounts of PUFA C20 and C22 carbon chains, namely omega-3 fatty acids.

The fatty acid profiles obtained in our study are presented in Table 2. The major saturated fatty acids of fresh crayfish are palmitic (18.10 %) and stearic (11.40 %) acids and the total saturated fatty acid ratio was found to be 36.43 %. After cooking, a decrease of 8 % in BC group samples, 2.25 % in SVC group samples and 12 % in MC group samples were determined in the ratio of basic saturated fatty acids of crayfish.

The major monounsaturated fatty acids of fresh crayfish were palmitoleic (4.26 %) and oleic (16.04 %) acids and the total monounsaturated fatty acid ratio was found to be 21.93 %. It was found that the total monounsaturated fatty acid (MUFA) ratio of all samples increased after cooking. High and long-term heat treatment applied to seafood during the cooking process may lead to a decrease in ω-3 PUFA concentration. Some researchers put forward that technological processes such as frying, smoking, canning, marinating, etc. negatively affect the omega 3 PUFA especially EPA and DHA concentrations (Pigott & Tucker,1987; Candela et al.,1997; Gladyshev et al.,2007; Kaya et al.,2008; Colakoglu et al.,2011). The major polyunsaturated fatty acids (PUFA) of fresh crayfish were linoleic (4.73%), arachidonic (7.70 %), eicosapentaenoic (7.75 %), and docosahexaenoic (2.26 %) acids and the ratio of total polyunsaturated fatty acids was found to be 24.67 %. It was determined that there was no significant change in the total polyunsaturated fatty acid ratio of all cooked samples except for the SVC group samples. After cooking process the highest

loss in total PUFA acids was around 23 % in the SVC group samples.

Ceylan et al. (2018) reported that the ratio of ω-3/ω-6 is considered as an important value for the loss of nutritional value of fish. Indeed, after the cooking processes were applied to the fresh crayfish, a slight decrease in the ω-3/ω-6 ratio was detected in the SVC and MC group samples ($p>0.05$), and an increase in the BC group samples ($p<0.05$) compared to the fresh crayfish.

The effects of ω-6 and ω-3 fatty acids, which are taken into the body with the foods in the diet, on human metabolism are known. Therefore, it is necessary and recommended for a healthy diet that the ω-6/ω-3 ratio taken into the body through diets is less than 1 (Simopoulos, 2001). Excessive intake of ω-6 polyunsaturated fatty acids with diets triggers the pathogenesis of many diseases in cardiovascular diseases, cancer, inflammatory and autoimmune system, while increasing ω-3 PUFA levels (low ω-6/ω-3 ratio) has a suppressive effect (Simopoulos, 2002). The balance of ω-6/ω-3 fatty acids is an important marker in reducing the risk of coronary heart disease (Simopoulos, 2008). The ω-6/ω-3 ratio of fresh crayfish was 1.14. It was determined that the ω-6/ω-3 ratio of the cooked BC, SVC, and MC samples were between 1.06 and 1.21. According to COMA (1994) the PUFA/SFA ratio should be above 0.45 and the ω-6/ω-3 ratio should not exceed 4.0 for human nutrition. In this study the PUFA/SFA ratio of all cooked crayfish samples were between 0.53 to 0.74.

Table 2. Fatty acid composition in fresh and cooked crayfish

Fatty acids (%)	**FC**	**SVC**	**BC**	**MC**
C12:0 Lauric acid	1.00±0.01^{a}	0.17±0.04^{b}	0.15±0.01^{b}	0.19±0.01^{b}
C14:0 Myristic acid	1.90±0.02^{a}	1.25±0.02^{b}	1.23±0.02^{b}	1.95±0.04^{a}
C15:0 Pentadecanoic acid	0.68±0.01^{a}	0.89±0.04^{b}	0.84±0.00^{c}	0.79±0.01^{c}
C16:0 Palmitic acid	18.10±0.13^{a}	21.29±0.13^{b}	20.40±0.05^{c}	20.20±0.13^{c}
C16:1 Palmitoleic acid	4.26±0.02^{a}	7.36±0.02^{b}	6.96±0.02^{c}	7.06±0.06^{c}
C17:0 Heptadecanoic acid	1.23±0.02^{a}	1.28±0.01^{a}	1.13±0.02^{b}	1.00±0.01^{c}
C18:0 Stearic acid	11.40±0.18^{a}	9.39±0.02^{b}	8.34±0.06^{c}	6.76±0.06^{d}
C18:1n9t Elaidic acid	0.46±0.01^{a}	0.48±0.01^{a}	0.41±0.02^{b}	0.37±0.00^{b}
C18:1n9c Oleic acid	16.04±0.16^{a}	13.34±0.10^{b}	13.71±0.06^{b}	14.72±0.00^{c}
C18:2n6 Linoleic acid	4.73±0.09^{a}	4.14±0.11^{b}	4.80±0.08^{a}	6.07±0.08^{c}
C20:0 Arachidic acid	1.00±0.02^{a}	0.84±0.04^{b}	0.72±0.00^{c}	0.54±0.01^{d}
C20:1 cis-Eicosenoic acid	1.19±0.05^{a}	1.07±0.01^{a,b}	0.99±0.04^{b,c}	0.84±0.04^{c}
C18:3n3 a-Linolenic acid	0.90±0.00^{a}	1.33±0.00^{b}	1.64±0.03^{c}	1.66±0.04^{c}
C21:0 Heneicosanoic acid	0.57±0.06^{a}	0.39±0.01^{a}	0.49±0.08^{a}	0.62±0.03^{b}
C20:2 cis-11.14-Eicodienoic acid	1.34±0.01^{a}	1.29±0.04^{a}	1.55±0.07^{b}	1.49±0.02^{c}
C22:0 Behenic acid	0.58±0.00^{a}	0.51±0.01^{b}	0.43±0.01^{c}	0.32±0.01^{d}
C20:3n6 cis-8.11.14-Eicosatrienoic acid	0.00±0.00*	0.14±0.08^{a}	0.19±0.02^{a}	0.21±0.02^{a}
C20:3n3 cis-11.14.17-Eicosatrienoic acid	0.00±0.00*	0.14±0.01^{a}	0.12±0.04^{a}	0.18±0.03^{a}
C20:4n6 Arachidonic acid	7.70±0.30^{a}	5.26±0.02^{b}	6.90±0.08^{c}	5.89±0.08^{d}
C20:5n3 cis-5.8.11.14.17 Eicosapentaenoic acid	7.75±0.01^{a}	5.11±0.07^{b}	7.33±0.01^{a}	6.20±0.44^{c}
C22:6n3 Docosahexaenoic acid	2.26±0.01^{a}	1.66±0.02^{b}	2.14±0.00^{c}	2.06±0.03^{d}
ΣSFA	36.43±0.28^{a}	35.99±0.11^{a}	33.71±0.10^{b}	32.35±0.22^{c}
ΣMUFA	21.93±0.13^{a}	22.24±0.06^{a}	22.06±0.02^{a}	22.99±0.11^{b}
ΣPUFA	24.67±0.20^{a}	19.05±0.27^{b}	24.66±0.10^{a}	23.74±0.57^{a}
Σω-3 PUFA	10.91±0.02^{a}	8.24±0.06^{b}	11.23±0.01^{a}	10.10±0.40^{a}
Σω-6 PUFA	12.43±0.21^{a}	9.53±0.16^{b}	11.89±0.02^{a}	12.16±0.14^{a}
Σω-3/ ω-6	0.88±0.01^{a}	0.87±0.01^{a}	0.95±0.01^{b}	0.84±0.02^{a}
Σω-6/ ω-3	1.14±0.02^{a}	1.16±0.01^{a}	1.06±0.00^{b}	1.21±0.04^{a}
ΣPUFA/SFA	0.68±0.00^{a}	0.53±0.00^{b}	0.74±0.01^{c,d}	0.74±0.02^{c,d}

*Statistical analyses are not applicable. Results are the mean ± standard deviation. $^{(a-d)}$Different letters on the same row between groups show statistical significance ($p<0.05$). FC, fresh crayfish; BC, boiled crayfish; SVC, sous vide cooked crayfish; MC, microwaved crayfish

Amino acid composition

Free amino acids do not only contribute to the immune system and energy metabolism in the human body, but also increase the flavor performance in foods. Amino acids generally give characteristic flavors such as sweet, sour, bitter and umami (Xu et al., 2016).

Seafood is a source of nutrients that are high in protein and rich in essential amino acids, beneficial to the human body. Amino acids are important biomolecules that serve as building blocks of proteins and intermediates in various metabolic pathways (Mohanty et al., 2014). The effect of the thermal process on the stability of muscle proteins is known in previous research. Changes in protein fractions of fish muscle during the thermal process are heat denaturation of proteins from fish muscle. The proteins in the skeletal muscle of red meat, fish, and poultry are quite similar and heat treatment affects these proteins in a similar way (Dutson & Orcutt, 1984; Hwang et al., 1997; Shirai et al., 1996; Rathod et al., 2022). Table 3 shows the amino acids in fresh and cooked crayfish. Among free amino acids in fresh crayfish (FC), the predominant one was glutamic acid, followed by alanine, aspartic acid, glycine, arginine, and tyrosine. These components may be responsible for taste-active ingredients such as abalone, which contains large amounts of taste-active amino acids such as glutamic acid, glycine, arginine and alanine reported by Hwang et al. (1997) or taste-active components such as glycine, arginine, proline, alanine and glutamic acid reported in Japanese spiny lobster by Shirai et al. (1996).

According to the research results, the total essential amino acid amounts of the cooked crayfish samples were found to be 8.87 g/100 g in the MC group, 7.80 g/100 g in the BC group and 7.60 g/100 g in the SVC group, respectively. A significant increase was detected in the total amino acid amounts of all cooked crayfish after cooking ($p<0.05$). There was an increase of 2.05 g/100g, 3.33 g/100g and 4.78 g/100g in the total amino acid amounts of the BC, SVC, and MC groups compared to the FC group respectively. Accordingly, it is clearly seen that the highest increase in essential amino acids was in microwave cooked in the package. For the cooked crayfish samples, lysine and leucine amino acids are the major acids and their amounts (Table 3) were found to be the highest ($p<0.05$) compared to the fresh crayfish except for the lysine amino acid content of the SVC group sample.

Essential/Nonessential (EAA/NEAA) ratio was determined to be between 0.81-0.98. Iwasaki and Harada (1985) reported that the EAA/NEAA ratio of many fish is 0.70 on average. Although the EAA/NEAA ratio (0.81) in the cooked crayfish groups was the lowest in the SVC group, there were small changes in the total amino acid amounts of the protein and without loss of the nutritional value.

Table 3. Amino acid composition in fresh and cooked crayfish

Amino acids (g/100g)	FC	BC	SVC	MC
Aspartic acid	0.88±0.01[a]	0.99±0.01[b]	1.56±0.02[c]	1.50±0.04[d]
Glutamic acid	1.75± 0.01[a]	1.76±0.10[a]	2.78±0.06[b]	2.57±0.10[c]
Serine	0.55±0.01[a]	0.83±0.00[b]	0.87±0.01[c]	0.80±0.01[d]
Glycine	0.85±0.00[a]	0.81±0.00[b]	0.93±0.00[c]	0.94±0.05[d]
Histidine*	0.51±0.01[a]	0.66±0.00[b]	0.66±0.00[b]	0.61±0.00[c]
Arginine	0.78±0.00[a]	0.89±0.00[b]	0.91±0.01[b]	0.99±0.00[c]
Threonine*	0.56±0.00[a]	0.72±0.00[b]	0.81±0.00[c]	0.74±0.00[b]
Alanine	1.06± 0.03[a]	0.92±0.00[b]	1.06±0.06[a]	1.08±0.03[a]
Proline	0.59±0.00[a]	0.59±0.00[a]	0.69±0.01[b]	0.75±0.02[c]
Tyrosine	0.55±0.00[a]	1.16±0.12[b]	0.63±0.00[c]	0.98±0.00[d]
Valine*	0.61±0.01[a]	0.68±0.01[b]	0.76±0.01[c]	0.77±0.00[c]
Methionine*	0.37±0.00[a]	0.44±0.00[b]	0.53±0.00[c]	0.50±0.00[d]
Isoleucine*	0.72±0.00[a]	0.88±0.01[b]	0.93±0.01[c]	0.93±0.00[c]
Leucine*	1.04±0.01[a]	1.09±0.04[b]	1.30±0.04[c]	1.30±0.04[c]
Phenylalanine*	0.60±0.00[a]	0.66±0.00[b]	0.80±0.00[c]	0.80±0.00[c]
Lysine*	2.09±0.09[a]	2.44±0.49[b]	1.59±0.02[c]	2.99±0.07[d]
Tryptophane*	0.19±0.00[a]	0.23±0.01[b]	0.22±0.01[b]	0.23±0.01[b]
ΣEAA	6.69±0.12[a]	7.80±1.19[b]	7.60±0.08[c]	8.87±0.12[d]
ΣNEAA	7.01±0.06[a]	7.95±0.23[b]	9.43±0.17[c]	9.61±0.25[d]
ΣEAA/NEAA	0.95±0.02[a]	0.98±0.05[b]	0.81±0.05[c]	0.92±0.50[d]

*Essential amino acids (EAA). Results are the mean ± standard deviation.[(a-d)]Different letters on the same row between groups show statistical significance ($p<0.05$). FC, fresh crayfish; BC, boiled crayfish; SVC, sous vide cooked crayfish; MC, microwaved crayfish

Mineral contents

Table 4 shows the mineral content of fresh and cooked crayfish. The predominant elements among 14 minerals analyzed were calcium, zinc, magnesium, selenium, potassium, phosphorus, sodium, mangan, litium, iron, copper, chromium, molybdenum**,** and barium for fresh crayfish. The Ca, Zn, Mg, Se, K, P, Na, Mn, Li, Fe, Cu, Cr, Mo and Ba contents of fresh crayfish was determined as 289.7, 426.4, 178.6, 16.6, 144.2, 230.9, 190.5, 4.5, 1.0, 31.3, 36.1, 0.7, 0.6 and 0.7 mg/kg^{-1} respectively. After all cooking processes, the quantity of these macro and microelements significantly decreased and the most loss of mineral matter was in the sodium ($p<0.05$). Furthermore, a significant increase in iron and copper content of the MC group samples were determined after the cooking process ($p<0.05$). The impact of cooking process on the decreasing mineral contents of many seafood has been reported in previous studies (Gokoglu et al., 2004; Ersoy et al., 2006; El-Kholie et al., 2012; Hosseini et al. 2014). It has been reported that a remarkable decrease was observed in the Na, Ca, Mg, and P content of fish in particular ($p<0.05$). Noticeable amounts of phosphorous and zinc appear to have been lost in cooked crayfish by all cooking methods. A similar situation exists for the magnesium amounts of cooked crayfish, regardless of cooking methods. Gall et al. (1983) reported on the content of magnesium and phosphorus were found to be consistent with the results of our study. Meanwhile, a significant decrease was detected in the amount of iron (Fe) in the crayfish cooked by boiling and sous-vide method**s**, while a significant increase was detected in the crayfish cooked in the microwave. Depending on the cooking method significant differences were found in the copper (Cu) amounts of the crayfish both as increase and decrease ($p<0.05$). It was determined that the copper amount of crayfish cooked by the sous vide resulted in a decrease but it increased in the boiling method. Tamari and Tsuchiya (2004) point out that the lithium content of fish, especially anchovies and sardines, is a good source for lithium intake and may be beneficial for

patients with manic-depressive psychosis. In our study, the lithium content of fresh crayfish was found to be 0.97 mg/kg^{-1}. After the cooking processes, an increase in the lithium content of cooked crayfish was determined in boiling and microwave cooking methods. Molybdenum, which is widely distributed in nature, is one of the essential elements necessary for life. The toxic effect of molybdenum is especially related to the copper level (Hicsonmez, 2010). In this study, a slight increase in the molybdenum (Mo) amount was detected in boiled cooked crayfish after cooking. However, this increase is at a level that does not create a toxic effect. A slight decrease in molybdenum amounts were detected in sous vide and microwave cooked crayfish ($p<0.05$). Chromium (Cr), cobalt (Co), and barium (Ba) elements are found in trace amounts in foods, excess amounts cause toxic effects (Watanabe et al., 1997; Yilmaz et al., 2010). Although these elements were detected in trace amounts in cooked crayfish; Ba, Co and Cr amounts were found to be highest in microwave cooked crayfish samples.

Table 4. Mineral content in fresh and cooked crayfish

Minerals (mg/kg^{-1})	FC	BC	SVC	MC
Calcium (Ca)	289.70±7.50^{a}	257.30±50.00^{b}	272.40±11.45^{c}	144.60±9.40^{d}
Zinc (Zn)	426.40±5.22^{a}	271.40±4.16^{b}	301.50±0.71^{c}	309.00±3.04^{d}
Magnesium (Mg)	178.60±15.30^{a}	140.60±14.30^{b}	143.40±11.70^{c}	157.70±17.70^{d}
Selenium (Se)	16.62±0.16^{a}	16.45±0.14^{a}	16.42±0.06^{a}	15.53±0.21^{a}
Potassium (K)	144.20±11.90^{a}	144.80±8.50^{b}	178.60±4.20^{c}	195.00±1.50^{d}
Phosphorus (P)	230.90±4.60^{a}	173.60±4.60^{b}	181.20±2.66^{c}	185.60±5.63^{d}
Sodium (Na)	190.50±0.22^{a}	37.02±0.06^{b}	41.85±1.04^{c}	86.90±0.60^{d}
Manganese (Mn)	4.46±0.02^{a}	3.26±0.07^{b}	3.54±0.08^{b}	10.53±0.22^{c}
Lithium (Li)	0.97±0.00^{a}	1.03±0.00^{b}	0.91±0.00^{a}	1.53±0.01^{c}
Iron (Fe)	31.29±0.58^{a}	29.88±0.39^{b}	28.29±0.19^{b}	347.90±12.54^{c}
Copper (Cu)	36.12±0.54^{a}	65.07±0.30^{b}	29.51±0.31^{c}	44.84±0.23^{d}
Chromium (Cr)	0.74±0.03^{a}	0.67±0.02^{b}	0.63±0.03^{b}	1.83±0.08^{d}
Molybdenum (Mo)	0.63±0.04^{a}	0.71±0.05^{b}	0.58±0.03^{c}	0.28±0.02^{d}
Barium (Ba)	0.67±0.03^{a}	2.54±0.08^{b}	0.81±0.05^{c}	8.17±0.05^{d}
Σ	1551.80±46.14^{a}	1144.33±82.67^{b}	1199.64±32.51^{c}	1509.41±51.23^{d}

Results are the mean ± standard deviation. $^{(a-d)}$Different letters on the same row between groups show statistical significance ($p<0.05$). FC, fresh crayfish; BC, boiled crayfish; SVC, sous vide cooked crayfish; MC, microwaved crayfish

Vitamin content

Fish and fish products contain all the necessary vitamins for human nutrition (Alexander et al., 2006; Thorat, 2017; Reames, 2012). Table 5 shows vitamin B_1, B_2, B_3, B_6, B_{12} and E profiles of fresh and cooked crayfish. Vitamin B_{12}, B_3, and E were found to be in high amounts: 1.02 µg/100g, 2.43 and 3.49 mg/100g in fresh crayfish respectively. There was a significant decrease in all vitamin contents except B_{12} of cooked crayfish samples compared to fresh crayfish. After the cooking process, a significant increase was determined in the vitamin B_{12} content of the crayfish in boiled and sous vide cooked. Vitamin B_{12} is an important water-soluble vitamin.

It is found in varying amounts in animal tissues and products (Chhatbar & Velankar, 1979). Water-soluble vitamins (niacin, thiamine, riboflavin, pyridoxine, and cobalamine) are the most abundant vitamins in aquatic organisms. Vitamin B_{12}

(cobalamine) is especially abundant in oily fish and crustaceans (Venugopal, 2018). In our study, loss of vitamin B_3 and E amounts were determined after all cooking processes ($p<0.05$).

While the boiling process causes a decrease in vitamin E and group B (B_1, B_2, B_3, B_6) vitamins, there is no loss in the amount of vitamin B_{12}. It was determined that the highest amount of B_{12} among the cooked crayfish samples was found in sous-vide cooked crayfish. This result related to B vitamins is compatible with the report of Ersoy & Ozeren (2009). Vitamins are sensitive to losses during heat treatment. Washing before processing or cooking causes the loss of water-soluble vitamins. In addition, the combination of oxygen, heat and light or the effect of each separately causes vitamin losses (Venugopal, 2018).

Table 5. Vitamin content of fresh and cooked crayfish

Sample	Vitamin B_1 (thiamin) (mg/100g)	Vitamin B_2 (riboflavin) (mg/100g)	Vitamin B_3 (niacin) (mg/100g)	Vitamin B_6 (pyridoxine) (mg/100g)	Vitamin B_{12} (cobalamin) (µg/100g)	Vitamin E (α-tocopherol) (mg/100g)
FC	0.03 ± 0.00^{a}	0.09 ± 0.00^{a}	2.43 ± 0.07^{a}	0.08 ± 0.00^{a}	1.02 ± 0.13^{a}	3.49 ± 0.01^{a}
BC	0.02 ± 0.00^{a}	0.06 ± 0.00^{b}	2.01 ± 0.13^{b}	0.07 ± 0.00^{a}	1.15 ± 0.06^{a}	3.15 ± 0.02^{b}
SVC	0.04 ± 0.00^{b}	0.08 ± 0.00^{c}	1.63 ± 0.04^{c}	0.09 ± 0.00^{a}	1.40 ± 0.07^{b}	2.77 ± 0.09^{c}
MC	0.03 ± 0.00^{a}	0.08 ± 0.00^{c}	1.80 ± 0.06^{d}	0.08 ± 0.00^{a}	1.01 ± 0.04^{a}	2.41 ± 0.03^{d}

Results are the mean ± standard deviation. $^{(a-d)}$Different letters on the same column between groups show statistical significance ($p<0.05$). FC, fresh crayfish; BC, boiled crayfish; SVC, sous vide cooked crayfish; MC, microwaved crayfish

Conclusion

The results obtained from the study revealed that crayfish has high protein content, low ω-6/ω-3 ratio, suitable EAA/NEAA ratios in terms of nutritional value, is rich in Ca, Zn, Mg, Se, K, P, Na, Fe and Cu, significant B_3 and vitamin E content, and it is a valuable food source. Because of the appropriate EAA/NEAA ratio (0.81–0.98) in cooked crayfish, it is a high-quality protein source. Both fresh and cooked crayfish due to their low ω-6/ω-3 ratios (1.06-1.21) are good indicators for healthy nutrition. The cooking methods with the least loss of vitamins and minerals were sous vide and microwave cooking methods. Consequently, it had been determined that sous vide and microwave cooking were the most suitable cooking method in terms of preserving the nutritional quality of crayfish.

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Ethical approval

The authors declares that this study complies with research and publication ethics.

Data availability statement

The authors declare that data are available from authors upon reasonable request.

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