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The impacts of fish protein isolate addition on the nutritional and quality properties of chicken frankfurter during cold storage (4 °C)

Auswirkungen der Zugabe von Fischeiweißisolat auf die ernährungsphysiologischen Eigenschaften und die Qualitätsmerkmale von kalt gelagerten (4 °C) Hühnerwürstchen

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Summary

It is important to maintain the sensory quality and shelf-life of the final product, while increasing the nutritional quality of the meat product and reducing the cost of production. It was aimed to combination of fish protein isolate prepared from discard fish and chicken meat for frankfurter production to provide both add value to the discard fish and to enlarge the nutritional quality of the final product. The amount of chicken meat used in frankfurter sausages was reduced by 10%, 20% and 30%, and pony fish protein isolates were added instead. Peroxide, free fatty acid and TBA values were determined below the acceptable limits during storage for 47 days, while all sausage groups were found to reach to the limit of non-consumption on day 26 according to TVB-N and TVC values. However, according to the sensory evaluations, the 30%FPI group had a shelf life of 33 days, while the 20%FPI and 10%FPI groups had a shelf life of 40 days and the 0%FPI (FPI-free) group had a shelf life of 47 days. The least textural deterioration was observed in 20%FPI group, however, texture feature of 10%FPI group was the most close to control group. The addition of fish protein isolate to chicken sausages has improved the nutritional quality in respect to protein content, DHA level and n6/n3PUFA ratio and they can be stored for 26 days in cold conditions like control group.

Keywords: Fish protein isolate, texture, frankfurter, fatty acid composition, shelf life

Zusammenfassung

Es ist wichtig die sensorische Qualität und die Haltbarkeit des fertigen Produktes zu erhalten, während man die ernährungsphysiologischen Eigenschaften eines Fleischprodukts verbessert und die Produktionskosten senkt. Im Rahmen dieser Studie wurde für die Produktion von Frankfurtern gezielt eine Kombination von Hühnerfleisch und aus überschüssigen Fischen gewonnenem Fischproteinisolat verwendet, um sowohl den Wert der andernfalls verworfenen Fische zu steigern, als auch den Nährwert des Endprodukts zu steigern. Die Menge an Hühnerfleisch in den Würstchen wurde um 10%, 20% bzw. 30% reduziert und durch entsprechende Mengen an Ponyfisch-Proteinisolat ersetzt. Während einer Lagerung über 47 Tage wurden Peroxid, freie Fettsäuren und TBA-Werte unterhalb der zulässigen Grenzwerte ermittelt. Die TVB-N und TVC-Werte hingegen überschritten am 26. Tag die Grenzwerte. Basierend auf den sensorischen Bewertungen hatte die 30% FPI-Gruppe eine Haltbarkeit von 33 Tagen, während die 20% FPI- und 10% FPI-Gruppen eine Haltbarkeit von 40 Tagen hatten und die 0% FPI-Gruppe eine Haltbarkeit von 47 Tagen aufwies. Die geringste Verschlechterung der strukturellen Qualität im Laufe der Lagerung wurde in der 20% FPI-Gruppe beobachtet, jedoch war die 10% FPI-Gruppe hinsichtlich der Textur der Kontrollgruppe am ähnlichsten. Die Zugabe von Fischproteinisolat zu Hühnerwürstchen hat den Nährwert in Bezug auf den Proteingehalt, den DHA-Gehalt und das Verhältnis von n6/n3PUFA verbessert und sie können unter gleichen Bedingungen wie bei der Kontrollgruppe für 26 Tage gekühlt gelagert werden.

Schlüsselwörter: Fischproteinisolat, Textur, Frankfurter, Fettsäure-Zusammensetzung, Haltbarkeit

Introduction

Changes in family lifestyle are reflected in demands for nutritious foods that are convenient to prepare. Therefore, there is a growing interest for consumption of ready-to-eat foods enriched with seafood proteins or lipids, because of their beneficial health effects. Fish protein isolate is a kind of protein concentrate which can prepared from discard and seafood processing by-product. Isolation of fish muscle protein with acid or alkali method, which is shown as an alternative to the surimi production, is a convenient method that allows the use of the fish to be processed as a whole (Nolsøe and Undeland 2009). This method permits easy removal of materials not intended for human consumption, such as scales and bones. During the pH shifting process, fish muscle proteins are made highly soluble at acidic or alkaline pH and then recovered by isoelectric precipitation by adjusting the pH to approximately 5.5 (Özyurt et al. 2015a). Fish protein isolates from discard or seafood processing by-products can be used as a functional ingredient such as food additives, gelling agent and emulsifier (Park 2009; Özyurt et al. 2015b). Although pH shifting method presents efficient recovery of high quality fish protein, there have been scarce information about their usage in food products. It is possible that many functional food products could be derived using fish protein isolate.

Frankfurther-type sausage is a minced meat based product manufactured from ground meat such as chicken, pork, beef with added preservatives and flavours, and consumed around the world. Besides sensory attributes which is well accepted by especially kids, their low cost and ease of cooking compared to other foods have made them popular. Therefore, it is very important to maintain as much as possible the sensory quality and shelf life of the final product when the nutritional quality of frankfurther is improved. On the other hand, for meat products retailers, it is important to reduce the production costs while meeting the sensory needs of consumers. Animal protein isolates have become more important for this reason because the sources of plant protein can rise sensory problems that cause a decrease in meat taste (Petrášová et al. 2018).

The interest for discard and seafood by-products valorisation has been increase for a number of reasons, including economic, health and environmental aspects in recent years. Food and agriculture organisation of the United Nations asserts that every year almost a quarter of the total marine catch (estimated 20 million tonnes of fish) are discarded (FAO 2017). Ponyfish, which is accepted as discard fish because of small size, largely exist in Mediterranean and its consumption is rejected because of processing difficulties. Thus, in this study, it was aimed to combination of fish protein isolate and chicken meat for frankfurter production to provide both add value to the discard fish and to enlarge the nutritional quality of the final product.

Materials and methods

Recovery of fish protein and preparation of chicken frankfurter

Whole ponyfish *(Equulites klunzingeri)* were used for preparing fish protein isolates were approximately 3.65±1.5 g in weight. Protein isolates were produced by using alkali-aided process according to the method of Hultin and Kelleher (Hultin and Kelleher 2001) with slight modifications. Fish samples were supplemented with 1:6 cold distilled water and homogenized for 1 min on a Waring blender (Waring Products, Torrington, Connecticut, USA). The pH of the solution was adjusted to 11 by adding 2 M NaOH and then homogenates were centrifuged at 13000 x g (Sigma 16 SK, Germany) for 20 min at 4°C. This process distinguished soluble proteins from neutral lipids and solid substances in fish such as connective tissue and bone. A double layer of cheesecloth was used for filtration of the middle phase. The pH of the middle phase was adjusted to 5.5 by adding 2 M HCl for isoelectrically precipitating ponyfish proteins. The precipitated protein was then centrifuged for 20 min at 13000 x g at 4°C to set the aggregated proteins. The sediments of the second centrifuging step which were called as fish protein isolates (FPI) were collected and stored at –18°C for one month until the time of production of frankfurter.

Fresh chicken crumbs used in frankfurter production was obtained from a national company and stored frozen –18°C for one month. In the study, natural salted sheep sausage casing was used and these casings were supplied from a commercial company (Ergenç, Gaziantep). Polyamide based packages (Polinas, Manisa, Turkey) were used as packing material and prepared frankfurters were packaged with vacuum packaging machine (RV50 Reepack, Italy). Chicken frankfurter dough was divided into 4 groups; the control group was without any FPI addition, and for the other 3 groups chicken crumbs were substituted by 10%, 20% and 30% with FPI. When preparing the frankfurter formulation content, the amount and kind of ingredients was determined in accordance with national standards for meat sausage (TSE 2009). The all ingredients used for production of frankfurters were added in the four treatments at the same proportions. The formulation of frankfurters with different ratio of the fish protein isolate is as below.

Ingredients: Chicken crumb (67% for 0%FPI, 60.3% for 10%FPI, 53.6 for 20%FPI and 46.9 for 30%FPI), Fish protein isolate (0% for 0%FPI, 6.7% for 10%FPI, 13.4 for 20%FPI and 20.1 for 30%FPI), Oil (10 % sunflower oil + beef fat for all groups), Starch (2.55% for all groups), Salt (1.85% for all groups), Sugar (0.13% for all groups), Ginger (0.04% for all groups), Allspice (0.11% for all groups), Red pepper (0.11% for all groups), Black pepper (0.14% for all groups), Coriander (0.11% for all groups), Soy flour (2.55% for all groups), Sodium nitrate (0.01% for all groups), Sodium polyphosphate (0.2% for all groups), Liquid smoke (0.2% for all groups) and Ice (15% for all groups).

Fish protein isolates and chicken crumbs were thawed overnight in a refrigerator at 4°C and all ingredients mixed in dough machine. Afterwards, the frankfurter dough was stuffed into a casing and heating process were applied at 90°C for 20 min. After cooling with cold showering, the casings were removed and the frankfurthers vacuum packed and stored at cold storage (4°C) until sensory rejection. From each group, vacuum packaged frankfurters were selected by random and all analyses were performed in triplicates on day 0, 5, 12, 19, 26, 33, 40, 44 and 47.

Proximate composition and fatty acid methyl ester (FAME) analysis

Moisture and crude ash content of 0%FPI, 10%FPI, 20%FPI and 30%FPI groups samples were detected in an oven at 103°C and 550°C, respectively until the weight became constant. Lipid content was performed according

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to procedure of Bligh and Dyer (1959) and crude protein was found by Kjeldahl's method (AOAC 1998).

The extracted frankfurter oil samples were put into a small tube and converted to their FAMEs. This procedure was conducted by *trans*-methylation using 2 M KOH in methanol and *n*-hexane according to Ichihara et al (Ichihara et al. 1996) with minor modification. 10 mg of extracted oil sample was dissolved in heptane (2 ml), followed by 2 M methanolic KOH (4 ml) and vortexed. After the centrifugation at 4000 rpm for 10 min, the heptane layer was taken for gas chromatography (GC) analyses.

The fatty acid profile was analyzed using a GC Clarus 500 (Perkin-Elmer, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m × 0.32 mm ID 0.25 lm BP20 0.25 UM, USA). The oven temperature was 140°C, held for 5 min, raised to 200°C at a rate of 4°C min^{-1} and then to 220°C at a rate of 1°C min^{-1}, while the injector and detector temperatures were set at 220°C and 280°C, respectively. The sample size was 1 µl, and the carrier gas was controlled at 16 ps. The split ratio was 1:50. Fatty acid peaks were identified by comparing the retention times of FAME with the standard 37component of the FAME mixture. Three replicate GC analyses were performed, and the results were expressed in GC area % as mean value ± standard deviation.

Chemical analyses of frankfurters

The total volatile base nitrogen (TVB-N) content of frankfurters was detected by the Antonocopoulos (1973), and expressed as mg TVB-N per 100 g frankfurter. The value of thiobarbituric acid reactive substance (TBARs) was performed according to method of Tarladgis et al. (1960) in frankfurters and the results expressed as TBARs value, mg of malondialdehyde (MA) per kg. Peroxide value (PV), expressed in milliequivalents of peroxide oxygen per kilogramme of oil, and was determined according to method of AOCS.14 Free fatty acid (FFA) contents, expressed as % of oleic acid were done by the AOCS method (AOCS 1994). pH was determined in the homogeneous mixtures of frankfurter and distilled water (1:10, w:v), using a pH meter (Metler-Toledo, Switzerland).

Physical analyses of frankfurters

Texture profiles of frankfurter samples were measured using a texture analyser TA.XT2i (Stable Micro Systems, Godalming, Surrey, and U.K). Frankfurter pieces (1.5 cm height and 2 cm diameter) were prepared from each of the stored frankfurter groups and subjected to a 2-cycle compression. Before TPA analysis, sliced frankfurter samples were dried with filter paper and a flat plate aluminium cylinder probe (P/25) was attached to a 50 N load cell. The specified settings of the test were: compressed to 70% original height through a 2-bite mechanism with a cross-head speed for 2.0 mm/ min.; test speed 1 mm/s; pre-test and post-test speed 2.0 mm/s (Comunian et al. 2014). For determining variations of textural deterioration among groups, TPA curves were obtained and the main parameters of texture: hardness, resilience, springiness and cohesiveness values were measured and as secondary parameter gumminess and chewiness values were calculated depend on them. Gumminess was determined by multiplication of hardness and cohesiveness, another parameter, chewiness value was calculated with multiplication of gumminess and springiness values as previously outlined by Bourne (Bourne 1978). The data were processed by the program Texture Exponent 32 software (Stable Micro Systems, U.K.). All presented data are means from triplicate analysis of each sample.

During storage period, instrumental colour variations were analysed a method carried out by Calder (Calder 2003). Colour parameter of sliced frankfurter sausages were measured used the CIE $L^*a^*b^*$ system with CM-500 chromameter (Konica Minolta, Osaka, Japan). Before colour analysis, white and black tiles were using for sensor standardised. L^* (lightness), a^* (redness/ greenness) and b^* (blueness/yellowness) parameters were quantified. The Hue angle and Chroma (C*) parameters were calculated as follows:

$$\text{Hue} = (a^{*2} + b^{*2})^{1/2}$$

$$\text{Chroma (C*)} = \text{Arctan}\ (b^*/a^*)$$

All the measurements were carried out in three replicates on transversally cut sections of the frankfurter sausages.

Microbiological analyses

Frankfurter sausage (10 g) was collected aseptically in a stomacher bag and mixed with 90 mL of ringer solution and then homogenised using a stomacher for 3 min for the determination of total aerobic and psychrophile counts (log CFU per g). Further decimal dilutions were made up to 10^{-8} and then 0.1 mL of each dilution was pipetted onto the surface of plate count agar. All plates were performed in triplicate and incubated for 2 days at 30°C for total aerobic count and 10 days at 5°C for total psychrophile counts (ICMSF 1982; FDA/BAM 2001). Lactic acid bacteria count was detected using MRS (De Man, Rogosa-Sharpe) agar with incubated at 30°C for 2 days.

Total coliform bacteria count was determined according to two pour plating methods of FDA (FDA 1998) using Violet Red Bile Agar (VRBA, Oxoid, CM0107, Hampshire, England). Petri dishes included one millilitre aliquots of each dilution and VRBA were incubated for 24 h at 30°C. Mould-yeast count was observed on PDA (Potato Dextrose Agar) medium incubated for 5 days at 25°C.

Sensory analysis

The measurement of the freshness of frankfurter (colour, texture, odour and taste) was assessed according to a hedonic scale from 5 to ≤1 was used (Fernández et al. 2002). A score of 5 represents 'very good' while ≤1 represents 'very bad'. Each assessment was carried out by a minimum of eight trained panellists.

Statistical analysis

A general linear model, one-way ANOVA, was used to determine significant differences ($p<0.05$) among frankfurter sausages with different formulation. Multiple comparisons were carried out by the Duncan test. Statistical application was performed with the software SPSS version 19 (SPSS, Chicago, Illinois, USA).

Results and discussion

Proximate composition and fatty acid profiles of frankfurters

Proximate and fatty acid compositions of frankfurter sausages prepared with different rates of fish protein isolate are given in Table 1. Protein, lipid, moisture and crude ash ratio were found to be 17.33%, 11.05%, 70.78% and 1.19% for raw chicken crumbs; and 25.03%, 4.50%, 69.65% and 0.22% for fish protein isolate, respectively (data not shown

TABLE 1: *Proximate composition and fatty acid profiles of frankfurter sausage.*

	0%FPI	10%FPI	20%FPI	30%FPI
Moisture	70.95±0.28^{b}	71.78±0.56^{a}	69.65±0.47^{c}	71.54±0.50^{ab}
Protein	13.32±0.24^{b}	15.90±0.16^{ab}	16.16±1.35^{ab}	16.48±0.19^{a}
Lipid	12.31±0.57^{a}	11.20±0.44^{ab}	10.23±0.25^{b}	9.94±0.91^{b}
Crude ash	1.92±0.05^{ab}	1.68±0.43^{b}	2.19±0.14^{a}	1.92±0.22^{ab}
C12:0	0.03±0.01^{b}	0.03±0.01^{b}	0.03±0.00^{ab}	0.04±0.00^{a}
C14:0	0.65±0.13^{b}	0.69±0.16^{b}	0.79±0.02^{ab}	1.04±0.04^{a}
C16:0	15.23±0.27^{ab}	15.21±0.25^{b}	15.68±0.01^{ab}	15.78±0.11^{a}
C17:0	0.27±0.21^{a}	0.38±0.10^{a}	0.39±0.01^{a}	0.57±0.03^{a}
C18:0	7.14±0.61^{b}	7.44±0.77^{ab}	7.48±0.13^{ab}	8.67±0.23^{a}
C20:0	0.14±0.01^{b}	0.14±0.00^{b}	0.10±0.01^{c}	0.17±0.00^{a}
C22:0	0.27±0.04^{a}	0.27±0.00^{a}	0.27±0.00^{a}	0.28±0.00^{a}
C24:0	0.03±0.00^{a}	0.03±0.01^{a}	0.02±0.00^{a}	0.03±0.00^{a}
∑SFA	23.75±0.73^{b}	24.18±1.28^{b}	24.74±0.17^{ab}	26.57±0.40^{a}
C14:1	0.09±0.00^{b}	0.09±0.00^{b}	0.11±0.00^{a}	0.11±0.00^{a}
C16:1	1.94±0.13^{ab}	1.89±0.06^{b}	2.15±0.02^{a}	2.06±0.06^{ab}
C17:1	0.17±0.02^{b}	0.17±0.03^{b}	0.19±0.01^{b}	0.24±0.01^{a}
C18:1n7	1.31±0.05^{a}	1.34±0.13^{a}	1.48±0.00^{a}	1.38±0.02^{a}
C18:1n9	33.54±0.36^{a}	33.27±0.73^{ab}	32.05±0.05^{bc}	31.07±0.40^{c}
C20:1n9	0.06±0.01^{b}	0.06±0.01^{b}	0.24±0.01^{a}	0.22±0.00^{a}
C22:1n9	0.58±0.02^{b}	0.64±0.11^{ab}	0.91±0.04^{a}	0.92±0.19^{a}
∑MUFA	37.67±0.80^{a}	37.45±0.50^{a}	37.11±0.02^{a}	35.99±0.15^{b}
C18:2n6	34.21±0.38^{a}	34.02±1.42^{a}	32.50±0.16^{ab}	30.67±0.25^{b}
C18:3n3	1.39±0.18^{a}	1.35±0.11^{a}	1.29±0.05^{a}	1.14±0.00^{a}
C18:3n6	0.15±0.01^{a}	0.16±0.01^{a}	0.16±0.01^{a}	0.15±0.01^{a}
C20:2	0.04±0.04^{ab}	0.05±0.01^{ab}	0.07±0.00^{a}	0.02±0.01^{b}
C20:3n6	0.02±0.00^{b}	0.02±0.00^{c}	0.04±0.00^{b}	0.05±0.00^{a}
C20:5n3	0.03±0.08^{d}	0.15±0.08^{c}	0.34±0.03^{b}	0.51±0.04^{a}
C20:4n6	0.14±0.01^{c}	0.14±0.00^{c}	0.17±0.00^{a}	0.16±0.00^{b}
C22:6n3	0.08±0.00^{d}	0.36±0.03^{c}	0.84±0.07^{b}	1.24±0.16^{a}
∑PUFA	36.51±0.05^{a}	36.31±1.30^{a}	35.67±0.12^{ab}	34.20±0.45^{b}
n3	1.5	1.86	2.47	2.89
n6	34.52	34.34	32.87	31.03
n6/n3	23.01	18.46	13.31	10.74

Superscripts with different letter in the same row are significantly different (p<0.05). The values are expressed as mean ± standard deviation

in table). It was determined that the group with the highest content of protein content was 30%FPI group prepared with 30% fish protein isolate and the group with lowest protein content was 0%FPI group prepared with 100% chicken crumbs. Similarly, lipid contents of frankfurters also decreased with the addition of FPI especially in 30%FPI group. According to the proximate analysis, enriched frankfurter sausage with FPI had more desirable composition compared to the chicken frankfurter.

The main fatty acids of the sausages prepared with chicken crumbs (0%FPI) and fish protein isolate at different ratios (10%FPI, 20%FPI and 30%FPI) were palmitic acid (C16: 0), stearic acid (C18: 0), oleic acid (C18: 1n9) and linoleic acid (C18: 2n6). Many researchers have reported similar results for the fatty acid composition of chicken meat-based sausages (Abdulhameed et al. 2014; Jeun-Horng et al. 2002). The level of total saturated fatty acids in all groups was detected in range of 23.75–26.57%. Groups 0%FPI, 10%FPI and 20%FPI (37.11–37.67%) were found to be similar in terms of total monounsaturated fatty acids (MUFA), whereas the MUFA content of 30%FPI group (35.99%) were found to be significantly lower than the other groups (p<0.05). The high MUFA value observed in all groups in the current study was thought to be derived from the liquid oil added to sausage pastes. Among the total polyunsaturated fatty acids, linoleic acid (C18:2n6) and linolenic acid (C18:3n3) in all groups were the highest value. As it is known, linoleic acid content is the main fatty acids of the chicken meat component, and in this study it was also found higher in the groups with high chicken meat content. In this study, it was detected that in the 30%FPI group, significant amounts of decosahexanoic acid (C22:6n3) were also present compared to the 0%FPI, 10%FPI and 20%FPI groups. It can be thought that the decrease in total PUFA in 30%FPI group was also due to low linoleic acid content.

The ratio of n6:n3 fatty acid, which may cause some consequences for pediatric neurodevelopment and adverse health effects, has increased in western diets during the last few decades. Although the traditional human diet has a ratio of n6:n3 of about 1:1, current Western diets are described by a ratio of around 15–20:1, reflecting inadequete intake of omega 3 fatty acids and excessive intake of omega 6 fatty acids (Simopoulos 2008). In this study, the n6:n3 PUFA ratio of the sausages prepared with chicken crumbs (0%FPI) and fish protein isolate at different ratios (10%FPI, 20%FPI and 30%FPI) were found as 23.01, 18.46, 13.31 and 10.74, respectively. It was obviously seen that the addition of fish protein isolate was enhance the nutritional value of frankfurter sausages.

Chemical evaluation of frankfurters

Initial pH values for all groups ranged from 6.04 to 6.16 (Table 2). During cold storage, the highest pH value was found in group 0%FPI at day 12 (6.89), but there was no statistically significant difference between groups at the end of storage (P> 0.05). A gradual increase or fluctuations in pH in vacuum packaged sausages were observed other studies during storage (Garcia et al. 2010; Kumar et al. 2011; Henning et al. 2016). Previous results demonstrate that the pH of meat products is altered by the presence of dietary fiber. During storage, increases in pH in sausage probably due to the accumulation of basic compounds such as ammonia, derived from microbial action (Nychas et al. 1998). Özer et al. (2012) found that the pH values of sausages prepared from thornback ray packed in vacuum in cold storage were between 5.82 and 6.31, and Tirloni et al. (2015) reported that the pH values of the frankfurter sausages prepared from Atlantic salmon were between 6.38 and 6.47. It was also found that in this study the pH values in all groups were within acceptable limit values (6.80-7) for fish based products according to Oehlenschlager (1992).

The amount of TVB-N, which shows an increase with activity of endogenous enzymes and spoilage bacteria, are given in Table 2. At the initial day of storage, the TVB-N value determined in the range of 5.54–10.94 mg/100g increased significantly (36.82–39.67 mg/100g) and exceeded the acceptable limit (35 mg/100g) in all groups on the 26th day according to clasification of Botta et al. (1994). Dinçer (2008) recorded that TVBN value for sausage prepared with rainbow trout was in range of 39.90–45.22 at 21th day. Similar to current study, Mendes and Gonçalves (2008) and Soccol et al. (2005) reported that rapid declines in TVB-N contents of vacuum packaged products in could be seen.

At the initial day of storage, the lowest TBA value was observed in frankfurther prepared with 100% chicken crumbs, and the highest TBA value was observed in frankfurther with 30% fish protein isolat (P <0.05). While fluctuations in TBA values were observed during the storage period, it was determined that an increase in the TBA

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TABLE 2: *Chemical evaluation of frankfurter sausage during storage period.*

Storage time (days)	0%FPI	10%FPI	20%FPI	30%FPI
pH				
0	6.16±0.02^{a6}	6.11±0.01^{b7}	6.04±0.01^{d7}	6.07±0.01^{c7}
5	6.82±0.04^{a2}	6.62±0.02^{b2}	6.54±0.04^{c3}	6.48±0.02^{d2}
12	6.89±0.03^{a1}	6.80±0.02^{c1}	6.88±0.01^{ab1}	6.84±0.02^{b1}
19	6.37±0.06^{b4}	6.46±0.00^{a3}	6.38±0.02^{b4}	6.42±0.03^{ab3}
26	6.18±0.02^{c6}	6.37±0.02^{a4}	6.28±0.04^{b5}	6.34±0.01^{a4}
33	6.11±0.01^{d7}	6.39±0.02^{a4}	6.29±0.01^{b5}	6.17±0.02^{c6}
40	6.17±0.03^{c6}	6.22±0.04^{c6}	6.70±0.11^{a2}	6.45±0.04^{b2}
44	6.51±0.03^{a3}	6.21±0.02^{c6}	6.15±0.01^{d6}	6.30±0.04^{b5}
47	6.31±0.03^{a5}	6.30±0.03^{a5}	6.33±0.03^{a45}	6.33±0.02^{a4}
TVB-N (mg/100g)				
0	6.91±0.01^{c6}	5.54±0.03^{d5}	10.94±0.17^{a78}	10.12±0.92^{b6}
5	10.46±0.68^{ab5}	9.73±0.05^{b4}	10.63±0.37^{ab8}	11.29±0.80^{a6}
12	11.97±0.90^{ab4}	10.78±0.55^{b4}	12.56±0.54^{a67}	12.04±1.06^{ab56}
19	16.03±1.10^{c2}	20.84±2.00^{b2}	23.39±1.70^{a3}	19.01±0.71^{b3}
26	38.68±1.00^{ab1}	39.67±2.36^{a1}	37.15±0.82^{b2}	36.82±0.11^{b2}
33	11.05±0.78^{b45}	10.09±0.51^{b4}	42.21±1.95^{a1}	43.57±2.88^{a1}
40	13.56±0.53^{a3}	12.75±1.23^{a3}	12.94±0.48^{a6}	13.62±0.47^{a45}
44	16.09±0.92^{ab2}	13.91±1.13^{c3}	17.71±1.47^{a4}	15.25±0.09^{bc4}
47	16.02±0.91^{b2}	21.56±0.06^{a2}	14.64±1.15^{bc5}	13.91±0.67^{c45}
PV (mEq/kg)				
0	2.71±0.14^{c6}	3.51±0.49^{b23}	5.51±0.03^{a2}	3.99±0.22^{b2}
5	3.63±0.46^{a345}	3.50±0.39^{a23}	3.65±0.20^{a4}	3.58±0.34^{a23}
12	1.97±0.12^{b7}	2.38±0.32^{ab5}	2.67±0.27^{a5}	2.86±0.24^{a4}
19	4.50±0.46^{a2}	3.82±0.40^{ab2}	3.88±0.34^{ab4}	3.48±0.15^{b234}
26	7.44±0.17^{a1}	2.67±0.24^{d45}	4.81±0.42^{c3}	5.55±0.51^{b1}
33	4.24±0.43^{b23}	3.10±0.23^{c34}	6.13±0.48^{a1}	3.46±0.15^{c234}
40	3.87±0.32^{b234}	3.95±0.13^{b12}	4.04±0.44^{b4}	5.34±0.66^{a1}
44	3.28±0.58^{b456}	4.40±0.08^{a1}	3.78±0.35^{ab4}	3.88±0.16^{ab23}
47	3.02±0.08^{ab56}	2.73±0.26^{b45}	2.19±0.25^{c5}	3.31±0.23^{a34}
FFA (% of oleic acid)				
0	1.29±0.50^{b5}	1.47±0.12^{b5}	1.64±0.11^{b5}	2.55±0.08^{a5}
5	1.90±0.18^{c34}	2.12±0.13^{c23}	2.51±0.16^{b34}	2.97±0.28^{a234}
12	3.16±0.27^{a1}	2.53±0.14^{c1}	3.11±0.02^{ab12}	2.73±0.27^{bc45}
19	2.43±0.23^{b2}	2.41±0.34^{b12}	3.35±0.18^{a1}	3.79±0.29^{a1}
26	1.91±0.14^{c34}	1.78±0.15^{c4}	2.74±0.21^{b34}	3.28±0.23^{a2}
33	2.19±0.15^{b23}	2.35±0.08^{b123}	2.83±0.27^{a23}	3.10±0.04^{a23}
40	1.51±0.18^{c45}	2.08±0.05^{b3}	3.16±0.24^{a12}	3.11±0.17^{a23}
44	1.87±0.14^{c34}	2.17±0.23^{bc23}	2.44±0.21^{ab4}	2.77±0.13^{a345}
47	2.02±0.08^{c23}	1.76±0.04^{d45}	2.49±0.18^{b34}	2.68±0.07^{a45}
TBA (mg MA/kg)				
0	0.46±0.03^{c4}	0.82±0.05^{b34}	1.36±0.07^{b23}	1.67±0.04^{a34}
5	0.47±0.05^{d4}	0.79±0.03^{c4}	1.39±0.05^{b2}	1.90±0.19^{a1}
12	0.46±0.03^{d4}	0.95±0.14^{c2}	1.50±0.09^{b1}	2.07±0.09^{a1}
19	0.60±0.02^{c2}	0.82±0.04^{b34}	0.88±0.07^{b6}	1.62±0.10^{a4}
26	0.55±0.04^{c3}	0.77±0.05^{b4}	1.36±0.05^{a23}	1.36±0.19^{a5}
33	0.72±0.04^{d1}	0.90±0.04^{c23}	1.15±0.07^{b4}	1.66±0.13^{a34}
40	0.60±0.02^{c2}	1.04±0.02^{b1}	1.00±0.17^{b5}	1.77±0.03^{a23}
44	0.53±0.01^{c3}	0.92±0.08^{b2}	1.27±0.09^{a3}	1.28±0.18^{a5}
47	0.48±0.04^{d4}	0.74±0.06^{c4}	1.36±0.04^{b23}	1.82±0.06^{a2}

Superscripts with different letter (a–d) in the same row indicate significant differences of the parameter with respect to the research groups ($p<0.05$). Superscripts with different number (1–8) in the same column indicate significant differences of the parameter with respect to the storage days ($p<0.05$). The values are expressed as mean ± standard deviation

values of 30%FPI group was observed. However, it was determined that the TBA values observed during storage in all experimental groups were below the limit values (3–4 mg MA/kg) specified by Smith (2017) Tang et al. (2001) reported that TVBN values of sousages prepared with chicken, whiting and mackarel were 1.60, 1.55 and 25.55 mg/kg MA at 10th day, respectively. It was obviously seen that the differences in theese values were caused by the type and proportion of fish used in sausage making. The initial PV values in all groups were in range of 2.71–5.51 mEq/kg (Table 2). Although fluctuations in PV values were observed during the storage periods, PV value did not increase at end of the storage time (2.19–3.31 mEq/kg). It can be thought that this may be due to the reducing of lipid oxidation by decreasing oxygen from the medium with vacuum packing (Mbarki et al. 2009). Connell (1995) stated that seafood can not be used for human consumption when the PV value exceeds 10 mEq/kg. In this study, it was seen that all the groups were not able to reach these limit values stated during the storage period.

Hydrolytic degradation in foods is formed by hydrolytic enzymes such as lipase and leads to the formation of free fatty acids (FFA). Since the free fatty acids formed tend to be rapidly oxidized, increases in the amount of free fatty acids in the foods are not desirable. In this study, the initial FFA value was observed in the range of 1.29–2.55% in all groups. However, FFA value was found as 1.76–2.68% at the end of the storage period. Although the increases and decreases in FFA values during storage were observed in this study, it was determined that the recorded values were within the range of 1–7% recommended for fish oils (Bimbo 1998).

Physical evaluation of frankfurters

Colour is one of the key quality attributes, which clarifies the consumer acceptability and marketability of many minced meat and fish products (Sachindra and Mahendrakar 2010). Figure 1 shows the effect of adding different rates of FPI on the colour values of chicken frankfurter during refrigerated storage. At day 0, there were no significant differences in the L^* (lightness) values for all groups. The significant reduction was initiated on day 12 and maintained and reached the least values at the end of storage. This decreasing could be explained by pH values of meat products are not stable thought storage that cause to lightness value variations (Abbasi and Samadi 2014). Fish protein addition with 10% and 20% rates led to high lightness value that is desirable than 10% FPI adding and without FPI adding groups.

CIE a^* value (redness) is accepted as an indicator for the evaluation of sausage type meat products (Dvorak et al. 2001). In current study, the highest a^* value at the initial day of storage was found in group 30% FPI with 2.61, however the lowest values during storage was also determined in this group. The redness values decreased with increasing storage period an all groups. The reduction of redness may signify a discoloration of product making it undesirable to consumers (Kim et al. 2013). Shabanpour and Etemadian (2016) pointed out that addition of proteins to food product has significant effect on the a^* value caused by of haem proteins concentration. Yellowness (b^*) value is another important colour parameter. The variations of control and FPI added groups are presented in Figure 1. The initial b^* values were change between12.23–12.37. Some fluctuations were noted during storage and there was obvious decline at the end of storage. Mancini and Hunt (2005) reported that the decrease of yellowness is referred to the oxidation of myoglobin. The highest b^* value was observed in 30% FPI group. This could be explain due to include higher rate of fish protein isolate that is known sensitive to oxidation and colour losses. The higher yellowness of meat product perceived as a negative feature for the consumers acceptability (Pereira et al. 2011).

The differences of chroma value are given in Figure 1. All groups have higher chroma value (12.50–12.64) in the early storage time than latest days (11.99–12.52). Chroma

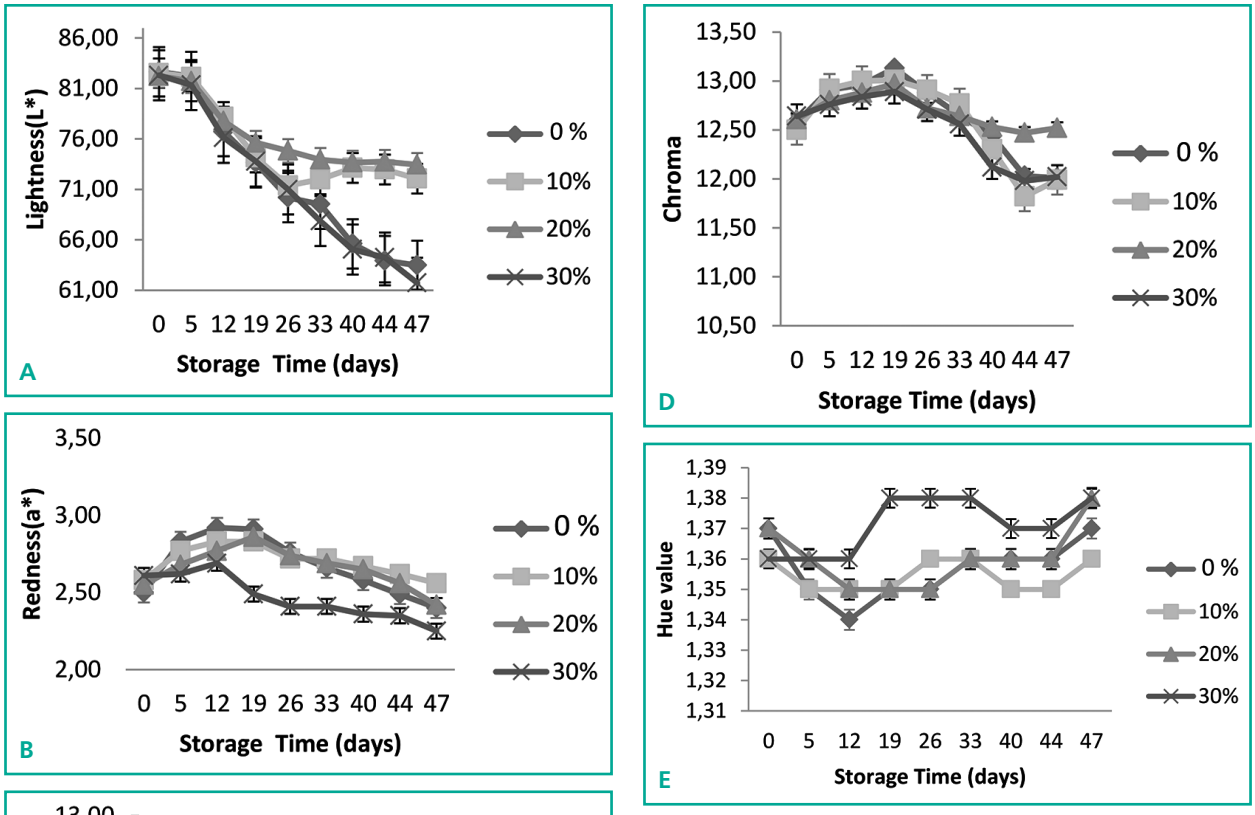

FIGURE 1: *Colour changes of frankfurter sausages during storage period.*

value signifies the colour saturation and higher chroma value represents the purer colour. Refrigerated storage conditions and being sensitive of fish products to microbial changes could be reason for reduction of chroma value. Cuttle et al. (2001) demonstrated that the loos of chroma value can be related to bacterial activity. The hue angle of fortified frankfurter sausages ranged from 1.36 to 1.37, and the without any adding FPI group (0%FPI) which was 1.36 at the beginning of storage (Fig.1). During refrigerated storage, the hue values were instable and there was slightly variance between groups, though the difference was non-significant ($p > 0.05$).

Textural properties of food play a key role in consumer acceptance. While there are many instrumental analyses to determine the textural property of food, the most commonly used and successful method is texture profile analysis (TPA). Like other meat products, the textural properties of sausages have been the subject of much research and texture is used as a quality indicator for ingredients and final product quality (Bourne 2002). Textural properties of all frankfurter sausage groups are tabulated in Table 3. For emulsion products, such as sausages and pate, hardness is a key parameter in consumer preference. Initial hardness values were 3251.23, 4234.19, 4408.39 and 4246.87 g measured for 0%FPI, 10%FPI, 20%FPI and 30% FPI, respectively. These results corroborate the findings of Shao et al. (2016) who found that hardness values of heat-generated gels obtain from chicken breast increased from 1725.64–3411.54 g as the amount of protein increased. Among FPI added groups, while 10% and 20% FPI groups had positive effect on hardness of frankfurter sausages, 30% FPI group effect adversely. Muguruma et al. (2003) outlined that hardness of sausages was improved with different kinds of biopolymers made from whey protein isolate, soybean protein and casein on chicken sausages texture. Hardness values decreased prominently in all groups from the 12th day of storage onwards. Similarly, Smith (2017) highlighted that hardness value of smoked chicken sausage was substantially decreased during storage at 2°C.

Chewiness reflects tenderness characteristics and has a cubic response that expressed as gumminess and springiness of the product (Caine et al. 2003; Horita et al. 2014). Initial chewiness values were varied from 2024.43 to 2085.71 gmm and these values increased as 2062.21–2196.79 gmm. (Table 3). While at the beginning of storage, chewiness values of all groups are slightly different from each other, addition of FPI has effected these values throughout the storage and the variations became considerably for each groups at the end of storage. This variance could be justified by different response of chicken and fish proteins to heating. Liu (2012) indicated that fish proteins are less fixed to heat-related changes and sensitive to heating of chicken proteins gels effect rheological and water holding properties. Furthermore, Li et al. (2018) stated that higher chewiness values related to pH and water-binding ability of proteins which is negatively related to consumer preferences.

Cohesiveness value of food is considered to be another important parameter. Yang et al. (2010) indicated that co-

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hesiveness reflects the rate of is a degree of difficulty in breaking down the interior surface of the sausages. In this study, cohesiveness value scales were reported from 0.65 to 0.67 up to 26.th day of storage and showed some fluctuations for all groups toward the end of the storage period. While 10%FPI, and 20%FPI groups had lower, 30%FPI group had higher cohesiveness value compared the control group (0%FPI) at the end of storage (Table 3). Higher cohesiveness value of meat products has negative effect to consumer preference (Chorbadzhıev et al. 2017).

Gumminess described as the food product of hardness multiplied by the cohesiveness and used to simulate the energy needed to disintegrate food pieces (Bourne 2002; Prabpree and Pongsawatmanit 2011). Consistent with the literature, in the current study, initial gumminess value of frankfurter sausage groups were found 2167.50 to 2865.455.50 g and these values were considerably decreased through storage. The latest gumminess values ranged from 1874.46 to 2157.07 g (Table 3). These results corroborate the findings of Rahmanifarah et al. (2013) who found that gumminess values of fish sausage were vary from 1400–2200 g.

Adhesiveness is defined as the stickiness of the sample to the probe and is similar to the sensory assessment "stickiness to mouth" (Das et al. 2006). The adhesiveness of all frankfurter sausage groups were shown at Table 3. Initial adhesiveness values were ranged between –2.57 and –2.89 gs, and the final adhesiveness value were observed from –2.96 to –3.32 gs. The results of the study presented here agree with the results of Dinçer (2008) who reported that the adhesiveness of fish sausage from rainbow trout *(Onchorynchus mykiss)* during the cool storage varies from –0.43 gs to –3.85 gs. Pereira et al. (2011) stated that the adhesiveness value cannot be high in sausage-type products cause of sausage should be have a smooth, and firm surface properties.

Resilience is known that the capacity of the food to regain its original position. While there was no significant differences among initial resilience values (0.19 g), significant alterations were observed in frankfurter sausage groups at the end of storage (0.29–0.57 g). These results are in agreement with the research done by Khansole (2016) who determined resilience values as between 0.16 and 0.36 g for chicken sausages during refrigerated storage.

Similar to quantity of resilience, springiness define as the ability of the products to return to its primary state after the first compression (Paker et al. 2015). The springiness values variations of control and FPI enriched groups are shown in Table 3. The values ranged between 0.80–0.89 mm, the significance decrease started at the 26th day of storage and progressed during the rest of the storage period. The results are in keeping with previous observa-

TABLE 3: *Textural evaluation of frankfurter sausage during storage.*

Days	Hardness	Chewiness	Cohesiveness	Gumminess	Adhesiveness	Resilience	Springiness	Groups
0	3251.23±1.03^{d1}	2024.43±2.68^{c3}	0.67±0.02^{a2-3}	2167.50±50.32^{c1}	–2.57±0.55^{a1}	0.19±0.02^{a3}	0.88±0.01^{a1}	0%FPI
	4234.19±0.18^{c1}	2024.49±0.39^{c3}	0.65±0.00^{b2-3}	2752.22±0.12^{b1}	–2.60±0.02^{a1}	0.19±0.01^{a3}	0.89±0.01^{a1}	%10 FPI
	4408.39±0.88^{a1}	2032±1.53^{b3}	0.65±0.00^{b2-3}	2865.45±0.57^{a1}	–2.58±0.01^{a1}	0.19±0.01^{a3}	0.89±0.01^{a1}	%20 FPI
	4246.87±0.98^{b1}	2085.71±1.61^{a3}	0.65±0.01^{ab2-3}	2774.62±24.00^{b1}	–2.89±0.01^{b1}	0.19±0.01^{a3}	0.89±0.00^{a1}	%30 FPI
5	3051.89±2.14^{c1}	2033.69±1.96^{c3}	0.65±0.01^{a3}	1993.90±17.57^{d1-2}	–2.60±0.02^{a1-2}	0.20±0.01^{a3}	0.88±0.01^{a1}	0%FPI
	4053.61±62.54^{b1}	2035.04±0.42b^{c3}	0.65±0.00^{a3}	2634.84±40.65^{c1-2}	–2.62±0.01^{a1-2}	0.19±0.01^{a3}	0.80±0.14^{a1}	%10 FPI
	4308.68±14.42^{a1}	2037.54±1.57^{b3}	0.65±0.00^{a3}	2800.64±9.37^{a1-2}	–2.59±0.01^{a1-2}	0.20±0.02^{a3}	0.88±0.01^{a1}	%20 FPI
	4108.20±2.55^{b1}	2087.37±2.85^{a3}	0.65±0.01^{a3}	2684.03±24.37^{b1-2}	–2.87±0.03^{b1-2}	0.19±0.01^{a3}	0.88±0.01^{a1}	%30 FPI
12	2839.26±43.20^{d1-2}	2041.54±2.62^{b2-3}	0.65±0.00^{a3}	1845.52±28.08^{d1-2-3}	–2.68±0.04^{b1-2}	0.21±0.01^{a3}	0.88±0.01^{a1}	0%FPI
	3810.05±11.30^{c1-2}	2072.37±26.58^{a2-3}	0.65±0.00^{a3}	2476.53±7.35^{c1-2-3}	–2.65±0.04^{ab1-2}	0.21±0.01^{a3}	0.86±0.03^{a1}	%10 FPI
	4236.03±27.28^{a1-2}	2037.51±4.67^{b2-3}	0.65±0.00^{a3}	2753.42±17.13^{a1-2-3}	–2.59±0.01^{a1-2}	0.20±0.02^{a3}	0.88±0.00^{a1}	%20 FPI
	4009.63±6.63^{b1-2}	2091.95±3.17^{a2-3}	0.65±0.00^{a3}	2606.26±4.31^{b1-2-3}	–2.88±0.88^{c1-2}	0.20±0.02^{a3}	0.89±0.01^{a1}	%30 FPI
19	2805.38±8.50^{d1-2-3}	2045.28±3.45^{b2-3}	0.65±0.01^{a3}	1832.87±20.06$^{d1-2-3-4}$	–2.71±0.02^{c1-2}	0.20±0.02^{a3}	0.88±0.01^{a1}	0%FPI
	3308.57±5.62^{c1-2-3}	2091.11±6.83^{a2-3}	0.65±0.01^{a3}	2161.61±20.36$^{c1-2-3-4}$	–2.68±0.02^{b1-2}	0.20±0.02^{a3}	0.88±0.01^{a1}	%10 FPI
	4098.93±9.39^{a1-2-3}	2038.13±3.11^{b2-3}	0.65±0.00^{a3}	2664.30±6.10$^{a1-2-3-4}$	–2.61±0.01^{a1-2}	0.20±0.01^{a3}	0.88±0.00^{a1}	%20 FPI
	3489.93±67.26^{b1-2-3}	2097.16±2.74^{a2-3}	0.65±0.01^{a3}	2280.05±45.35$^{b1-2-3-4}$	–2.89±0.01^{d1-2}	0.19±0.04^{a3}	0.89±0.01^{a1}	%30 FPI
26	2776.53±19.62^{d2-3}	2125.10±4.86^{b1-2-3}	0.67±0.03^{ab2-3}	1869.16±67.290^{d2-3-4}	–2.98±0.02^{b2-3}	0.23±0.02^{bc2-3}	0.78±0.01^{b2}	0%FPI
	2925.01±34.43^{c2-3}	2139.15±2.92^{a1-2-3}	0.68±0.01^{ab2-3}	1998.71±23.69^{c2-3-4}	–2.97±0.02^{b2-3}	0.26±0.02^{b2-3}	0.77±0.01^{b2}	%10 FPI
	3834.94±333.50^{a2-3}	2045.96±10.66^{c1-2-3}	0.64±0.01^{c2-3}	2467.16±32.90^{a2-3-4}	–2.64±0.01^{a2-3}	0.20±0.01^{c2-3}	0.87±0.02^{a2}	%20 FPI
	3064.39±33.31^{b2-3}	2142.96±3.11^{a1-2-3}	0.71±0.01^{a2-3}	2165.63±41.07$^{cd2-3-4}$	–2.99±0.03^{b2-3}	0.36±0.03^{a2-3}	0.73±0.02^{c2}	%30 FPI
33	2640.79±51.23^{c3}	2134.29±3.99^{c1-2}	0.68±0.02^{b1-2-3}	1786.98±55.373^{d-4}	–3.08±0.03^{c3-4}	0.26±0.01^{b2-3}	0.73±0.03^{b2-3}	0%FPI
	2904.41±7.11^{b3}	2147.24±6.29^{b1-2}	0.67±0.02^{b1-2-3}	1936.19±56.34^{c3-4}	–3.00±0.02^{b3-4}	0.26±0.01^{b2-3}	0.74±0.03^{b2-3}	%10 FPI
	3511.38±13.61^{a3}	2048.85±0.41^{d1-2}	0.68±0.02^{b1-2-3}	2376.10±58.97^{a3-4}	–2.76±0.03^{a3-4}	0.21±0.03^{c2-3}	0.84±0.03^{a2-3}	%20 FPI
	2843.81±39.44^{b3}	2169.28±2.92^{a1-2}	0.72±0.02^{a1-2-3}	2047.17±41.45^{b3-4}	–3.15±0.05^{d3-4}	0.37±0.03^{a2-3}	0.70±0.1^{b2-3}	%30 FPI
40	2622.12±20.75^{d3}	2154.31±4.53^{c1}	0.70±0.02^{a2-3}	1826.93±53.31^{c3-4}	–3.07±0.03^{b3-4}	0.26±0.04$^{bc1-2-3}$	0.71±0.01^{b3-4}	0%FPI
	2901.47±2.53^{b3}	2199.11±2.53^{a1}	0.68±0.03^{a2-3}	1973.04±78.32^{b3-4}	–3.06±0.03^{b3-4}	0.29±0.02^{b1-2-3}	0.72±0.01^{b3-4}	%10 FPI
	3473±86±13.43^{a3}	2053.77±1.75^{d1}	0.64±0.02^{b2-3}	234.99±79.06^{a3-4}	–2.82±0.02^{a3-4}	0.23±0.01^{c1-2-3}	0.78±0.03^{a3-4}	%20 FPI
	2748.72±31.06^{c3}	2176.53±1.24^{b1}	0.71±0.01^{a2-3}	1951.50±26.93^{b3-4}	–3.16±0.02^{c3-4}	0.42±0.03^{a1-2-3}	0.64±0.03^{c3-4}	%30 FPI
44	2627.24±6.92^{c3}	2162.88±5.95^{c1}	0.68±0.02^{ab1-2}	1795.25±38.85^{c3-4}	–3.13±0.03^{b3-4}	0.31±0.02^{b1-2}	0.66±0.02^{b4-5}	0%FPI
	2837.02±36.46^{b3}	2206.07±4.59^{a1}	0.69±0.04^{ab1-2}	1956.49±97.73^{b3-4}	–3.17±0.04^{b3-4}	0.32±0.02^{b1-2}	0.64±0.03^{b4-5}	%10 FPI
	3272.61±19.40^{a3}	2061.16±1.41^{d1}	0.66±0.03^{b1-2}	2159.55±85.87^{a3-4}	–2.91±0.06^{a3-4}	0.26±0.01^{c1-2}	0.74±0.02^{a4-5}	%20 FPI
	2616.38±17.30^{c3}	2182.55±2.01^{b1}	0.73±0.03^{a1-2}	1901.48±89.43^{bc3-4}	–3.22±0.03^{c3-4}	0.49±0.01^{a1-2}	0.59±0.02^{c4-5}	%30 FPI
47	2603.46±3.95^{c33}	2171.49±3.76^{c1}	0.72±0.03^{b1}	1874.46±76.80^{c3-4}	–3.22±0.04^{bc4}	0.34±0.01^{b1}	0.63±0.01^{b5}	0%FPI
	2778.01±23.76^{b3}	2181.89±3.05^{b1}	0.71±0.03^{b1}	1962.76±54.75^{bc3-4}	–3.18±0.01^{b4}	0.35±0.02^{b1}	0.60±0.02^{c5}	%10 FPI
	3172.05±27.72^{a3}	2062.21±1.77^{d1}	0.68±0.02^{b1}	2157.07±62.19^{a3-4}	–2.96±0.02^{a4}	0.29±0.02^{c1}	0.71±0.01^{a5}	%20 FPI
	2587.16±5.06^{c3}	2196.79±5.88^{a1}	0.78±0.01^{a1}	2026.59±28.56^{b3-4}	–3.32±0.12^{c4}	0.57±0.02^{a1}	0.55±0.01^{d5}	%30 FPI

Superscripts with different letter (a–c) indicate significant differences of the parameter with respect to the research groups ($p<0.05$). Superscripts with different number (1–5) indicate significant differences of the parameter with respect to the storage days ($p<0.05$). The values are expressed as mean ± standard deviation

tional studies carried out by Lazo et al (2017) who demonstrated that springiness value 0.80mm. Addition of protein led to better hardness and springiness of products but the concentration of added protein is important (Tobin et al. 2013).

Results of texture measurement showed that textural deterioration of frankfurter sausages increased with storage time. However, there were no significant differences among groups in terms of chewiness (2024-2097gmm), springiness (0.88–0.90mm), adhesiveness (–2.57–2.96gs), resilience (0.19–0.21g) and cohesiveness (0.65–0.69) values until the 26th day of storage. At the end the least textural deterioration was observed in 20% FPI group. However, texture feature of 10% FPI group was the most close to control group.

Microbiological evaluation of frankfurters

According to the food codex on sausages, it has been reported that the amount of yeast-mold in meat products should be less than $1x10^2$ log cfu/g (Anonymous 2010). Yeast and mold were not found in all frankfurter sausage groups during the storage period. *Enterobacteriaceae,* a psychrotolerant, can grow at refrigeration temperatures; on the other hand they can not compete well with other saprophytic gram negative bacteria. The total *Enterobacteriaceae* count for all groups during storage is shown in Figure 2. There were no count for the *Enterobacteriaceae* at the initial time of storage, but it was found in range of 2.68–2.97 log cfu/g at 5th day of storage and increased during storage time in all groups.

The aerobic and psychrophile bacteria counts of control group (0%FPI) and fish protein isolate added groups (10%FPI, 20%FPI and 30%FPI) were in range of 2.63–3.08 log cfu/g and 2.15–.80 log cfu/g respectively. The aerobic and psychrophile bacteria count was found to increase regularly during storage in all sausage groups (Fig 2.). According to the International Commission on Microbiological Food Standards (ICMSF), seafood products reported a TVC limit of 10^6. The number of aerobic bacteria in frankfurter sausage groups (0%FPI, 10%FPI, 20%FPI and 30%FPI) on the 26th day of storage were found as 5.96, 5.76, 6.23 and 5.30 log cfu/g, respectively. Ciekure et al. (2016) detected high bacterial load in the cold smoked sausage samples sold in the market (5.26 log cfu/g). It was reported that the total number of mesophilic and psychrophile bacteria in commercially sold chicken sausages varied from 7.14 to 7.28 log cfu/g and from 7.72 to 7.87 log cfu/g, respectively, and that 80% of these chicken sausages were microbiologically inconsumable (Álvarez-Astorga et al. 2002). In the present study it was determined that sausages could not be consumed microbiologically after 26th day for all groups.

Changes in total lactic acid bacteria (LAB) counts during cold storage of vacuum packaged sausage groups are shown in Figure 2. Lactic acid bacteria, which has anaerobic and aero-tolerant properties, constitute an im-

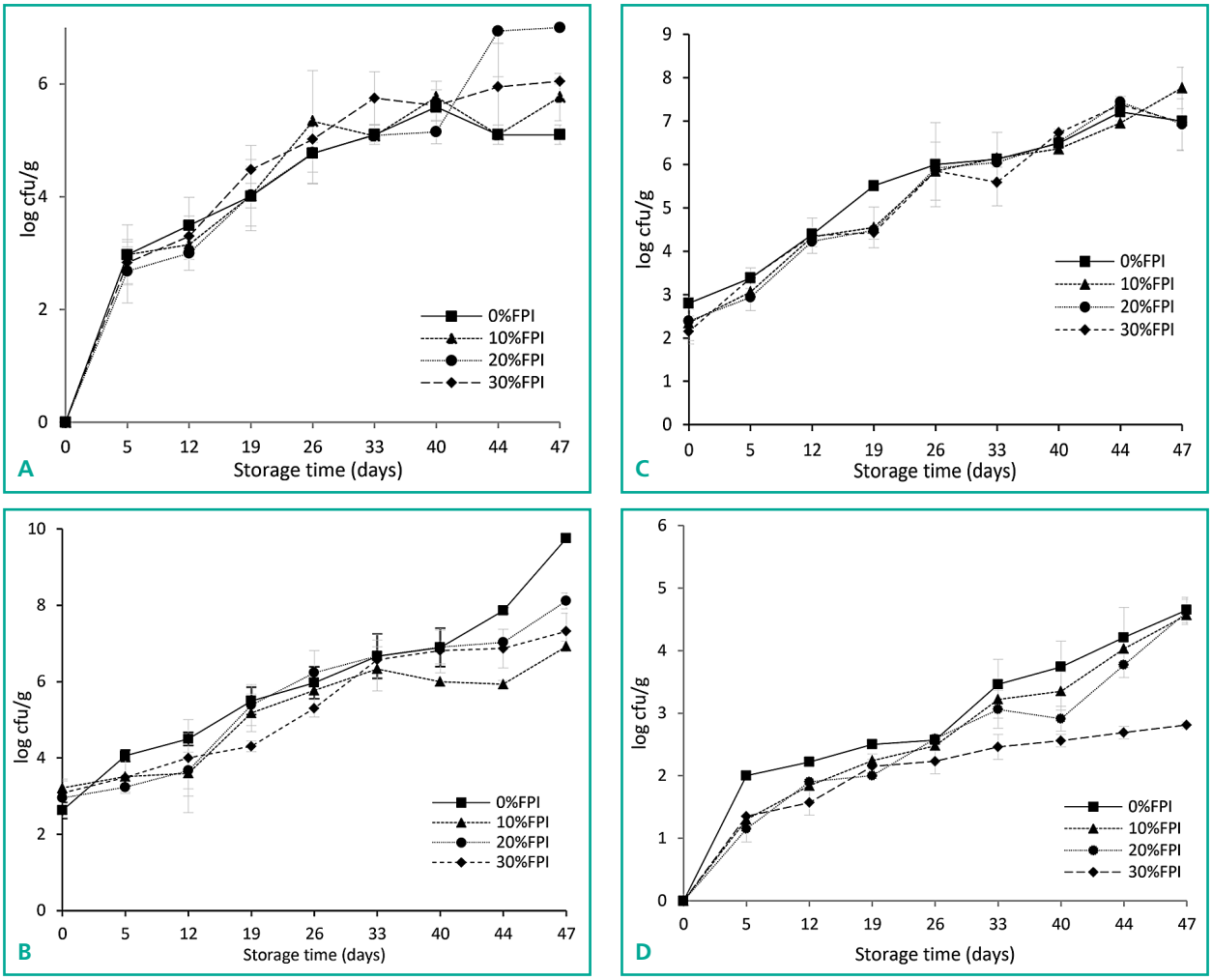

FIGURE 2: *Microbiological changes in frankfurter sausages, A: total Enterobacteriaceae count, B: total anaerobic bacteria count, C: total psychrophile bacterial count D: total lactic acid bacteria count.*

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portant part of the microbial population in vacuum-packed sausages (Kleerebezem et al. 2010; Özdemir 1997). Although, lactic acid bacteria were not found in any sausages at the initial day of storage, it was found as 2.00 log cfu/g in control group, 1.30 log cfu/g in 10%FPI group, 1.15 log cfu/g in 20%FPI group and 1.35 log cfu/g in 30%FPI group on the 5th storage day. It was observed that the LAB counts increased during storage, but the highest increase was observed in the control group (4.65 log cfu/g). Gokoglu et al. (2010) also found that LAB increased during storage in sausages stored in modified atmospheric and vacuum packages, and that the LAB counts in sausages stored at 30% CO_2 / 70% N^2 atmosphere reached 8.31 log cfu/g at the end of storage.

Sensory assessment

As a result of the sensory evaluation, appearance, colour, texture, odour and flavour parameters of frankfurter sausages were scored as 5 for all groups until 19th days of cold storage period (Table 4). Panellists did not reported any negative sensory quality assessment for fish protein isolate addition groups. It was observed that all sensory parameters decrease from the 19th day of storage. When the sensory parameters were generally evaluated, it was recorded that sensory acceptance for 30%FPI, 20%FPI, 10%FPI and 0%FPI group was 33, 40, 40 and 47 days, respectively. In this study, it was seen that the results of microbiology analysis did not show a parallel with the results of sensory evaluation. According to the microbiological data, all the study groups were on the 26th day to the limit of consumption, but the panellists couldn't notice in that days. Similarly, Özoğul et al. (2004) stated that modified atmospheric packed sardines reached acceptable limit in respect to microbiological quality before the day when panellists sensorial rejected it. In this study, it was thought that the reason for the panellists to diagnose sensory impairment late might be that the aroma of smoke used in the sausage paste was derived from the bad taste and smell masking.

TABLE 4: *Sensory evaluation of frankfurter sausage during storage.*

Storage time (days)	Appearance	Colour	Texture	Odour	Flavour	Group
0	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	0%FPI
	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	10%FPI
	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	20%FPI
	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	30%FPI
5	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	0%FPI
	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	10%FPI
	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	20%FPI
	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	30%FPI
12	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	0%FPI
	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	10%FPI
	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	20%FPI
	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	30%FPI
19	4.75±0.35^{a1}	4.75±0.35^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	4.88±0.35^{a1}	0%FPI
	4.50±0.71^{a1}	4.50±0.71^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	4.88±0.35^{a1}	10%FPI
	4.50±0.71^{a12}	5.00±0.00^{a1}	5.00±0.00^{a1}	5.00±0.00^{a1}	4.75±0.38^{a1}	20%FPI
	4.50±0.71^{a12}	4.50±0.71^{a12}	3.75±0.35^{b2}	5.00±0.00^{a1}	4.81±0.26^{a1}	30%FPI
26	4.00±0.00^{a2}	4.00±0.00^{a2}	4.00±0.00^{a2}	3.00±0.00^{c2}	3.44±0.32^{b2}	0%FPI
	4.00±0.00^{a2}	4.00±0.00^{a2}	4.00±0.00^{a2}	3.50±0.00^{b2}	3.94±0.18^{a2}	10%FPI
	4.00±0.00^{a2}	4.00±0.00^{a2}	4.00±0.00^{a2}	4.00±0.00^{a2}	4.00±0.00^{a2}	20%FPI
	4.00±0.00^{a2}	4.00±0.00^{a2}	3.50±0.00^{b2}	3.90±0.22^{a2}	3.81±0.26^{a2}	30%FPI
33	4.00±0.00^{a2}	4.00±0.00^{a2}	3.00±0.00^{a3}	3.00±0.00^{d2}	3.38±0.23^{a2}	0%FPI
	4.00±0.00^{a2}	4.00±0.00^{a2}	3.00±0.00^{a3}	3.50±0.00^{c2}	3.50±0.38^{a3}	10%FPI
	4.00±0.00^{a2}	4.00±0.00^{a2}	3.00±0.00^{a3}	4.00±0.00^{a2}	2.88±0.23^{b3}	20%FPI
	3.50±0.00^{b2}	4.00±0.00^{a2}	2.50±0.00^{b3}	3.80±0.27^{b2}	2.69±0.26^{b3}	30%FPI
40	3.50±0.00^{a3}	3.50±0.00^{a3}	3.00±0.00^{a3}	3.00±0.00^{a2}	3.25±0.27^{a2}	0%FPI
	3.00±0.00^{b3}	3.00±0.00^{b3}	2.00±0.00^{b4}	3.00±0.00^{a3}	2.69±0.46^{b4}	10%FPI
	3.00±0.00^{b3}	3.00±0.00^{b3}	1.50±0.00^{c4}	3.00±0.00^{a3}	2.63±0.44^{b3}	20%FPI
	3.00±0.00^{b3}	3.00±0.00^{b3}	1.50±0.00^{c4}	2.50±0.00^{b3}	2.31±0.65^{b4}	30%FPI
44	3.00±0.00^{a4}	3.00±0.00^{a4}	3.00±0.00^{a3}	3.00±0.00^{a2}	3.00±0.00^{a3}	0%FPI
	3.00±0.00^{a4}	3.00±0.00^{a4}	2.00±0.00^{b4}	2.50±0.00^{b4}	2.31±0.26^{b5}	10%FPI
	2.50±0.00^{b3}	2.50±0.00^{b3}	1.50±0.00^{c4}	2.00±0.00^{c4}	2.19±0.37^{b4}	20%FPI
	2.50±0.00^{b3}	2.50±0.00^{b3}	1.00±0.00^{d5}	2.00±0.00^{c4}	1.63±0.52^{c5}	30%FPI
47	3.00±0.00^{a4}	3.00±0.00^{a4}	2.50±0.00^{a4}	2.50±0.00^{a3}	2.50±0.00^{a4}	0%FPI
	2.00±0.00^{b4}	2.00±0.00^{b4}	2.00±0.00^{b4}	2.00±0.00^{b5}	1.88±0.23^{b6}	10%FPI
	1.50±0.00^{c4}	1.50±0.00^{c4}	1.50±0.00^{c4}	1.50±0.00^{c5}	1.19±0.26^{c5}	20%FPI
	1.50±0.00^{c4}	1.50±0.00^{c4}	1.00±0.00^{d5}	1.50±0.00^{c5}	1.19±0.26^{c6}	30%FPI

5 = Very good, 4 = Good, 3 = Medium, 2 = Bad, 1 = Very poor – Superscripts with different letter (a–d) indicate significant differences of the parameter with respect to the research groups ($p<0.05$). Superscripts with different number (1–8) indicate significant differences of the parameter with respect to the storage days ($p<0.05$). The values are expressed as mean ± standard deviation.

Conclusion

At the end of the study, fish protein isolate added groups showed higher protein content, DHA value and more acceptable ratio of n6/n3 PUFA. According to the results of the chemical analysis, peroxide (PV), free fatty acid (FFA) and thiobarbituric acid (TBA) values of all groups of frankfurter were found to be below acceptable limits during storage for 47 days but TVB-N value exceeded the acceptable limit after 26th day. Similarly, there were no considerable differences among groups in terms of some textural properties (chewiness, springiness, adhesiveness, resilience and cohesiveness values) until the 26th day of storage. In regards of microbiological quality, all sausage groups were also found to be unacceptable after the 26th day of storage. However, it was recorded that the results of sensory analysis did not support these results. This study leads to the conclusion that the protein extracted from the ponyfish, which is a discard fish, have a potential to improve the nutritional value of chicken frankfurter without affecting other quality features in the negative way.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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