Rheological and microstructural properties of beef sausage batter formulated with fish fillet mince

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Abstract Rheological properties and microstructure of beef meat sausage batter, incorporated with different percentages of fish fillet mince (5 %, 20 %, 35 % and 50 %), were investigated and compared to the control (0 % fish). By increasing the proportion of fish fillet mince to the sausage formula up to 35 % and 50 %, hardness was increased by 40 % and 16 %, respectively, ($P < 0.05$), whereas, cohesiveness and springiness showed no significant differences ($P > 0.05$). In terms of temperature sweep test, storage modulus (G') of control sample faced a substantial drop from 10 °C to 58 °C, corresponding to the lowest magnitude of G' at its gelling point (~58°), but completed at around 70 °C, as same as the other treatments. Whereas the gelling point of batter sample with 50 % fish mince remained at nearly 42 °C, which was remarkably lowest among all treatments, indicating the better gel formation process. SEM micrographs revealed a previous orderly set gel before heating in all treatments whereas after heating up to 90 °C gel matrices became denser with more obvious granular pattern and aggregated structure, specifically in sample with 50 % fish mince. In conclusion, addition of fish mince up to 50 % into beef sausage formula, positively interacted in gel formation process, without diminishing its rheological properties.

Keywords Sausage · Fish mince · Texture profile analyses · Rheological properties · Microstructure

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Introduction

Due to modern dietary trends, there has been an ever-increasing shift towards the consumption of the oldest types of ready-to-eat meat products known as sausages. In this regards, the majority of emulsified sausages purchased by consumers are a product of pork, beef and chicken. However, there is evidence that consumers' preference has been changed to alternative products that are healthier, nutritious, convenient and rich in variety (Herrero et al. 2008). It has been revealed that seafood is an excellent source of nutrients such as vitamins, mineral, and a major source of omega-3 fatty acids, which are substances necessary for the human body, and also reduce the risk of malignant disease from cancer to heart disease.

Numerous investigations have already been conducted to examine the functional properties of meat emulsion and fish sausages separately by means of dynamic rheological tests (Yongsawatdigul and Park 2003) mainly by monitoring the storage modulus (G') and loss modulus (G'') during gel formation process. Jafarpour and Gorczyca (2009) examined rheological characteristics and microstructure of fish surimi and kamaboko gel (Jafarpour and Gorczyca 2009). They indicated that Alaska Pollock and Threadfin bream kamaboko showed greater storage modulus than Common carp kamaboko, as measured by temperature sweep test. Dynamic rheological (temperature sweep) tests recorded a similar pattern of sol-gel transition temperatures depending on the storage modulus (G') parameter for all surimi tested.

Obviously, regardless of type of meat, rheological properties of protein gels are heavily affected by different processing parameter such as pH and setting conditions and also formulation ingredients. Chan et al. (2011) examined the functional and rheological properties of proteins in frozen Turkey breast meat and reported that in terms of dynamic viscoelastic

behavior, low, normal, and high pH meat had the same myosin denaturation temperature (Chan et al. 2011). For example, Álvarez et al. (2012) carried out a study on textural and viscoelastic properties of pork frankfurters containing canola-olive oils, rice bran, and walnut (Álvarez et al. 2012). They stated that vegetable oil addition favored gel network formation, and when combined with walnut extract showed the highest improvement of gel elasticity which was confirmed by scanning electron micrographs. Savadkoochi et al. (2013), by studying the rheological behavior of MDMs gel and referring to the linear viscoelastic range data by running a frequency sweep test, labeled the MDMs as the weak gel (Savadkoochi et al. 2013). In 2013, Zhang and his colleagues investigated the effects of starches on the textural, rheological, and color properties of surimi-beef gels with microbial transglutaminase and reported that the gel with potato starch showed the highest gel strength (Zhang et al. 2013). However, in terms of the sol-gel transition stage, the highest and lowest storage modulus belonged to the surimi-beef mixture containing 3 % corn starch and 3 % tapioca starch, respectively. In terms of meat protein type Liu et al. (2013) investigated the rheological behavior of fish and chicken surimi gel at low and high heating regimes (Liu et al. 2013). Esturk and Park (2014) investigated the dynamic rheological responses of actomyosin during temperature sweep test from cold temperate and warm water fish species and reported that apart from Alaska Pollock, activation of endogenous thermostable proteases caused considerable hydrolysis of actin and myosin (Esturk and Park 2014).

Based on the review of literatures, we observed that some researchers have conducted studies on textural and rheological properties of fish and meat products, separately; however there is no report on exploring the textural, rheological properties and microstructure of blended red meat sausages with fish meat. Thus, the present study was designed to produce blended red meat sausages containing different percentages of fish fillet mince and therefore to investigate the textural, rheological and microstructural characteristics of the resultant raw and heated emulsion sausages.

Materials and methods

Ingredients

Frozen skinless Talang queenfish (*Scomberoides commersonnianus*) fillets were purchased from Abzei talae company (Booshehr, Iran), and stored at -18°C at the processing plant for further experiments. Other ingredients were prepared by Kalleh Company, Amol-Iran, as sausage preparation procedure was carried out at R&D section of this company.

Frankfurter sausage preparation

Frankfurter sausages were prepared based on 70 % beef meat as the major component and treatments were assigned as 5 %, 20 %, 35 % and 50 % fish mince samples as were replaced to the meat portion in sausage formula and compared to the control one (0 % fish). The other added ingredient were 0.5 % fresh garlic, 12.0 % vegetable oil, 10.2 % ice/water, 1.6 % salt, 0.4 % sodium poly phosphate, 0.05 % ascorbic acid, 0.01 % sodium nitrite, 0.25 % black pepper, 0.1 % coriander seed, 0.25 % nut, 2.0 % wheat starch, 2.0 % isolated soy protein, 0.1 % smoke powder and 0.5 % sugar. After addition of fish mince, the moisture content of formula was adjusted to 78 %, in order to evaluate the effect of fish mince on sausage more accurately. The sausages were prepared in a research and development pilot plant according to industrial procedures. The frozen fish fillet (Talang queenfish) and beef meat were transferred to a grinder machine (Seydelamn, AU 300, Germany), separately, then the mixture was placed in a cutter machine (model DMS 45C, Germany) and blended following addition of sodium chloride, sodium polyphosphate, and ice/water. Blending was performed at a speed of 1500 rpm, then at a higher speed of 3000 rpm for six min. Thereafter, the homogenized paste was stuffed into a synthetic polyamide casing (Poshesh Navid, Iran) with a 24 mm diameter, then samples were kept in a cooking room (Reich, Touch smoker TS750, Germany) with a programmed temperature time cooking schedule until the core temperature of sausages reached 72°C . The temperature of the products was monitored using a thermocouple probe positioned in the geometric center of the sausages and cooking was completed in about 90 min. After cooking, sausages were chilled quickly with a cold water shower and then they were stored at 4°C overnight, until further analysis.

Proximate analysis

Approximately 5 g sample was homogenized with 50 mL of distilled water ($\text{pH} = 5.8$) and its pH was determined using a pH meter (Methrom standard pH-meter). Fat, protein, ash, total carbohydrate, and moisture of the sausages were measured using official AOAC methods (AOAC 2000).

Texture profile analysis (TPA)

Textural properties of cooked sausages were evaluated by using a texture analyzer (Brookfield CT3 Texture analyzer, USA) according to the procedure described by Bourne (2002). Before analysis, cooked samples were equilibrated at room temperature for approximately 90 min. Afterward, for each treatment three cylindrical samples (25 mm height) were cut from cooked sausages and placed on the flat plate of the

texture analyzer and axially compressed by a cylindrical plunger ($\varnothing = 50$ mm) adapted to a 10 Kg load cell at a deformation rate of 1.0 mm/s. Previous trials established a compression limit of 50 % of the original height for samples and the average was recorded. The parameters determined were hardness (N), cohesiveness, adhesiveness (N/s), elasticity (%), and gumminess. All determinations were performed based on an average of three replicates per treatment (Bourne 2002).

Dynamic rheological measurements

Apart from several processing factors such as speed of knives, temperature and time of cutting (which were kept constant in this study), the ingredients in formulation can cause inconsistency in meat batters sausage. Therefore, in order to study the actual rheological behavior of red meat with fish mince, after addition of NaCl (Merck, Darmstadt, Germany), the incorporated mince paste was blended and before addition of other ingredients, 20 g of mince paste was taken and kept at 4 °C during overnight. Dynamic viscoelastic behavior of raw meat emulsions were tested in duplicate samples which were carried out by the method described by Jafarpour and Gorczyca (2009) with using a controlled-stress rheometer (Anton paar Rheometer, MCR 302 Series, Austria) equipped with a 20 mm parallel plate geometry, and the gap between the two plates was adjusted to 1 mm. A fine already set paste was used for dynamic viscoelastic behavior measurement. For each test, 3 g of the well-mixed batter samples were loaded on the bottom plate of the rheometer and allowed to equilibrate at temperature (10 °C) for 5 min, and then thermal sol-gel transformation was ramped between 10 to 90 °C at a constant rate of 1 °C/min by circulating water. Temperature was precisely controlled and monitored. The exposed sample perimeter was covered with a layer of silicon oil to prevent dehydration. The gelation measurements were monitored under controlled stress, (50 Pa) and constant frequency of 1 Hz, both based on the linear viscoelasticity range (LVR, 0.1–1000 Pa and 0.1–100 Hz, respectively). The results were expressed as the storage modulus (G' , a measure of elastic property), loss modulus (G'' , a measure of viscous property), and tangent delta ($\tan \delta$; G''/G').

Scanning electron microscopy (SEM)

Microstructure of sausages prepared with different fish mince substitution formulation was determined using a scanning electron microscope (KYKY-EM 3200 SEM, China). Samples with a thickness of 2 to 3 mm were fixed with 2.5 % (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 2 h. The samples were then rinsed for 1 h in distilled water before being dehydrated in ethanol with serial concentrations of 50 %, 70 %, 80 %, 90 % and 100 % (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater, KYKY, SBC-12, China).

The specimens were visualized with a scanning electron microscope (SEM) at an acceleration voltage of 15 KV.

Statistical analysis

By application of SPSS software package, data were subjected to the analysis of variance (One-Way ANOVA), and the Levene's test was used to assess the equality of variance in different samples. The significance of difference between means was measured at confidence level of 95 % (probability 0.05) by the Tukey test.

Results and discussion

Proximate compositions

Results from the proximate analysis are illustrated in Table 1. As expected, there was no changes in terms of moisture and pH of beef sausage after addition of different percentages of fish fillet mince, ($P > 0.05$). However, some parameters such as protein, carbohydrate and subsequently ash content showed significant differences among the means ($P < 0.05$). According to the results, increasing the proportion of fish fillet mince in the sausage formula up to 20 %, did not change the protein percentage compared to the control one ($P > 0.05$). The highest protein content was measured in the treatment containing 50 % fish mince ($P < 0.05$). The same trend was observed in terms of carbohydrate content whereby sample with 50 % fish mince gave the highest carbohydrate content compared to other samples ($P < 0.05$). Furthermore, apart from the sample with 5 % fish mince, substitution of red beef meat with fish fillet mince caused lower amount of lipid and higher ash content in the sausage formula ($P < 0.05$).

Texture profile analyses (TPA)

Table 2 shows the TPA results of cooked beef sausages with different percentage of fish mince substitution. According to the data, addition of fish fillet mince to the sausage formula at lower concentrations up to 20 %, lowered significantly the hardness of sausage's texture ($P < 0.05$), whereas, at 35 % and 50 % substitution, hardness was increased by 40 % and 16 %, respectively, compared to the control ($P < 0.05$). According to Youssef and Barbut (2010), increasing of protein level in cooked Frankfurter batter could increase significantly the texture resistance against the compression force. These authors, in their previous study (2009), also stated that increase in hardness can be contributed to either the smaller fat globule sizes or higher protein content in the sausage gel network. In our study, it was not the case as the protein content of 35 % fish mince was close to the 20 % fish mince, however, its hardness was more than twice of the later. Hence, rather than

Table 1 Proximate analysis and pH of beef sausage after addition of different percentages of fish fillet mince

Treatment	Moisture*	Protein	Lipid	Carbohydrate	Ash	pH
BF ₁	68.63 ± 0.92	14.10 ± 0.17 ^c	14.33 ± 0.15 ^d	1.90 ± 0.11 ^c	1.89 ± 0.01 ^d	6.27 ± 0.02
BF ₂	67.90 ± 0.10	14.16 ± 0.15 ^c	14.26 ± 0.25 ^d	1.96 ± 0.15 ^c	1.93 ± 0.01 ^d	6.30 ± 0.05
BF ₃	67.70 ± 0.20	14.30 ± 0.20 ^{bc}	14.56 ± 0.20 ^c	2.16 ± 0.05 ^{bc}	2.23 ± 0.02 ^c	6.25 ± 0.04
BF ₄	67.66 ± 0.15	14.70 ± 0.17 ^b	14.63 ± 0.11 ^b	2.50 ± 0.00 ^a	2.38 ± 0.00 ^b	6.22 ± 0.02
BF ₅	67.50 ± 0.00	15.63 ± 0.15 ^a	14.36 ± 0.11 ^a	2.40 ± 0.17 ^{ab}	2.51 ± 0.02 ^a	6.21 ± 0.01

* (Mean ± SD) Mean, after an average of triplicate trial ± standard deviation at ($p < 0.05$); BF₁: 100 % red meat, BF₂: 5 % fish fillet mince, BF₃: 20 % fish fillet mince, BF₄: 35 % fish fillet mince, BF₅: 50 % fish fillet mince

Different superscripts in the same column indicated a significant difference ($P < 0.05$) according to a one-way ANOVA and Tukey test

protein content, there are other parameters such as the quality of protein-protein bonds in the emulsion, that could potentially enhance or diminish the textural quality of the resultant sausage. The sample with 35 % fish mince showed the highest hardness value, however according to Dincer and Cakli (2010), higher values for parameters measured in texture profile (hardness, cohesiveness, springiness, and chewiness) do not necessarily mean better quality as was the case in this study based on the rheological tests (see dynamic rheological properties section).

Dincer and Cakli (2010) examined the texture quality of sausage prepared from fresh and frozen fish fillets and reported the lowest and highest mean values of hardness as 44.5 N and 52.8 N, respectively, for the fresh group and 16.1 N and 35.7 N, respectively, for the frozen group (Dincer and Cakli 2010). Accordingly, from the results of current study, the hardness value of sausage containing 50 % fish mince (56.36 N) was close to the highest value of that from the Dincer and Cakli's study. Accordingly, it is obvious that addition of fresh fish mince into beef sausage formula can enhance its textural quality, through formation of more protein-protein bonds and creation of a firmer thermo-irreversible gel network during heating process.

In terms of adhesiveness, nearly the same trend was observed as the lowest value (0.20 N/s) was recorded for samples with 5 % and 20 % fish mince ($P < 0.05$) and the highest adhesiveness was recorded for those with 35 % and 50 % fish

mince. Their correspondent values were significantly higher than that of the other treatments including the control one ($P < 0.05$). More importantly, the cohesiveness of all treatments containing fish fillet mince was improved significantly ($P < 0.05$) compared to the control (Table 2), while no significant differences were observed within treatments containing different percentages of fish fillet mince ($P > 0.05$), confirming that higher hardness value does not necessarily correlate with higher texture quality. Furthermore, it was noticed that springiness of cooked sausages was not affected by presence of different percentages of fish fillet mince in the formulation ($P > 0.05$). The gumminess values showed nearly the same trend as the hardness as it is calculated directly by multiplying of hardness and springiness parameters, i.e. the higher hardness, the higher gumminess values (Table 2). Based on the literatures, there are many other factors that can influence the texture properties of protein gel networks such as protein nature (Liu et al. 2013), moisture content, fat type and percentage (Carballo et al. 1993), additive ingredients, etc. In other words, fluctuations in protein and water content of sausage emulsion can affect the emulsification ability of the formulation with consequent decrease in TPA parameters such as hardness, springiness and cohesiveness (Daros et al. 2005). For instance, Álvarez et al. (2012) who evaluated the textural quality and microstructure characteristics of frankfurters, stated that the presence of vegetable oils in the sausage formulation was found to reduce significantly the

Table 2 TPA results of beef meat sausage formulated and replaced with different percentages of fish fillet mince

Treatment	Hardness (N)	Adhesiveness (N/s)	Cohesiveness	Springiness (%)	Gumminess
BF ₁	48.43 ± 1.02 ^c	0.35 ± 0.05 ^c	0.51 ± 0.01 ^b	0.86 ± 0.01	24.82 ± 1.06 ^d
BF ₂	36.45 ± 2.15 ^d	0.30 ± 0.10 ^c	0.68 ± 0.01 ^a	0.88 ± 0.01	24.84 ± 1.46 ^d
BF ₃	31.69 ± 4.49 ^d	0.20 ± 0.10 ^c	0.62 ± 0.73 ^a	0.85 ± 0.01	19.73 ± 2.31 ^c
BF ₄	68.03 ± 2.16 ^b	1.20 ± 0.00 ^b	0.60 ± 0.01 ^{ab}	0.87 ± 0.02	40.85 ± 1.32 ^b
BF ₅	56.36 ± 4.02 ^a	1.50 ± 0.10 ^a	0.63 ± 0.02 ^a	0.84 ± 0.01	35.64 ± 1.39 ^a

Mean, after an average of triplicate trial ± standard deviation at ($p < 0.05$); BF₁: 100 % red meat, BF₂: 5 % fish fillet mince, BF₃: 20 % fish fillet mince, BF₄: 35 % fish fillet mince, BF₅: 50 % fish fillet mince

Different superscripts in the same column indicated a significant difference ($P < 0.05$) according to a one-way ANOVA and Tukey test

hardness and gumminess compared to the one made with 20 g/100 g back-fat, concluding “that the composition and characteristics of fat are highly influential on the textural properties of meat products such as frankfurters. In addition, Savadkoshi and his colleagues (2013) investigated the textural properties of cooked emulsions containing different percentages of chicken skeleton MDM and reported that lower protein contents (mainly myosin) of thigh compared to neck MDMs is responsible for reduction of the cohesiveness of cooked emulsions.

According to the literatures, to make the sausage emulsion, comminution of meat proteins in combination with salt addition and subsequent heating is necessary, which in turn drastically alter the structure of the meat system. In this regards, several interconnected factors such as the ionic strength, salt concentration and subsequent amount of myofibrillar proteins that are extracted into the water-phase during comminution and blending influence the quality of the meat network (Comfort and Howell 2003).

Dynamic rheological properties

Shear stress sweep test

The linear viscoelastic range of sausage emulsions was determined using a shear stress sweep, in which the sample was sinusoidally deformed by increasing the shear stress from 0.1 to 1000 Pa at a constant frequency (1 Hz) at 10 °C (Figure not shown). The initial corresponding values of storage modulus (G') and loss modulus (G'') stood at 10^3 to 10^4 Pa, respectively. Based on the results, storage and loss moduli progressed independent of applied shear stress up to 100 Pa whereas the magnitude of G' emulsion sausages was higher than the viscous component. The same trend of G' and G'' during the LVR test has been postulated by previous researches (Jafarpour and Gorczyca 2009; Ross-Murphy 1995). Considering the $G'' > G'$ as indication of linear viscoelastic behavior of viscous materials, the dominance of storage modulus over loss modulus may be due to the partially self-association and overlapping of protein network which consequently will form a weak gel. However, by increasing the applied stress, the remaining interactions will not be able to stand further strain and the network structure breaks apart. Similarly, in this study, by increasing the applied shear stress, prior to the crossover point, the correspondent G' curve descended while the G'' one ascended and finally crossed at the point of 700 Pa for control treatment and approximately 1000 Pa for the rest of samples. Accordingly, beyond the crossover point, the dynamic behavior of emulsions changed as the internal proteinous bonds were ruptured and the strength of remained bonds were reduced, hence the viscous component became higher than the

elastic component. Thus a shear stress of 50 Pa was applied for running the temperature sweep test as was in the LVR of stress sweep test.

Frequency sweep

In this study, to examine the viscoelastic properties of raw emulsions, the frequency sweep test was performed from 0.1 to 100 Hz (Figure not shown). According to the results, storage and loss moduli exhibited a dependency on the frequency over the entire frequency range, showing a similar pattern by shifting upward by increasing the frequency and finally their G' and G'' crossed over at 100 Hz. Other researchers have reported nearly the same behavior of G' and G'' during frequency sweep test of protein gels (Ferry 1980; Jafarpour and Gorczyca 2009a; Karaman et al. 2011; Savadkoochi et al. 2013). The area of stability of emulsions can be related to the “plateau zone” during the test which was in agreement with the Savadkoochi et al. (2013) results. In terms of a well-structured system, the gel exhibits a constant G' on a frequency sweep analysis (Fukushima et al. 2007). In this study, the storage modulus value was higher than the loss modulus, while both of these were highly dependent on frequency, indicating the dominance of elastic component over the viscous component in the tested emulsions that could be classified as a weak gel (Karaman et al. 2011). However, Fukushima et al. (2007) evaluating the frequency sweep of three fish paste from walleye pollock, white croaker and threadfin bream at temperature range of 5–30 °C, reported the dependency of G' and G'' on frequency amplitude and referred these fish paste as a sol not even a weak gel system which is known as *suwari* gel. This result was evidenced by SDS-PAGE pattern where there was no sign of fish myosin heavy chain depolymerization up to 30 °C.

Storage modulus

Typical rheograms of storage (G') modulus of sausage emulsion containing of different percentages of fish fillet mince are shown in Fig. 1. Accordingly, an obvious difference was recorded in G' graph of sausage emulsion containing fish mince compared to the one from the control indicating the differentiation in thermodynamic properties and thermal stability of these two types of proteins (Fukushima et al. 2007). By running the temperature sweep test, the G' of control sample encountered a substantial slope from 10 °C up to 58 °C (valley shape), corresponding to the lowest magnitude of G' at its gelling point (~58 °C), in comparison with the other treatments. According to the literatures, two thermal steps can be distinguished in gelation of myosin; firstly at range of 30 °C to 50 °C, corresponding to the aggregation of the globular heads of myosin, and secondly involves structural changes in the helix form of the myosin tail at above 50 °C (Tornberg

Fig. 1 Dynamic viscoelastic rheograms of storage (G') modulus of beef meat comprising different percentage of fish fillet mince plus 2 % NaCl during temperature sweep test at 50 Pa shear stress and 1 Hz frequency, from 10 to 90 °C

2005). In this study, not only the gelation step was not observed at 30 °C, but also, a drop was recorded at 50 °C, mainly because the substrate was composed of incorporated beef meat and fish mince and was not pure myosin substrate. The drop stage in the rheograms of treatments is likely correspondent to the effect of rising temperature on protein unfolding which increase the fluidity of the “semi-gel structure”. Moreover, Esturk and Park (2014), postulated that in terms of fish protein, the drop of storage modulus (at around 46 °C to 50 °C) may be contributed to the unfolding of light meromyosin (tail) (LMM) and heavy meromyosin (HMM) chains, based on work by Sano et al. (Sano et al. 1989). Some other researchers reported the presence of heat-stable proteases in fish protein that activates at higher temperatures (50–60 °C) and causes the autolytic degradation of fish protein components, therefore, decreasing the storage modulus during temperature sweep test (Yongsawatdigul and Park 2003).

In the current study, the gelling point of sample with 50 % fish mince was recorded at nearly 42 °C, which was remarkably the lowest among all the samples, but completed at around 70 °C, the same as other treatments. According to Hermansson (1986), meat gelation is a phenomenon influenced by unfolding and refolding of polypeptide chains initiating by denaturation process at which molecular structure of proteins changes without alteration of amino acid sequence. Subsequently, by reaggregation of the denatured protein molecules an orderly gel network forms, mainly due to cross-

linking of myosin filaments, which turn the viscous sol to a thermo-irreversible elastic gel (Debusca et al. 2013).

Apart from the 5 % fish mince sample, by addition of fish mince to the sausage formula, the G' showed only a negligible decrease up to its gelling point (no valley shape in the rheograms).

After gelling point at 58 °C, the G' showed a shoulder pattern, irrespective of fish mince percentage in the sausage formula, indicating a thermo-irreversible sol-gel transition stage, resulting in a viscoelastic protein gel network, mainly by formation of hydrophobic interactions and covalent disulfide bonds (Esturk and Park 2014; Yin and Jae 2014; Zhang et al. 2013). In this state, hydrophobic interactions and disulfide bonds are involved (Savadkoobi et al. 2013) as the polypeptide chains unfold its helical structure and expose its inner surface to the surrounding aqueous environment in order to reduce the randomness and increase the stability of the system. At relatively higher temperature (>50 °C), the extent of protein unfolding increases which in turn enhances such interactions, corresponding to a 3D elastic gel network (Lanier et al. 2005).

The plateau zone in the G' rheograms started from around 70 °C up to 90 °C, indicating the completion of gel formation stage. Obviously, from around 60 °C to 90 °C, the G' value of emulsions was affected by addition of different percentages of fish fillet mince as it was higher in terms of emulsions containing fish mince compared to the one from the control.

Hence, beef sausage batter showed lower ability to form protein film around the oil droplets to make a strong gel matrix by cross-linking of either protein-protein or protein-lipid-protein sections, which resulted in a less elastic protein gel. These results are in accordance with the TPA parameters such as hardness. Furthermore, according to the rheograms, the G' magnitude was predominant over the G'' one during the temperature sweep test for all tested treatments, corresponding to the higher elasticity component than the viscous one.

Phase angle

To examine the viscous and elastic components of sausage emulsions, the changes in phase angle against the temperature was plotted in Fig. 2. Phase angle graphs of all samples exhibited the same trend during heating regime. Accordingly, the Phase angle graphs moved upwards constantly from 10 °C to around 42 °C, indicating the enhancement of viscous component of emulsion due to protein unfolding process (Yongsawatdigul and Park 2003). In other words, all proteins showed a proper ability to solubilize and become ready to make a “hydrodynamic mass” at the initial stage of gel forming process. Afterward, these graphs of $\tan \delta$ moved downward from 40 to 42 °C and reached its minimum value up to the 90 °C, demonstrating the increase of elastic component of the resultant gel by heating and formation of protein bonds mainly due to hydrophobic interactions and covalent disulfide bonds. According to the literature, $\tan \delta$ is

proportional to the energy dissipated/stored per cycle under an oscillatory shear strain test. Hence, an absolute elastic material would demonstrate $\delta = 0^\circ$, whereas in case of an absolute viscous material δ would stand at 90° . According to Farahnaky et al. (2010), in terms of $\tan \delta$, there are three classifications in order to distinguish between polymer and emulsion systems; very high $\tan \delta$ (dilute solutions), between 0.2–0.3 (amorphous polymers) and near 0.1 (glassy crystalline polymers) (Farahnaky et al. 2010).

SEM micrographs

In this study, SEM of sausage emulsions revealed a previous orderly set gel before heating in all treatments (Fig. 3 a-e). This observation supports the theory that low temperature setting can cause gelation in meat batters by formation of cross-linking between myosin heavy chains induced by endogenous transglutaminase (TGase) (Kumazawa et al. 1995; Seki et al. 1990) as well as the thermal formation of non-covalent bonds and disulfide bonds (Hossain et al. 2011). However, species kind and habitat temperature heavily influence the setting response and TGase mediated cross-linking of myosin heavy chain reaction (Araki and Seki 1993). After heating up to 90 °C, gel matrices became denser with more obvious granular pattern, resembling a cauliflower shape, particularly in red meat sausage treatment (control). Álvarez et al. (2012), investigating the modori phenomenon in Sardine surimi, asserted that a typical surimi gel exhibits a fibrillar

Fig. 2 Phase angle graphs of beef meat paste comprising different percentage of fish fillet mince plus 2 % NaCl during temperature sweep test at 50 Pa shear stress and 1 Hz frequency, from 10 to 90 °C

Fig. 3 SEM micrographs of beef meat comprising different percentage of fish fillet mince plus 2 % NaCl before heating at 90 °C (A-E) and after heating at 90 °C for 30 min (A-E'); A:100 % red meat, B:5 % fish fillet mince, C: 20 % fish fillet mince, D: 35 % fish fillet mince, E: 50 % fish fillet mince

structures, whereas application of modori temperature (>40 °C) cause the disappearance of such a pattern by showing the aggregation of globular surfaces which resembles a loose granulated mass (Álvarez et al., 2012). These authors

postulated that application of high temperature during setting can coagulate the proteins by formation of hydrophobic interactions and disulfide bonds, which in turn inhibit further reorganization of proteins to form a fibrillar structure which is the

characteristic of a thermo-irreversible viscoelastic gel network. In the current study, such a microstructure, i.e. the globular structure of proteins connected by strands exposing a dense and ordered streaming pattern was observed in the 35 % treatment at higher magnification ($\times 1000$). Conversely, such a microstructure was correspondent to the weaker gel network, confirmed by dynamic rheological tests. On the other hand, in the 50 % treatment, the gel matrix exposing coarser aggregated structures was responsible for the firmer gel with superior quality in terms of thermodynamic behavior based on the storage modulus and phase angle.

According to the SEM micrographs, fat particles were perfectly covered with the proteinaceous layers before heating, indicating the capability of all treatments (without and with fish mince) in producing high quality emulsions. The fat particles were also finely distributed over the gel network after heating (Fig. 3 a-e). Referring to Barbut et al. (1996), who evaluated the effect of different heating on meat batter containing NaCl and Tri-polyphosphate at different levels, reported an organized matrix arrangement prior to cooking, which became denser with thicker junction zones and lesser pore size after heating, resulting a rigid and consolidated gel network (Barbut et al. 1996). These authors also stated that at 40 °C, the gel matrix became denser partly due to expansion and swelling of fat globules, whereas by reaching to 100 and 115 °C (simulating a canning operation) the protein matrix showed higher porosity due to fat and gelatin squeezing out of the structure. However, increasing the fat concentration of sausage emulsion can diminish its texture quality after heating, as was investigated by Carballo et al. (1993). Carballo and his colleagues reported that addition of up to 20 % fat to the sausage emulsion lowered the “effective concentration” of protein which in turn reduced the formation of membrane around the fat globules, whereas, at a lower fat concentration (5 %) the fat particles perfectly integrated among the protein matrix by bonding to the protein strands (Carballo et al. 1993).

According to Tornberg (2005) “high penetration force for gels usually reflect highly aggregated junction points in the gel, as was shown for blood plasma gels, and good water-holding gels suggesting a fine-stranded gel with a higher amount of junction points per volume unit.”, (Tornberg 2005). Jafarpour and Gorczyca (2009) studied the microstructure of surimi and they carried out a quantitative comparison of the surimi gel networks by measuring the number and average area of the polygonal structures in the gel networks. These authors associated the superior quality of surimi with its compactness gel matrix and the compactness was quantified in terms of higher number and smaller polygonal structures.

Similarly, Arfat and Benjakul (2012) reported that in terms of Kamaboko gel, higher interconnected three-dimensional protein networks results in a more compact and denser gel

network with finer strands, which are coincidental with the higher breaking force and higher water holding capacity (Arfat and Benjakul 2012).

Conclusion

Addition of fish fillet mince into the beef sausage batter not only did not interfere with the mechanism of gel formation during heating, but also improved it, which was confirmed by TPA, rheology (storage modulus, phase angle) tests and SEM micrographs. Sausages containing 35 % and 50 % fish mince showed greater texture parameters such as hardness, cohesiveness and subsequent gumminess. Furthermore, in terms of temperature sweep tests, it was revealed that addition of 50 % fish mince into sausage batter caused a remarkable improvement in rheology properties and gel formation process. All samples showed a preexistence of a protein structure in raw state (i.e. 25 °C) which was strengthened by the gelation step upon heating. In other words, by addition of fish fillet mince, sol-gel transition state occurred by gradual density enhancement of gel matrices as temperature was raised to 90 °C. Therefore, it is recommended to enhance the texture quality of emulsified beef meat sausages by addition of fish fillet mince up to 50 % (w/w) without lowering its textural and rheological properties.

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