

## Improvement of Gel Properties of Salt-Reduced Fish Sausage by Adding Food Additives

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### Abstract

Low-salt seafood products are gaining popularity for their lower health risks, while the food industry faces technical challenges in improving its quality. In Vietnam, an average of 100g of sausage contains 1.5g of NaCl, equivalent to 600 mg of sodium, nearly half of the recommended daily salt intake and 2/3 of the sodium needs of children from 4 to 6 years old. This study aimed to assess the effect of sodium chloride (NaCl) reduction on texture of fish sausages and improve their texture by food additives. Fish sausage were manufactured by blending surimi with different concentrations of four food additives and other ingredients, and the quality characteristics were evaluated. The gel strength of fish sausage reduced with reduction of salt concentration. egg white powder (EW), whey protein concentrate (WPC), kappa carrageenan ( $\kappa$ -carrageenan), transglutaminase (TGase) are effective in improving the texture of reduced-salt sausages. The addition of 1% EW and 1% WPC, 0.9%  $\kappa$ -carrageenan and 0.3% TGase results in the gel strength of sausage increasing to 1.25- time. In particular, a 0.9%  $\kappa$ -carrageenan and 0.3% TGase added sausage exhibited the higher gel strength and water holding capacity among the sausages.

Keywords: Whey protein, egg white, kappa carrageenan, transglutaminase, gel strength, salt-reduced sausage.

### 1. Introduction

Sodium chloride (NaCl) is the most commonly used ingredient in sausages because it improves texture, enhances both flavors and taste, and lowers the water activity and inhibiting microbial proliferation to extend shelf life of final products. However, the high sodium concentration in Emulsion-type sausage is a concern, since excessive sodium salt intake from food is related to many health risks, for example, increasing chances of developing hypertension, cardiovascular disease and stroke. According to World Health Organization (WHO) guidelines, the recommended daily sodium salt intake is below 5 g/person; but this value exceeds 9.4 g/person for Vietnamese, seriously affecting people's health. Food concerns about sodium consumption have encouraged government organizations and the food industries to search for methods to reduce sodium chloride. However, salt reduction is a great challenge due to its fundamental role in the manufacture of meat and fish products such as the ability to solubilize the meat proteins, increase the hydration and water retention capacity, and improve the yield and juiciness of the product during and after cooking.

The emulsion-type fish sausages are characterized by a stable, finely chopped mixture of fish muscle, fat, water, salt and other ingredients such as fillers, stabilizers, antioxidants or spices depending

on the desired product. The fish muscle protein is responsible for the stable matrix formed during cooking in fish sausage and are categorized into three types such as myofibrillar protein, sarcoplasmic proteins and muscle proteins. The myofibrillar proteins account for 70-80% of total protein contents. Surimi is a concentrated myofibrillar protein that contains approximately 55-60% myosin and is the intermediate product for making fish sausage [1]. Gelation is an important step in forming the desired texture for fish sausage from surimi.

Salt is used to extract myofibrillar protein during surimi processing to obtain the desired texture and taste. Without the addition of salt, it would not be possible for myosin heavy chain (MHC) to aggregate due to the lack of myosin solubilization and unfolding [1]. Salt (2-3 g/100g NaCl) is usually added to solubilize the myofibrillar proteins [2], and the solubilized expanding proteins form a viscous protein paste in the form of a continuous matrix. After thermal aggregation and cross-linking, surimi paste develops into fine three-dimensional solid-like networks and forms an elastic gel, thus giving full play to the functional properties of fish sausage during cooking. The decrease in salt concentration adversely affects the extractability and solubility of surimi proteins, resulting in a poor gel structure and mechanical properties of fish sausage. A reducing sodium chloride content not only decrease in the perceived saltiness and

overall flavor intensity but also the water holding capacity and gel strength of the sausage decrease.

As society advanced, more and more consumers have become increasingly conscious of eating healthily. Consequently, the fish process products industry must match that trend and work toward reducing salt in processed foods. However, it might be a great challenge for fish sausage product industry since salt plays a vital role in protein gelation.

Our objectives were to reduce salt in fish sausage and improve texture of salt-reduced fish sausage by using food additives.

## 2. Materials and Methods

### 2.1. Materials

Surimi (mechanically deboned, washed and stabilized fish flesh) is widely used as an intermediate product for a variety of constituted seafoods, including kamaboko, chikuwa, satsuma-age, hanpen, fish sausage, crab legs and imitation shrimp products. In this research, frozen surimi was obtained from Long Hai Company at Tinh Gia - Thanh Hoa, Vietnam (Water: 76%; pH: 6.5) was used as raw material for fish sausage. Samples were packed in a polystyrene box and immediately transported to the laboratory. Frozen surimi was cut and into 1kg blocks. Surimi were vacuum-packed and kept at -18 °C until used.

Whey protein concentrate (WPC 80), egg white powder (EW), kappa carrageenan ( $\kappa$ -carrageenan) and transglutaminase (TGase) were obtained from Ba Dinh company-Vietnam.

### 2.2. Methods

#### 2.2.1. Preparation of fish sausage

Frozen surimi was thawed at room temperature for 1 h and cut into small pieces and an appropriate amount was weighed and transferred to the cutting mixer machine. The surimi was then chopped for 1 minute. Salt, spices, different food additives and ice (final moisture of surimi was adjusted to 78%) were added and blended in a mixer at high speed for 2 minutes. The temperature was maintained below 10 °C throughout the preparation. The paste was carefully stuffed into PE casing (30 mm diameter), sealed and incubated in water bath at 40 °C for 30 min followed by heating at 90 °C for 30 min. The gels were cooled immediately for 15 min in ice water and were refrigerated at 4-6 °C overnight to set prior to further analyses.

#### Design the experiments

To research effect of salt concentration on fish sausage texture, salt (0%, 0.5%, 0.7%, 0.85%, 1.0% and 1.2%) were added during surimi chopping to make fish sausage following the preparation steps above.

To improve the salt-reduced fish sausage, the functional ingredients were added into surimi as fish sausage gelation enhancers such as whey protein, egg white protein, konjac oligo-glucomannan, TGase, and modified crosslinked starch. Surimi, NaCl 0.85%, different additives and ice were chopped following the preparation steps above

#### 2.2.2. Gel strength measurement

Gel strength (GS) was determined as described by Oujifard Amin [3]. The refrigerated samples were allowed to reach room temperature (20-25 °C) prior to testing by texture analyzer (TA-XT plus, Texture Technologies Corp, NY, USA). Samples were cut to a length of 30 mm with a knife. Texture profile analysis was done using a spherical probe (Probe, N0 P/5S) at room temperature (20 °C), flowed by a procedure using a texture analyzer. The machine was set up at 75% deformation with 1 mm/sec crosshead speed, and 5 kg full scale. Then, a puncture test was carried out by penetrating the sample to breaking point with metal probe equipped with 5-mm-diameter spherical head using the speed of 1 mm/s. Finally, breaking force (g) and breaking deformation (mm) were evaluated. Each measurement was replicated 5-6 times. The gel strength (g.cm) was equal to the breaking force (g) multiplied by the breaking deformation (cm).

#### 2.2.3. Water-holding capacity analysis

The water-holding capacity (WHC) was determined according to the method of Wang *et al* [4]. The surimi gel pieces were accurately weighed as  $m_1$  and then wrapped up with filter paper. The gel samples were transferred to 5 centrifuge tubes according to 5 consecutive days and stored at 4 °C. Each day, this tube is centrifuged at 2500 rpm for 15 min. Amount of water that release from samples during cool storage and centrifuge process absorbed in filter paper. The filter paper in the samples were removed after centrifugation. The completed gel was weighed as  $m_2$ .

The WHC was calculated according to equation:

$$\text{WHC (\%)} = \left(1 - \frac{m_1 - m_2}{m_1}\right) \times 100 = \frac{m_2}{m_1} \times 100 \quad (1)$$

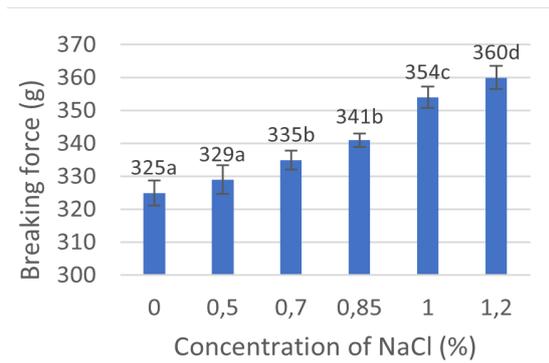
## 3. Results and Discussion

### 3.1. Effect of Salt Concentration on Gel Strength of Fish Sausage

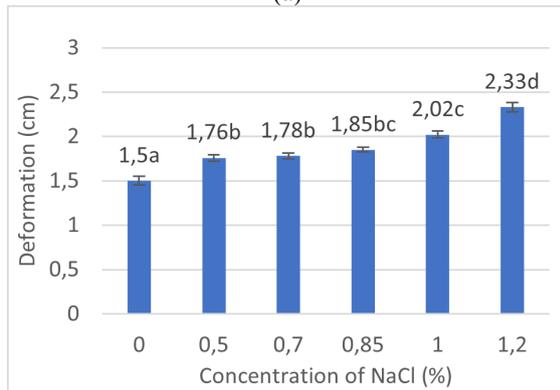
Salt concentration of 1.2% is suitable taste of food and makes them more enjoyable so salt (0%, 0.5%, 0.7%, 0.85%, 1.0% and 1.2%) were added to make fish sausage. The gel strength of sausage at different salt concentrations was presented in Fig. 1.

The breaking force, deformation and gel strength of fish sausage gels gradually increased with the rise in salt concentration (0%, 0.5%, 0.7%, 0.85%, 1%). There was significant difference in these properties of sausage gel when the addition of NaCl of 1.2% with

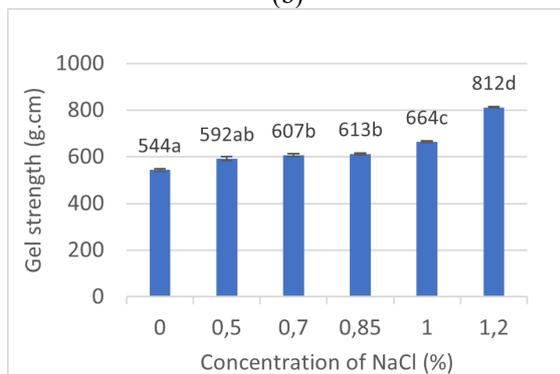
highest gel strength. According to Núñez-Flores *et al.* [2], in the absence of salt and hence of myosin unfolding and solubilization, there was no MHC polymerization and so surimi gel were characterized by highly aggregated proteins, reduced elasticity and poorly stabilized protein network characteristics of a low-quality gel. On the other hand, in gel made with added salts, MHC polymerization occurred, and the gelation resulted in a well-stabilized protein network with good physicochemical properties. Decreases in salt content depressed protein solubility and the strength of fish sausage gels.



(a)



(b)



(c)

Fig. 1. The breaking force (a), deformation (b) and gel strength (c) of fish sausage at different salt concentration

The salt concentration of 0.85% was chosen with the objective to reduce 30% salt concentration in sausages. At this salt concentration, the gel strength was 613 g.cm. Texture is the most important criterion for sausages, hence texture of salt- reduced fish sausage can be enhanced by adding food additives.

### 3.2. Effect of Food Additives on Texture of Salt - Reduced Fish Sausage

The functional ingredients were added into surimi as fish sausage gelation enhancers including whey protein, egg white protein, konjac oligo-glucumannan, TGase, and modified crosslinked starch. The effect of food additives on textural properties of salt-reduced fish sausage were presented the following.

- *Effect of whey protein concentrate on gel strength and water holding capacity of salt - reduced fish sausage*

Whey proteins exhibit particular functional properties, such as solubility, viscosity, water binding, emulsification and gelation that are desirable in a food system [3]. The effect of whey protein concentrate (WPC) addition on the gel strength of salt-reduced fish sausage is presented in Fig. 2.

The addition of 1%, 2%, 3% WPC increased the gel strength to 132%, 115%, 113% as compared with that of the control sample. The gel strength of sausage reached the highest at 1% of WPC. These results suggests that the addition of WPC improved the textural properties of gels due to whey protein concentrate being functional filler. However, the gel strength of sausage decreased at 4% of WPC. This might be the result of myofibrillar protein concentration reduction due to high WPC and water content to adjust moisture to 78%.

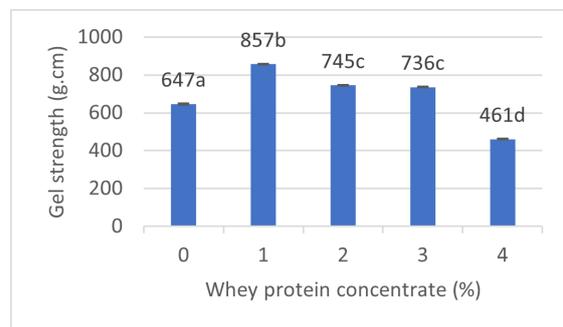


Fig. 2. Effect of WPC on gel strength of fish sausage

The sausages with the addition of whey protein at the concentration of 3% and 4% had lower water holding capacity than other treatments (Fig. 3). Water holding capacity is strongly influenced by the three-dimensional structure of the myofibril protein. Myofibril proteins provide an open space for water

immobilization, so protein denaturation will have an effect on shrinking the space.

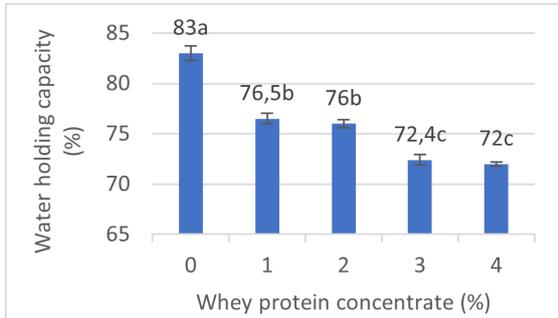


Fig. 3. Effect of whey protein concentration on water holding capacity of salt-reduced fish sausage

- Effect of egg white on gel strength and water holding capacity of salt - reduced fish sausage

Egg white has also been used to improve gel quality, inhibiting the softening of the gel by acting as an enzyme inhibitor [7]. The effect of EW addition on the gel strength of salt-reduced fish sausage was presented in Fig. 4.

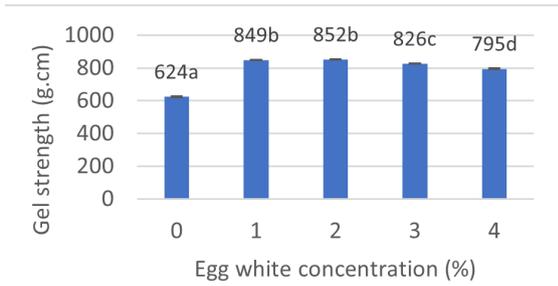


Fig. 4. Effect egg white concentrations on salt-reduced fish sausage gels

Fig. 4 shows the result of the gel strength for fish sausage gels containing EW at 0% - 4%. The addition of EW significantly affected the gel strength ( $p \leq 0.05$ ). As shown in Fig. 4, the highest gel strength was observed in sausage gel with 1-2% EW and then decreased when concentration of EW was higher than 2%. The increase was 36% compared to that of the control. These results suggested that the addition of EW improved the textural properties of gels due to the surface SH groups of EW protein. This enhanced gel network formation by forming S-S bonds with other protein molecules. However, the addition of EW resulted in decrease in the water holding capacity of sausage (Fig. 5)

The results showed that sausages with the addition of EW had a water holding capacity of 68%-76.3% and water holding capacity decreased with increasing EW dosage. These results are similar to A N F Rahma *et al.*, 2023 who reported that surimi with the addition of EW had lower water holding capacity [8].

- Effect of kappa carrageenan on gel strength and water holding capacity of salt - reduced fish sausage.

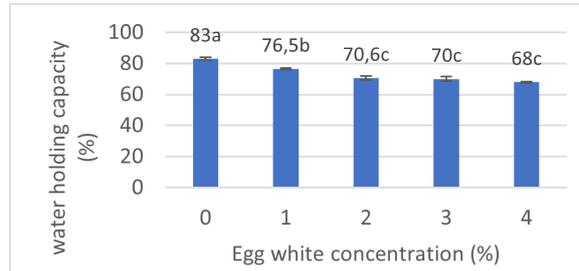


Fig. 5. Effect of egg white concentration on water holding capacity of salt-reduced fish sausage

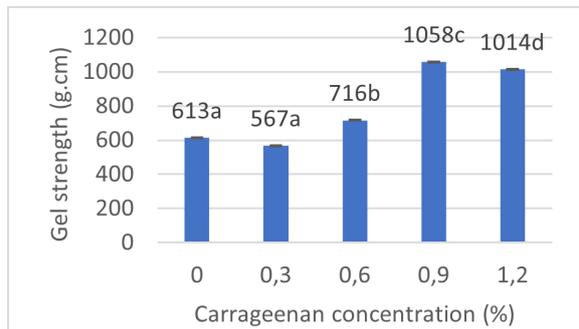


Fig. 6. Effect  $\kappa$ -carrageenan concentration on gel strength of salt-reduced fish sausage gels

Kappa carrageenan ( $\kappa$ -carrageenan) is one of the essential ingredients in many foods processing, which plays a prominent role in stabilizing and regulating gelling characteristics. It is reported that  $\kappa$ -carrageenan can improve the rheological properties, water holding capacity, textures, and microstructure of gel, which further modify the quality of final products [9]. The effect of  $\kappa$ -carrageenan addition on the gel strength of salt-reduced fish sausage was presented in Fig. 6.

The results in Fig. 6 shows that the gel strength of sausage increased as the concentration of  $\kappa$ -carrageenan increased. The addition of 0,3%, 0,6%, 0,9%, and 1,2%  $\kappa$ -carrageenan increased the gel strength to 1.17, 1.72 and 1.66 times respectively compared with the control sample and reached the highest at 0,9% of carrageenan. These results indicate that the addition of carrageenan improved the textural properties of the gel. The myofibrillar protein and  $\kappa$ -carrageenan unfolded after heating, exposing the internal groups of protein and helices of  $\kappa$ -carrageenan. During the cooling period,  $\kappa$ -carrageenan was filled into the protein network.  $\kappa$ -carrageenan promotes hydrophobic interactions and the formation of disulfide bonds, resulting in a more ordered and crosslinked fish protein network gel structure.

As shown in Fig. 7, compared to the control sample, the WHC of the sausage samples significantly increased with the growth in  $\kappa$ -carrageenan

concentration ( $p < 0.05$ ).  $\kappa$ -carrageenan dry powder is often added during the chopping process and make the pre-suspension of  $\kappa$ -carrageenan in water enables its swelling and increases its surface area, which enables the formation of a stronger gel network during heating process, which entraps more water in the network.

- Effect of Transglutaminase enzyme concentration on gel strength and water holding capacity of salt-reduced fish sausage.

Transglutaminase (TGase), which induces the formation of a non-disulfide covalent bond called gamma-Glu-epsilon-Lys isopeptides, is activated during a setting process. It plays a crucial role in the setting of surimi gel, leading to an improvement in gel formation. The effect of TGase on the gel strength of salt-reduced fish sausage was presented in Fig. 8.

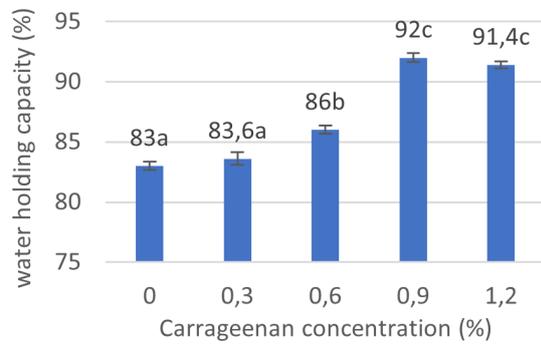


Fig. 7. Effect of  $\kappa$ -carrageenan concentration on water holding capacity of salt-reduced fish sausage

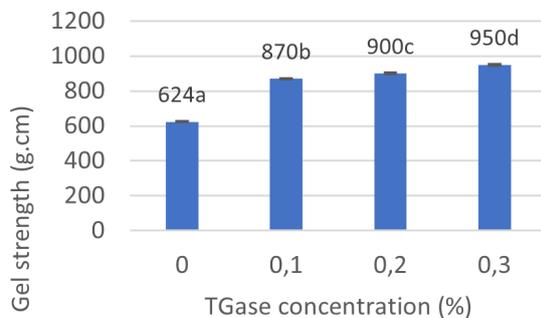


Fig. 8. Effect TGase concentrations gel strength of salt-reduced fish sausage gels

The result in Fig. 8 shows that gel strength of fish sausage adding different TGase concentration had a significant effect on the gel strength of the product. The gel strength of sausage increased as the concentration of TGase rose, reaching its peak value at 0.3 % TGase. These results are similar with Seighalani *et al.*, 2016 and Ping-Hsiu Huang *et al.*, 2022, who reported that breaking force and deformation of surimi gels increased when TGase increased up to 0.3% [11, 12].

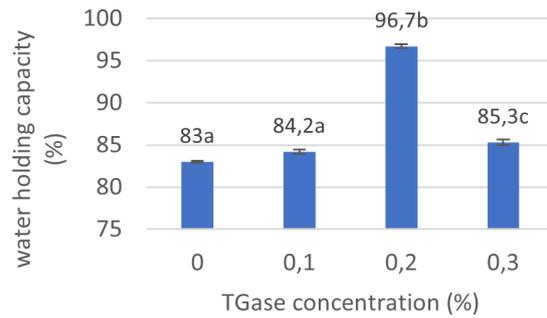


Fig. 9. Effect TGase concentrations on water holding capacity of salt-reduced fish sausage gels

Transglutaminase catalyzes the intramolecular and intermolecular cross-linking of proteins to form new proteins, so the space network structure formed by the catalysis of transglutaminase can hold a large amount of water, it can prevent the water shrinkage of products during processing. As shown in Fig. 9, WHC of sausages increased with the increase in TGase concentration. However, the addition of TGase at a level 0.3% resulted in a decrease in the WHC. This might be cause the  $\epsilon$ -( $\gamma$ -glutamyl)-lysine covalent cross-links were formed in excess, the protein-water interaction decreased, leading to significant water loss and a disrupted formation of compact gel [13].

### 3.3. Effect of Different Food Additives at Optimum Concentration on Textural Properties of Salt-Reduced Fish Sausage

The breaking force (g) was used to represent gel hardness, the breaking deformation represented the gel elasticity. The gel strength (g.cm) was equal to the breaking force (g) multiplied by the breaking deformation (cm) and was used to evaluate gel quality. Fig. 10 showed that the deformation of samples adding WPC and EW increased to 1.25 times compared to the control. However, their breaking force did not significantly increased. This showed that WHC and EW improved the elastic of sausage. The equivalence in deformation of the sausages adding of egg white and whey protein (Fig. 10b) had a similar structure with the 1.2% NaCl sample (Fig. 1b). The same goes for breaking force (Fig. 10a and Fig. 1a) and gel strength (Fig. 10c and Fig. 1c).

Addition of  $\kappa$ -carrageenan at 0.9% and TGase at 0.3% increased both breaking force and deformation of fish sausage gel. The addition of carrageenan at 0.9% exhibited the highest gel strength.

The water-holding capacity of surimi-based products is critical in determining the quality of surimi-based products and how well they hold water [14, 11]. The effect of different food additives at optimum concentration on water holding capacity is presented in Fig. 11.

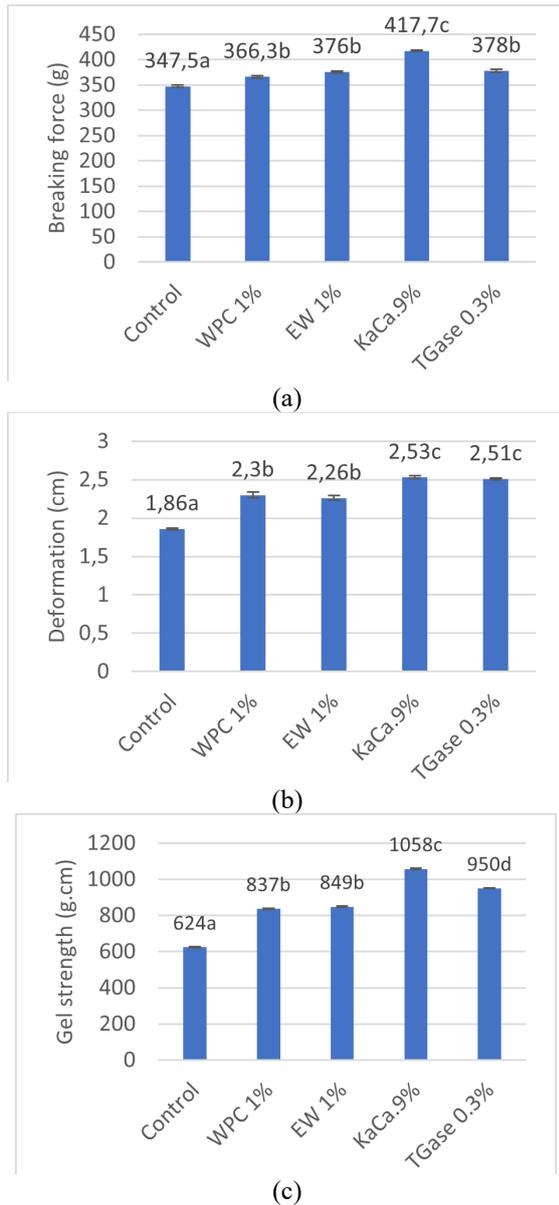


Fig. 10. Effect of different food additives at optimum concentration on breaking force (a), deformation (b), and gel strength (c) of salt-reduced fish sausage

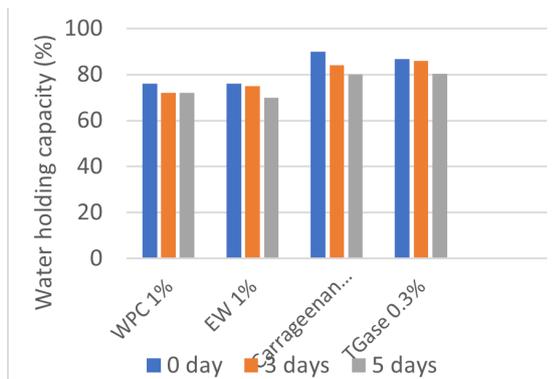


Fig. 11. Effect of different food additives at optimum concentration on water holding capacity of fish sausage.

The addition of EW to surimi can decrease the water holding capacity of fish sausage during storage. This might be due to during heating, gelation of EW and mixed-fish emulsion occurred, resulting in adhesion between the egg white protein and the fish protein. As a result, the gelation of the sausage became brittle and easily breakable and water was easily lost. A decrease in water holding capacity of fish sausage adding 1% of whey protein was also observed in samples during storage. The addition of 0.3% TGase improved the water holding capacity of sausages due to the stable structure of the gel network and its water entrapment ability.

The solid character of  $\kappa$ -carrageenan gels arises from the formation of a three-dimensional network, which extends continuously through the entire volume and entraps the dispersed. The interactions of three-dimensional network include van der Waals, forces, hydrogen bonds, charge transference, ionic, hydrophilic and hydrophobic. Consequently, gels containing  $\kappa$ -carrageenan exhibited higher water holding capacity. The addition 0.9% of  $\kappa$ -carrageenan to the fish sausage resulted in a increase in WHC comparing with control and other additives.

#### 4. Conclusion

Textural properties of salt-reduced fish sausage can be improved by addition of 1% WPC, 1% EW, 0.9%  $\kappa$ -carrageenan, 0.3% TGase, pre-incubate at 40 °C for 30 min before heating at 90 °C for 10 min. Gel strength and water holding capacity significantly increased with the addition of  $\kappa$ -carrageenan and TGase. From our studies, kappa carrageenan and TGase demonstrated a great potential as a textural enhancer for salt-reduced fish sausage.

#### Acknowledgements.

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