Carp Muscle Protein Patterns and Textural Properties as Affected by Starch Additions to The Mince Protein Gels Made From Wild Grass Carp (*Ctenopharyngodon Idella*), Silver Carp (*Hypophthalmichthys Molitrix*) and Bigmouth Buffalo Carp (*Ictiobus Cyprinellus*)

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RESEARCH

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ABSTRACT

To compare the effects of the type of carp meat gel and starch addition on the gel property, two major invasive carp species (grass carp and silver carp) and a native freshwater fish (bigmouth buffalo) were used for preparing fish meat paste and fish meat gel. Six different types of starches were assayed for their gelatinization profile and added to partially replace fish meat paste at different concentrations (0, 2, 4, and 6 g/100 g) for making fish meat gel on equal moisture (80 %) and salt (3 %). Bigmouth buffalo had higher-intensity bands of tropomodulin (38.8 Kda) and tropomyosin α (33-37 kDa) than the other two fishes. Grass carp exhibited lower band intensity at 47.9 KDa, and the myosin light chain at 15.9 KDa was missing in both of the fish meat and fish meat paste. The result of the texture analysis is in line with the temperature sweep test data, confirming the higher plasticity of silver carp gel than that of either bigmouth buffalo or grass carp. The meat paste of silver carp had a significantly higher G' (storage modulus) value at Peak 2 (77 ℃) than the other two fishes, in contrast, bigmouth buffalo had a significant higher G' value at peak 1 (48 ℃). The breaking force (611.8 g) and deformation distance (11.7 mm) of silver carp cooked meat gel were significantly higher than those of grass carp and bigmouth buffalo. The addition of starches to the grass carp or silver carp paste lowered the breaking force of the cooked gels in a dose-responsive manner as compared to the control without adding starch ($p<0.05$), but no differences were found between the bigmouth buffalo paste with the same starch except the samples containing Firmtex™. Pearson correlation coefficient analysis showed that Myosin light chain-1 (18.5 kDa band) was correlated to loss factor and gel strength among three kinds of fishes.

Key Words: carp; starch; gel strength; fish meat paste; fish meat gel.

INTRODUCTION

The invasive Asian carp species, silver carp (*Hypophthalmichthys molitrix*) and grass carp (*Ctenopharyngodon idella*) have proliferated greatly in the water basin of Mississippi river and its tributary waters since 1980s [1-3]. The Bigmouth buffalo (*Ictiobus cyprinellus*) is a North American fish species belonging to the family Catostomidae is considered as carp [4]. These wildly grown carps can grow up to 80-100 lbs in sizes (different from the aquacultured carps raised in some countries) and create the ecology of the rivers that threaten the survival of other fish species. Several US state governments along the Mississippi river have offered assistance programs to eliminate these wild carps. Therefore, creating demand for the wild carps by utilizing the wild carp meat may provide a solution for the removal of carps from US rivers and lakes [5]. Carps are long and bony and American consumers are not familiar with eating carps. Producing surimi-like products provide a potential means for utilization of the cheap sources of carp protein. Understand the protein composition may help in the utilization of carp meat. Our preliminary studies showed that surimi-like gels made from carps are firm. Therefore, it is imperative to study how starch additives change the textural properties of carp surimi-like gels.

However, there is no comparison of protein patterns and starch-blended textural properties between fish meat and fish meat gel produced from wild grass carp, silver and bigmouth buffalo.

In addition to being a texture modification, addition of starches to the meat system may reduce cost. Both fish meat paste and starch are subjected to changes in properties during the cooking process. Rheological changes of paste during heating can be characterized by the textural properties of visco-electricity in terms of reading the storage modulus (G'), and modulus (G") during thermal gelation [6] while the physical changes during the starch heating process can be observed in terms of viscosity. The pasting properties of starches during heating and cooling processes are typically characterized by gelatinization temperature, gelatinization maximum, setback, breakdown, and final viscosity affects the quality of final starch-based products [7]. Adding starches to the meat paste may enhance water holding and change the visco-elestic properties of the cooked gel. Tapioca cassava native starch, tapioca derived starch acetate (E1420), tapioca derived distarch phosphates (E1412), Novation 1900 (comprise of corn, waxy corn, potato, tapioca and rice), Firmtex™ (modified waxy corn, E1442 (hydroxypropyl distarch phosphate)) and N-Hance 59 (native potato starch) are commonly used food starches in the food industries. Their effect on carp protein gel formation has not been studied. Identifying the best starches for modifying carp protein gel will contribute to the utilization of carp meat.

The formation of the three-dimensional network by protein during heating is generally thought to be due to the denaturation of the protein molecules and interaction of the denatured molecules to form cross-linkages [8]. During the heating process, starch interacts with the proteins affects the gel texture [9, 10] To study how protein patterns and starch addition affected textural properties, protein profile of grass carp, silver carp, and bigmouth buffalo was determined by electrophoresis of meat and mince paste. The rheological properties of carp mince and the RVA profile of starch were individually observed during the heating process. And the breaking force, deformation distance and gel strength were observed on heated mixture of carp mince and starches.

MATERIALS AND METHODS

Fish meat and starch

Fresh-harvested wild grass carp (*Ctenopharyngodon idella*), bigmouth buffalo carp (*Ictiobus cyprinellus*) and silver carp (*Hypophthalmichthys molitrix*) were obtained from the Two Rivers Fisheries (Wickliffe, KY) and buried under crushed ice during transportation to the walk-in cooler of the pilot plant in Department of Food Science, Nutrition and Health promotion at MSU. Fish meat was cut into 3 mm cubes and washed 3 times at the ratio of 4:1 as described by Jafarpour & Gorczyca (2008)[11]. The mince was dewatered by wrapping and squeezing with cheesecloth and then ground with a LEM grinder (Big Bite,

West Chester, OH) at 4℃. Cryoprotectants (4 g/100 g sucrose, 4 g/100 g sorbitol, and 0.3 g/100 g sodium tri-polyphosphate) were incorporated into the paste and homogenized by a Hobart mixer (Troy, Ohio , USA) at lowest speed (speed one) for 2 min, then increase to speed two for 2 min and then to highest speed (speed three) for 20 min. The resulting fish meat paste was placed into a bag with one pound each, and frozen at -80 ℃ until use. Native starch, distarch phosphate and starch acetate (Vedan Vietnam Dong Nai, Vietnam) were made from tapioca cassava root. Novation 1900 (made from a mixture of corn, waxy corn, potato, tapioca and rice,), Firmtex™ (made from waxy corn starch), and N-Hance 59 (native potato starch) were kindly obtained from the Ingredion Incorporate (Bridgewater, NJ).

SDS-PAGE analysis of the three species of carp

Fish meat were ground and mixed with phosphate buffer solution (pH 8.5, 1:10 w/v) and shake for 1 hr with an orbital shaker at 4 °C, and then centrifuged at 2795 g for 10 min to obtain the supernatant. The protein content of supernatant was determined by Bradford assay (Bradford, 1976). The protein content was diluted to 2 mg/mL with phosphate buffer solution, and 0.5 mL of diluted samples was mixed with 0.5 mL sodium dodecyl sulfate (SDS) sample buffer containing beta-mercaptoethanol [12] After boiling for 10 min, 8 µL of the cooled solution containing equivalent of 8 µg proteins were loaded onto a gradient gel (8-16%). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the procedure of Laemmli (1970). The SDS-PAGE gels were washed to clean up the background stain, scanned and analyzed using a Molecular Imager (Bio-Rad Chemidoc™ XRS+, Hercules, California).

Dynamic rheological measurements

To compare the dynamic rheological properties of paste made from fish meat (silver carp, grass carp, and bigmouth buffalo), fish meat paste was prepared by mixing with 3 % NaCl and adjusted to 80 % moisture content after moisture determination as previously described [13].

Dynamic rheological measurements were performed on an Anton paar dynamic rheometer (MCR 502, Anton Paar, Houston, TX, USA). A parallel-plate geometry of 25 mm diameter and normal force of 1 N was set for measurement. The fish meat paste was placed between the parallel plates of the rheometer. The excess samples protruding beyond the upper plate were removed. After rest for 2 min to ensure both thermal and mechanical equilibrium, samples were heated at a speed of 1°C/min from 10 to 90 °C with 0.1% shear strain (γ) and 10 Hz frequency. Storage modulus (G') was recorded from the temperature sweep test. To prevent moisture loss during measurement, silicone oil was gently applied to the edge of parallel plate. Three replicates of each sample were measured.

Starch gelatinization profiles

Pasting properties of 6 % (w/w) starch were determined using a rapid visco-analyzer (RVA, RVA 4500, Perten Instruments, Hägersten Sweden). Starch and water were added to achieve a total weight of 25 g containing 6 % (w/w) starch. The dispersions were kept at room temperature for a further 30 min to hydrate the starch. The pasting profile of the sample and agitation speeds of the paddle were monitored during the thermal treatment, programmed as follows: increasing the temperature from 35 ℃ to 95 ℃ at a heating rate of 1.5 ℃/min, holding the temperature at 95 ℃ for 10 min, decreasing the temperature to 50 ℃ at 1.5 ℃/min. Agitation speed of paddle was started at 960 rpm for the first 10 s and kept constant at 160 rpm until the end of the experiment [7]. Pasting profiles were evaluated in triplicate for the average values of pasting parameters.

Preparation of fish meat gels

Sample preparation was based on equal moisture (80 %), and salt (3 %) following homogenization for further 3 min as described by Tan et al. (2019) [13]. The fish meat paste was stuffed into the cylinders (60 mL capacity, 10 cm in length and 3 cm in diameter), which were placed on a stainless-steel rack and covered by a glass ball on top, to prevent evaporation during heating process [13]. The rack with the cylinders were then submerged vertically in a 40 °C

water bath for 15 min, and then in a 90 °C water bath for 20 min. After cooking, the fish meat paste was converted to fish meat gels in the cylinders, which were immediately cooled in an iced water bath (2 °C) for 10 min, and then stored at 4 °C overnight before texture analysis.

Measurement of texture properties

A texture analyzer (TA-XT2i, Stable Micro Systems, UK) was used to evaluate the textural properties of fish meat gels. Gels were equilibrated to room temperature for 1 h and then cut into cylinders (25 mm in height) as described by Meng et al., (2016) [14]. Determination of the breaking force (g) and the deformation distance (mm) was conducted with a cylindrical probe (8 mm in diameter) traveling from the surface of the gel to the point of breakage used to at the speed of 60 mm/min. Both the breaking force and deformation are reported for the first force peak [15]. The gel strength was calculated by

$$\text{Gel strength} = \text{Breaking force} \times \text{Deformation}$$

Statistical Analysis

For measurements of texture, color and cooking loss, experiments were replicated three times. Data were subject to analysis of variance using XLStat (2015, Addinsoft USA, New York, NY). Significant differences among means were determined by the Tukey honestly significant difference (HSD) test using probability level of 0.05. Pearson's correlation coefficient analyses were determined. Significance levels were defined using $P < 0.05$.

RESULTS AND DISCUSSION

Protein profile of grass carp, silver carp, and bigmouth buffalo meat

The protein distribution patterns of three species of fish are shown in Figure 1. Proteins were extracted from the fresh fish meat and fish meat paste to investigate the processing effect on protein compositions. No significant processing effect was observed. For the three kinds of fish, myosin heavy chain (MHC), actin, tropomodulin, α-tropomyosin, β-tropomyosin and three myosin light chains (18.5, 16.8 and 13.5 KDa) were all found in the protein profile (Table 1). However, the protein distribution patterns of three species of fish were different [16-19].

Grass carp exhibited lower band intensity at 47.9 KDa, and the 15.9 KDa myosin light chain protein band of grass carp was absent compared with other 2 species. Most of the bands of bigmouth buffalo were similar to that of grass carp and silver carp, except the tropomodulin (38.8 KDa) band was darker and tropomyosin α (33-37 KDa) was darker and in a lower position in bigmouth buffalo carp as compared to that of the silver and grass carp.

To our best knowledge, no muscle protein profile of bigmouth buffalo had been ever reported. Recently, MHC (200 KDa), actin (45 KDa), troponin-T (35 KDa), myosin light chain (21 KDa), tropomyosin (40 KDa) were identified in protein of silver carp [20, 21]. As to grass carp, MHC (approximately 200 KDa), three myosin light chains, tropomyosin β and tropomyosin α (33-37 KDa) had been reported by Tao et al., (2007) and Yang et al., (2014) [18, 22]. To our best knowledge, our study is the first time to report the different of the myofibril protein compositions of bigmouth buffalo, silver and grass carp.

Dynamic rheological properties

Sol-gel transition thermographs of the three kinds of fish meat paste are shown in Figure 2 to describe their thermal transition behavior. All the three kinds of fish meat paste showed two peaks of G' during the heating process from 10 °C to 90 °C, however, the temperature range of silver carp peak 1 (34.7 °C) was significantly lower than that of grass carp (46.0 °C) and bigmouth buffalo (48.0 °C) as shown in Table 2. The peak 1 G' value (8680 [Pa]) of bigmouth buffalo is significantly higher than the other two carps. To our best understanding, no rheological study was performed on bigmouth buffalo. The characteristically high G' value on peak 1 might influence the texture of fish meat gel made of bigmouth buffalo. No significant differences were observed between the peak 2 temperature of three fish species.

The silver carp had a significant higher G' value at peak 2 and final temperature of 90 °C, when compared with

the other two fish species. In contrast, bigmouth buffalo had a significantly higher G' value at peak 1. Because G'>G'' (loss modulus) in the whole temperature range, the paste and gel showed the consistency as a solid [23]. The loss factor (tan δ, the ratio of the viscous property to the elasticity, data not shown) of fish meat paste made from grass carp, silver carp and bigmouth buffalo meat, declined after the first G' peak and decreased the values continuously with the increase of temperature. This phenomenon indicated more cross-linked covalent reactions were formed during this range of thermal treatment. Because the first G' peak of thermograph seems to depend on the fish species, Jafarpour & Gorczyca (2009) [6]summarized G' first peaks at 43, 44 and 52 °C for flying fish myosin, carp myosin and Alaska pollock surimi, respectively. Abdollahi et al., (2017) reported the first peak temperature of the silver carp myosin existed at 48 °C. Tao et al., (2007) reported the first peak of grass carp myosin ranged from 38 to 44°C.

Starch pasting properties

Figure 3 presents the gelatinization properties for native starch (tapioca), starch acetate (tapioca), distarch phosphate (tapioca), Novation 1900, Firmtex™ and N-Hance 59 measured by RVA, and the values of the parameters of gelatinization temperature, gelatinization maximum, setback, breakdown, and final viscosity are summarized in Table 3 according to the evaluation method as described by Agudelo et al. (2014) [24]. All the three kinds of tapioca derived starches had significantly higher gelatinization temperatures among the six samples. The viscosity at the peak of the RVA profile (gelatinization maximum) is considered as the equilibrium point between swelling and rupture of starch granules during heating [25, 26] Novation 1900, Firmtex™ and N-Hance 59 had much higher gelatinization maximum due to use the raw material like waxy corn and potato starches. Further disruption of starch granules and leaching out of starch molecules caused a decrease in viscosity. Therefore, the breakdown measures the susceptibility of gelatinized starch to disintegration due to the loss of starch granule integrity and subsequent disruption, leading to a reduction of the pasting viscosity [7]. The breakdown curve had been correlated to the gel stability during cooking of starch [27]. N-Hance 59 showed a significantly higher breakdown value than the other starches.

In general, the RVA setback obtained from the measurement occurs not only due to the degree of re-association of gelatinized starch (particularly amylose) molecules during cooling, but also due to the simple kinetic effect of cooling on viscosity. The setback value indicates short-term retrogradation of starch. Firmtex™ was similar to distarch phosphate (tapioca) which has higher setback viscosity.

Effects of different starches and fish meat on the textural properties of cooked fish meat gels

The results of breaking force (g) of the grass carp, silver carp, and bigmouth buffalo fish meat gel are shown in Figure 4. The breaking force (611.8 g) of silver carp gel were significantly higher than those of grass carp (323.7 g) and bigmouth buffalo (420.4 g). The results of textural properties were in line with the G' value of peak 2 and the final temperature in dynamic heological assay where the G' of silver carp gel was significantly higher than those of grass carp and bigmouth buffalo (Table 2). As summary of the results from several research groups showed, the breaking force of silver carp meat gel ranged from 300-560 g [20-21, 28-30]. As to grass carp meat gel, the breaking force was ranged ranging from 297-502 g [31, 32]. Our results were in line with these previous reports. The addition of starches to the grass carp meat gel had a significantly destructive effect on the breaking force values compared to the control ($p<0.05$). The breaking forces of grass carp meat gel containing starches decreased significantly compared with that without starches ($p<0.05$). Meanwhile, there were significant decrease in the breaking force with Novation 1900 and Firmtex™ when starch levels further increase from 2% to 6% ($p<0.05$). At the level of 6 % added starch, the breaking force of grass carp gel with Novation 1900 (109.7 g) and Firmtex™ (118.0 g) were significantly lower than those with tapioca derived starch including native

starch (151.6 g), distarch phosphate (155.0 g) and starch acetate (181.1 g). In silver carp gel, the breaking force decreased as the starch concentration increased. At the same additive level, difference existed between different kinds of starch. Different from grass carp, at the same starch level silver carp gel with added tapioca derived starch did not have higher breaking force than non-tapioca-derived starch. In silver carp gel, the breaking force decreased as the tapioca native starch, tapioca acetate starch, or Novation 1900 concentration increased. To our best knowledge, this study is first to report the textural and dynamic rheological properties of fish meat gel made from bigmouth buffalo. For all the bigmouth buffalo gel with addition of starches, the breaking force was significantly lower than the control ($p<0.05$), however, no difference was found between the bigmouth buffalo gel with the same starch species at the level 2, 4 and 6% except the samples with Firmtex™.

The results of deformation distance (mm) of grass carp, silver carp, and bigmouth buffalo gel are shown in Figure 5. The deformation (11.7 mm) of silver carp gel were significantly higher than those of grass carp gel (9.6 mm) and bigmouth buffalo (8.3 mm). Summarized the results from several research groups, the deformation of silver carp gel was ranging from 9.0-14.3 mm [20, 28-30, 21]. As to grass carp gel, the deformation distance was reported 6.7-10.0 mm [31, 32]. Our results were in line with these previous reports (9.6 mm). The deformation of all grass carp gel with starch were significantly lower than control ($p<0.05$) except those with 4% distarch phosphate and Novation 1900 (9.4 mm). At the level of 6 % added starch, the deformation of grass carp gel with Novation 1900 (6.6 mm), Firmtex™ (6.6 mm) and N-Hance 59 (7.7 mm) were significantly lower than those with tapioca derived starch including native starch (8.4 mm), distarch phosphate (8.9 mm) and starch acetate (9.4 mm). No significant difference of deformation was found between silver carp gel with 0, 2, 4 and 6% tapioca distarch phosphate. The deformation of silver carp with 2% N-Hance 59 (12.9 mm) was higher than control (11.7 mm). On the contrary, the deformation of bigmouth buffalo gel was increased by the addition of starch except N-Hance 59.

The results of gel strength (g × mm) of grass carp, silver carp, and bigmouth buffalo gel are shown in Figure 6. The impact of the addition of starch or modified starch on gel strength (g × mm) were different among grass carp, silver carp and bigmouth buffalo as the values of breaking force and deformation mentioned above.

In the mixture of meat paste, the starch gelatinized during heating through granule swelling, disruption of crystalline regions, loss of birefringence, increase of viscosity, fragmentation of the granules. Although the relationships between starch and protein in fish-meat gels have not been fully elucidated, it had been proposed that that the starch granules bound in the proteins had a “packing effect” on the protein due to the internal pressure [33]. The starch granules embedded in the gel not only competed the water bound in the protein to swell and gelatinize, but also exerted pressure against the protein which had already started gelation. In contrast, the growth of the starch granules may be limited by the gelatinized protein and the shortage of water. From our results, we know that gelatinization of all kinds of starch occurs above 60 °C, while the rheology changes of fish meat paste occurs at a temperature range lower than 60 °C. This makes us believe that the extrusion of the “packaging effect” of fish meat paste weights more than that of starch. Compared to grass carp and silver carp, bigmouth buffalo gel has two special features; the starch concentrations have little effect on breaking force, and deformation increased with elevated starch concentration. The fact that G' value of bigmouth buffalo at peak 1 was found to be significantly higher than the other two fishes might help to explain the two-phenomenon found in this study and by indirect confirmation that the "packing effect" model is reasonable. Further investigating is needed to decide whether its protein composition resulted in higher peak 1 value of storage modulus, less starch influence on breaking force, or the opposite effect in deformation.

For commercialized surimi-like products, the textural quality was significantly different ($p<0.05$) for each brand. Huda et al. (2000) [34] stated hardness ranged between 0.2 - 1.6 kg between fish balls with different

brands. The varying proximate compositions of surimi like products are mainly due to the different formulations used for fish balls production. Fish meat (or surimi) and salt are indispensable for making fish balls. Other additives include starch (wheat flour, potato starch, modified corn starch, Corn starch, sago starch or tapioca starch), baking powder, mono-sodium glutamate, vegetable oil, sugar, sodium pyrophosphate, or permitted flavor enhances might be used on surimi like products as additives. Besides, storage time also increased the breaking force of the cooked control sample [35]. Transglutaminase treatment appears to improve and maintain the texture-quality of fish products [36].

Carp paste gel can be made into fish ball and imitation crab stick [35, 37]. The range of the breaking force of the fish ball and the imitation crab stick in the literature is between 200 to 800 g and 160 to 210 g, respectively. The deformation range is 10-14 and 6.5 to 8 mm, respectively. Comparing these results, we believe that the carp, in combination with different fish and starch, can be used as a raw material for fish balls and imitation crab sticks. We purchased two fish ball products from adjacent Asian market to compare surimi-like products with carp meat gel by analysis breaking force and deformation. The breaking force (g) of product A (containing threadfin breams, modified potato starch, tapioca starch, salt, mono-sodium glutamate, sugar and garlic) and the product B (containing threadfin breams, potato starch, soybean oil, salt, dextrose, non-fat dry milk, mono-sodium glutamate, spices, powdered egg white, and sodium phosphate) were 472.65±49.29 and 1382.73.65±99.61, respectively. We believe that carp meat gel can also be adjusted to be similar to commercial products through a variety of additives.

Fish is predominantly perceived as a healthy food that reduces risk for coronary heart disease, which corroborates scientific evidence [38]. Fish is filled with omega-3 polyunsaturated fatty acids (PUFAs) in addition to the proteins with high biological value, certain minerals and vitamins [39]. The American Heart Association recommends eating fish at regular ration as part of a healthy diet [40]. Nevertheless, the fishery resources on the earth are facing depletion, and how to effectively use them as health promotion is the issue we face [41]. In this report, meat gel produced by three kinds of carp in combination with starch was in line with market products. Our study promotes the use of carp as a healthy food.

Correlationship of protein compositions with rheological characteristics and texture

For investigating the potential relationships of carp protein profiles with rheological characteristics and texture, Pearson's correlation coefficient analyses were performed between protein compositions (myosin heavy chain, actin, tropomodulin, β-tropomyosin, myosin light chain-1 and myosin light chain-2) and the rheological properties of meat paste (G' [Pa] peak 1, G' [Pa] peak 2, G' [Pa] final and Loss Factor (tan δ)) and the texture of the meat gel made by three kinds of fish (breaking force (g), deformation (mm) and gel strength (g X mm)). The results from the correlation study (Table 4) showed that both loss factor and gel strength have a significant correlation ($r = 0.99$, $P < 0.05$) between the content of myosin light chain-1. Some of the coefficients were high, but the level of significance was low, which might be due to the small number of sample size (fish species). To our best knowledge, no report was found about the effect of protein compositions on texture of gel. Because the species of fish determines the protein compositions [42], understanding the effect of protein compositions on the texture of gel will be helpful for adjusting the taste of food by using different combination of fishes. Because of depleting fishery resources, maximizing the use of fishery products will contribute to the sustainable development of fisheries [41]. On the utilization of fish catch for the production of surimi and fish gel products, the growth of the surimi industry could be enhanced with a breakthrough [43]. There is a need to increase the fish sample size by sampling in different seasons in the future to confirm these relationships.

Zhang et al., (2013) [44]suggested the first peak of G' profile reflected the cross-linking effect between proteins molecules through hydrogen bonds, and the drop of G' value after the first peak was due to endogenous proteolytic

enzymes, disaggregation of actin-myosin network structure, denaturation of myosin tail and the heat-rupture of hydrogen bonds. Kong et al. (2016) further summarized the formation of fish meat gel during heating into three-phase processes. The temperatures ranging from 0 to 40 °C is corresponding to the denaturation of myosin heavy chain (MHC), its unfolding and polymerization without the formation of non-disulfide covalent crosslinks. The temperatures ranging from 40-70 °C is related to the subunits dissociation and further unfolds of myosin light chain. Because 70 °C is near the end point of heat treatment, myosin light chain might plays an important role in gel formation, we would like to have more studys on the role of myosin light chain on rheological characteristics and texture in the future.

CONCLUSION

The SDS-PAGE analysis, texture/puncture, and dynamic rheological assay revealed the differences between the paste and gel made from the meat of silver carp, grass carp, and bigmouth buffalo and explained the highest gel strength of silver carp gel. The results of the texture analysis is in line with the temperature sweep test data of the rheological analysis, confirming the higher plasticity of silver carp gel than that of either bigmouth buffalo or grass carp. The effects of the adding starch or modified starch to breaking force and deformation were different between the gels made from grass carp, silver carp and bigmouth buffalo, implying that different food products may be made from the carp fish meat proteins and deserve continuous investigation.

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Tables

Table 1. Protein profile of grass carp, silver carp, and bigmouth buffalo meat.

Protein	MW (kDa)	bigmouth buffalo meat	bigmouth buffalo paste	silver carp meat	silver carp paste	grass carp meat	grass carp paste
Myosin heavy chain	200.5	9.69±0.32 c	9.41±0.21 c	14.98±0.07 b	10.49±0.31 c	16.54±0.69 a	15.94±1.02 ab
Actin	41.2	13.45±0.90 ab	11.94±0.81 b	11.99±0.76 b	14.26±0.76 a	1.32±0.05 c	1.82±0.09 c
Tropomodulin	38.8	4.55±0.22 c	5.22±0.42 c	4.34±0.02 c	4.99±0.22 c	13.84±.063 b	16.52±0.92 a
β-tropomyosin	36.5	5.83±0.43 a	5.77±0.85 a	6.35±0.34 a	5.87±0.28 a	5.83±0.25 a	6.37±0.33 a
α-tropomyosin	33.5	0.90±0.06 b	1.79±0.14 a				
Myosin light chain-1	18.5	3.81±0.09 ab	2.37±0.02 c	4.03±0.10 a	3.61±0.16 b	1.86±0.12 d	2.28±0.14 c
Myosin light chain-2	16.8	3.84±0.17 cd	2.43±0.05 e	4.21±0.04 c	3.26±0.15 d	6.37±0.33 b	3.61±0.56 a

Different letters in each raw indicated significant difference between starch at $p<0.05$.

Table 2. Storage modulus (G') peaks and loss factor (tan δ) of grass carp, silver carp, and bigmouth buffalo measured by rheometer.

	temperature range (°C)		G' [Pa]			Loss Factor (tan δ)
	peak 1	peak 2	peak 1	peak 2	final	final
grass carp	46.0±0.5 a	78.4±0.7 a	6613±939 a	12748±2671 b	10279±33 b	0.074±0.008 a
silver carp	34.7±1.2 b	77.0±1.7 a	6785±735 a	17764±2516 a	17784±2778 a	0.082±0.003 a
bigmouth buffalo	48.0±1.0 a	77.0±1.0 a	8680±729 b	13825±957 ab	13283±928 b	0.073±0.004 a

Table 3. Gelatinization parameters of native starch (tapioca), starch acetate (tapioca), Distarch phosphate (tapioca), Novation 1900, Firmtex™ and N-Hance 59 measured by RVA.

	Visc(cP)				
	Sample Gelatinization temperature (°C)	Gelatinization maximum	Breakdown viscosity	Setback viscosity	Final viscosity
native starch (tapioca)	64.5±0.3b	705.3±25.4a	336.7±9.7b	259.7±19.7a	628.3±32.8a
starch acetate (tapioca)	60.9±0.1c	908.7±19.6a	236.7±18.2ab	505.0±26.4b	1177.0±55.9b
Distarch phosphate (tapioca)	66.6±0.2a			718.0±63.2c	1515.7±138.9c
Novation 1900	60.1±0.1d	1815.3±125.1c	222.0±40.0a	529.7±55.9b	2123.0±96.3e
Firmtex™	57.2±0.1f	1465.3±93.8b	258.3v±55.9ab	1014.3±26.5d	2221.3±82.7e
N-Hance 59	59.0±0.2e	2274.7±27.8d	901.0±29.6c	431.7±57.2b	1805.3±41.8d

Pasting profiles were evaluated in triplicate for the average values of pasting parameters.

Different letters in each column indicated significant difference between starch at $p<0.05$.

Table 4. Pearson correlation coefficient analysis between protein compositions and Storage modulus (G') peaks and loss factor (tan δ) of grass carp, silver carp, and bigmouth buffalo measured by rheometer, and breaking force (g), deformation (mm) and gel strength (g X mm).

Protein	G' [Pa] peak 1 N=3	G' [Pa] peak 2 N=3	G' [Pa] final N=3	Loss Factor (tan δ) N=3	breaking force (g) N=3	deformation (mm) N=3	gel strength (g X mm) N=3
Myosin heavy chain	-0.79	-0.69	-0.78	-0.36	-0.64	0.25	-0.38
Actin	0.54	0.89	0.93	0.64	0.86	0.08	0.66
Tropomodulin	-0.67	-0.81	-0.87	-0.51	-0.76	0.08	-0.53
β-tropomyosin	-0.79	-0.69	-0.77	-0.36	-0.64	0.25	-0.38
Myosin light chain-1	-0.23	0.94	0.90	0.99*	0.96	0.78	0.99*
Myosin light chain-2	-0.79	-0.68	-0.76	-0.34	-0.63	0.26	-0.36

** Significant at 0.05 level.

Figures

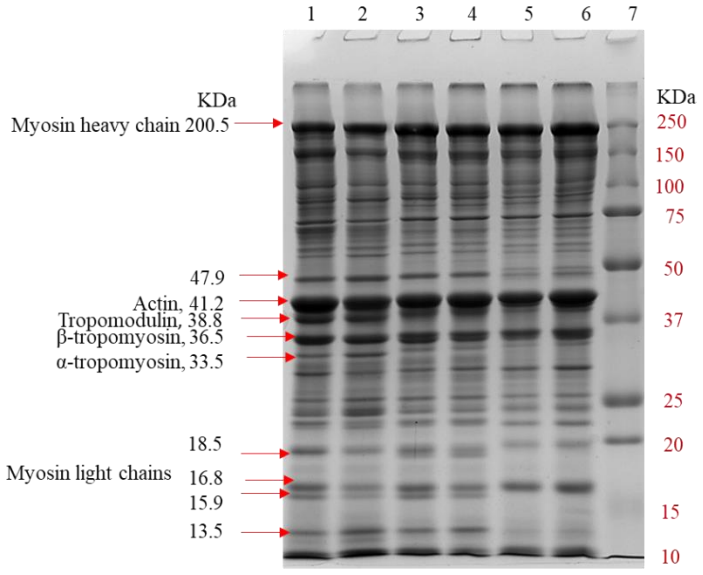


Figure 1. SDS-PAGE analysis of the meat and the corresponding paste.

Lane 1: bigmouth buffalo meat; Lane 2: bigmouth buffalo paste; Lane 3: silver carp meat; Lane 4: silver carp paste; Lane 5: grass carp meat; Lane 6: grass carp paste; Lane 7: molecular mass marker.

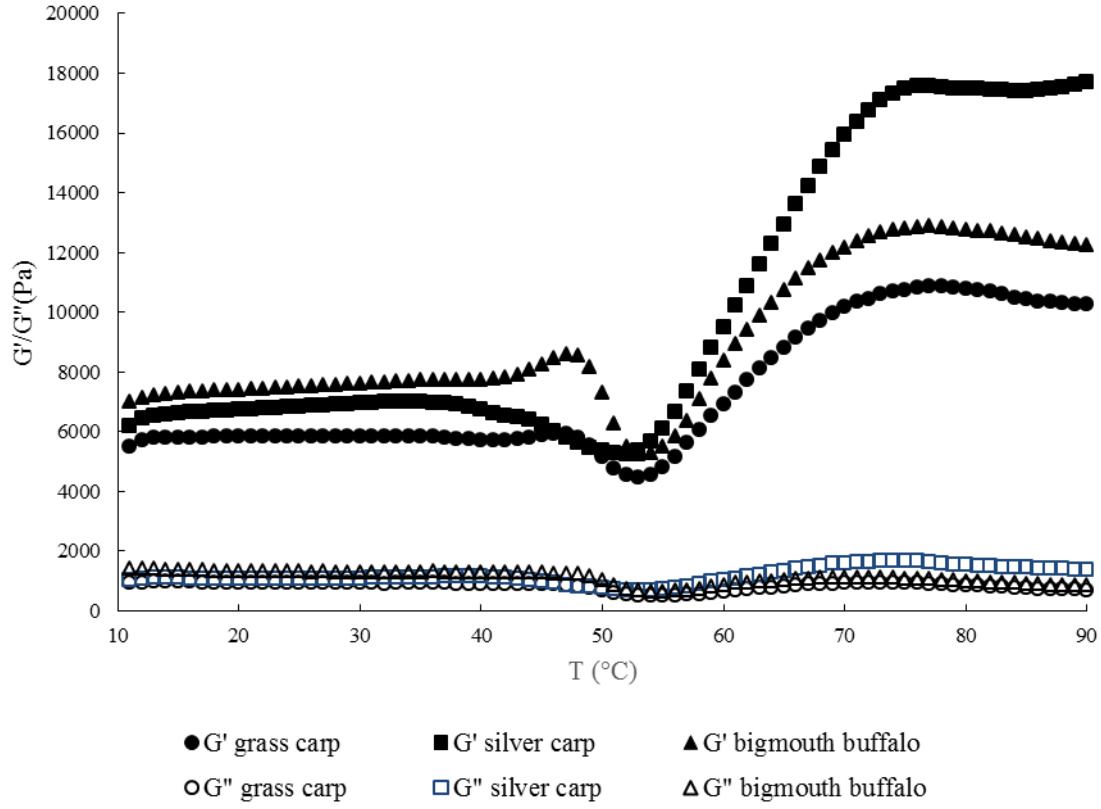


Figure 2. Storage modulus (G') and loss modulus (G") of paste made of three kinds of fish (grass carp, silver carp and bigmouth buffalo).

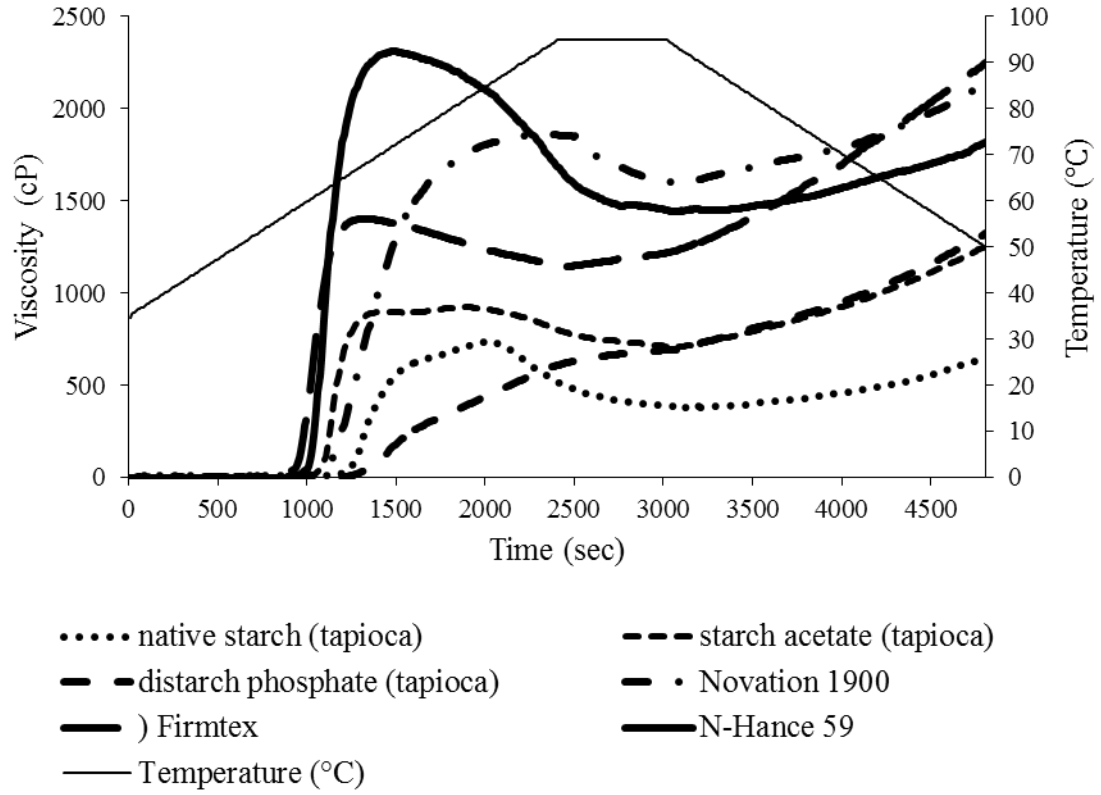


Figure 3. Gelatinization properties of (a) native starch (tapioca), (b) starch acetate (tapioca), (c) distarch phosphate (tapioca), (d) Novation 1900, (e) Firmtex™ and (f) N-Hance 59 measured by RVA.

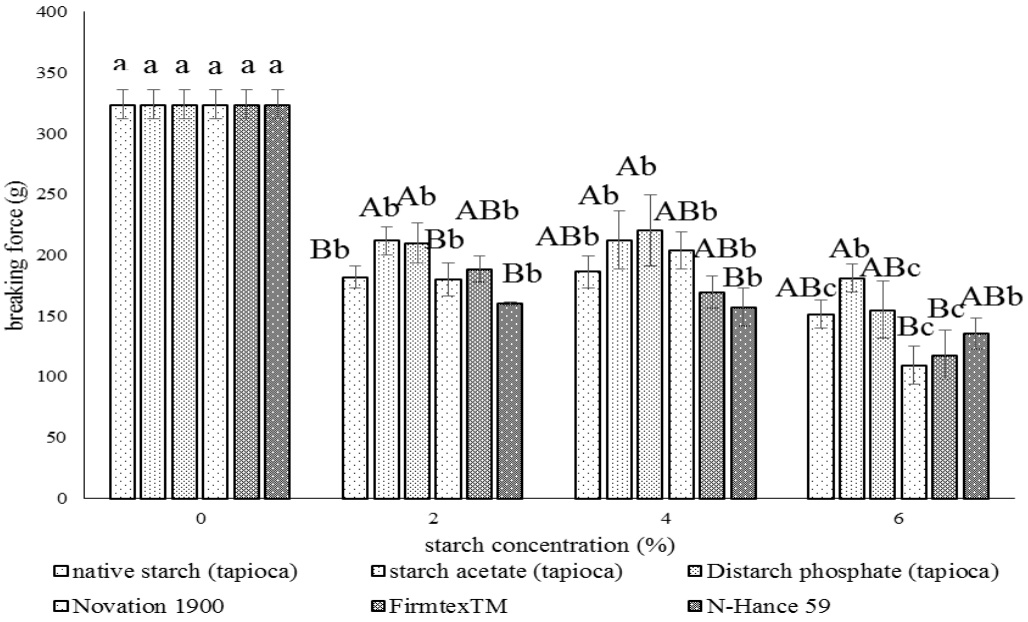


Figure 4. Breaking force of cooked gel made of the combination of three kinds of meat ((a) grass carp, (b) silver carp, and (c) bigmouth buffalo) and six kinds of starches (native starch (tapioca), starch acetate (tapioca), distarch phosphate (tapioca), Novation 1900, Firmtex^{TM} and N-Hance 59).

Different lower-case letters indicated differences ($p < 0.05$) among levels of one kind of starch addition ($p < 0.05$). Different capital letters indicated differences ($p < 0.05$) among six kind of starch addition of same starch concentration.

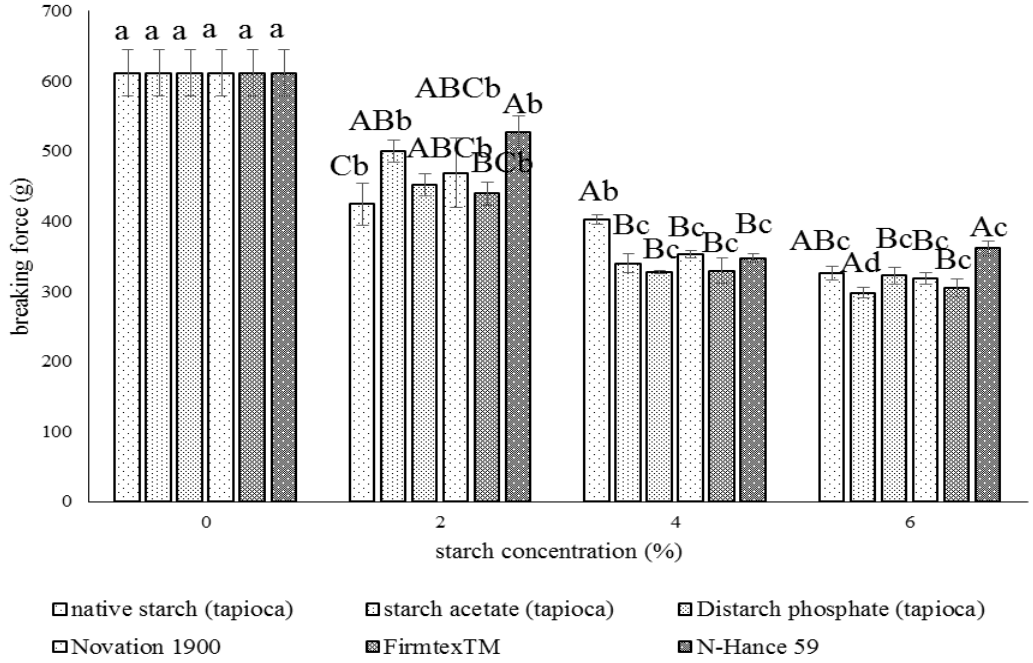
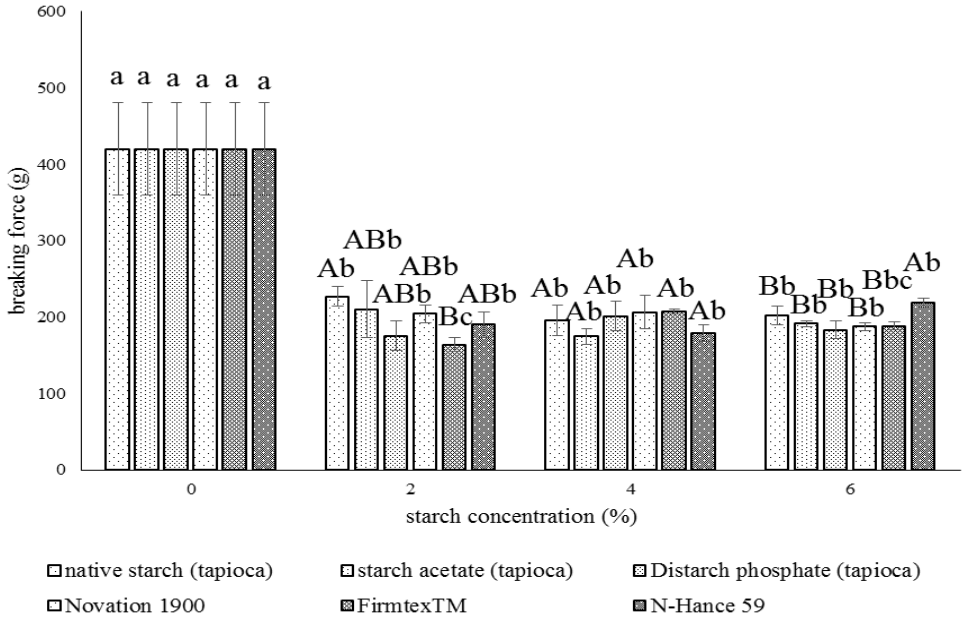


Figure 4. (Continued)

Different lower-case letters indicated differences ($p < 0.05$) among levels of one kind of starch addition ($p < 0.05$). Different capital letters indicated differences ($p < 0.05$) among six kind of starch addition of same starch concentration.



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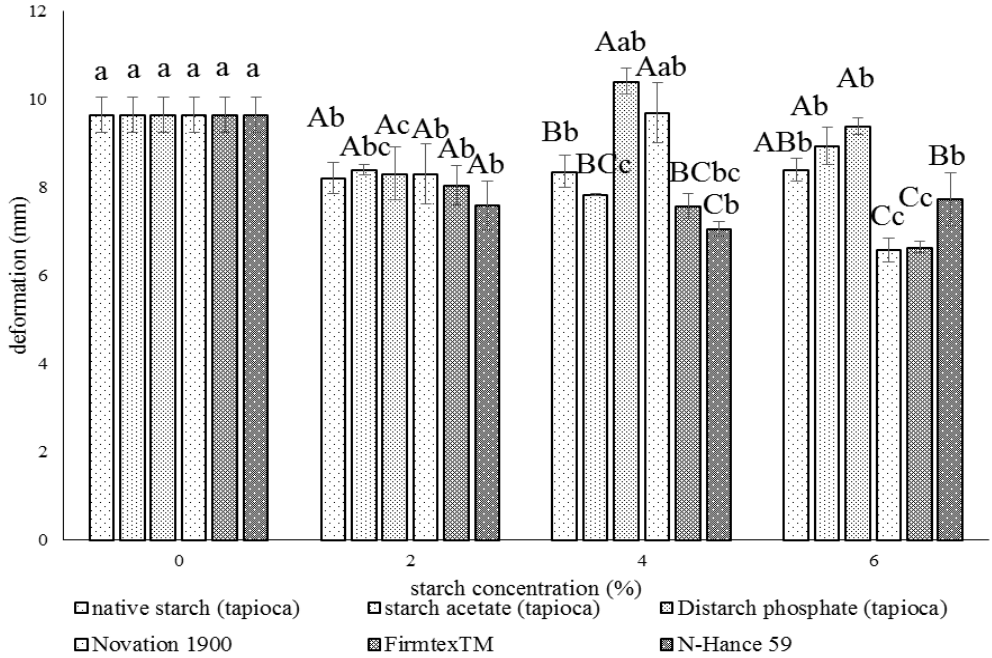

Figure 5. Deformation of cooked gel made of the combination of three kinds of meat ((a) grass carp, (b) silver carp, and (c) bigmouth buffalo) and six kinds of starches (native starch (tapioca), starch acetate (tapioca), distarch phosphate (tapioca), Novation 1900, Firmtex^{TM} and N-Hance 59).

Different lower-case letters indicated differences ($p < 0.05$) among levels of one kind of starch addition ($p < 0.05$). Different capital letters indicated differences ($p < 0.05$) among six kind of starch addition of same starch concentration.

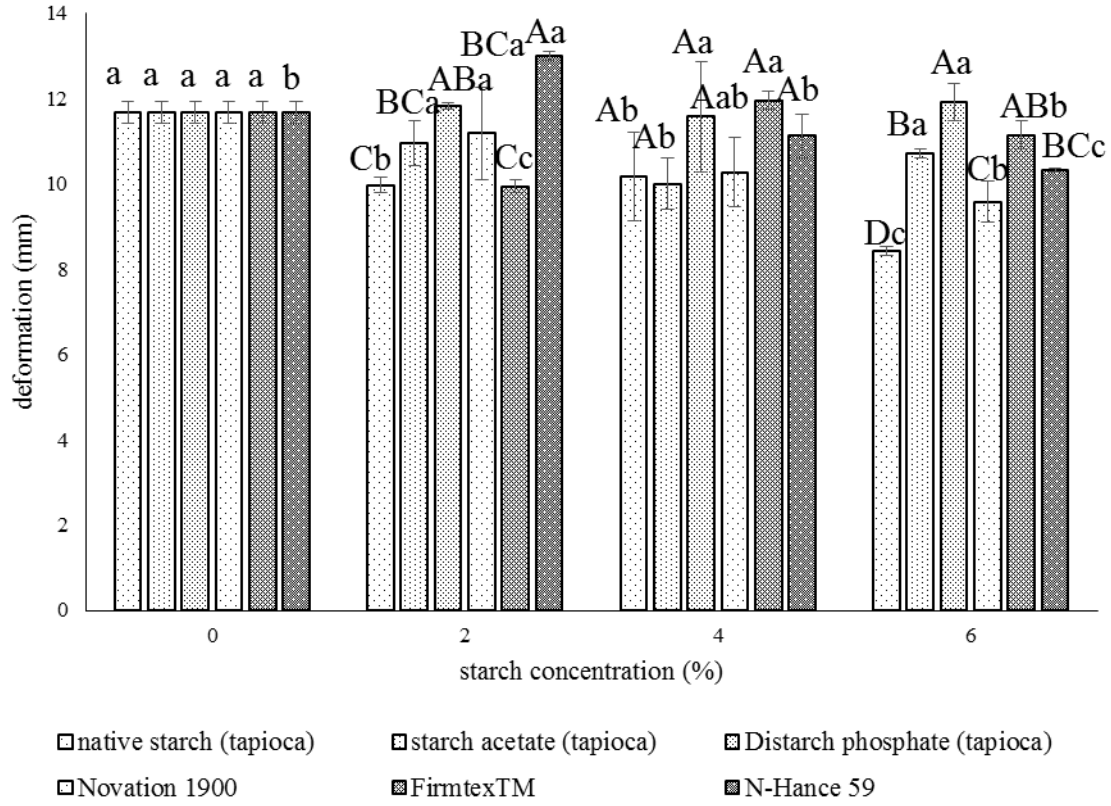

Figure 5. (Continued)

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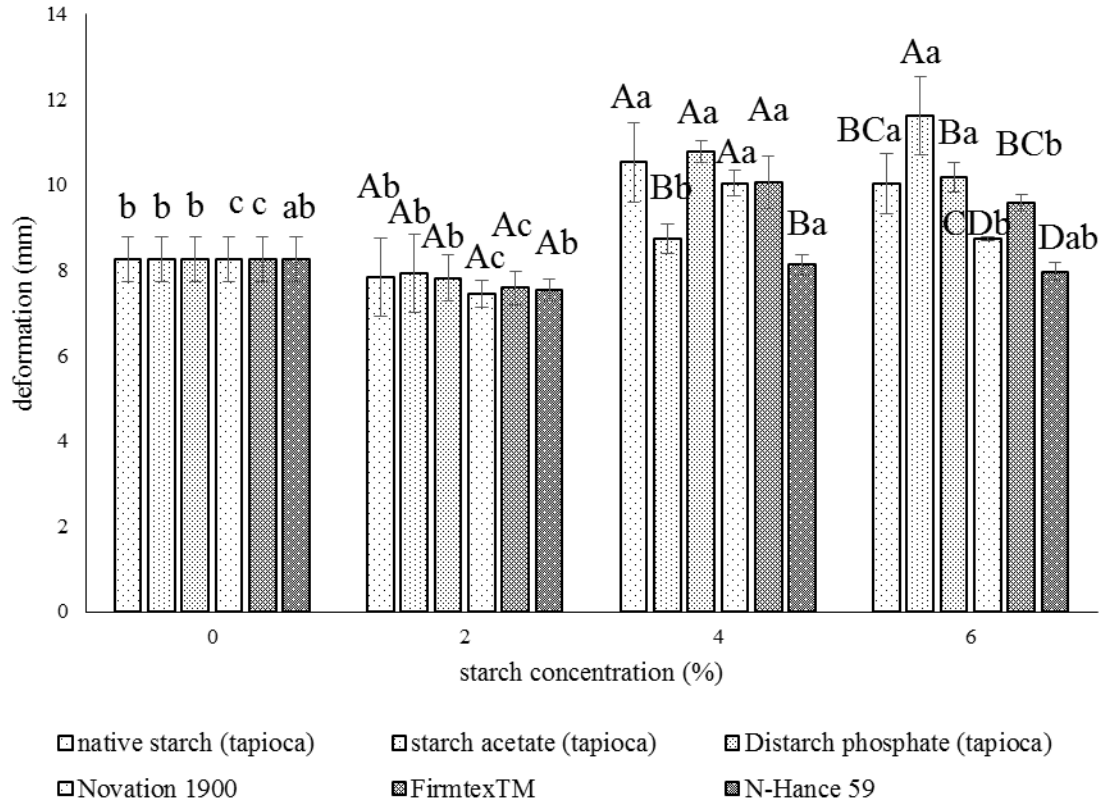


Figure 5. (Continued)

Different lower-case letters indicated differences ($p < 0.05$) among levels of one kind of starch addition ($p < 0.05$). Different capital letters indicated differences ($p < 0.05$) among six kind of starch addition of same starch concentration.

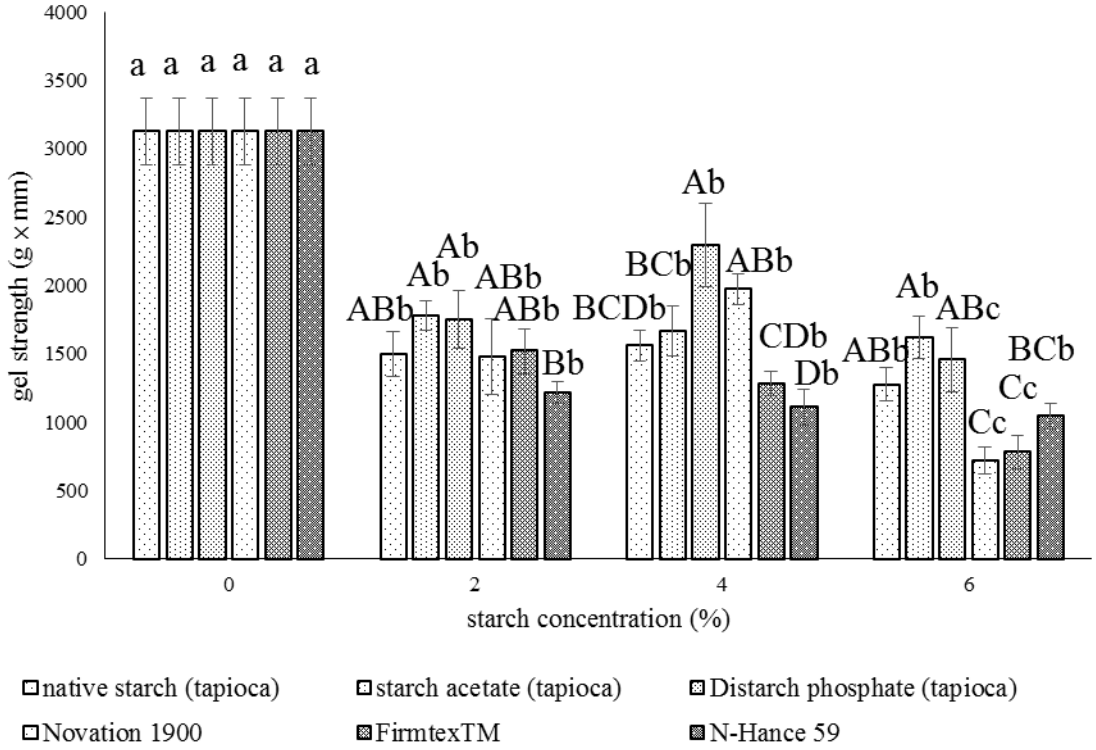


Figure 6. Gel strength (c) of cooked gel made of the combination of three kinds of meat ((a) grass carp, (b) silver carp, and (c) bigmouth buffalo) and six kinds of starches (native starch (tapioca), starch acetate (tapioca), distarch phosphate (tapioca), Novation 1900, Firmtex™ and N-Hance 59).

Different lower-case letters indicated differences ($p < 0.05$) among levels of one kind of starch addition ($p < 0.05$). Different capital letters indicated differences ($p < 0.05$) among six kind of starch addition of same starch concentration.

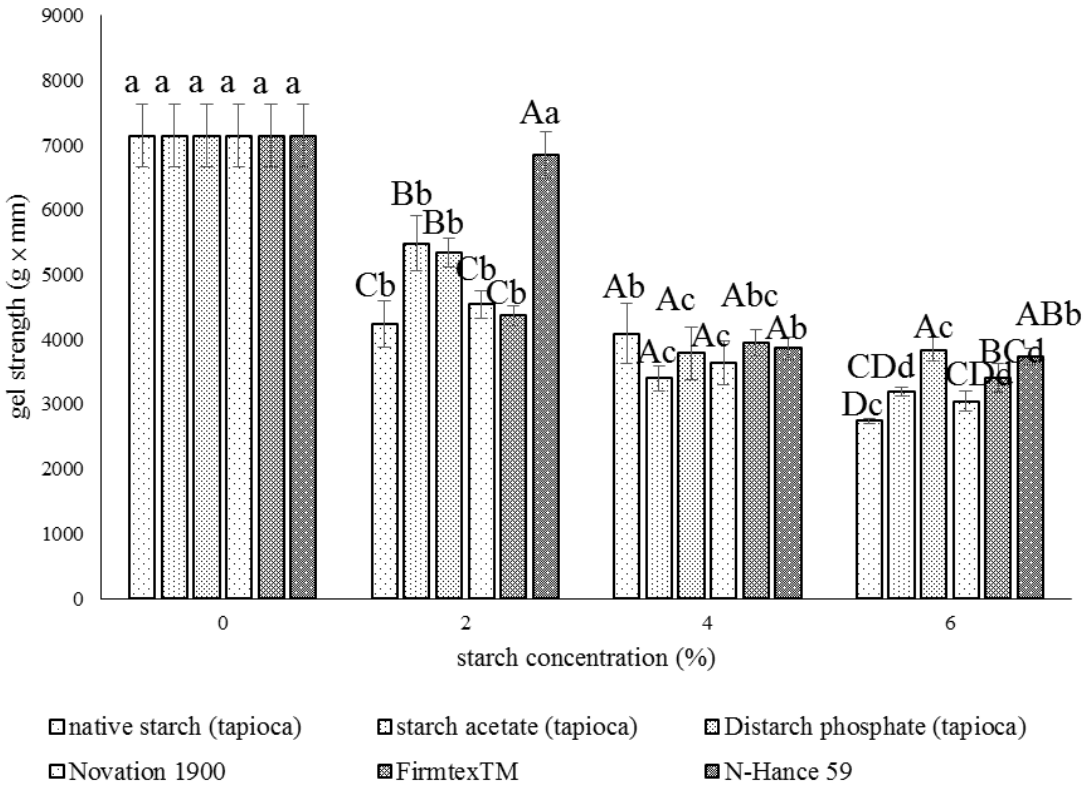
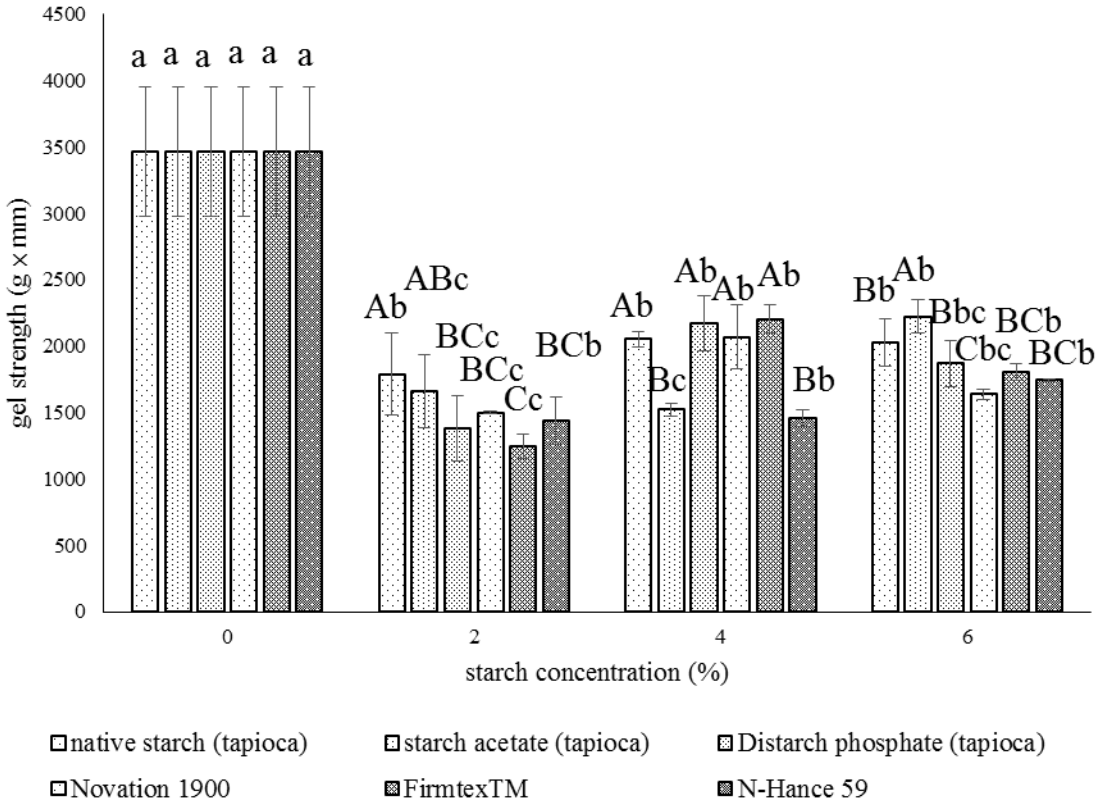


Figure 6. (Continued)

Different lower-case letters indicated differences ($p < 0.05$) among levels of one kind of starch addition ($p < 0.05$). Different capital letters indicated differences ($p < 0.05$) among six kind of starch addition of same starch concentration.



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Different lower-case letters indicated differences ($p < 0.05$) among levels of one kind of starch addition ($p < 0.05$). Different capital letters indicated differences ($p < 0.05$) among six kind of starch addition of same starch concentration.

PEER REVIEW

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