

Performances of Cold-Set Binders, Food Hydrocolloids, and Commercial Meat Binder on the Physical and Chemical Characteristics of Tilapia Fish Balls

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Abstract

The overall objective of this study is to determine the effect of hydrocolloid additives in reformed fish products and to compare the performances by testing chemical and physical properties of the restructured samples. There are nine treatments in this study including control samples. The eight types of meat binders include cornstarch, commercial meat-binder, carrageen an, methylcellulose, Activa® RM, plasma powder FG+, plasma powder FG and sodium alginate. The results showed that Activa® RM and FG+ and FG could provide satisfactory binding properties in fish balls. There was no significant difference among all cooked samples moisture ($p < 0.05$). Raw treatments had slightly higher moisture than cooked treatments. Samples treated with Activa® RM had the highest WHC for cooked samples, while methylcellulose had the lowest WHC and cooking yield. All other binder treatments samples had higher cooking yield than that of the control. Samples treated with sodium alginate had the lowest pH values for both cooked and raw samples. There were no significant differences detected for water activity for both raw and cooked samples. Samples treated with Activa® RM, FG+ and FG treated samples had the best puncture, texture, hardness, springiness. In summary, Activa® RM, FG+ and FG treatments performed well for all parameters, and sodium alginate, methylcellulose, and meat binder treatment did not show advantages when compared with the control.

Introduction

Traditional restructured technology involves adding salt and phosphate in comminuted meat with the aid of mechanical force, which can extract meat myofibrillar proteins that can hold large amount of water. Phosphate has a similar function to the most effective water-binding agents in processed meat, such as binders and stabilizers. However, addition salt and phosphate in to meat products could cause health hypertension and high blood pressure health issues. Several cold-set techniques have been developed in order to meet the consumer demand for various restructured meats. The techniques include using polysaccharides [1-3] pearl E and pearl F, blood plasma fractions [4], and microbial transglutaminase enzyme preparations [5]. The functions of binders and stabilizers in meat products are to form various cuts of meat into affordable and acceptable innovative muscle food productions. Based on the United States Department of Agriculture (USDA) definition, binders are used to thicken or to improve texture, consistency and sensory scores of meat. Stabilizers are food additives that contribute an optimal finished meat system and provide value-added qualities to meat system applications. It also can improve finished product stability, provide consistent texture and viscosity, and make food products firmer. Currently, there are many products available in the supermarket, which are made by binding comminuted meat products along with spices, seasonings, and stabilizer in to one cohesive product. Various binders are available to meat processors. Some binders are proteins, such as soy protein isolate, pea protein, wheat protein, milk casein ate, gelatin, and egg protein. Some binders are derived from enzymes, such as transglutaminase and beef fibrin. Some binders contain little or no protein, such as fibers, flours, and starches. Hydrocolloids are another type binder that is widely used into meat products, which have been employed by the meat industry to function as gelling agents, stabilizers, or thickening agents. These hydrocolloids can be dissolved or dispersed into aqueous solution, which can increase viscosity or gel formation [6]. Most of these hydrocolloids come from either plant (polysaccharides) or animal sources (protein). Many reports have addressed the applications of polysaccharides in the meat industry as meat binders. Polysaccharides are comprised of three subgroups, including non-ionic, anionic and cationic. Nonionic polysaccharides include hydroxyethyl cellulose and dextrin; anionic polysaccharides include xanthenes gum, carrageen an, guar gum, alginate and Carboxy Methyl Cellulose (CMC); cationic polysaccharides include arginine hydrochloride and chitosan [6,7]. The first cold-set binding system is a sodium alginate system. The three most common ingredients in alginate binding or gelling systems are alginate salt, a calcium source, and acidulant or sequestrate, such as encapsulated. Lactic acid or Glucono Delta Lactones (GDL) [8]. The calcium source in an alginate binding system should be added at the last stage of the process to avoid pre-

gelation during processing. The interaction between calcium and monomer units would develop the polysaccharide-meat protein into a composite gel, which is a thermo-irreversible gel [5]. The function of acidulate or sequestrant in this system is to modify the reaction rate and to control the hydration rate and gel setting time or to accelerate the release of calcium [9,10]. The setting temperature and time for this system is usually 0-4°C overnight. Once the gel system is formed, the gel interacts with myofibrillar protein. These are mainly electrostatic interactions between the anionic group on alginate and positively charged group on protein. No report has been made about whether the functionality of myofibrillar protein could be improved by conjugation with alginate. Therefore, the grade of alginate, calcium sources, and sequestering agents and their ratios must be used appropriately in order to develop the overall desired texture for different food products. The second cold-set binding system is blood plasma fraction- Fibrimex blood plasma. This type of binding agent relies on the physiological clot forming action of the plasma proteins fibrinogen and thrombin. The available commercial binder is Fibrimex[®], which is produced by the Dutch Company Sonac BV. Its binding action is based on the transformation of fibrinogen into fibrin by the action of thrombin. The fibrin interacts with collagen to bind the meat pieces and develops restructured meat products [11]. When Fibrimex[®] is mixed with water, it forms the binder solution. This solution can then be applied to the surface of meat pieces; the thrombin enzyme converts fibrinogen into fibrin. Fibrin molecules develop cross-linked gel by the function of transglutaminase enzyme in the fibrinogen. Transglutaminase enzyme can connect and develop cross-linking between fibrin and collagen in meat. Therefore, this cold-set system has a big advantage if used in the muscle meat containing higher collagen, such as beef forequarter [4]. The third cold-set binding system is pearl meat cold-set binder. Pearl F is white powder with carbohydrate, protein, and bone ash mix. It is used to bind seam-boned muscle and large meat pieces. Pearl E is a protein active meat binder that can be used in binding small size pieces of raw meat. Pearl E is developed by Earle Products Old, Australia. Pearl F is developed by Chiba Flour Milling Co. Ltd, Japan [12]. The fourth cold-set binding system is microbial transglutaminase enzyme (protein-glutamine γ -glut amyl transferase). Transglutaminase is an enzyme that catalyzes the covalent cross-link gel formation with different types of proteins. MTGase catalyzes covalent bonds between the ϵ -amino group of lysyl residues and the γ -carboxamide group of glutamyl residues of adjacent proteins [13]. The role of MTGase in catalyzing the cross-linking of myosin heavy chains has been investigated, but no clear reaction mechanism has been summarized. It has a wide active pH range from 4.0 to 9.0 and the active temperature is 0-70°C with the optimal activity at 55°C. When applying MTGase into cold water fish muscle, the optimal temperature is in the range of 25-30°C [13]. Fish muscle contains an endogenous transglutaminase (TGase) of its own. Sufficient calcium ions in fish muscle promote the endogenous TGase to be activated and can develop gel at low temperature. Activa is a product that contains microbial transglutaminase (MTGase) and sodium caseinate [14]. The function of sodium caseinate is as a substrate to increase cross-linking in the meat product. The MTGase catalyzes the acyls to form covalent cross-linking in protein and peptides, most of time this occurs between glutamine and lysine residues. This helps the protein aggregation and gelation to occur. Transglutaminase has been used in pork, beef, and chicken. Some reports point out that MTGase applications are

influenced by meat species [14]. MTGase interacts with muscle protein to produce thermo-stable gels at temperatures below 30°C. Many researchers have investigated the cold set binder in meat products. Ensor and others [15] concluded that the use of alginate and calcium system binder could improve quality of restructured beef texture and reduce formulation costs. Moreno and others [13] addressed that alginate and MTGase were very suitable as binding ingredients for fish, Alginate has been extensively studied in restructured meat products, but there is limited research on fish products. MTGase has been widely used in pork, beef, chicken, and several studies were reported on the application in lamb, fish, and seafood products. Moreno and others [16] used sodium alginate and microbial transglutaminase to homogenize and bind small fish muscle pieces into restructured fish products for frozen storage. Lennon and others [4] investigated the cold set binding agents including Texor, Fibrimex, alginate, and Activa EB to reform steaks. Serrano and others [17] used transglutaminase and sodium caseinate as binding agent to bind different amounts of walnuts with meat to form restructured steaks. Se Avila and others performed research on cold set binder plasma on dry ham. The gelling capacity of fish proteins in comminuted fish products is one of their most important functional properties. The myofibrillar protein of fish can form a firm gel, and the main gel-forming protein in fish is myosin. Myosin plays an important function for the development of the elasticity properties of gels. The gelling properties of protein in surimi products have been commercially utilized to produce imitation shellfish meat. However, there are very few studies on comparing the performances of fish products in the presence of different types of binders. The aim of this study was to investigate the behavior of different restructured fish products with different hydrocolloids and cold set binders during chilled storage. These parameters compared include cooked meat color and raw meat color at different storage period, water holding capacity, water activity, raw and cooked meat pH puncture test and TPA test.

Materials and Methods

Prepare samples

Sway filets (Beaver Street Fisheries Inc., FL, and USA) were purchased from a local grocery store. The vacuum-packed filets were sold as frozen products. The fish were farm raised, and produced in Vietnam. There were a total of nine treatments. There was not any binder added to the control treatment; treatment 2: minced fish with 5.0% corn starch; treatment 3: minced fish with 2.0% Meatbinder (GRINDSTED[®] meat binder 2-555, Danisco, KS); treatment 4: minced fish with 1.5% carrageenan (GRINDSTED[®] Carrageenan, Denison, KS); treatment 5: minced fish with 1.5% methylcellulose (Dow, Michigan); treatment 6: minced fish with 1.0% Activa[®] RM; treatment 7: minced fish with 0.7% plasma powder FG+ (Sonac, USA); treatment 8: minced fish with 0.7% plasma powder FG (Sonac, USA); and treatment 9: minced fish with 0.4% (w/w) encapsulated lactic acid (IFP Incorporated, MN), 0.3% (w/w) calcium carbonate (Micro white Codex 50, IMERYS, GA) and 0.8% (w/w) sodium alginate (FD155, Danisco, KS). Fish samples were thawed under refrigeration temperature at 4°C overnight. The semi-thawed filets were cut into small pieces, and transferred to a food processor (Cuisinart[®] Prep 9[®] 9-Cup Food Processor, Model DLC-2009CHBM), and blended for 2 minutes. Meat pieces around the food process walls

were scraped off with a rubber spatula to ensure even blending. Meat binders were manually sprinkled into the paste, then the binder was covered with paste before further mixing in the food processor, and blending was continued for another 3 minutes. Except for the sodium alginate system, in which limited grinding processes were used after adding the encapsulated lactic acid. The fish balls were scooped out with a tablespoon and shaped between the palms of the hands. To avoid sticking, both hands wet with cold water. There were about 12-15 fish balls made in each tray. The trays were covered with stretch wrap film to avoid moisture loss. Trays were placed into a refrigerator overnight to set the gel. Between each treatment, the food processor was cleaned thoroughly, and dried with paper towels prior to next treatment preparation. The pH, aw, and moisture of raw fish balls were measured on the first day. After overnight storage, the fish balls were cooked in a 70°C water bath. The pH, aw, WHC, and moisture of both cooked and raw fish balls were measured on the second day. The cooked both puncture and TPA tests were evaluated after cooling the fish balls to room temperature. The raw puncture and TPA tests were evaluated on the same day as cooked texture measurements.

pH analysis

10 grams raw fish samples were placed into 90 ml water in stomacher bag and homogenized with the stomacher. The pH of the slurry was measured by using a Fisher Accumet Model 230A pH/ion meter (Fisher Scientific Inc., Salt Lake City, UT). The cooked meat samples were blended in a blender for 15 seconds. The pH measurements of both raw and cooked samples were determined. The pH meter was calibrated using pH buffers 4.00 (SB 101-500, Fisher Scientific, Fair Lawn, NJ) and 7.00 (SB 107-500, Fisher Scientific, Fair Lawn, NJ). The probe was placed into the sample homogenate and allowed to equilibrate for one minute before the pH reading was recorded. All pH readings were performed in triplicate.

Water activity analysis

Water activity (aw) of homogenized raw and cooked fish samples were measured with an Aqua Lab (Series 3, Decagon Devices, Inc.). The Aqua Lab was warmed up 15 minutes before use. All aw cooked and raw readings were performed in triplicate.

Water holding capacity analysis

The water-holding capacity method was based on previous studies with slight modification. The cooked fish balls were minced with a food processor. Ten grams of minced cooked samples were placed into 40 ml tubes containing 20 ml of 0.6 M sodium chloride solution, and the tube was vortexed (VotexGeniz 2 TM Cat. No.12-812 Model G 250, Fisher Scientific, McGraw, IL) for 1 minute to ensure even distribution. The tubes were placed into a 4°C refrigerator for 15 minutes prior to centrifugation. The centrifuge (Sorvall RC-5B, Beverly, MA) was turned on 30 minutes prior to use. The tubes were centrifuged at 7000 rpm at 4°C for 15 minutes. After centrifugation, the liquid lost during centrifugation was collected. The sample was allowed to stand for 1 minute so the liquid could drain. Only the liquid was decanted and solid meat particles were kept in the tube. The WHC of cooked sample was calculated as the ratio of the water remaining after centrifugation to the initial content of meat sample, using the following formula. The WHC (%) was determined by using the following equation:

$$WHC \% = 100 \times \left(\frac{W1 - W2}{W3} \right)$$

Where W1 represents solution added into the sample, g

W2 represents solution removed after, g

W3 represents the meat samples mass, g

Cooking yield analysis

Ten fish balls of each treatment were placed in clear reclosable zipper bags for cooking. During cooking, the bags were zipped to avoid moisture loss. The samples were heated for about 90 minutes in a water bath (70°C ± 1°C) until internal temperature reached 70°C, which was monitored with copper-constantan thermocouples. The copper-constantan thermocouples were inserted into the center of the fish ball before cooking. The water bath was preheated approximately 30 minutes until the water bath temperature reached 70°C. After reach the desired internal temperature was reached, the cooked fish balls were placed on paper towels and cooled for 15 minutes at room temperature. The weight (Mettler Toledo Scales, Model: MS 3001S 103, Switzerland) of the fish balls were recorded before and after cooking, and cooking yield was calculated using the following equation:

W1 represents the weight before cooking, g

W2 represents the weight after cooking, g

TPA Analysis

The TPA was carried out using a texture profile analyzer (TA-XT Express, Stable Micro Systems Ltd.). After the fish balls were cooked, their weights were recorded for the cooking yield. The fish balls were cooled to room temperature before performing Texture Profile Analysis (TPA). A 5 kg load cell was applied at a crosshead speed of 1 mm/s. A double compression cycle test was performed with up to 50% compression of the original portion height with an aluminum cylinder probe 5-cm diameter. A gap of 5 seconds was allowed to elapse between the two compression cycles. Once tests were finished, the following parameters would be recorded, including hardness, springiness, adhesiveness, cohesiveness, chewiness, resilience, and gumminess.

Puncture test analysis

After the fish balls were cooled to room temperature, the puncture test was performed. A Stevens-LFRA Texture analyzer was used to penetrate the approximate 2 cm diameter fish ball. The diameter of the spherical probe was 0.635 cm. The penetrating speed was 2.00 mm/sec. The highest value throughout puncturing was recorded. Six samples per treatment were measured. Samples were removed from refrigerated conditions and centrally placed underneath the probe. Tests were performed at ambient environment.

Moisture analysis

Moisture content determination applied the method from AOAC with modifications. About 3.0 g of raw paste and cooked fish sample was placed in an aluminum tray and placed in a vacuum oven at 80°C for 24 hours under 23kPa pressure, and cooled to room temperature in desiccators prior to taking final weights. Three samples per

treatment were measured. Moisture (%) was calculated using the following equation:

W1 represents the weight before drying, g

W2 represents the weight after drying, g

Objective color measurement

The raw meat and cooked fish ball was minced with a food processor. The minced samples were transferred in to a Petri dish, and color measurements were taken from the bottom dish. The raw color was measured with Hunter Mini Scan XE plus colorimeter (Hunter Associated Laboratory, Inc, Reston, VA) in the same manner as cooked minced samples. The colorimeter was calibrated with a standard black tile and white tile as recommended by the manufacturer. The samples were measured at three locations for L*, a* and b* values. The instrumental color of L*, a* and b* color spectrum were recorded. Where L* represents the total light reflected on a scale ranging from 0 = black to 100 = white, while a* represents the amount of red (positive values) and green (negative values), and b* values represents the amount of yellow (positive values) and blue (negative values).

Statistical analysis

Data were analyzed using the GLM procedure of SAS (SAS institute, 2002) by generating an analysis of variance (ANOVA). The model includes the main effects of binder treatments and storage days for both raw and cooked fish balls. For other parameters, the main effects of treatments were analyzed. Comparisons among means were performed using SAS Turkey of SAS 9.4. P values less than 0.05 (P<0.05) were considered statistically significant. The MEANS procedure was employed to analyze data and treatment.

Results and Discussion

pH analysis

The data demonstrated that fish balls treated with 1.5% methylcellulose and 1.5% carrageenan had the highest pH values among these treatments (Table 1). Fish balls treated with sodium alginate and encapsulated lactic acid had significantly lower pH

Table 1: Cooked and raw pH and A_w measurements for raw and cooked fish balls treated with meat binders and stored at 4°C.

Treatment	Attributes			
	Cooked pH	Raw pH	Cooked a_w	Raw a_w
Control	9.26 ^{ab}	8.96 ^a	0.977 ^a	0.972 ^a
5.0% Cornstarch	9.20 ^{ab}	9.09 ^a	0.978 ^a	0.980 ^a
1.2% Meatbinder	9.03 ^{ab}	8.71 ^a	0.975 ^a	0.962 ^a
1.5% Carrageenan	9.49 ^a	9.35 ^a	0.975 ^a	0.975 ^a
1.5% Methylcellulose	9.45 ^a	9.27 ^a	0.975 ^a	0.969 ^a
1.0% Activa [®] RM	9.31 ^{ab}	9.17 ^a	0.980 ^a	0.970 ^a
0.7% FG+	9.36 ^{ab}	9.22 ^a	0.976 ^a	0.962 ^a
0.7% FG	9.26 ^{ab}	9.10 ^a	0.980 ^a	0.976 ^a
0.8% Sodium Alginate	8.74 ^b	8.56 ^a	0.975 ^a	0.967 ^a

^{a-b}Means in same column with different superscripts are significantly different (p<0.05).

values when compared with samples treated with carrageenan and methylcellulose. The analyzed results stated that there was no statistically significant difference between samples treated with sodium alginate and control; however, both raw and cooked samples treated with sodium alginate had the lowest pH values among all treatments. The raw fish balls were set overnight before cooking. The encapsulated lactic acid are small beads of acid surrounded by a lipid coating, and the acid was gently blended into the fish mixing in order to avoid disrupting the lipid coat. When calcium ions were introduced into the alginate system, the encapsulated lactic acid helped to slowly release calcium and control the gel development rate and setting time. After 24 hours setting, it formed the thermo-irreversible gel system. The raw pH values were measured after 24 hours setting, which can explain the lower pH values of sodium alginate samples compared with other treatments.

Water activity analysis

No significant differences (P>0.05) were observed among treatments regarding water activities (a_w) (Table 1). The water activity of the raw meat sample lies in the range of 0.962 to 0.980. After formulated with binders, due to their high moisture contents, the water activity of the product did not show significantly drop. Values still ranged from 0.975 to 0.98. Except for samples treated with carrageenan and cornstarch, other treated fish ball samples decreased in a_w after cooking. The accuracy of a_w equipment is +/- 0.003 and repeatability is +/- 0.002. In general, the raw of fresh meat and fish has the highest a_w at 0.99, the raw of cooked meat is around 0.91-0.98. A_w value is used toffee or available water in food systems. Dissolved substances could reduce values of water activity. In this study, all binders are used to binder water in product and bind meats together. The water activity was not affected by the binders.

Moisture analysis

The results for both raw and cooked moisture measurements are shown in (Table 2). There was no significant difference (P>0.05) in cooked moisture among most samples with different treatments. Except for the cornstarch treatment, the moistures for binder-treated samples were consistently lower (P>0.05) than control samples. Cooked samples treated with cornstarch had the lowest moisture among treatments and were significant lower (P<0.05) than control samples.

Cooked Water Holding Capacity Analysis

The WHC percentages among different treatments showed significantly different results (P<0.05) (Table 2). The samples treated with Activa[®] RM had much higher (P<0.05) WHC values than control samples. Samples treated with methylcellulose had significantly lower (P<0.05) WHC values when compared with the control treatment. The cooked samples treated with meat binder, FG+, FG and sodium alginate had similar WHC (P>0.05). The cooked samples treated with carrageenan and cornstarch showed similar WHC values (P>0.05) as the control samples. The samples treated with Activa[®] RM showed significantly higher WHC values (P<0.05) than the control treatment, and methylcellulose treatments had significantly lower WHC (P<0.05) when compared to the control. In this experiment, the centrifugation method was used to determine WHC. Preliminary experimental results (data not shown) demonstrated that the

Table 2: Cooked WHC and cooking yield, and moisture for both raw and cooked fish balls, treated with meat binders and stored at 4°C.

Treatment	Attributes			
	Cooked Moisture (%)	Raw Moisture (%)	Cooked WHC (%)	Cooking Yield (%)
Control	83.68 ^a	85.49 ^a	85.17 ^{bc}	88.44 ^{bc}
5.0% Cornstarch	80.52 ^a	80.96 ^b	82.30 ^{cd}	93.36 ^a
1.2% Meatbinder	84.79 ^a	84.29 ^{ab}	91.72 ^{ab}	93.14 ^a
1.5% Carrageenan	82.96 ^a	83.60 ^{ab}	87.00 ^{abc}	94.17 ^a
1.5% Methylcellulose	83.23 ^a	84.56 ^{ab}	76.41 ^d	84.91 ^c
1.0% Activa® RM	83.08 ^a	84.29 ^{ab}	95.03 ^a	90.42 ^{ab}
0.7% FG+	84.03 ^a	84.33 ^{ab}	91.87 ^{ab}	91.83 ^{ab}
0.7% FG	83.61 ^a	84.21 ^{ab}	92.82 ^{ab}	92.45 ^{ab}
0.8% Sodium Alginate	82.21 ^a	84.36 ^{ab}	92.34 ^{ab}	92.67 ^{ab}

^{a-d} Means in same row with different superscripts differ significantly ($P < 0.05$).

centrifugation method for raw fish paste is not appropriate due to development of fish gelation. The fish muscle is broken down with different degrees of integrity during chopping and blending processing, which extracts myofibrillar protein from fish muscle. The comminuted fish incorporated with sodium chloride solution in cold environmental developed properties similar to surimi. The fish proteins were separated from centrifugation processing, and retained its gel forming ability, so the centrifugation method cannot be used for raw minced fish WHC measurements.

Table 2 results showed the ability of binder to uptake added water in meat when combined with sodium chloride. This method attempts to mimic the practical industry processing when salt is added as an ingredient. It is concluded that samples treated with methylcellulose had the lowest WHC while samples treated with Activa® RM had the highest WHC ability compared with control samples.

Cooking yield analysis

In general, there was a positive relationship between cooking yield and water holding capacity. The samples treated with methylcellulose showed the lowest cooking yield percentage. There was no statistical difference between control and methylcellulose treatments ($P > 0.05$), however, the control treatment showed 3.5% cooking percentage higher than that of methylcellulose treatment. Samples treated with methylcellulose had lower cooking yield than control samples. From a meat processor's point of view, this is undesirable. All other treatments showed consistently higher than control treatment. The cornstarch, meat binder, and carrageenan treatments showed significantly higher cooked yield than the control treatment. Activa® RM, FG+, FG and sodium alginate showed similar cooking yields ($P > 0.05$) as the control. However, there was a 1-4% increase in cooking yield, which would still be important to meat processors. The higher cooking yield from binder treatments may be due to the water binding capacity of all meat binders except for methylcellulose.

Cooked and raw meat samples puncture test analysis

For puncture or penetration tests, the forces of deformation are used to test muscle binding or sample hardness. Small cylinders, balls, needles, and cones are used to penetrate sample to imitate mouth bite. Two parameters were displayed as the results of the test: peak load and final load in units of grams. The peak load is the highest

load value recorded during the test. The final load is the last load recorded prior to the probe returning to its original position. In this experiment, only peak loads were recorded. It provides the hardness of meat samples. In this study, a 6.35 mm spherical ball probe was used. A ball probe is typically used in samples that are not consistent or are not completely flat. Since it is difficult to make exactly the same size and same shape fish balls, the ball probe was selected in this study. The puncture value results for both raw and cooked fish balls are shown in (Table 3). Samples treated with Activa® RM showed significantly higher puncture values ($P < 0.05$) than the rest of treatments. Both raw and cooked samples treated with meat binder showed lower values than other binder treatments, including control samples. Some binder-treated samples had significantly higher value ($P < 0.05$) than samples treated with meat binder and some samples had slightly higher values ($P > 0.05$) than samples treated with meat binder. The control treatments showed the second lowest hardness among treatments. Cooked samples treated with Activa® RM and carrageenan showed significantly higher puncture values than the rest of samples ($P < 0.05$), Activa® RM showed slightly higher hardness than samples treated with carrageenan ($P > 0.05$) (Table 3). Control, methylcellulose, and sodium alginate treated samples had similar hardness. These three samples had higher hardness than samples treated with meat binder, but less hardness than samples treated with 0.7% FG+ and 0.7% FG.

Table 3: Puncture tests for both raw and cooked fish balls treated with different meat binders.

Puncture Test	Attributes	
	Raw Meat	Cooked Meat
Control	22.50 ^{bc}	168.6 ^{cd}
5.0% Cornstarch	26.59 ^b	217.9 ^{bc}
1.2% Meatbinder	18.50 ^c	134.7 ^d
1.5% Carrageenan	22.33 ^{bc}	337.5 ^a
1.5% Methylcellulose	29.17 ^b	162.6 ^{cd}
1.0% Activa® RM	37.67 ^a	340.2 ^a
0.7% FG+	28.67 ^b	263.0 ^b
0.7% FG	28.58 ^b	257.7 ^b
0.8% Sodium Alginate	25.08 ^{bc}	160.5 ^{cd}

^{a-d} Means in same column with different superscripts differ significantly ($p < 0.05$).

Table 4: Objective raw meat color L* values for fish balls treated with different meat binders and stored at 4°C for 5 days.

Attribute	Treatment	Storage Time			
		Day 0	Day 1	Day 3	Day 5
Raw L*	Control	75.87 ^{a,y}	71.68 ^{ab,y}	68.68 ^{b,y}	67.69 ^{b,z}
	5.0% Cornstarch	81.51 ^{a,w}	80.4 ^{ab,w}	79.57 ^{bc,w}	78.64 ^{c,w}
	1.2% Meatbinder	80.41 ^{a,wx}	76.88 ^{b,wx}	77.06 ^{b,w}	75.88 ^{b,wx}
	1.5% Carrageenan	75.47 ^{a,y}	71.36 ^{b,y}	70.32 ^{bc,y}	68.51 ^{c,z}
	1.5% Methylcellulose	80.74 ^{a,wx}	76.68 ^{a,wx}	75.99 ^{a,wx}	76.16 ^{a,wx}
	1.0% Activa® RM	77.34 ^{a,xy}	73.19 ^{b,xy}	71.74 ^{bc,y}	70.63 ^{c,yz}
	0.7% FG+	77.59 ^{a,wx,y}	73.59 ^{b,xy}	72.49 ^{bc,xy}	69.97 ^{c,yz}
	0.7% FG	76.09 ^{a,y}	71.96 ^{b,y}	71.65 ^{bc,y}	69.6 ^{c,xy}
	0.8% Sodium Alginate	80.83 ^{a,wx}	77.12 ^{ab,wx}	77.51 ^{ab,w}	73.16 ^{b,xy}

^{a-d} Means in same row with different superscripts differ significantly (P < 0.05).
^{w-z} Means in same column with different superscripts differ significantly (P < 0.05).

Cooked and raw objective meat color measurement

On the initial day, control treatments and samples treated with carrageenan were darker (P<0.05) for raw meat samples, when compared to samples treated with meat binder, methylcellulose, sodium alginate, and similar with samples treated with Activa® RM. Except the lightness of methylcellulose treatment did not significantly change (P>0.05), all other samples became darker as storage time increased (Table 4). Cornstarch and methylcellulose treatments showed the highest lightness from day 0 to day 5. This might be due to the fact that adding cornstarch into samples increases the lightness. That is also true for methylcellulose. Methylcellulose is a white powder, which can increase meat lightness when added into minced fish. In contrast, carrageenan powder is a yellowish color. It reduces lightness after being formulated into fish samples. Sodium alginate, methylcellulose, cornstarch and meat binder treated samples had more lightness than control samples (P<0.05). Except for Activa® RM treatment, there was no significant difference in redness (a*) detected during storage period from day 0 to day 5 for raw fish samples (Table 5). However, when only comparing

Table 5: Objective raw meat color a* values for fish balls treated with different meat binders and stored at 4°C for 5 days.

Attribute	Treatment	Storage Time			
		Day 0	Day 1	Day 3	Day 5
Raw a*	Control	3.59 ^{a,x}	2.45 ^{a,xy}	2.03 ^{a,xyz}	1.69 ^{a,x}
	5.0% Cornstarch	2.66 ^{a,xy}	2.68 ^{a,xy}	2.69 ^{a,xy}	2.77 ^{a,x}
	1.2% Meatbinder	2.06 ^{a,y}	1.44 ^{a,y}	1.32 ^{a,z}	1.76 ^{a,x}
	1.5% Carrageenan	2.09 ^{a,y}	1.78 ^{a,xy}	1.8 ^{a,xyz}	1.76 ^{a,x}
	1.5% Methylcellulose	1.99 ^{a,y}	1.75 ^{a,y}	1.72 ^{a,yz}	1.79 ^{a,x}
	1.0% Activa® RM	2.58 ^{a,xy}	1.94 ^{ab,xy}	1.66 ^{b,yz}	2.03 ^{ab,x}
	0.7% FG+	2.51 ^{a,xy}	1.98 ^{a,xy}	1.74 ^{a,xyz}	2.13 ^{a,x}
	0.7% FG	3.02 ^{a,xy}	2.65 ^{a,xy}	2.53 ^{a,xyz}	2.78 ^{a,x}
	0.8% Sodium Alginate	3.43 ^{a,xy}	3.06 ^{a,x}	2.96 ^{a,x}	2.78 ^{a,x}

^{a-d} Means in same row with different superscripts differ significantly (P < 0.05).
^{w-z} Means in same column with different superscripts differ significantly (P < 0.05).

Table 6: Objective raw meat color b* values for fish balls treated with different meat binders and stored at 4°C for 5 days.

Attribute	Treatment	Storage Time			
		Day 0	Day 1	Day 3	Day 5
Raw b*	Control	8.53 ^{a,wx,yz}	7.33 ^{b,y}	6.35 ^{c,y}	5.97 ^{c,x}
	5.0% Cornstarch	9.06 ^{a,wx}	9.32 ^{a,v}	9.25 ^{a,v}	9.39 ^{a,uv}
	1.2% Meatbinder	7.90 ^{a,z}	7.02 ^{ab,y}	6.85 ^{b,y}	7.34 ^{ab,wx}
	1.5% Carrageenan	10.95 ^{a,u}	10.6 ^{ab,u}	10.51 ^{ab,u}	9.98 ^{b,u}
	1.5% Methylcellulose	8.18 ^{a,yz}	8.53 ^{a,vw}	8.15 ^{a,w}	8.19 ^{a,vw}
	1.0% Activa® RM	8.31 ^{a,xyz}	7.50 ^{ab,xy}	6.94 ^{b,xy}	7.06 ^{b,wx}
	0.7% FG+	9.21 ^{a,vw}	8.28 ^{ab,wx}	7.91 ^{b,wx}	8.02 ^{b,vw}
	0.7% FG	9.51 ^{a,v}	8.80 ^{ab,vw}	8.52 ^{ab,vw}	8.41 ^{b,uvw}
	0.8% Sodium Alginate	8.71 ^{a,wx,y}	8.71 ^{a,vw}	8.56 ^{a,vw}	7.95 ^{a,vw}

^{a-d} Means in same row with different superscripts differ significantly (P < 0.05).
^{w-z} Means in same column with different superscripts differ significantly (P < 0.05).

day 0 and day 5, except for cornstarch treatments, the fish balls from all treatments insignificantly decreased the redness. For the cornstarch treatment, the redness slightly changed during 5 days of storage. Control samples decreased redness more sharply than other treatments. It started with the highest initial redness, and ended with the lowest redness. Therefore, compared with control treatments, all binders could efficiently protect the redness of meat samples. There was no significant b* value (yellowness) reduction for cornstarch, methylcellulose, and sodium alginate samples as storage period increased from day 0 to day 5. For the rest of the treatments, the b* value decreased as storage time increased (Table 6). Among these treatments, meat binder samples showed the lowest yellowness values compared with all other treatments for all storage days. The yellowness was significantly lower (P<0.05) in some days and little bit lower in other days. Carrageenan and samples had higher yellowness than other treatments; this is due to the yellow color of carrageenan itself, which can increase the yellowness of samples (Tables 7 and 8).

Cooked Meat L*, a* and b*

After meat samples were cooked in a water bath for 30 minutes,

Table 7: Objective cooked meat color L* values for fish balls with different binders and stored at 4 °C for 5 Days.

Attribute	Treatment	Storage Time	
		Day 1	Day 5
Cooked L*	Control	76.15 ^{a,yz}	76.23 ^{a,x}
	5.0% Cornstarch	79.59 ^{a,uv}	78.48 ^{b,vw}
	1.2% Meatbinder	81.35 ^{a,u}	80.37 ^{b,x}
	1.5% Carrageenan	74.88 ^{a,z}	74.01 ^{b,y}
	1.5% Methylcellulose	78.98 ^{a,wx}	77.62 ^{a,wx}
	1.0% Activa® RM	76.62 ^{a,y}	76.29 ^{a,x}
	0.7% FG+	77.43 ^{a,y}	76.53 ^{b,x}
	0.7% FG	77.61 ^{a,xy}	76.43 ^{b,x}
	0.8% Sodium Alginate	80.83 ^{a,uv}	79.96 ^{b,uv}

^{a-d} Means in same row with different superscripts differ significantly (P < 0.05).
^{w-z} Means in same column with different superscripts differ significantly (P < 0.05).

Table 8: Objective cooked meat color a* values for fish balls with different binders and stored at 4 °C for 5 Days.

Attribute	Treatment	Storage Time	
		Day 1	Day 5
Cooked a*	Control	-7.69 ^{a,z}	-0.89 ^{a,xyz}
	5.0% Cornstarch	-5.34 ^{a,xyz}	-0.64 ^{a,x}
	1.2% Meatbinder	-0.62 ^{a,xyz}	-0.67 ^{a,xy}
	1.5% Carrageenan	-0.38 ^{a,wx}	-0.55 ^{b,x}
	1.5% Methylcellulose	-0.49 ^{a,xyz}	-0.62 ^{b,x}
	1.0% Activa® RM	-0.70 ^{a,yz}	-1.06 ^{b,z}
	0.7% FG+	-0.77 ^{a,z}	-1.01 ^{b,yz}
	0.7% FG	-0.48 ^{a,wxy}	-0.61 ^{b,x}
	0.8% Sodium Alginate	-1.69 ^{a,w}	-0.20 ^{a,w}

^{a-d} Means in same row with different superscripts differ significantly (P < 0.05).

^{w-z} Means in same column with different superscripts differ significantly (P < 0.05).

Table 9: Objective cooked meat color b* values for fish balls with different binders and stored at 4 °C for 5 Days.

Attribute	Treatment	Storage Time	
		Day 1	Day 5
Cooked b*	Control	9.37 ^{a,yz}	9.41 ^{a,xy}
	5.0% Cornstarch	9.69 ^{a,xyz}	9.56 ^{a,xy}
	1.2% Meatbinder	9.22 ^{b,z}	9.40 ^{a,y}
	1.5% Carrageenan	10.49 ^{a,x}	10.32 ^{b,w}
	1.5% Methylcellulose	9.62 ^{a,xyz}	9.60 ^{a,xy}
	1.0% Activa® RM	9.38 ^{a,yz}	9.16 ^{a,y}
	0.7% FG+	9.88 ^{a,xy}	9.67 ^{a,wxy}
	0.7% FG	10.11 ^{a,wx}	10.09 ^{a,wx}
	0.8% Sodium Alginate	9.54 ^{b,yz}	9.71 ^{a,wxy}

^{a-d} Means in same row with different superscripts differ significantly (P < 0.05).

^{w-z} Means in same column with different superscripts differ significantly (P < 0.05).

cooking yield, texture, pH, moisture, and WHC were evaluated. A blender was used to grind fish balls for 15 seconds and the ground fish samples were placed into Petri dishes, covered with lids. The cooked meat color was measured using colorimeter through a Petri

Table 10: Cooked TPA for fish balls with different binder treatments.

Cooked TPA	Treatment	Attribute						
		Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
	Control	1588.3 ^{ef}	-15.17 ^{ab}	0.956 ^a	0.510 ^{bcd}	810.9 ^e	773.2 ^c	0.411 ^b
	5.0% Cornstarch	1858.7 ^{de}	-24.10 ^b	0.952 ^{ab}	0.504 ^{cd}	937.6 ^c	894.3 ^c	0.384 ^{bc}
	1.2% Meatbinder	2125.8 ^{cd}	-24.04 ^b	0.904 ^{bc}	0.497 ^d	1057.2 ^{bc}	955.1 ^{bc}	0.364 ^c
	1.5% Carrageenan	3226.1 ^a	-2.01 ^a	0.833 ^d	0.456 ^e	1477.8 ^a	1239.3 ^a	0.320 ^d
	1.5% Methylcellulose	1188.1 ^f	-1.58 ^a	0.880 ^{cd}	0.438 ^e	521.8 ^d	458.8 ^d	0.320 ^d
	1.0% Activa® RM	2775.8 ^{ab}	-4.78 ^a	0.963 ^a	0.531 ^{ab}	1477.3 ^a	1421.6 ^a	0.492 ^a
	0.7% FG+	2579.6 ^{bc}	-5.99 ^a	0.938 ^{ab}	0.526 ^{abc}	1360.4 ^a	1277.6 ^a	0.458 ^a
	0.7% FG	2473.8 ^{bc}	-3.67 ^a	0.922 ^{abc}	0.515 ^{bcd}	1274.1 ^{ab}	1175.6 ^{ab}	0.466 ^a
	0.8% Sodium Alginate	1791.1 ^{de}	-26.53 ^b	0.945 ^{ab}	0.454 ^a	976.3 ^c	921.6 ^{bc}	0.387 ^{bc}

dish lid. The grinder was clean thoroughly before preparing the next treatment. When compared, all cooked meat color changed from day 0 to day 5. All meat sample treatments decreased in lightness during the storage period. There were no significant differences in lightness detected for control samples or those treated with methylcellulose or Activa® RM (P>0.05). For other treatments, the cooked lightness values changed significantly (P<0.05). However, overall, the lightness of samples decreased as storage time increased. The same trends were observed with raw carrageenan and treatments when compared with cooked b* values (yellowness) (Table 9). The cooked samples treated with carrageenan showed the highest yellowness value among treatments, and had significantly higher yellowness than samples treated with control, meat binder, Activa® RM and sodium alginate. Meatbinder samples had the lowest yellowness values among all treatments. When compared with yellowness on day 0 and day 5, except for meat binder and sodium alginate treatments, there were no yellowness changes among samples (P >0.05).

Cooked and raw TPA analysis

Carrageenan samples had the highest hardness, adhesiveness, gumminess, and chewiness parameters, and had the lowest springiness and cohesiveness parameters (Table 10). Samples treated with 1.0% Activa® RM, 0.7% FG+ and 0.7% FG had similar hardness (P>0.05). Compared with samples treated with control and cornstarch, there was no advantage found (P>0.05) when adding cornstarch as a binder. However, there were slightly harder than control samples. Samples treated with methylcellulose had the softest texture among treatments. Except for samples treated with methylcellulose, all other binder treatments had a firmer texture than the control treatment. Therefore, except for methylcellulose, all these binders can be applied into fish balls to improve the hardness texture. The 1.0% Activa® RM, 0.7% FG+ and 0.7% FG treatments had less stickiness, more cohesiveness, more gumminess, more chewiness, and more resilience than control treatments. Compared with cooked fish ball samples, the raw TPA data demonstrated that 1.0% Activa® RM (2182.7 g) had the firmest texture among all treatments (Table 11). It was 7 times the hardness of the control samples (319.0 g). Next, samples treated with 0.7% FG+ and 0.7% FG treated samples had firmer textures than control samples. When compared with control samples, sodium alginate system, cornstarch, and meat binder did not show any advantage. Samples treated with 1.0% Activa® RM had the lowest adhesiveness, followed by samples treated with 0.7% FG+ and 0.7% FG. Samples treated with 1.0% Activa® RM showed the highest

Table 11: The TPA values for raw fish balls with different binder treatments.

Raw TPA Treatment	Attribute						
	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Control	319.0 ^e	-222.3 ^b	0.541 ^c	0.311 ^d	99.3 ^d	55.3 ^e	0.072 ^e
5.0% Corn Starch	333.6 ^e	-517.22 ^d	0.800 ^{ab}	0.383 ^c	127.7 ^d	102.2 ^{de}	0.074 ^e
1.2% Meatbinder	327.4 ^e	-338.2 ^c	0.734 ^b	0.326 ^d	106.7 ^d	78.7 ^{de}	0.069 ^e
1.5% Carrageenan	546.6 ^d	-112.4 ^{ab}	0.475 ^c	0.283 ^d	154.8 ^{cd}	73.4 ^{de}	0.107 ^d
1.5% Methylcellulose	396.1 ^e	-425.7 ^{cd}	0.860 ^a	0.486 ^a	194.1 ^c	169.2 ^c	0.150 ^c
1.0% Activa® RM	2182.7 ^a	-8.67 ^a	0.762 ^{ab}	0.416 ^{bc}	907.7 ^a	691.7 ^a	0.320 ^a
0.7% FG+	693.9 ^c	-48.7 ^a	0.529 ^c	0.301 ^d	207.1 ^c	110.4 ^d	0.141 ^c
0.7% FG	914.9 ^b	-12.2 ^a	0.729 ^b	0.399 ^c	366.3 ^b	267.6 ^b	0.255 ^b

Table 12: Relationship among raw and cooked tpa hardness and puncture tests.

Pearson Correlation Coefficients, N=81							
Prob> r under H0: Rho=0							
	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Hardness	1	0.116	-0.166	0.178	0.973	0.924	0.275
Adhesiveness	0.116	1	-0.355	-0.337	0.043	-0.014	0.135
Springiness	-0.16	-0.355	1	0.687	0.009	0.184	0.604
Cohesiveness	0.178	-0.337	0.687	1	0.384	0.499	0.753
Gumminess	0.973	0.043	0.009	0.384	1	0.983	0.445
Chewiness	0.924	-0.014	0.184	0.499	0.983	1	0.556
Resilience	0.275	0.135	0.604	0.753	0.445	0.556	1

Table 13: Relationship among cooked TPA parameters.

Pearson Correlation Coefficients, N=81							
Prob> r under H0: Rho=0							
	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Hardness	1	0.542	0.039	0.154	0.989	0.963	0.879
Adhesiveness	0.542	1	-0.594	-0.467	0.454	0.361	0.61
Springiness	0.039	-0.594	1	0.852	0.162	0.28	0.122
Cohesiveness	0.154	-0.467	0.852	1	0.29	0.391	0.324
Gumminess	0.989	0.454	0.162	0.29	1	0.992	0.891
Chewiness	0.963	0.361	0.28	0.391	0.992	1	0.876
Resilience	0.879	0.61	0.122	0.324	0.891	0.876	1

gumminess and chewiness among treatment samples. For sodium alginate treated samples, all parameter values were higher than control samples. Control treated samples had the softest texture among all treatments. Hardness is the force required to break food samples into pieces during first bite or the maximum force of first compression. 1.0% Activa® RM, 0.7% FG+ and 0.7% FG of raw samples had the top three highest hardness values among nine treatments. For cooked samples, the top four highest hardness values were achieved with carrageenan and, 1.0% Activa® RM, 0.7% FG+ and 0.7% FG treatments. There is positive correlation between hardness of cooked samples and hardness of raw samples (Table 12). The correlation coefficient is 0.695. The value of a correlation coefficient ranges between -1 and 1. The greater the absolute value of a correlation coefficient, the stronger the linear relationship. While the correlation coefficient between

hardness and punctures values of cooked samples was 0.367 (Table 12). Adhesion is to measure stickiness of food products. A higher value means the food is stickier. For raw samples, the top three low adhesive products were samples treated with 1.0% Activa® RM, 0.7% FG+ and 0.7% FG. For cooked samples, the top three low adhesive products were samples treated with carrageenan and methylcellulose and 1.0% Activa® RM samples. Springiness is interchangeable with the term “elasticity”. A higher value means the food is stickier. It describes the remaining structural integrity to spring back. It also describes how well the food samples spring back after they are deformed during the first compression. In general, the more the product is destroyed, the lower the springiness value. Control samples and samples treated with Activa® RM had the highest springiness among cooked samples. Samples treated with sodium alginate and methylcellulose had the

Table 14: Relationship among raw tpa parameters.

Pearson Correlation Coefficients, N=81				
Prob> r under H0: Rho=0				
	Raw-Puncture Test	Cooked-Puncture Test	Cooked Hardness	Raw Hardness
Raw-Puncture Test	1	0.309	0.18	0.199
Cooked-Puncture Test	0.309	1	0.367	0.101
Cooked Hardness	0.18	0.367	1	0.695
Raw Hardness	0.199	0.101	0.695	1

highest springiness for all raw samples. Gumminess applies only to semi-solid products and chewiness applies only to solid products. In this experiment, only chewiness values were considered and investigated. Chewiness is the energy required to break down the solid food products. It has highly positive correlation with hardness. The correlation coefficients between gumminess and chewiness with hardness are 0.973 and 0.924 respectively (Table 13). Activa[®] RM has the largest chewiness among nine treatments for both cooked and raw meat samples. Cohesiveness was calculated as the ratio between that the area of work during the second compression and the area of work during the first compression (Area 2/Area 1). It is defined as how well the product can withstand the second deformation relative to its resistance under the first deformation. In food systems, the cohesiveness is the energy or the number of times the food to can be broken down until it can be swallowed. The correlation coefficient between hardness and cohesiveness is 0.178 for raw fish balls (Table 14). It was concluded that the hardness and springiness of foods were uniformly distributed on an evaluation scale. The results may be opposite for cohesiveness.

Conclusions

This study showed that samples treated with Activa[®] RM and FG+ and FG produced satisfactory binding in fish balls. These three binders can result in higher cooking yield, hardness texture, and maintain both cooked and raw fish ball lightness during storage period. Considering overall parameters evaluated in this study, it is concluded that Activa[®] RM binder showed the best functionality or performance, following with FG+ and FG treatments. Samples treated with sodium alginate performed at medium level. Moreover, studies showed that salt could inhibit alginate from forming a gel with meat protein. A sodium alginate system is not suitable for products with salt. Samples treated with meat binder and methylcellulose showed the worst performance.

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