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PHYSICOCHEMICAL PROPERTIES OF SURIMI POWDER MADE FROM THREADFIN BREAM (*NEMIPTERUS JAPONICUS*) WITH VARIOUS DRYOPROTECTANTS ADDED

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ABSTRACT

Surimi powder and different dryoprotectants (control, sucrose, sorbitol, lactitol, maltodextrin, trehalose, polydextrose and palatinit) were produced, and the physicochemical properties were measured. The control failed to form a gel, whereas the treated samples exhibited high gel formation (1.00-2.67%). All treated samples showed improved water holding capacity (up to ±267%) relative to the control. The addition of dryoprotectant also improved the emulsifying and foaming properties compared to the control. Ca^{2+}–ATPase activity, sulfhydryl content and gel electrophoresis results suggested that the protein in the control sample was denatured during the drying process whereas the addition of dryoprotectant successfully prevented protein denaturation. Thus, the addition of dryoprotectant particularly polydextrose can successfully maintain the physicochemical properties of threadfin bream surimi powder better than sucrose.

Keywords: Surimi powder; dryoprotectant; physicochemical properties; protein denaturation.

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1. INTRODUCTION

Surimi powder, the dry form of surimi is a useful raw material for making various fish-based products due to its good physicochemical properties [1]. Surimi powder offers many advantages compared to frozen surimi such as ease of handling, more convenient storage and usefulness in dry mixtures [2]. Freeze-dried surimi powder has better characteristics than powder produced by the spray-drying method [3] and oven-drying method [4]. For example, in [3] found that freeze-dried surimi made from saithe (Pollachius virens) had better emulsifying capacity (~85%) compared to oven-dried surimi (~60%). They also reported that freeze-dried surimi had higher water holding capcity (WHC) (~350%) than oven-dried surimi (~330%). In [4] reported that freeze-dried surimi made from threadfin bream (Nemipterus japonicus) had higher WHC (20 ml H_2O/g protein) than oven-dried surimi (6 ml H_2O/g protein).

The drying process can cause denaturation of proteins due to the aggregation of protein when water is removed from the matrix [5]. The sugars and other polyols used as a cryoprotectant in the freezing process when making frozen surimi also can be used in the drying process when making surimi powder [6]. In this case, these compounds are referred to as dryoprotectants [7]. The incoporation of sucrose as a commercial cryoprotectant imparts a sweet taste in surimi products, which many consumers dislike [8]. A sugar with low sweetness can be used as an alternative to overcome this problem. In [9] evaluated the use of sucrose, lactitol, sorbitol, maltodextrin, trehalose, polydextrose and palatinit as cryoprotectants in frozen surimi. However, the effectiveness of these compounds as dryoprotectants when making surimi powder has yet to be reported.

In this study, freeze-dried surimi powder made from threadfin bream and different dryoprotectants (sucrose, lactitol, sorbitol, maltodextrin, trehalose, polydextrose and palatinit) were produced, as was a control with no dryoprotectant. This goal of this study was to evaluate the effect of these dryoprotectants on the physicochemical properties of the different treatments relative to the control. The following analyses were conducted: proximate composition, whiteness, gel formation, WHC, emulsifying and foaming properties, Ca^{2+}–ATPase activity, sulfhydryl (SH) content and gel electrophoresis.

2. RESULTS AND DISCUSSION

2.1. Proximate Composition

Table 1 shows the proximate composition of the control surimi powder and the samples with dryoprotectant added. Protein constituted the largest portion of all samples, followed by moisture content. The control sample had the highest protein content. For the treated samples, carbohydrate, fat and ash respectively were the next most common constituents whereas the order was ash, fat and carbohydrate for the control sample. The control sample had the highest amount of protein after drying because dryoprotectant was not present, but it had the lowest amount of carbohydrate. In [10] reported that surimi powder made from threadfin bream with 3.5% sucrose added had protein, moisture, ash, fat and carbohydrate contents of 72.9, 5.6, 2.2, 1.9 and 17.4% repectively. The lower protein and higher carbohydrate contents found in the current study are due to the higher amount of dryoprotectant used (6%).

Table 1. Proximate composition of the control surimi powder and the samples with dryoprotectant added

Sample	Water (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
Control	5.07±0.04^{a}	90.94±0.92^{a}	1.23±0.16^{a}	2.04±0.12^{a}	0.72±0.90^{a}
Sucrose	5.11±0.02^{a}	67.61±0.42^{b}	1.09±0.60^{b}	1.84±0.02^{a}	24.35±0.42^{b}
Sorbitol	5.14±0.10^{a}	67.42±0.48^{b}	1.10±0.02^{b}	1.84±0.03^{a}	24.50±0.54^{b}
Lactitol	5.12±0.07^{a}	67.45±0.34^{b}	1.07±0.02^{b}	1.83±0.01^{a}	24.53±0.33^{b}
Maltodextrin	5.08±0.04^{a}	67.51±0.12^{b}	1.08±0.04^{b}	1.86±0.04^{a}	24.47±0.12^{b}
Trehalose	5.09±0.07^{a}	67.53±0.39^{b}	1.09±0.06^{b}	1.86±0.04^{a}	24.43±0.36^{b}
Polydextrose	5.10±0.05^{a}	67.52±0.40^{b}	1.09±0.07^{b}	1.87±0.04^{a}	24.42±0.39^{b}
Palatinit	5.11±0.02^{a}	67.45±0.33^{b}	1.07±0.04^{b}	1.88±0.03^{a}	24.49±0.36^{b}

(Means in each column with different superscript letters are significantly different at $P < 0.05$)

The Food and Agriculture Organization (FAO) defines FPC (fish protein concentrate) as any stable fish preparation intended for human consumption, in which the protein is more concentrated than in the original fish [11]. Surimi powder with a protein content of at least 65% is considered to be FPC type A, thus the surimi powder produced in this study can be classified as such.

2.2. Whiteness, Gel Formation and WHC

Table 2 lists the whiteness values for control and treated samples. Whiteness is an index used to rate the general appearance of surimi. The control sample had a significantly lower whiteness value ($P < 0.05$) than all treated samples (i.e., 2–3 points lower than other samples). In [7] reported that freeze-dried surimi powder with 2.5% sucrose had a ~3 point higher whiteness value than freeze-dried surimi powder without additives. Among the treated samples in the current study, the whiteness value ranged from 81.30 to 82.77. The whiteness value of freeze-dried surimi made from threadfin bream by [4] was about 87.85, and that produced by [10] had a whiteness value of about 83.93. The different whiteness values found among studies may be due to different years and seasons during which the studies were conducted.

Table 2. Whiteness, gel formation and WHC of the control surimi powder and the samples with dryoprotectant added

Sample	Whiteness	Gel Formation (%)	WHC (ml H_2O/g Protein)
Control	79.81±0.12^{d}	*np	18.53±0.61^{d}
Sucrose	82.54±0.03^{ab}	2.67±0.58^{a}	39.66±0.94^{bc}
Sorbitol	81.30±0.19^{c}	2.00±0.00^{ab}	41.91±1.20^{abc}
Lactitol	82.53±0.04^{ab}	1.67±0.58^{bc}	36.04±10.59^{c}
Maltodextrin	82.52±1.41^{ab}	1.67±0.58^{bc}	43.19±1.56^{abc}
Trehalose	82.22±0.03^{ab}	1.00±0.00^{c}	45.81±1.21^{ab}
Polydextrose	81.60±0.05^{bc}	1.00±0.00^{c}	49.60±1.80^{a}
PalatinitPalatinit	82.77±0.01^{a}	1.67±0.58^{bc}	42.16±6.45^{abc}

(Means in each column with different superscript letters are significantly different at $P < 0.05$. *np = not perfomed/failed to form gel)

Among the treated samples in this study, the sorbitol and polydextrose treatments had significantly lower ($P < 0.05$) whiteness values than the palatinitpalatinit treatment. Glucose which is a reducing sugar in polydextrose might have contributed to the browning of the samples. No significant difference ($P > 0.05$) in the whiteness value was detected among the lactitol, sucrose, maltodextrin, trehalose and palatinitpalatinit treatments. These data suggest

that the choice of dryoprotectant to mix with surimi may affect the whiteness of surimi powder upon drying.

Gel formation and WHC data are also shown in Table 2. The control sample failed to form a gel in the gel formation test at all concentrations between 1 and 10% because the proeins in the control were denatured. In contrast, the treated samples gelled at concentrations between 1 and 2.67%. Samples that gelled at lower concentrations had higher gelling properties. The sucrose treatment required a significantly higher ($P < 0.05$) concentration for gel formation to occur compared to the other treated samples (except for sorbitol). Thus, the sucrose treatment which gelled at a concentration of 2.67% had the lowest gelation properties among the treated samples. The gel formation ability of surimi powder with sucrose added was lower than that of freeze-dried threadfin bream surimi powder reported previosly by [10]. There was no significant difference ($P > 0.05$) in gel formation among the lactitol, maltodextrin, trehalose, polydextrose and palatinit treatments which gelled at concentrations of 1-1.67%. This finding suggests that use of a low sweetness sugar results in higher gelation properties than use of sucrose/sorbitol.

The control sample had the lowest WHC (Table 2). The drying process can cause denaturation of proteins due to the aggregation of protein when water is removed from the matrix [5]. However, the addition of dryoprotectant improved the WHC of surimi powder, as the treated samples had significantly higher ($P < 0.05$) WHC than that of the control. In [3] also reported that the WHC of freeze-dried surimi made from saithe with sugar added (~350%) was higher than that of freeze-dried surimi without sugar added (~320%).

The WHC values of the treated samples in this study were higher than those reported by [10] for freeze-dried surimi made from threadfin bream (19.5 ml H_2O/g protein). In [4] reported that the WHC of freeze-dried threadfin bream surimi powder was 20 ml H_2O/g protein, which is lower than the WHC found in this study. The higher WHC in this study likely was due to the higher amount of dryoprotectant (6%) used, which had a better protective effect on the protein compared to 3.5% sucrose to produce surimi powder by [4, 10]. The WHC values in this study also were higher than those for oven-dried surimi powder treated with a low sweetness sugar (2.60-3.00 ml H_2O/g protein) reported by [12]. Previous studies also reported that freeze-dried

surimi powder exhibited higher WHC than spray-dried surimi powder [3] and oven-dried surimi powder [4]. Thus, WHC is clearly influenced by the addition of dryoprotectant [7, 13], drying method [3-4] and fish species [10].

The polydextrose treatment exhibited the highest WHC among the treated samples, and the value was 267% higher than that of the control sample. No significant differences ($P > 0.05$) in WHC were detected among the sorbitol, lactitol, trehalose, maltodextrin and palatinit treatments. These dryoprotectants improved the WHC by 226–247% relative to the control. The sucrose and lactitol treatments had significantly lower ($P < 0.05$) WHC than the polydextrose treatment. In [14] suggested that polydextrose may be substituted interchangeably for the sucrose and/or sorbitol now commonly used in surimi production.

2.3. Emulsifying Properties

Fig. 1 and 2 show the emulsifying capacity and emulsifying stability respectively. The emulsifying capacity was higher as the concentration of surimi powder increased. The control sample did not have emulsifying capacity until the concentration reached 1.5%, but at 2% concentration the emulsifing capacity was 9.25%. Moreover, the control sample did not have emulsifying stability at any of the concentrations tested. Denaturation of protein during drying in the control sample may be responsible for the loss of emulsifying capacity. In [3] reported that at 1% concentration, freeze-dried surimi powder made from saithe without sugar added (~50%) had lower emusifing capacity and stability than freeze-dried surimi powder with sugar added (~85%).

The optimum emulsion capacity of a protein occurs when the hydrophilic and hydrophobic proportions are in balance [15]. Denaturation of the protein can decrease the emulsification capacity of surimi powder, but adding sugar as a dryoprotectant maintains the hydrophilic residues after drying, resulting in improved emulsion [16]. Among the treated samples, those with sucrose and sorbitol added had quite similar emulsifying capacity at 0.5% concentration and at 1% concentration. These values were significantly lower ($P < 0.05$) than those of the other treated samples at 0.5% concentration and 1% concentration. The emulsifying capacity of the trehalose, polydextrose, and palatinit treatments at 1% concentration were quite similar to the results reported by [10], in which freeze-dried threadfin bream surimi powder had

emulsifying capacity > 80% at 0.8% concentration. Emulsifying capacity of the polydextrose and palatinit treatments was higher than that of freeze-dried surimi powder from saithe containing 5% sucrose and 0.2% phosphate, which was 82.5% at concentration of 1% [17]. At 1.5% concentration, all treated samples exhibited various emulsifying capacities (74.07-90.37%). No significant differences (P > 0.05) in emulsifying capacity were found among the trehalose, polydextrose and palatinit treatments at all concentrations.

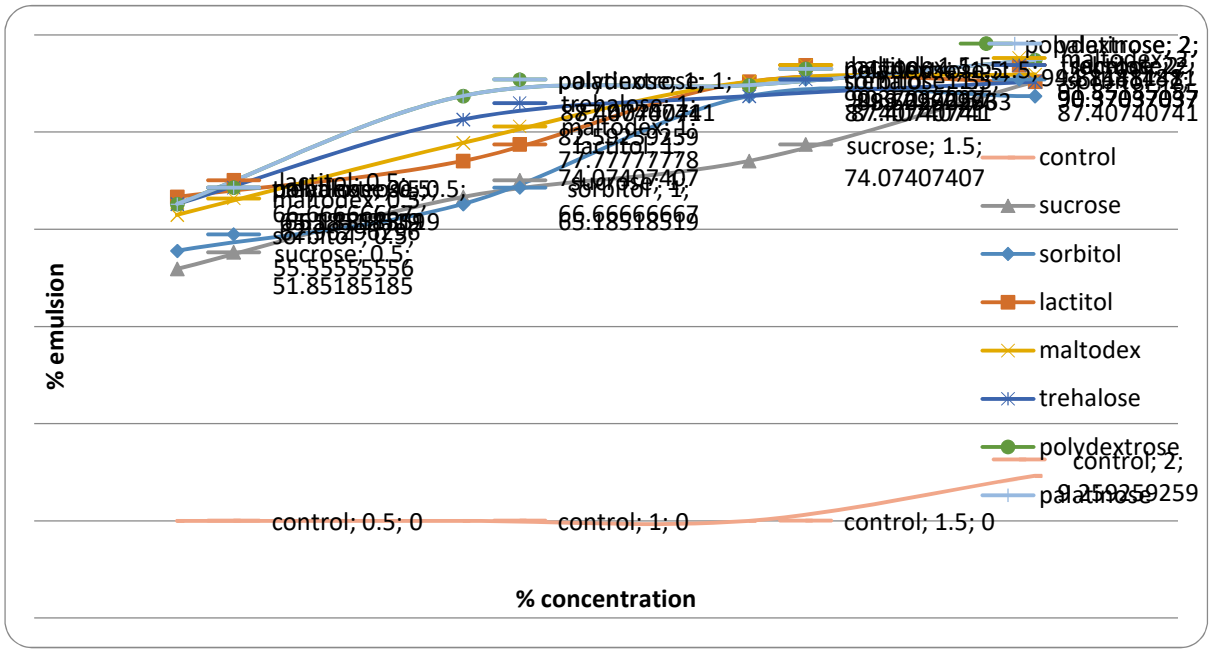

Fig.1. Emulsifying capacity of control and treated samples

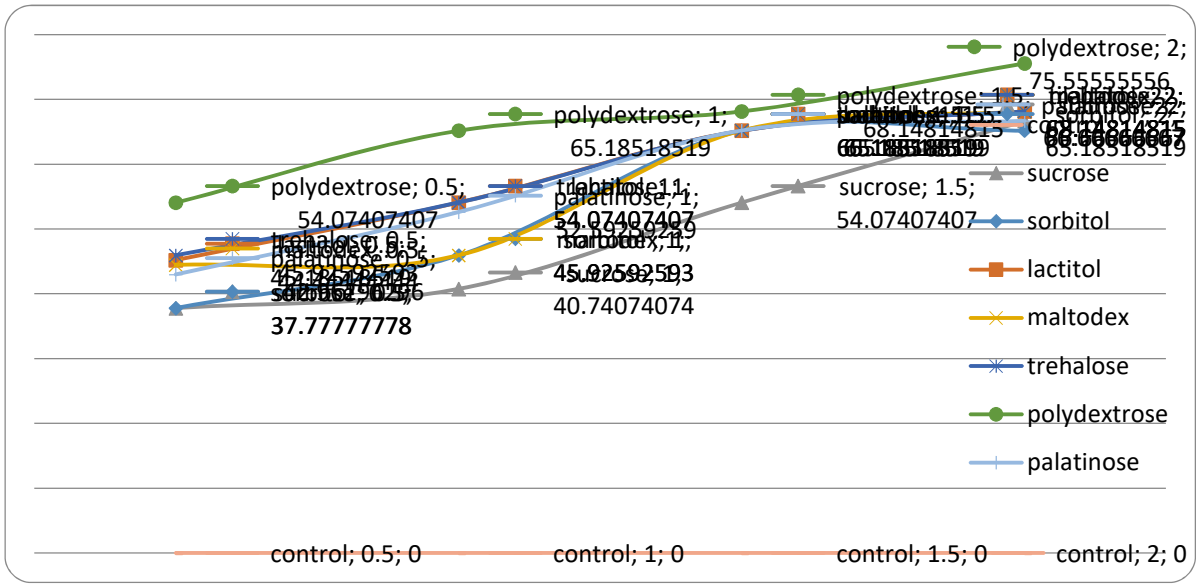

Fig.2. Emulsifying stability of control and treated samples

Emulsifying stability reflects the stability of an emulsion when heated. Fig. 2 shows that the treated samples had varying emulsifying stabilities at concentrations of 0.5 and 1%. At 2% concentration, the emulsifying stability of the polydextrose treatment was about 75.56%. This value is lightly lower than that reported in a previous study of freeze-dried surimi powder made from threadfin bream (82.8%) at 2% concentration [10]. However, at concentrations of 0.5, 1 and 2%, the polydextrose treatment exhibited significantly higher ($P < 0.05$) emulsifying stability than the other treated samples. This result suggests that polydextrose has a greater dryoprotective effect on surimi powder during the drying process, resulting in higher emulsifying stability after heating. Thus, freeze-dried surimi powder treated with polydextrose potentially could be used as an emulsifier.

2.4. Foaming Properties

Fig. 3 shows the foaming capacity and stability of control and treated samples. Denaturation of protein in the control sample is expected to cause loss of foaming properties. The control sample had only 1.67% foaming capacity and it lost its foam after 30 min, resulting in no foaming stability. The addition of dryopotectant succesfully increased the foaming properties of the surimi powder. The foaming capacities of surimi powder treated with lactitol, maltodextrin, trehalose, polydextrose and palatinit (40–45%) were significantly higher ($P < 0.05$) than those of the sucrose (32%) and sorbitol (33%) treatments. The foaming capacities of surimi powder treated with lactitol, maltodextrin, trehalose, polydextrose and palatinose in this study were higher than that reported by [10] which is 35%.

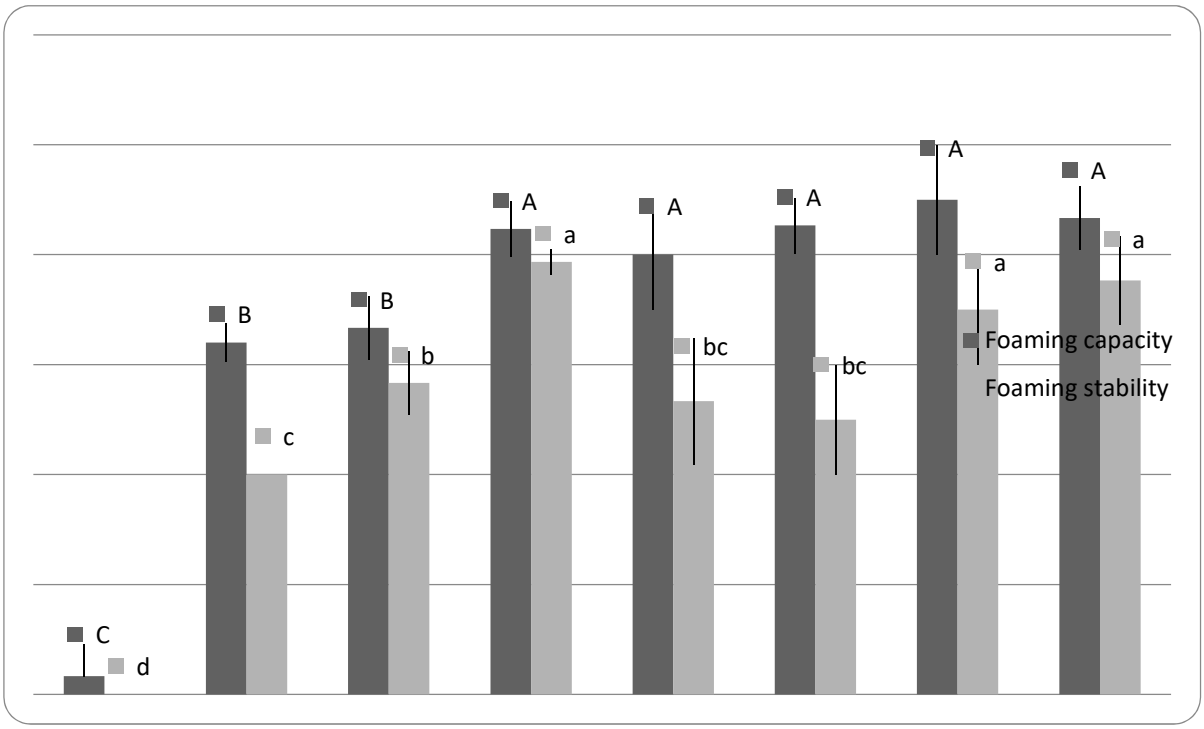

Fig.3. Foaming capacity of control and treated samples (Means in each bar with different superscript letters are significantly different at $P < 0.05$)

After 30 min of monitoring, the foam volume of the samples decreased. The samples with lactitol, polydextrose and palatinit added had the highest foaming stability among the treated samples (39, 35, and 37%, respectively). The addition of sucrose which is commonly used commercially as a cryprotectant, resulted in much lower foaming stability (about 20%). Better foaming properties of surimi powder are related to the stability of the fish protein [3, 10]. The results of this study suggest that the foaming properties of surimi powder vary depending on the dryoprotectant added. In [3] reported that foaming stability of surimi powder also depends on the drying process used, as freeze-dried surimi powder had higher foaming stability than spray-dried surimi powder. In [9] suggested that the addition of polydextrose had a better protective effect on surimi during frozen storage than the addition of sucrose, lactitol, sorbitol, maltodextrin, trehalose and palatinit.

2.5. Ca^{2+}–ATPase Activity

Table 3 shows the Ca^{2+}–ATPase activity and SH content of control and treated samples. Ca^{2+}–ATPase activity often is used as a sensitive indicator of the extent of myosin unfolding (denaturation) and aggregation [18]. The control sample had the lowest Ca^{2+}–ATPase activity.

This result indicates that aggregation of myosin occurred in the control sample during the drying process, as a large amount of water was removed from the matrix. This protein denaturation explains the loss of gel formation, low WHC and poor emulsifying and foaming properties of the control sample.

Table 3. Ca^{2+}–ATPase activity and SH content of the control surimi powder and the samples with dryoprotectant added

Sample	Ca^{2+}–ATPase Activity	SH Content
Control	116.27±8.45^{g}	7.73±0.94^{d}
Sucrose	310.50±17.93^{f}	9.13±0.03^{c}
Sorbitol	374.41±12.87^{e}	11.13±0.03^{b}
Lactitol	503.08±7.41^{c}	10.76±0.30^{b}
Maltodextrin	379.47±4.85^{e}	9.16±0.74^{c}
Trehalose	463.06±17.25^{d}	10.95±1.61^{b}
Polydextrose	602.76±18.00^{a}	12.80±0.41^{a}
Palatinit	539.51±8.58^{b}	11.77±0.02^{ab}

(Means in each column with different superscript letters are significantly different at $P < 0.05$)

The addition of dryoprotectants provided protection against drastic reduction of Ca^{2+}–ATPase activity. The polydextrose treatment had the highest Ca^{2+}–ATPase activity, followed by the palatinit and lactitol treatments (Table 3). The sucrose treatment had the lowest Ca^{2+}–ATPase activity among the treated samples. The Ca^{2+}–ATPase activities measured in this study were higher than that reported by [13] for active fish protein powder (~155 μg Pi/mg protein/10 min). The addition of polydextrose provided the greatest protection from myosin aggregation because it drastically reduced the amount of water lost during the drying process. In [9] found the highest Ca^{2+}–ATPase activity in frozen surimi treated with polydextrose (127.8 μg Pi/mg protein/10 min) compared to samples treated with sucrose, lactitol, sorbitol, maltodextrin, trehalose and palatinit (75.0-119.2 μg Pi/mg protein/10 min) after 6 months of frozen storage. In [19] also reported that polydextrose was highly effective at preventing a decrease in Ca^{2+}–ATPase activity after 8 weeks of storage compared with glucose syrup and sucrose/sorbitol in the actomyosin of rainbow trout.

2.6. SH Content

A decrease in Ca^{2+}–ATPase activity coincides with a decrease in SH [20]. In the current study, the surimi powder without dryoprotectant had the lowest SH content among all samples tested. This loss of SH content likely was caused by denaturation of protein in the control sample. In [21] reported that freeze drying led to a substantial decrease of free SH content in fish protein powder. Decreased free SH content is accompanied by an increase in disulfide bond content as a result of the oxidation of SH groups or disulfide interchange [22].

The addition of dryoprotectant succesfully prevented the decrease of SH content of surimi during the drying process. The SH contents of the treated samples were significantly higher ($P < 0.05$) than that of the control sample. This result suggests that the addition of dryoprotectant is important for alleviating the denaturation of threadfin bream protein during drying. Among the treated samples, the polydextrose and palatinit treatments had the highest SH content and did not differ from each other ($P > 0.05$). However, only the polydextrose treatment had a significantly higher ($P < 0.05$) SH content than the sucrose, lactitol, sorbitol, maltodextrin and trehalose treatments. The SH content of the polydextrose treatment was 40% higher than that of the sucrose treatment. In [9] reported that the SH content of frozen surimi made from threadfin bream ranged from 7.39 to 15.71 mol SH/10^5g protein during 6 months of frozen storage, and these values are slightly higher than those observed for the surimi powder samples in this study. This indicates that the drying process resulted in higher oxidation of SH compared to the frozen storage process.

2.7. SDS Gel Electrophoresis

Fig. 4 shows the protein banding patterns of control and treated samples. The major protein bands in the studied samples were myosin heavy chain (MHC) (~200 kDa) followed by actin (~43 kDa). The oxidation of these myofibrillar proteins, as measured by loss of ATPase activity and SH content, was in good agreement with the SDS-PAGE data. The MHC band was missing in the control sample due to denaturation of myofibrillar proteins. The actin band also exhibited decreased intensity in the control sample. It can be concluded that the drying process caused the disappearance of the MHC and actin bands.

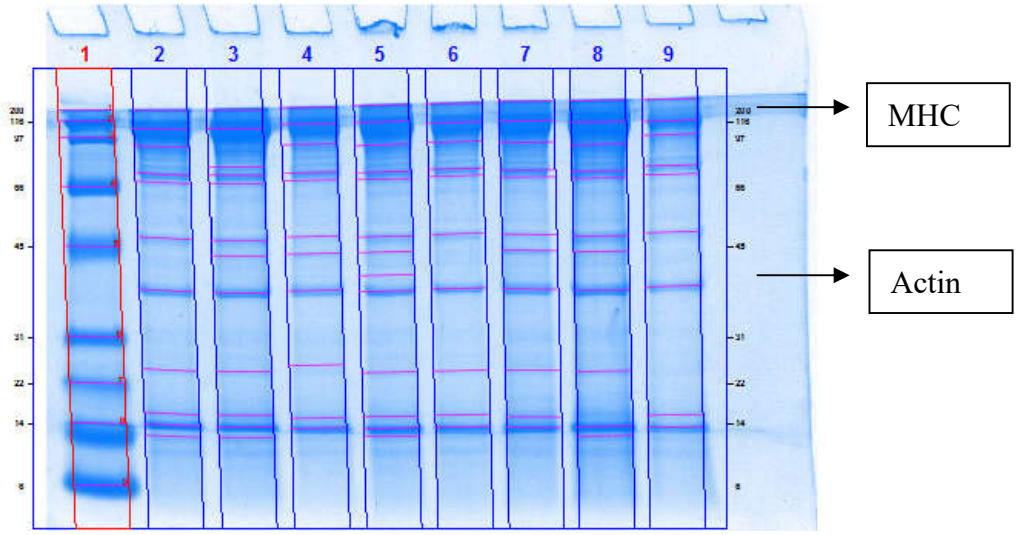

Fig.4. SDS-PAGE of control and treated samples (Lane 1 = standard; MHC, myosin heavy chain, L2 = polydextrose, L3 = palatinit, L4 = sucrose, L5 = sorbitol, L6 = maltodextrin, L7 = trehalose, L8 = lactitol, L9 = control)

In [23] reported that the MHC band disappeared during frozen storage of ling cod (*Ophiodon elongatus*) surimi without sugar added because the myosin component had undergone aggregation and insolubilization. In contrast, the sample with cryoprotectant added maintained the MHC band. The SDS-PAGE data in this study also showed that the addition of dryoprotectant effectively prevented the aggregation of myosin during drying, as the MHC and actin bands of all treated samples appeared very clearly in the gels.

3. EXPERIMENTAL

3.1. Surimi Powder Preparation

Surimi powder was prepared following the method described by [10]. Frozen surimi blocks made from threadfin bream with different dryoprotectants (sucrose, lactitol, sorbitol, maltodextrin, trehalose, polydextrose and palatinit) and phosphate added to constitute 6% and 0.3% of the total wet weight respectively were produced in a local surimi manufacturing facility located in Perak, Malaysia. Frozen surimi without dryprotectant added was produced as a control sample. The frozen blocks were transported by refrigerated truck to the laboratory and stored at -18 °C. Frozen surimi blocks were sliced into 20 x 1 x 10 cm sections using a meat bone saw (Powerline, Norwalk, CT, USA). Sliced frozen surimi samples then were freeze dried (Labconco Freeze Dry System, Kansas City, MO, USA) at a pressure of 0.05 mm

Hg in the chamber (condensing plate temperature: -40 °C) for 72 h until the moisture content reached ±5%. The surimi samples were milled using a miller (UiTM, Selangor, Malaysia) for 10 sec and then sieved using a 28 mm screen mesh. The surimi powder then was vacuum packed (Audionvac VMS 133, Hogeweyselaan, The Netherlands) and kept at 6 ^{o}C for analysis.

3.2. Proximate Composition

Proximate composition of the sample was determined using standard procedures of the Association of Official Analytical Chemists [24]. Moisture content was determined using the oven method, and crude protein content was determined using the Kjedahl method. Fat content was measured with the Soxhlet method and ash content was determined using the dry ashing method. Carbohydrate content was calculated by difference.

3.3. Whiteness, Gel formation and Water Holding Capacity (WHC)

Color was analyzed following [25] using a colorimeter (Minolta Spectrophotometer, Model CM-3500d, Osaka, Japan). L* (lightness), a* (redness) and b* (yellowness) were measured. Whiteness was calculated using the following equation from [26]:

$$\text{Whiteness} = 100 - [(100 - L^*)2 + a^*2 + b^*2]0.5$$

Gel formation was analyzed following the method of [27]. A series of ten 10 mL surimi powder solutions in water were made at concentrations of 1-10%. Solutions were mixed in a vortex mixer (Stuart, Bibby Scientific Limited, Stone, Staffordshire, UK) for 5 min. The tubes were heated at 90 °C for 30 min in a water bath (Wisebath® fuzzy control system, Daihan Scientific, Seoul, Korea) and then placed in a cold room for 30 min. The lowest concentration at which a sample did not fall down or slip from an inverted test tube was designated as the gel formation concentration. Gel formation was measured in triplicate per concentration for each treatment from two batches of drying.

The WHC of surimi powder samples was analyzed following [27]. One gram of surimi powder was added to 40 mL of 3% NaCl solution in a 50 mL centrifuge tube. The sample was homogenized for 5 min using a vortex mixer (Stuart, Bibby Scientific Limited) and then centrifuged for 5 min at 7500 g (Hettich Universal 30 RF centrifuge, Tuttlingen, Germany). The supernatant was poured through a funnel into a 50 mL calibrated measuring cylinder. The

volume of the supernatant was subtracted from the original 40 mL and the result was reported as mL of H2O held by 1 g of protein.

3.4. Emulsification Properties

To measure emulsifying capacity, an amount of surimi powder was added to 25 mL of distilled water and 25 mL of corn oil to give a final concentration of about 0, 0.5, 1, 1.5 and 2% [10]. The mixture was blended (Waring Commercial blender, Stamford, CT, USA) for 1 min and transferred to a 50 mL calibrated centrifuge tube and centrifuged (Hettich Universal 30 RF) at 7500 g for 5 min. Emulsifying stability was determined by the same procedure except that before the sample was centrifuged, the emulsion was heated for 30 min at 90 ^{o}C in a water bath (Wisebath® fuzzy control system) followed by cooling in tap water for 10 min. The emulsion capacity and emulsion stability were calculated using the following formula [28].

Emulsifying properties = (volume after centrifugation/original volume) x 100

3.5. Foaming Properties

Two grams of surimi powder were added to 100 mL of distilled water and blended at high speed in commercial blender (Waring Commercial blender) for 1 min. The mixture was transferred carefully into a 250 mL calibrated beaker to measure the volume. The foam capacity was calculated as the volume of the mixture after blending compared to the original volume. The foaming stability was calculated as the ratio of the foam capacity after 30 min divided by the original foam capacity [27].

3.6. Actomyosin Extraction and Ca^{2+}–ATPase Activity

Actomyosin was prepared according to the method of [29]. Surimi powder (0.2 g) was rehydrated with 0.8 g of chilled distilled water. The sample was homogenized in 10 ml of chilled 0.6 M KCl (pH 7.0) for 4 min in a vortex mixer (Stuart, Bibby Scientific Limited), and the sample container was placed on ice. Each 20 sec of homogenization was followed by a 20 sec rest interval to avoid overheating during extraction. The homogenate was centrifuged at 8370 g for 30 min at 4 oC (Hettich Universal 30 RF). Three volumes of chilled distilled water were added to precipitated actomyosin. Actomyosin pellets were collected by centrifugation at 8370 g for 20 min at 4 ^{o}C, and then dissolved by stirring in an equal volume of chilled 0.6 M

KCl (pH 7.0). Undissolved debris was removed by centrifugation at 8370 g for 30 min at 40 ^{o}C. Actomyosin was kept on ice during all analyses. The actomyosin samples were used to measure Ca^{2+}–ATPase activity and SH content and for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

The Ca^{2+}–ATPase activity was measured following the method of [30]. Approxmately 0.2 mL of actomyosin was diluted in a reaction solution (7.6 mM ATP; 15 mM CaCl2; 150 mM KCl; and 180 mM Tris-HCl, pH 7.4) at room temperature and allowed to react for 10 min. To stop the reaction, 1.0 mL of 10% trichloroacetic acid was added. The mixture was subsequently centrifuged at 3500 g for 5 min (Hettich Universal 30 RF) at 25 oC. A 1.0 mL sample of the supernatant then was reacted with 3.0 mL of 0.66% ammonium molybdate in 0.75 N sulfuric acid. A 0.5 mL aliquot of freshly prepared 10% FeSO4 in 0.15 N sulfuric acid was then added. This reaction was performed for 2 min for color development. The absorbance was read at 700 nm in a UV-vis spectrophotometer (UV-160A, Shimadzu, Kyoto, Japan) to determine the ATPase activity. NaH2PO4 was used to prepare the standard curve for phosphate calculation.

3.7. SH Content

The procedure used to measure the SH content was previously described by [30]. A 0.5 mL aliquot of actomyosin was mixed with 1.0 mL of a urea and sodium dodecyl sulfate (SDS) solution (8.0 M urea, 3% SDS and 100 mM phosphate buffer with a final pH of 7.4). The titration of SH was initiated by the addition of 0.5 mL of 5, 5'-dithio-bis (2-nitrobenzoic-acid) (DTNB) reagent (10 mM DTNB in 0.1 M phosphate buffer with a final pH of 7.4). A sample blank was run with 0.5 mL of phosphate buffer without DTNB. A reagent blank was run with only water. The absorbance was read at 420 nm in a UV-vis spectrophotometer (UV-160A, Shimadzu) after sitting for 15 min at room temperature. The concentration of SH was calculated using a molar extinction coefficient of 11400 M-1cm-1.

3.8. SDS-PAGE

SDS-PAGE was performed in a Mini-PROTEAN®Tetra Cell (Bio-Rad, Hercules, CA, USA) according to the method of [31]. A 12% resolving gel and a 4% stacking gel were used. The sample buffer consisted of 0.5 M Tris-HCl pH 6.8, SDS, bromophenol blue and glycerol in deionized water. Sample preparation was conducted by mixing protein samples with loading

buffer in a 1:2 ratio and heating the mixture to 37 ^{o}C for 60 min. An SDS-PAGE broad-range molecular weight standard (6.5 to 200 kDa) (Bio-Rad) was used. The running buffer consisted of 12 mM Tris base, 192 mM glycine and 0.1% SDS (w/v) in deionized water. The voltage used to run the gel was 200 V for 90 min. Gels were then immersed in fixative solution (deionized water containing 40% methanol and 10% acetic acid) for 15 min and stained overnight with a coomassie blue stain buffer. The gel then was destained with a destaining buffer (1% acetic acid) for 2 h on a shaker. The gels were visualized using a Bio-Rad Molecular imager® Gel DocTM XR+ with Image LabTM software (serial number 721BR03679).

3.9. Statistical Analysis

SPSS software (SPSS 17.0 for Windows, SPSS Inc, Chicago, IL, USA) was used to evaluate the data. All analyses were performed in triplicate and all experiments were replicated twice. Analytical variation was established through one way analysis of variance (ANOVA). Data are reported as mean ± standard deviation. Comparison of means was performed using Duncan's multiple range test with a level of significance of 0.05.

4. CONCLUSION

The addition of dryoprotectant to surimi powder generally provides protection from protein denaturation during the drying process. However, each of the seven dryoprotectants tested performed differently. Sucrose had the lowest dryoprotective effect, whereas polydextrose provided the best protection (the highest WHC, emulsifying stability, Ca^{2+}–ATPase activity, and SH content). Thus, polydextrose may be used as an alternative to the traditional sucrose/sorbitol in commercial surimi production. It is a nonsweet compound that can be applied to surimi powder production when sweetness is undesirable, and it provides a sugar substitute with diminished caloric value. Palatinit and trehalose also successfully provided dryoprotection in surimi powder.

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