GEL-FORMING PROPERTIES OF RED TILAPIA (OREOCHROMIS NILOTICUS) SURIMI AS AFFECTED BY SELECTED ADDITIVES

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By

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To my beloved family

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Surimi and surimi-based fish product are native to Japan; however, its consumption has spread to many parts of the world. Gel-forming ability of surimi is the main indicator for its quality, which is affected by factors such as the addition of food grade additives and fish species. Therefore, the objectives of this study were to identify the gel-forming properties of red tilapia surimi, to improve the physical, thermal, rheological and chemical properties of surimi gels by the addition of different concentrations of sodium pyrophosphate (SPP) (in combination with 2.5% $CaCl_2$), transglutaminase (TGase) and chitosan. The physico-chemical characteristics of the surimi gel were evaluated based on the kamaboko gel which were prepared by a 2-stage heating at 40°C for 20 min and

90°C for 30 min. Different levels of transglutaminase ranging from 0.10 to 0.50%, chitosan ranging from 0.50 to 1.5%, and SPP ranging from 0.0 to 0.04% (in combination with $CaCl_2$) were added to the surimi before the preparation of kamaboko gels. The kamaboko gel without any additives was used as the control. An increasing trend of Ca^{2+}-ATPase activity was obtained in the surimi and kamaboko gel compared to the fish mince. The values for fish mince, surimi and kamaboko gel of red tilapia were 0.17 ± 0.04, 0.27 ± 0.01 and 0.20 ± 0.05 µmol Pi/mg protein/min, respectively. The hardness and chewiness were significantly ($p<0.05$) different between the fish mince and kamaboko gels. Significant increase in maximum storage modulus (G') was observed in the kamaboko and surimi compared to the fish mince. The intensity of myosin heavy chain (MHC) band in the kamaboko gel was higher than the fish mince and surimi. No significant ($p>0.05$) changes were observed in the intensity of actin band of fish mince, surimi and kamaboko. The differential scanning calorimetry (DSC) thermograms of fish mince showed three peaks at 41.0°C, 54.5°C and 72.0°C; however, only two peaks were obtained at 40.2°C and 74°C in surimi and 41.5°C and 72.5°C in kamaboko gel. A smaller enthalpy change (ΔH) of the myosin peak was obtained from the kamaboko gel as compared to the fish mince and surimi, but no significant ($p>0.05$) difference was observed in the ΔH of actin for the fish mince, surimi and kamaboko. The type and levels of the additives had significant effect ($p<0.05$) on the breaking force and deformation of the samples. Samples containing 0.30% TGase showed the highest breaking force and deformation. The addition of TGase at > 0.30% and that of chitosan > 1.25% resulted in the decrease in the breaking force and deformation of the kamaboko gels. Gels with 0.30% TGase, 1.25% chitosan and 0.05% SPP separately had lowest expressible content among treatments. Different levels of additives had significant effect on the gel whiteness. Gels with 0.50% TGase showed the highest value of whiteness. Additives used had significant effect on the myosin heavy chain band intensity, but no marked changes in the actin band intensity were observed. The MHC band almost disappeared with the addition of TGase at 0.30%. Significant increase in the G'_{max} was observed in gels with additives compared to the control gel. Addition of additives had significant effect on the ΔH, maximum transition temperature (T_m) of the myosin and ΔH of actin ($p<0.05$) but slight differences between the T_m of actin was observed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN PENAMBAHAN ADITIF KEATAS CIRI-CIRI PEMBENTUKAN GEL SURIMI IKAN TILAPIA MERAH (*OREOCHROMIS NILOTICUS*)

Oleh

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Surimi dan produk berasaskan surimi berasal dari Jepun; walau bagaimanapun, pengambilan surimi dan produk surimi telah berkembang dibanyak negara di dunia. Keupayaan pembentukan-gel surimi ialah penunjuk utama kualiti, yang dipengaruhi oleh faktor seperti aditif gred makanan dan spesis ikan. Oleh itu, objektif kajian ini ialah untuk mengenalpasti ciri pembentukan-gel surimi ikan tilapia merah, dan memperbaiki sifat fizikal, terma, reologi dan kimia gel surimi dengan penambahan kepekatan yang berbeza bagi natrium pirofosfat (SPP) (dalam kombinasi dengan 2.5% $CaCl_2$), transglutaminase (TGase) dan chitosan. Ciri fiziko-kimia gel surimi dinilai berdasarkan gel kamaboko, yang telah disediakan melalui 2-peringkat pemanasan iaitu pada 40°C selama 20 minit dan 90°C selama 30 minit. Tahap peratus yang berbeza transglutaminase berjulat

antara 0.10 hingga 0.50%, chitosan antara 0.50 hingga 1.5%, dan SPP antara 0 hingga 0.04% (dalam kombinasi dengan 2.5% $CaCl_2$) ditambah kepada surimi sebelum penyediaan gel kamaboko. Gel kamaboko tanpa bahan aditif tambahan telah digunakan sebagai kawalan. Trend peningkatan Ca^{2+}-ATPase diperolehi dalam surimi dan kamaboko gel berbanding dengan daging ikan cincang. Nilai untuk daging ikan cincang, surimi dan kamaboko gel merah tilapia ialah 0.17 ± 0.04, 0.27 ± 0.01 dan 0.20 ± 0.05μmol Pi /mg protein/min. Kekerasan dan keanjalan ($p<0.05$) berbeza antara ikan cincang dan gel kamaboko. Peningkatan maksimum penyimpanan modulus (G'_{max}) jelas dilihat dalam kamaboko dan surimi berbanding dengan daging ikan cincang. Intensiti myosin rantai berat (MHC) dalam gel kamaboko lebih tinggi daripada ikan cincang dan surimi. Hasil kajian mendapati tiada perubahan yang ketara ($p>0.05$) yang perolehi dalam intensiti aktin daging ikan cincang, surimi dan kamaboko. Perbezaan Imbasan Kalorimetri Beza (DSC) thermogram daging ikan cincang menunjukkan tiga tahap tertinggi iaitu pada 41.0°C, 54.5°C dan 72.0 °C; Manakala, hanya dua tahap tertinggi didapati pada 40.2°C dan 74°C bagi surimi dan 41.5°C dan 72.5°C bagi gel kamaboko. Perubahan entalpi (ΔH) tahap tertinggi myosin diperolehi daripada gel kamaboko berbanding ikan daging cincang dan surimi, tetapi tiada perbezaan ketara ($p>0.05$) dapat dilihat dalam ΔH aktin untuk ikan cincang, surimi dan kamaboko. Jenis dan tahap bahan aditif mempunyai kesan yang signifikan ($p<0.05$) pada daya perubahan bentuk sampel. Sampel yang mengandungi TGase 0.30% menunjukkan daya tinggi untuk mengubah bentuk. Penambahan TGase pada tahap >0.30% dan Chitosan >1.25% menyebabkan penurunan dalam daya perubahan kekerasan dan mengubah bentuk gel kamaboko. Gel dengan TGase 0.30%, 1.25% Chitosan dan 0.05% SPP secara berasingan mempunyai kandungan terendah dalam semua rawatan. Perbezaan tahap bahan aditif mempunyai kesan yang ketara ($p>0.05$) terhadap keputihan gel. Gel dengan TGase 0.50% menunjukkan nilai tertinggi keputihan. Bahan aditif yang digunakan mempunyai kesan yang besar terhadap intensiti myosin rantai, tetapi hasil kajian menunjukkan tidak terdapat tanda perubahan yang ketara. Band MHC hampir hilang hasil penambahan TGase pada 0.30%. Peningkatan yang signifikan dalam G'_{max} diperhatikan dalam gel bahan tambahan berbanding dengan gel kawalan. Penambahan bahan tambahan mempunyai kesan signifikan ke atas ΔH, peralihan suhu maksimum (T_m) myosin dan ΔH aktin ($p<0.05$), didapati hanya sedikit perbezaan antara aktin T_m.

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LIST OF ABBREVIATIONS

%	percent
<	Less than
>	More than
ΔH	Enthalpy
°C	Degree centigrade
µg	Microgram
µl	Microliter
µmol	Micromole
acp	Actin peak
ANOVA	Analysis Of Variance
AOAC	Association of Official Analytical Chemists
ATP	Adenosine-5'-Tri Phosphate
cm	Centimeter
Da	Dalton
DSC	Differential Scanning Calorimetry
EW	Expressible Water
G	Elastic modulus or storage modulus
g	Gram
G_{max}	Maximum storage modulus
hr	Hour
J/g	Joule/gram

kDa	Kilo Dalton
kg	Kilogram
M	Molar
mg	Milligram
MHC	Myosin Heavy Chain
min	Minute
ml	Milliliter
mm	Millimeter
mM	Millimolar
mp	Myosin peak
MTGase	Microbial Transglutaminase
MW	Molecular weight of protein marker
N	Newton
NAM	Natural Actomyosin
nm	Nanometer
Pa	Pascal
Pi	Inorganic phosphate
Rf	Relative migration distance
SD	Standard Deviation
SDS	Sodium Dodecyl Sulfate
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
sec	Second
SPP	Sodium Pyrophosphate

TGase	Transglutaminase
T_m	Maximum transition temperature
T_{max}	Maximum temperature
T_p	Transition temperature peak
TPA	Texture Profile Analysis
v	Volt
v/v	volume/volume
w/v	Weight/volume
W_f	Final weight
W_i	Initial weight

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CHAPTER 1

GENERAL INTRODUCTION

Surimi has long been a major and popular food ingredient in Japan in the early 1960s, and at present surimi-based products are known in Asian and Western countries alike. The development of the surimi industry was based on the Alaska Pollock or walleye pollock (*Theragra chalcogramma*). The surimi production in Asian countries such as Thailand, Malaysia, Vietnam, Myanmar and Indonesia is mostly based on the demersal species such as threadfin bream (*Nemipterus* spp), big eye snapper (*Priacanthus* spp) and lizardfish (*Saurida* spp). These fish are mainly used due to their abundance as raw materials and also due to the good characteristics of their flesh for the manufacture of high-quality surimi. Due to the reduction of demersal resources, the application of freshwater fish for surimi production has been increasing. Understanding the physical, rheological and thermal properties of the muscle protein is necessary for utilization of the freshwater fish for surimi production. Tilapia as a freshwater fish has recently become an important food product and a fast growing industry in many countries. The aquaculture of red tilapia (*Oreochromis* spp) increased to approximately 1.5 metric tones in 2009 around the world (FAO, 2009). Therefore, this fish could be a good choice as it is available and sufficient in quantity for fish processing throughout the world. The low fat and white colored meat of red tilapia are significant points to make it a good resource for surimi production.

Among all the properties of surimi, gel-forming ability is the most important indicator which can determine the surimi quality. Thermal and physical including rheological behavior of surimi gel provides good information on its quality. These properties are important because of their effect on protein molecules and subsequently, texture and sensory quality of the final product (Shaviklo *et al.*, 2010). Gelation of surimi that occurs through the heating process, involves the formation of cross-linking of myofibrillar proteins mainly myosin. The integrity of the myofibrillar proteins is an indicator of the quality of the fish meat for surimi production. This property can be influenced by the fish species, harvesting condition of fish and the freshness of the fish. Changes in the integrity of the myofibrillar proteins can further change the surimi quality.

The gel-forming ability of surimi can be enhanced by the addition of ingredients such as polysaccharides, enzymes and salts. Phosphates are known additives in surimi and fish products since they enhance functional properties of surimi and surimi-based products by increasing the pH which results in increasing water binding of the gel (Chang and Regenstein, 1997; Park, 2000). Since calcium ion is necessary for TGase activity are chelated by phosphates (Benjakul *et al.*, 2006), it can be expected that the addition of calcium chloride can overcome this problem and improve the gel forming properties of surimi.

In addition, some biopolymers such as cellulose, starch and chitosan have been reported to contribute to gel-forming properties. The effect of chitosan in improving the fish and fish products properties has been shown in previous studies (Kamil *et al.*, 2002; Benjakul, 2000; Mao and Wu, 2007). In the presence of chitosan, protein-polysacharide conjugates are formed between the reactive amino group of glocusamine and glutaminyl residue of myofibrillar proteins by transglutaminase enzyme (Benjakul *et al.*, 2000).

Endogenous transglutaminase plays an important role in cross-linking of proteins at low temperature of setting process (Lanier, 2000; Hemung *et al.*, 2008). Transglutaminase catalyses the acyl transfer reaction between the carboxylamide groups of peptide-bound glutamine residues and primary amines. This reaction is essential for the gel-formation and viscoelastic attributes of surimi-based products. Industrial applications of animal TGase have been intensively studied (Traoré and Meunier, 1991; Oh *et al.*, 1993). However, the TGase amount from animal is rare and the purification procedure is complicated which made it expensive to be used in the food industry. *Streptomyces* and *Streptoverticillium* species are another source of the TGase which are Ca^{2+}-indepent enzymes and have higher activity than TGase obtained from animal sources. In addition, production of TGase in high amount at a low cost has made it suitable for application in the food industry. This Microbial TGase (MTGase) has been found as a functional additive in gel formation of surimi (Ando *et al.*, 1989) and shows a better activity than fish TGase (Hemung *et al.*, 2008). It helps to form a gel network structure to improve the gel quality of the surimi (Jiang *et al.*, 2002;

Hsieh *et al.*, 2002; Ramirez *et al.*, 2007). Thus, in this study microbial transglutaminase was used.

In this study gel-forming properties of red tilapia surimi have been identified and sodium pyrophosphate (in combination with calcium chloride), transglutaminase and chitosan in different concentrations were added to the red tilapia surimi to investigate the effect of these additives on the textural, rheological and thermal properties of the red tilapia surimi. Hence, the objectives of this research are:

1) To identify the gel-forming ability of surimi from red tilapia.

2) To determine the effect of sodium pyrophosphate (in combination with 2.5% calcium chloride), transglutaminase and chitosan on the physical, thermal and rheological properties of surimi from red tilapia.

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