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DEVELOPMENT OF FISH BALL FROM A LOW GEL-FORMING ABILITY FISH (*Sphyraena jello*) AND SEAWEED SOLUTION

SHIRLEY KUEH

THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF FOOD SCIENCE WITH HONOURS (FOOD TECHNOLOGY AND BIOPROCESS)

SCHOOL OF FOOD SCIENCE AND NUTRITION, UNIVERSITI MALAYSIA SABAH

2010

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

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ABSTRACT

This study was carried out to develop fish ball from a low gel-forming ability fish (*Sphyraena jello*) and seaweed solution. Hedonic Test was performed and the best formulation was found to contain 5% *Eucheuma denticulatum* solution (3% w/w) based on fish flesh weight. *Kappaphycus alvarezii* solution was found to be less suitable in making fish ball due to darker colour and poor springiness. Proximate analysis showed that this sample contains 72.8 ± 0.0% moisture, 2.0 ± 0.0% ash, 16.9 ± 0.1% protein, 0.4 ± 0.0% crude fat, 0.1 ± 0.0% crude fiber, and 7.8 ± 0.1% carbohydrate. Comparison was made between the selected formulation and control sample during storage (-18 °C) studies. The expressible moisture and drip loss of fish ball with 5% *Eucheuma denticulatum* solution was less ($p < 0.05$) than the control sample. Texture Profile Analysis (TPA) result showed that towards fourteen weeks of frozen storage, the control sample became harder, less springy and chewier ($p < 0.05$) than fish ball with 5% *Eucheuma denticulatum* solution. The results indicate that *Eucheuma denticulatum* solution could retain the moisture and preserve the texture of fish ball. Sensory evaluation found that after eight weeks of storage, sample without *Eucheuma denticulatum* solution was darker, fishier, harder, less springy, less juicy and less acceptable ($p < 0.05$), whereas addition of 5% *Eucheuma denticulatum* solution was able to preserve the colour and juiciness of the fish ball. The springiness and hardness of this selected sample was higher ($p < 0.05$) than the fresh control. After frozen storage for eight weeks, negligible microbial load change was observed for fish ball with 5% *Eucheuma denticulatum* solution, as indicated by minimal pH change.

ABSTRAK

PEMBANGUNAN BEBOLA IKAN DARIPADA SEJENIS IKAN BERKEUPAYAAN PENJELAN RENDAH (*Sphyraena jello*) DAN LARUTAN RUMPAI LAUT

Kajian ini telah dijalankan untuk menghasilkan bebola ikan daripada sejenis ikan yang mempunyai keupayaan penjelan yang rendah (Sphyraena jello) dan larutan rumpai laut. Ujian hedonik telah dijalankan dan formulasi terbaik didapati mengandungi 5% larutan Eucheuma denticulatum (3% b/b) berasaskan berat isi ikan. Larutan Kappaphycus alvarezii didapati kurang sesuai dalam penghasilan bebola ikan disebabkan warna yang lebih gelap dan kurang kekenyalan. Analisis proksimat menunjukkan bahawa sampel tersebut mengandungi 72.8 ± 0.0% lembapan, 2.0 ± 0.0% abu, 16.9 ± 0.1% protein, 0.4 ± 0.0% lemak kasar, 0.1 ± 0.0% serabut kasar, dan 7.8 ± 0.1% karbohidrat. Perbandingan telah dibuat di antara formulasi terpilih and sampel kawalan semasa kajian mutu simpanan (-18 °C). Kehilangan lembapan dan kehilangan titisan bebola ikan dengan 5% larutan Eucheuma denticulatum adalah kurang ($p < 0.05$) daripada sampel kawalan. Keputusan Texture Profile Analysis (TPA) menunjukkan bahawa setelah empat belas minggu penyimpanan sejukbeku, sampel kawalan menjadi lebih keras, kurang kenyal, dan lebih susah dikunyah ($p < 0.05$) daripada bebola ikan dengan 5% larutan Eucheuma denticulatum. Keputusan ini menunjukkan bahawa 5% larutan Eucheuma denticulatum dapat mengekalkan kelembapan dan tekstur bebola ikan. Penilaian sensori mendapati bahawa selepas lapan minggu simpanan, sampel tanpa larutan Eucheuma denticulatum menjadi lebih gelap, lebih berbau ikan, lebih keras, kurang kenyal, kurang kejusan dan kurang diterima ($p < 0.05$), manakala penambahan 5% larutan Eucheuma denticulatum dapat mengekalkan warna dan kejusan bebola ikan. Kekenyalan dan kekerasan sampel terpilih ini adalah lebih tinggi ($p < 0.05$) daripada sampel kawalan yang segar. Selepas lapan minggu penyimpanan sejukbeku, perubahan beban mikrobial amatlah sedikit pada bebola ikan dengan 5% larutan Eucheuma denticulatum, seperti yang ditunjukkan oleh perubahan pH yang minimal.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BIB	Balanced Incomplete Block
ED	*Eucheuma denticulatum*
FAO	Food and Agriculture Organization
KA	*Kappaphycus alvarezii*
Min.	Minimum
Max.	Maximum
PCA	Plate count agar
PDA	Potato dextrose agar
pH	potential of hydrogen
SD	Standard deviation
SE	Standard Error
SPSS	Statistical Package for Social Science
TPA	Texture Profile Analysis

LIST OF SYMBOLS

°C	Degree Celsius
%	Percentage
CFU/ g	Colony forming units per gram
cm	Centimeter
g	Gram
ι	Iota
κ	Kappa
kg	Kilogram
km	Kilometers
ml	Milliliter
mm	Millimeter
mol/ l	Mole per liter
N	Normality
ν	Nu
ppm	Parts per million
pH	Potential of hydrogen
sec	second
±	Plus minus
<	Less than
>	Greater than

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Fish has been the main supply of cheap and healthy protein to a large percentage of the world's population. In most Asian countries, especially those in Southeast Asia, fish is a main protein of the diet (Hajeb *et al.*, 2009). Other than fresh fish consumption, Southeast Asians are very familiar with a vast variety of comminuted fish products, such as fish ball, fish cake, fish finger, fish burger, and imitation products (Huda *et al.*, 2000). The basic ingredients of fish ball are fish meat, starch, salt, sugar and water.

The fish ball and fish cake industry has been reported to be growing since the early 1980s in countries such as Singapore, Malaysia, China, and Thailand. Manufacturers are also looking at the export market, especially for frozen fish balls and cuttlefish balls, to Australia, Japan, and the United States (Boran and Kose, 2007). In fact, several manufacturers in Singapore have also invested overseas. They established several fish ball and fish cake factories in Malaysia and China (Morrissey and Tan, 2000).

Fish ball production started from small family-based enterprises. In recent years, many factories have invested in modern machineries to increase output. Good quality fish ball should possess white colour, no fish smell and soft but elastic texture (Huda *et al.*, 2000). Generally, the two forms of fish meat used in fish ball making are the fresh form whereby the fish mince is unwashed, and the surimi form whereby the fish mince is subjected to washing process (Huda *et al.*, 2000). In a study conducted by Kose *et al.* (2006) to compare surimi and unwashed fish mince, it was reported that the loss of yield in surimi was caused by the washing step whereas unwashed fish mince had a higher yield. However, surimi had a longer shelf life when compared to unwashed fish mince. It was also reported that washed mince had better sensory attributes after refrigerated storage while poorer sensory attributes such as darker colour, off-odours, and loss in firmness were observed in unwashed fish mince.

In general, commercial fish balls are made from surimi whereas traditional fish balls are made from unwashed fish mince. Because sucrose and sorbitol alone or a mixture of both is added to surimi as cryoprotectants, commercial fish balls have an undesirable sweet taste. Other than having high caloric value, the excessive sweet taste may limit the consuming population of surimi-based products (Xiong *et al.*, 2009). On the contrary, sugar is not added or only added in minimal amount to traditional fish ball solely for the purpose of seasoning or flavouring. As a result, not being sweet is the advantage of traditional fish ball over commercial fish ball made from surimi. Yet, the major disadvantage of traditional fish ball is the limited storage time especially under refrigerated storage.

Although the fish ball production industry has quite a lucrative market, the industry faces some problems in terms of fish ball processing and storage of the final product. The major problem faced is that fish balls cannot be subjected to prolonged storage and the use of preservatives is not allowed. Fish balls are usually stored at a temperature of 0 to 4 °C prior to distribution. Under such temperature, fish balls made from surimi can be stored for a week (Rokiah *et al.*, 1997). Several types of deterioration may occur during storage of surimi and unwashed mince. Above all, protein denaturation and aggregation occur during frozen storage of surimi (Park and Lin, 2005). It is conceivable that cold destabilization of myofibrillar proteins, resulting from a weakening of the intramolecular hydrophobic interactions that stabilize the protein structure, would be a major factor in the instability of fish proteins. During frozen storage, formation of ice crystals lead to a redistribution of water, resulting in an interruption of the hydrogen bonding system and the exposure of hydrophobic or hydrophilic zones which favours intramolecular interaction. Consequently, protein destabilizes, leading to protein denaturation and aggregation (Carjaval *et al.*, 2005). Another type of deterioration in fish ball is microbial growth. Prolonged storage of fish ball under refrigeration will lead to the growth of bacteria and yeast. In turn, these microbiological changes bring about watery fish ball surface and bad smell (Rokiah *et al.*, 1997). Development of fishy and rancid odours from lipid oxidation is another type of deterioration in fish mince. Such deterioration is particularly serious when the fish used has a high fat content (Hutlin *et al.*, 2005).

Although illegal, some manufacturers resort to the use of boric acid or borax to preserve fish ball and prolong storage (Rokiah *et al.*, 1997; Yiu *et al.*, 2008). Generally, boric acid is used to control starch gelatinization, enhance colour, texture and flavour (Yiu *et al.*, 2008). Considering the need of fish ball manufacturers, it is necessary to look for other alternatives that are safe, cheap, effective, able to improve fish ball texture, and prolong the storage of fish ball.

In this study, Barracuda (*Sphyraena jello*), or better known by its local names such as *alu-alu*, *titir*, or *kacang-kacang* is purposely chosen as the raw material as it was reported to have a generally lower gel forming ability (Guenneugues and Morrissey, 2005). This study will investigate to what extend seaweed solution can improve the gelling properties of fish balls made from *Sphyraena jello*. This fish species is also chosen based on its availability and price. *Alu-alu* has a high landing rate in Kota Kinabalu and its price is relatively cheap when compared to some other demersal fish species. The landing of this species at Kota Kinabalu is reported to be 956.96 metric tonnes in 2008 (Fisheries Department of Sabah, 2009b). In the Kota Kinabalu fish market, small to medium size *Sphyraena jello* is usually priced between RM 4 – 7 per kilogram.

Eucheuma denticulatum and *Kappaphycus alvarezii* were used in this study as they are the main red seaweeds used for the commercial extraction of iota carrageenan and kappa carrageenan. Iota-carrageenan forms elastic and soft gels whereas kappa carrageenan forms rigid and brittle gels (McHugh, 2003; van de Velde *et al.*, 2001; Villanueva *et al.*, 2004). Clear gels of iota-carrageenan are resistant to syneresis and hysteresis (Janaswamy and Chandrasekaran, 2001), and is freeze-thaw stable (McHugh, 2003). In contrast, kappa-carrageenan exhibit some synaeresis (McHugh, 2003).

Iota-carrageenans can alter the texture and water holding properties of restructured fish products as a result of their gel-forming capacity and their ability to interact with the myofibrillar protein due to their anionic nature (Montero and Perez-Mateos, 2002). Previous study found that the water binding or moisture retention properties of iota carrageenan correlated well with the rheological properties of meat mince (Brewer, 1989; Egbert *et al.*, 1991) and fish mince products (Da Ponte et al., 1985a; Filipi and Lee, 1998; Gómez-Guillén and Montero, 1996), whether in the fresh condition or subjected to frozen storage. Meanwhile, the effects of kappa carrageenan were investigated mostly in meat systems (Campo et al., 2009; McHugh, 2003; Pietrasik and Li-Chan, 2001).

While the use of refined carrageenan in muscle food systems are widely studied, the gelling of seaweed solution or its effect in food systems are not covered much by current investigations. Seaweed solution is chosen to be used in this study in order to widen the application of seaweed without going through troublesome and costly extraction of pure carrageenan from seaweeds. Previous study by Goh (2006), Ngu (2005), and Yeo (2007) found that seaweed solution prepared from heating of seaweed powder with water possesses gelling property.

1.2 OBJECTIVES

Considering various factors related to fish ball processing and the gelling effect of seaweed solution, the specific objectives of this study are as follows:

1. To determine the best formulation for fish ball made from barracuda (*Sphyraena jello*) with the addition of most suitable seaweed solution, either *Eucheuma denticulatum* or *Kappaphycus alvarezii*.
2. To determine the proximate composition of the product.
3. To study the effect of seaweed solution on the storage of frozen fish ball based on physicohemical analysis, microbiological analysis, Texture Profile Analysis (TPA), and sensory evaluation.

CHAPTER 2

LITERATURE REVIEW

2.1 FISH BALL

Regulation 167 in the Malaysian Food Act 1983 and Regulations 1985 (2005) defines fish ball as the fish product prepared from a mixture of fish with starch, with or without condiments and vegetables, and the mixture formed into balls. Regulation 167 also states that each fish ball shall contain not less than 50% of fish and may contain permitted flavour enhancer and permitted food conditioner. This product is rich in protein and is usually marketed in the cooked or fried form (Rokiah *et al.*, 1997).

Fish ball is known by different local names such as *Bebola* in Malaysia and Brunei, *Bakso* in Indonesia, *Bola-bola* in Philippines, and *Luk-chin Pla* in Thailand. Although fish ball is quite a popular food in Southeast Asia, the quality characteristics of the product vary among countries. For instance, fish balls in Singapore and Malaysia are typically whither in colour and more elastic, while those sold in the Philippines and Hong Kong have a firmer texture but darker colour and stronger fishy note (Park, 2005b).

2.1.1 Fish Ball Market Demand

In Singapore, fish ball, the most popular surimi-based product is used in a local application, *Yong Tau Foo*. Some 4 million people consume approximately 70 tonnes fish ball/ fish cake a day, resulting in about 6 kg per capita consumption. Similarly, fish ball consumption in Thailand is quite high. The figure amounts to about 12,000 tonnes a year (Park, 2005b).

In Malaysia, there are about 27 fish ball manufacturing factories. In 1996, fish ball production of Malaysia amounts to 7,874 tonnes (Huda *et al.*, 2000). Consumer demand for fish ball has seen a significant increase over the years as fish ball is a food widely acceptable by all races in Malaysia. Variety in the uses of fish ball also contributed to the demand for this product. Other contributing factor is the growth of the restaurant industry (Rokiah *et al.*, 1997).

2.1.2 Comparison between Commercial Fish Ball and Traditional Fish Ball

Generally, commercial fish ball and traditional fish ball are the two types of fish ball available in the Malaysian market. However, commercial fish balls are more commonly found. Traditional fish balls are usually found only at certain eateries, whereby the shop or stall owners prepare the fish balls themselves. Likewise, traditional fish balls are also home made simply for household consumption.

Commercial fish ball is slightly different from local traditional fish ball as most commercial fish balls are made from surimi. Typical ingredients used for fish ball, in addition to surimi, are salt, sugar, monosodium glutamate, starch and water. Surimi is stabilized myofibrillar proteins obtained from mechanically deboned fish flesh that is washed with water and blended with cryoprotectants (Park and Lin, 2005). Figure 2.1 shows the processing flow of commercial fish ball as described by Rokiah *et al.* (1997). Meanwhile, the basic ingredient of traditional fish ball such as fish mince, starch, salt, sugar and water are usually mixed together manually, transformed into the shape of balls and then allowed to cook in boiling water.

In surimi production, large amounts of water are used to remove the sarcoplasmic proteins, blood, fat and other nitrogenous compounds from the minced flesh (Park and Lin, 2005). Since traditional fish ball production does not involve washing and dewatering steps, the processing flow is simpler. Additionally, the percentage yield after processing steps of fish mince without washing is higher when compared to surimi. Loss in the yield of surimi is attributed to the washing steps (Kose *et al.*, 2006). Yet, without the removal of water soluble impurities, the storage time of traditional fish balls is quite limited. Kose *et al.* (2006) concluded that plain mince without washing has the poorest sensory attributes such as darker colour, off odours, and loss in firmness after refrigerated storage when compared to surimi and mince produced from boiled fish.

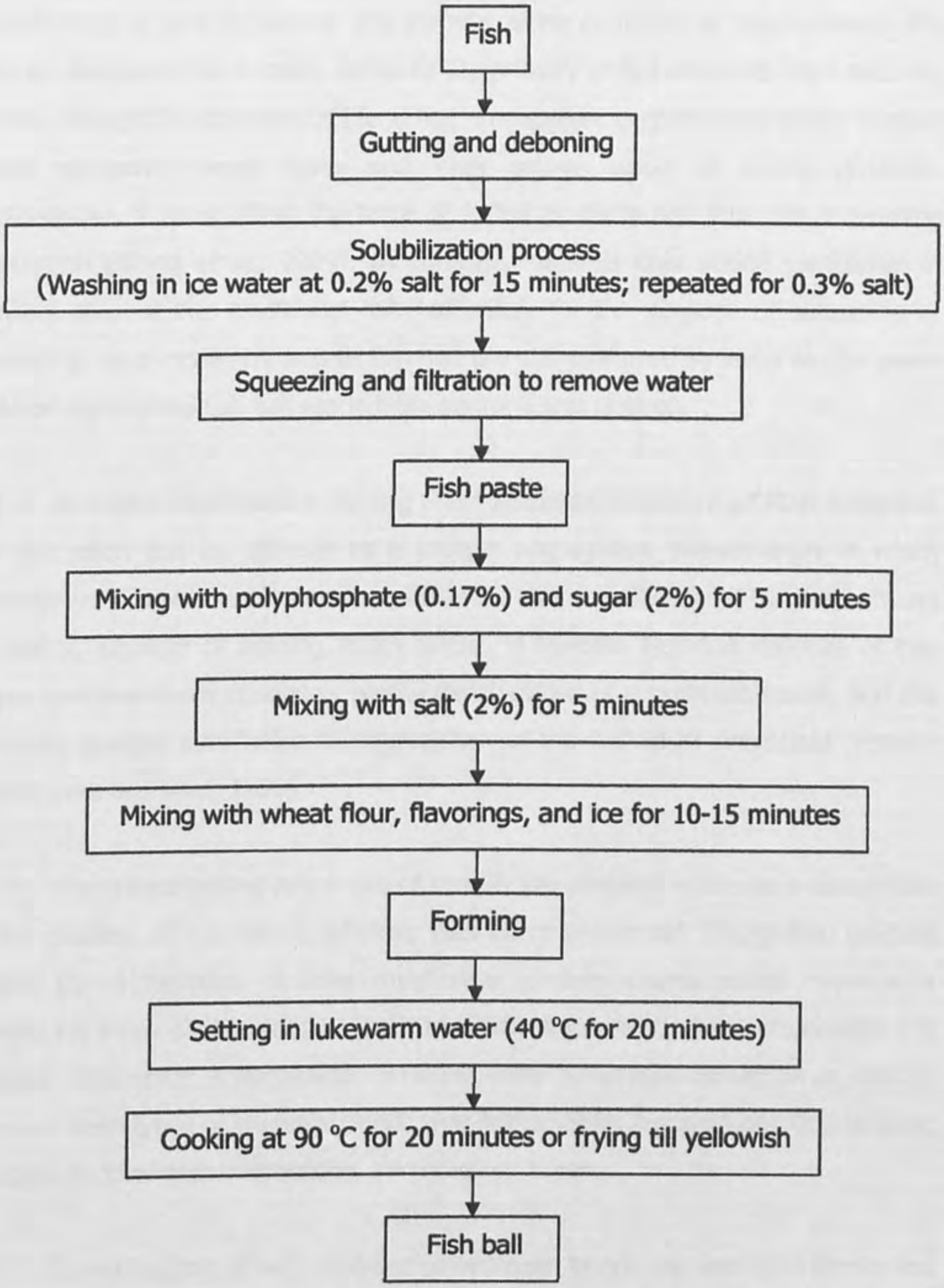

Figure 2.1: Processing flow of commercial fish ball.
Source: Rokiah *et al.* (1997).

Another distinct difference between the process flow of commercial fish ball and traditional fish ball is that no cryoprotectants such as sucrose, sorbitol, or sodium tripolyphosphate are used in traditional fish ball production. In the manufacture of surimi, sucrose and sorbitol, alone or mixed at approximately 9% w/w to dewatered fresh meat, serve as the primary cryoprotectants (Park and Lin, 2005). Though this commercial blend has an excellent cryprotective effect, it could cause excessive sweet taste and high caloric value in surimi products. Furthermore, it could affect the taste of surimi products and limits its consuming population (Xiong *et al.*, 2009). In contrast, sugar is only added sometimes in minimal amount into traditional fish ball solely for the purpose of seasoning or flavouring. As a result, traditional fish ball are still preferred by large as the sweet taste of commercial fish ball made from surimi is less desired.

2.1.3 Bonding Mechanism during Heat Induced Gelation of Fish Proteins

Gel formation can be defined as a protein aggregation phenomenon in which attractive and repulsive forces are so balanced that a well-ordered tertiary network or matrix, capable of holding much water, is formed. Gelation consists of two steps: *conformational change or partial denaturation of protein molecules, and the* following gradual association or aggregation of the individual denatured proteins (Matsumura and Mori, 1997).

The unique gelling properties of myofibrillar proteins is the main contributor to the gelation of fish mince, whether washed or unwashed. The gelling process entails the association of long myofibrillar protein chains which produce a continuous three-dimensional network in which water and other components are trapped. As a result, a viscoelastic gel is obtained (Sánchez-Gonzalez *et al.*, 2008). The four main types of chemical bonds that link proteins are hydrogen bonds, ionic linkages, hydrophobic interactions, and covalent bonds.

During heating, a large number of hydrogen bonds that maintain the folded protein structure are broken between the carbonyl and amide groups in the peptide backbone. This in turn allows the peptide backbone to become extensively hydrated and reduce the mobility of the water which it is in contact. Hydrogen bonds between proteins are more numerous when the gel is colder. As such, minced fish gels become firmer at colder temperatures (Lanier *et al.*, 2005). Hydrogen bonds between amino acids also stabilize the internal structure of individual protein molecules in water. The α-helix of native and partially denatured

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