DEVELOPMENT OF HIGH PROTEIN AND FIBRE FISH SNACKS INCORPORATED WITH COCONUT DREGS BY SINGLE-SCREW COOKING EXTRUDER

By

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Snacks are high in carbohydrates, sugar, salt, and fat but typically poor in protein and fibre content. Over-intake of high-calorie leads to a variety of health issues, including obesity and overweight, as highlighted in the National Health Surveys 2019 in Malaysia. As a result, there is a growing demand for healthy snacks, such as high-protein, high-dietary fibre, and low-fat snacks. Extrusion cooking can fulfil this need by providing a wide range of flexibility for processing healthier formulations in extruded expanded snacks. Round scad fish was chosen as the protein source because of its economic value and is under-utilised despite its high nutritional values. Coconut dregs, a by-product of the coconut milk industry, are high in dietary fibre and often used as low-value products, included in the snack formulation. Therefore, the objectives of this study were to develop extruded fish snacks with high nutritional values using different types of starch (tapioca, sago, and corn) at different percentages of starch (50, 67 and 75%), to study the effect of moisture content (13% and 18%) and addition of coconut dregs (0-20%) on the extruded fish snacks, to optimise the barrel temperature and screw speed of single screw cooking extruder on the extruded fish snacks incorporated with coconut dregs using response surface methodology (RSM) and to study the stability of optimised extruded fish snacks in three different types of packaging (polypropylene, aluminium, nylon) during accelerated shelf life study at 55 °C for 30 days. The results of the study revealed that a higher amount of starch (75%) increased the expansion ratio (2.50 ± 0.15, 2.26 ± 0.15, 2.55 ± 0.13) and fracturability (6.56 ± 0.2, 6.62 ± 0.01, 6.56 ± 0.20 N) but decreased the bulk density (221.86 ± 8.03, 187.39 ± 14.39, 198.88 ± 7.26 kgm^{-3}) and hardness of extrudates (62.17 ± 3.3, 23.52 ± 9.5, 69.54 ± 0.5 N) of tapioca, corn and sago starch extrudates, respectively. The findings indicated that the highest protein content (20.80%) was achieved with tapioca starch extrudates with 75% tapioca starch and 25% fish powder. This was followed by the inclusion of coconut dregs in the higher protein content of extruded fish snacks, which resulted in a lower expansion ratio, porosity, and crispiness but increased hardness and lightness. The addition of 20% coconut dregs contained the highest levels of total dietary fibre at feed moisture of 18%. The addition of coconut dregs increased the nutritional value of extruded fish snacks, but overall acceptance was low. Following that, the process optimisation by RSM was applied to produce high-protein and high-fibre extruded fish snacks with great potential for consumer acceptability in terms of hardness and fracturability properties. The optimal extrusion processing was achieved at 135 rpm for the screw speed and 180 °C for the barrel temperature. According to the hedonic score, consumers scored moderately like on the optimised high-protein and high-fibre extruded fish snacks incorporated with coconut dregs. For storage purposes, the physical and microbiological properties of high-protein and high-fibre extruded fish snacks incorporated with coconut dregs were stable during the 30 days of storage at accelerated temperature (55 ºC) in polypropylene, aluminium and nylon packaging, respectively. However, the peroxide value, which indicates the oxidation process, was significantly higher in polypropylene packaging than in nylon and aluminium packaging. The quality of the high-protein and high-fibre extruded fish snacks were maintained in the aluminium packaging for 5.6 months at an accelerated storage (55 ºC). In conclusion, this study may serve as a reference for the production of nutritious extruded snacks from fish and coconut dregs in the future. In addition, the study promoted usage of nutritious but underutilized round scad fish and low-value coconut dregs into a high value food product that will potentially help to boost consumption of healthy snacks among Malaysians.

Keywords: Coconut dregs, Extruded snacks, Fibre, Fish, Protein, RSM, Shelf-life, Single screw extruder,

SDG: GOAL 2 (Zero Hunger); GOAL 3 (Good Health and Well-being); GOAL 12 (Responsible Consumption and Production)

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PEMBANGUNAN SNEK IKAN TINGGI PROTEIN DAN SERAT YANG DITAMBAH DENGAN HAMPAS KELAPA MENGGUNAKAN TEKNIK PENYEMPERITAN

Oleh

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Snek mengandungi karbohidrat, gula, garam dan lemak yang tinggi tetapi lazimnya kurang kandungan protein dan serat. Pengambilan berlebihan kalori tinggi membawa kepada pelbagai isu kesihatan, termasuk obesiti dan berat badan berlebihan, seperti yang diketengahkan dalam Tinjauan Kesihatan Kebangsaan 2019 di Malaysia. Akibatnya, terdapat permintaan yang semakin meningkat untuk snek sihat, seperti protein tinggi, serat makanan tinggi dan snek rendah lemak. Memasak penyemperitan boleh memenuhi keperluan ini dengan menyediakan pelbagai fleksibiliti untuk memproses formulasi yang lebih sihat dalam snek kembang yang tersemperit. Ikan sardin dipilih sebagai sumber protein kerana nilai ekonominya dan kurang digunakan walaupun nilai pemakanannya tinggi. Hampas kelapa, hasil sampingan industri santan, mengandungi serat makanan yang tinggi dan sering digunakan sebagai produk bernilai rendah, termasuk dalam formulasi makanan ringan. Oleh itu, objektif kajian ini adalah untuk membangunkan snek ikan tersemperit dengan nilai pemakanan yang tinggi menggunakan pelbagai jenis kanji (tapioka, sagu, dan jagung) pada peratusan kanji yang berbeza (50, 67 dan 75%), untuk mengkaji kesan kelembapan. kandungan (13% dan 18%) dan penambahan hampas kelapa (0-20%) pada snek ikan tersemperit, untuk mengoptimumkan suhu tong dan kelajuan skru penyemperit masak skru tunggal pada snek ikan tersemperit yang digabungkan dengan hampas kelapa menggunakan permukaan tindak balas metodologi (RSM) dan untuk mengkaji kestabilan snek ikan tersemperit yang dioptimumkan dalam tiga jenis pembungkusan berbeza (polipropilena, aluminium, nilon) semasa kajian jangka hayat dipercepatkan pada 55 °C selama 30 hari. Hasil kajian menunjukkan bahawa jumlah kanji yang lebih tinggi (75%) meningkatkan nisbah pengembangan (2.50 ± 0.15, 2.26 ± 0.15, 2.55 ± 0.13) dan kebolehpatahan (6.56 ± 0.2, 6.62 ± 0.01, 6.56 ±) tetapi menurunkan ketumpatan pukal (221.86 ± 8.03, 187.39 ± 14.39, 198.88 ± 7.26 kgm-3) dan kekerasan extrudates (62.17 ± 3.3, 23.52 ± 9.5, 69.54 ± 0.5 N). Dapatan kajian menunjukkan bahawa kandungan protein tertinggi (20.80%) dicapai dengan penyemperit kanji ubi kayu dengan 75% kanji ubi kayu dan 25% serbuk ikan. Ini diikuti dengan kemasukan ampas kelapa dalam kandungan protein yang lebih tinggi bagi snek ikan tersemperit, yang menghasilkan nisbah pengembangan, keliangan, dan kerangupan yang lebih rendah tetapi meningkatkan kekerasan dan ringan. Penambahan 20% hampas kelapa mengandungi jumlah serat pemakanan tertinggi pada kelembapan makanan sebanyak 18%. Penambahan hampas kelapa meningkatkan nilai pemakanan snek ikan tersemperit, tetapi penerimaan keseluruhan adalah rendah. Berikutan itu, pengoptimuman proses oleh RSM digunakan untuk menghasilkan snek ikan tersemperit berprotein tinggi dan serat tinggi yang berpotensi besar untuk penerimaan pengguna dari segi sifat kekerasan dan kebolehpatahan. Pemprosesan penyemperitan optimum dicapai pada 135 rpm untuk kelajuan skru dan 180 °C untuk suhu penyemprit. Mengikut skor hedonik, pengguna mendapat markah sederhana seperti pada snek ikan tersemperit tinggi protein dan serat tinggi yang digabungkan dengan hampas kelapa. Untuk tujuan penyimpanan, sifat fizikal dan mikrobiologi snek ikan tersemperit berprotein tinggi dan serat tinggi yang digabungkan dengan hampas kelapa adalah stabil sepanjang 30 hari penyimpanan pada suhu dipercepatkan (55 ºC) dalam bungkusan polipropilena, aluminium dan nilon. Walau bagaimanapun, nilai peroksida, yang menunjukkan proses pengoksidaan, adalah lebih tinggi dengan ketara dalam pembungkusan polipropilena berbanding pembungkusan nilon dan aluminium. Kualiti snek ikan tersemperit protein tinggi dan serat tinggi dikekalkan dalam pembungkusan aluminium selama 5.6 bulan pada penyimpanan dipercepatkan (55 ºC). Kesimpulannya, kajian ini boleh menjadi rujukan kepada penghasilan snek tersemperit berkhasiat daripada ikan dan hampas kelapa pada masa hadapan. Di samping itu, kajian itu menggalakkan penggunaan ikan sardin yang berkhasiat tetapi kurang digunakan dan hampas kelapa bernilai rendah menjadi produk makanan bernilai tinggi yang berpotensi membantu meningkatkan pengambilan makanan ringan yang sihat di kalangan rakyat Malaysia.

Kata kunci: Hampas kelapa, Ikan, Jangka hayat, Snek tersemperit, Protein, Serat, RSM, Penyemperit skru tunggal

SDG: *GOAL 2 (Zero Hunger); GOAL 3 (Good Health and Well-being); GOAL 12 (Responsible Consumption and Production)*

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LIST OF ABBREVIATIONS

*a**	Redness
a_w	Water activity
*b**	Yellowness
ANOVA	Analysis of variance
d.b	Dry basis
g/cm^3	Gram per cubic centimeter
*L**	Lightness
m/s	Meter per second
min	Minute
mL	Milliliter
mg	Milligram
mm	Millimeter
PP	Polypropylene packaging
RPM	Rotations per minute
w/v	Weight by volume
WAI	Water absorption index
w/w	Weight by weight
WSI	Water solubility index
%	Percentage
°C	Degree Celsius

AL	Aluminium packaging
NY	Nylon packaging

CHAPTER 1

INTRODUCTION

1.1 Background

In most societies, people usually obtain their energy and nutrient requirements mainly from three planned meals (breakfast, lunch and dinner), which are often consumed with family, friends and colleagues at relatively predictable times and dedicated places (Bellisle, 2014). Moreover, the fast and increasing pace of life initiates the wide availability of convenience foods, especially in the era of modernisation and globalisation. Items that are palatable, nutritious, affordable, capable of being consumed quickly and preferably while being engaged in other activities, are desired by consumers (Mattes, 2018). The fast-paced lifestyle and increasing availability of convenience foods give rise to snacking behaviour.

The growth in snacking, particularly high fat, high sugar and salty snacks, also known as energy-dense foods, has been seen as a contributing factor to poor diet quality as compared to nutrient-rich foods, which are defined as foods with a high content of vitamins and minerals, such as vegetables, fruit, lean meat, and fish (Arenas-Jal et al., 2020; Domínguez Díaz et al., 2020; Alt et al., 2022). Snacks are typically consumed between main meals as a way to curb hunger and provide energy and nutrients to the body. Chaisuwan et al. (2019) reported that Malaysia had imported snacks foods at 65,729 tons in 2015 valued US$268 million at 10% growth rate per annum.

According to a survey by Nielsen (2014), over 80% of Malaysian consumers reported snacking at least once a day, with the most popular snack categories being biscuits, crackers, cakes, and snacks. Initiatives to improve the nutritional content of snack products was highlighted and showed demand for more nutritious options (Delcour & Poutanen, 2013; Arenas-Jal et al., 2020; Goh et al., 2020). Fish crackers continue to be a well-liked snack in Malaysia. Fish crackers are an excellent source of protein, particularly in the Malaysian state along the eastern coast (Nor et al., 2014). Fish crackers, also known as "keropok" in Malaysia, "kerupok" in Indonesia, “kaew krab pla” in Thailand, and “banh phong tom” in Vietnam, are a typical Southeast Asian snack food (Abdul Rashed et al., 2021)

Expanding tiny starch pellets is a common method for making commercial fish crackers (Chudasama et al., 2019). Fish cracker is made by mixing starch, minced fish, and water to make a dough, slices about 3 to 4 mm thick and dried in an oven. Then, the pellets can currently be prepared by frying them in oil, which could lead crackers to overly fatty, or by inflating them full of air (Chudasama et al., 2019). As stated in the Malaysia Food Act 1983 and Regulations 1985, fish crackers must be made using fish, starch, and a minimum of 12% protein content (basic mixture) (Food Act 1983 & Regulation, 1985). However, the high oil and saturated fat content of deep-fried foods has some negative impacts, including an increased risk of coronary heart disease, cancer, diabetes, and high blood pressure (Setyawan et al., 2013).

Beside fish crackers, other snacks available in market are in the form of extruded snacks whereas these snacks more focused on starch-rich products which are generally being nutrient dense (Stojceska et al., 2010; Hirth et al., 2014; Korkerd et al., 2016; Arribas et al., 2019; Beech et al., 2022). Extruded snacks in particular have attracted consumer attention due to their convenience and taste (Brennan et al., 2013; Nor et al., 2013; Yadav et al., 2018; Proserpio et al., 2020). Young people particularly favour these food products (Shah et al., 2019). However, extruded snacks are normally high in starch and dense in energy but low in nutritional content in terms of vitamins, proteins, and fibre as reviewed by Grasso (2020). Therefore, initiatives to improve the nutritional content of snack products was highlighted and showed demand for more nutritious options (Delcour & Poutanen, 2013; Arenas-Jal et al., 2020; Goh et al., 2020). Nutritious snacks are typically high in nutrients that are beneficial such as protein, fibre, vitamins, and minerals. In between meals, these snacks can give sustained energy, satiety, and essential nutrients (Marangoni et al., 2019).

Protein is a critical macronutrient for maintenance of normal bodily functions. The required daily protein intake varies by age, sex, and degree of daily activity (Akonor et al., 2021; Putra et al., 2021). It is essential for growth, repair, and maintenance of health. Low intake of animal-sourced proteins during late pregnancy is believed to be associated with low birth weight (Lonnie & Johnstone, 2020; Putra et al., 2021). Thus, a small quantity of fish can significantly improve the quality of dietary protein intake by complementing the essential amino acids compared to most terrestrial meats (Abraha et al., 2018). Hence, the use of round scad fish (*Decapterus madruasi*) to produce fish extruded snacks can be advantageous and the cost is lower compared to other fish species with a reasonable quantity of protein content (17-19 %) (LKIM, 2017).

Meanwhile, dietary fibre is comprised of plant-based carbohydrates that cannot be metabolised by digestive enzymes encoded in the human genome, such as amylase (Augustin et al., 2020; Cronin et al., 2021) and have many benefits on health (Lockyer et al., 2016; Li & Komarek, 2017; Daud et al., 2018; Gill et al., 2021). However, before dietary fibre was recognised as having nutritional importance, sources of fibre was formerly thought to be an indigestible component that could be quantified and labelled as "crude fibre." It was defined as the plant-based food residue left following extraction with solvent, dilute acid, and dilute alkali (Dai & Chau, 2017). Crude fibre is composed of digestion-resistant plant cell structural components such as lignin, pentosan, chitin, cellulose and others (Wahab et al., 2022).

To increase dietary fibre, by-product of food industry or agricultural waste that are presumed as low value can be fully utilized. Among many foods niche, the coconut milk industry produces ~41.5% of coconut meat press cake or coconut dregs after coconut milk is extracted. In Malaysia, the application of coconut dregs has never been documented for any meaningful purposes besides being discarded, though sometimes coconut dregs is fed to animal (Ng et al., 2010; Sulaiman et al., 2013). Therefore, the objective of this study was to develop a fish snack incorporated with coconut dregs using a single screw cooking extruder followed by optimising the process parameters (barrel temperature and screw speed) to produce snacks that were palatable and rich in protein and fibre. Furthermore, the storage stability of prepared fish snacks will be examined in this study.

1.2 Problem Statement

Extruded snacks, known for their convenience and taste, have predominantly focused on starch-rich products, which are generally nutrient-dense, as highlighted in studies by (Stojceska et al., 2010; Hirth et al., 2014; Korkerd et al., 2016; Arribas et al., 2019; Beech et al., 2022) while also attracting consumer's attention especially young people (Brennan et al., 2013; Nor et al., 2013; Yadav et al. 2018; Shah et al., 2019 and Proserpio et al., 2020). However, the National Health Survey in Malaysia have highlighted the rapid rise of obese adults, which can be an alarming problem that is growing among children (NHMS, 2019). Therefore, snacking options for Malaysian should be diversified to introduce healthier snacks to avoid further health problems associated with extruded snack food. The addition source of protein to feed material has been proven to be an effective way to increase the protein content of extruded snacks.

Several studies have investigated the utilisation of plant-based proteins to improve the protein content of extruded snacks, including chickpea, lentil, bean, soy, and pea proteins (Estrada-Girón et al., 2015; Singh et al., 2015; Olusegun et al., 2016; Elina et al., 2016; Philipp et al., 2017; Beck et al., 2018; Chan et al., 2019; Wang et al., 2019; Adeleye et al., 2020; Altaf et al., 2021). Additionally, several studies have looked into the addition of whey protein to extrudate snacks as reviewed by Day & Swanson (2013), Obradović et al. (2014); Rehal et al. (2017) and Mosibo et al. (2022). In addition, there are studies that have added fish as a source of protein (Singh et al., 2014; Goes et al., 2015; Ali et al., 2016, Pandi et al., 2019; Nkubana et al., 2020) because animal protein content is higher than plant protein. However, the addition of protein normally affects the appearance, texture, and taste of the extruded snacks. Starchy materials such as tapioca starch and corn starch may be used to improve the physical of the extrudate snacks incorporated with protein (Patel et al., 2016; Peksa et al., 2016; Fang et al., 2019; Syed et al., 2019; Téllez-Morales et al., 2022).

The low consumption of fibre-rich foods is a significant issue (NHMS, 2019), which is mostly due to the tastes and costs related to importing vegetables (Oo et al., 2021). To overcome this issue, researchers have investigated the use of food by-product, such as peels, husks, seeds, pomace, dregs, and stems, as a source of fibre in extruded products as reviewed by Borah et al. (2019), Offiah et al. (2019), Grasso (2020) and Leonard et al. (2020). The authors came to the conclusion that this strategy reduced the environmental impact of these by-products, as well as that extrusion processing offers an attractive way to incorporate their nutritional value into food systems (Alam et al., 2015; Rashid et al., 2015; Alam et al., 2016; Borah et al., 2016; Masli et al., 2018; Singha et al., 2018; Liu et al., 2019; Ribeiro Oliveira et al., 2020).

In Malaysia, coconut dregs from coconut milk production have a big potential for increasing the nutritional value of extruded snacks as dietary fibre (Rosni et al., 2020). However, an in-depth investigation needs to be done regarding the significant proportion of coconut dregs in extruded snacks to produce desired physicochemical properties of extruded snacks. Besides, feed moisture is also a crucial parameter, as the combination of high fibre and protein materials in extruded formulations will most likely affect the extrusion process behaviour as well as the extrudate's physical characteristic (Shelar & Gaikwad, 2019; Mazlan et al., 2020; Jabeen et al., 2022).

During extrusion processing, the quality of the finished product is dependent on numerous processing variables such as temperature, pressure, feed rate, and screw speed. Likewise, type of ingredients and the amount of moisture also have significant impact on quality of finished product (F. ul H. Shah et al., 2019). However, many studies on the optimisation of high-protein, high-fibre extruded snacks by twin screw extruder have been conducted compared to single screw extruder (Beck et al., 2018; Arribas et al., 2019; Liu et al., 2018; Nadeesha Dilrukshi et al., 2022; Shah et al., 2022). Therefore, these process parameters can be optimised by single screw extruder in order to investigate the effect of process parameter in production of nutritious extruded snacks that are well accepted by consumers.

Another critical issue to handle is during the storage of these products. These products are prone to physicochemical or microbial degradation if stored for prolonged time (Yadav et al., 2018a) especially when incorporated with fishery-derived ingredients mainly having an issue with lipid oxidation and fat rancidity. Light, oxygen, heat, heavy metals, the degree of unsaturation in lipids, and alkaline environments are some of the factors that promote oxidation (Shahmohammadi et al., 2016). Therefore, it must be kept in packaging that is adequate and resistant to moisture, gases, and light for a product to have a longer shelf life (Coradini et al., 2021).

Previous studies have already explored storage conditions and type of packaging to assure product quality during storage (Shaviklo et al., 2011; Alam et al., 2015; Dar et al., 2016; Ali et al., 2016; Sahu et al., 2018; Chikkanna et. al., 2020). Although a few studies on the shelf-life of the improvement extruded snacks in food quality have been reported (Shaviklo et al., 2011; Alam et al., 2015; Dar et al., 2016; Ali et al., 2016; Sahu et al., 2018; Chikkanna et. al., 2020), the storage stabilities of high protein, high fibre of fish extruded snacks incorporated with coconut dregs have not been investigated.

1.3 Hypothesis

H1: The type and amount of starch used in feed material have a considerable impact on the physicochemical properties of extruded fish snacks.
H2: The presence of coconut dregs and variations in feed moisture has significant effects on the physicochemical properties of extruded fish snacks.
H3: There is a substantial relation between barrel temperature and screw speed on the quality of extruded fish snacks by using coconut dregs, and response surface methods

can be utilised to discover the optimal combination of barrel temperature and screw speed.
H4: Different packing materials significantly affect the physicochemical and microbiological characteristics of extruded fish snacks incorporated with coconut dregs during an accelerated shelf-life study.

1.4 Objectives

i. To determine the effect of different types and percentages of starch (tapioca, sago, and corn) on the physicochemical properties of extrudates from the powder of round scad fish (*Decaptus madruasi*).
ii. To examine the impact of coconut dregs and feed moisture at various concentrations on the structural and functional qualities of extruded fish snacks.
iii. To optimise barrel temperature and screw speed of the extruder on fish snacks incorporated with coconut dregs by response surface methodology (RSM).
iv. To analyse the effect of different packagings on the physicochemical properties and microbiological qualities of extruded fish snacks throughout accelerated shelf life.

1.5 Thesis outline

Thesis is organised as follows:

Chapter 2 presents a review of snacks, types of snack processing and factors affecting extrusion. The raw materials involved in the production of extruded fish snacks and previous studies on the addition of coconut dregs in food applications are also discussed. Application of RSM to determine the optimal extrusion condition and shelf-life study of extruded snacks are also highlighted.

Chapter 3 describes the development of extruded fish snacks by using three types of starches at different levels that are commonly used in the production of extruded expanded snacks. The results on physical properties, protein and fat content, and acceptance of extruded fish snacks are discussed.

Chapter 4 investigates the effect of coconut dregs and variations in feed moisture on the physicochemical properties of extruded fish snacks. The chapter also presents and discusses results on the microstructure and sensory acceptance of extruded fish snacks incorporated with coconut dregs.

Chapter 5 presents the methodology and analysis for optimising the barrel temperature and screw speed of extruder on fish snacks incorporated with coconut dregs through

RSM. Furthermore, the chapter describes the composition, microstructure, and sensory acceptance of optimised high-protein, high-fibre extruded fish snacks.
Chapter 6 investigates and discusses the effects of various packaging methods on the physicochemical and microbiological qualities of extruded fish snacks throughout their accelerated shelf life.

Chapter 7 finally summarises and concludes the research in this study. Future works are also suggested at the end of this chapter.
The thesis outline is structured as shown in Figure 1.1, with the flow summarised as follows:

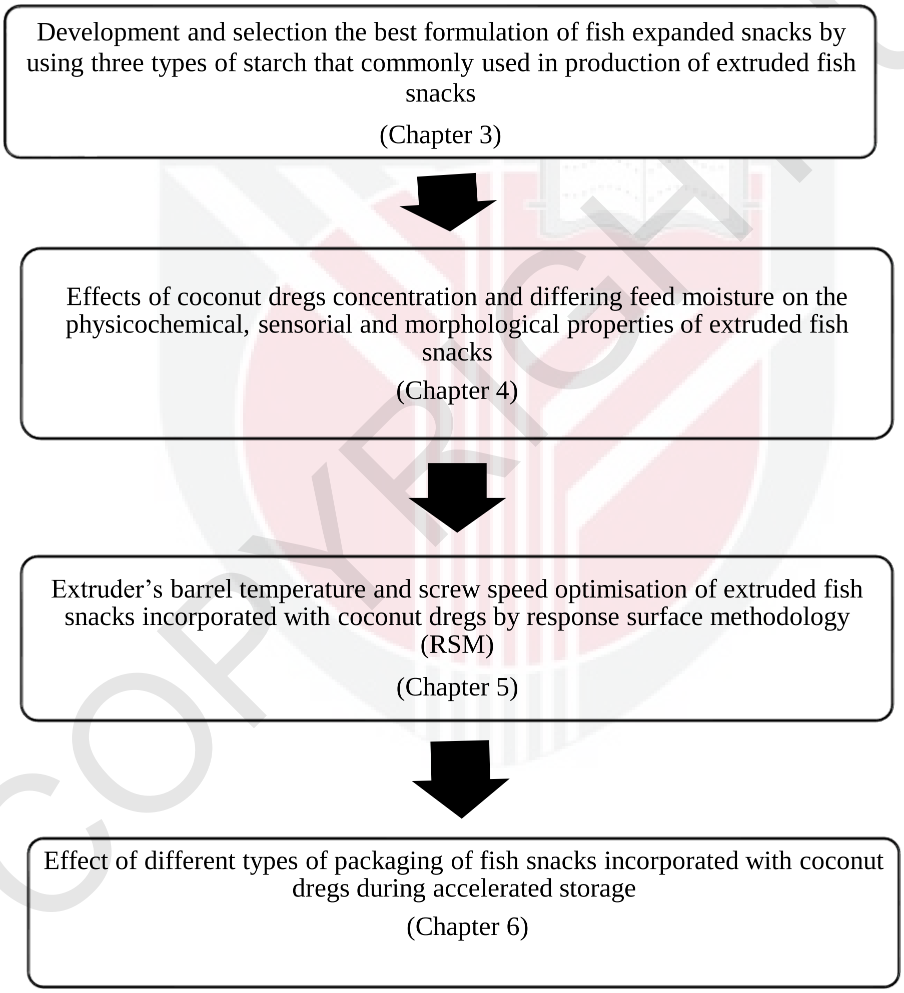

Figure 1.1: Flow diagram of thesis outline

1.6 Scope of study

The overall experiment flow is structured as shown in Figure 1.2 which illustrates the necessary research activities to develop high protein and high fibre extruded fish snacks. The first purpose of this study was to investigate the effect of different type of starch (tapioca, corn and sago) at different percentages (50, 67, 75%) on the physicochemical properties of extrudates from surimi powder of round scad fish (*Decaptus madruasi*). Following that, the effect of difference percentage of coconut dregs (0-20%) at different feed moisture (13 and 18%) on the high protein extruded snacks were examined. The third purpose was to optimise the processing parameter (barrel temperature and screw speed) of extrusion by applying RSM approach on the developed high protein and high fibre extruded fish snacks based on the results of the first and second objective. Based on the results of the third objective, the composition, sensory acceptance, purchase intention and morphology of optimized high protein and high fibre fish extruded snacks was discussed. The last objective was to analyse the effect of different packaging (polypropylene, aluminium and nylon) on the physicochemical properties and microbiological qualities of optimized high protein and high fibre extruded fish snacks during storage at accelerated temperature (55 °C) for 30 days.

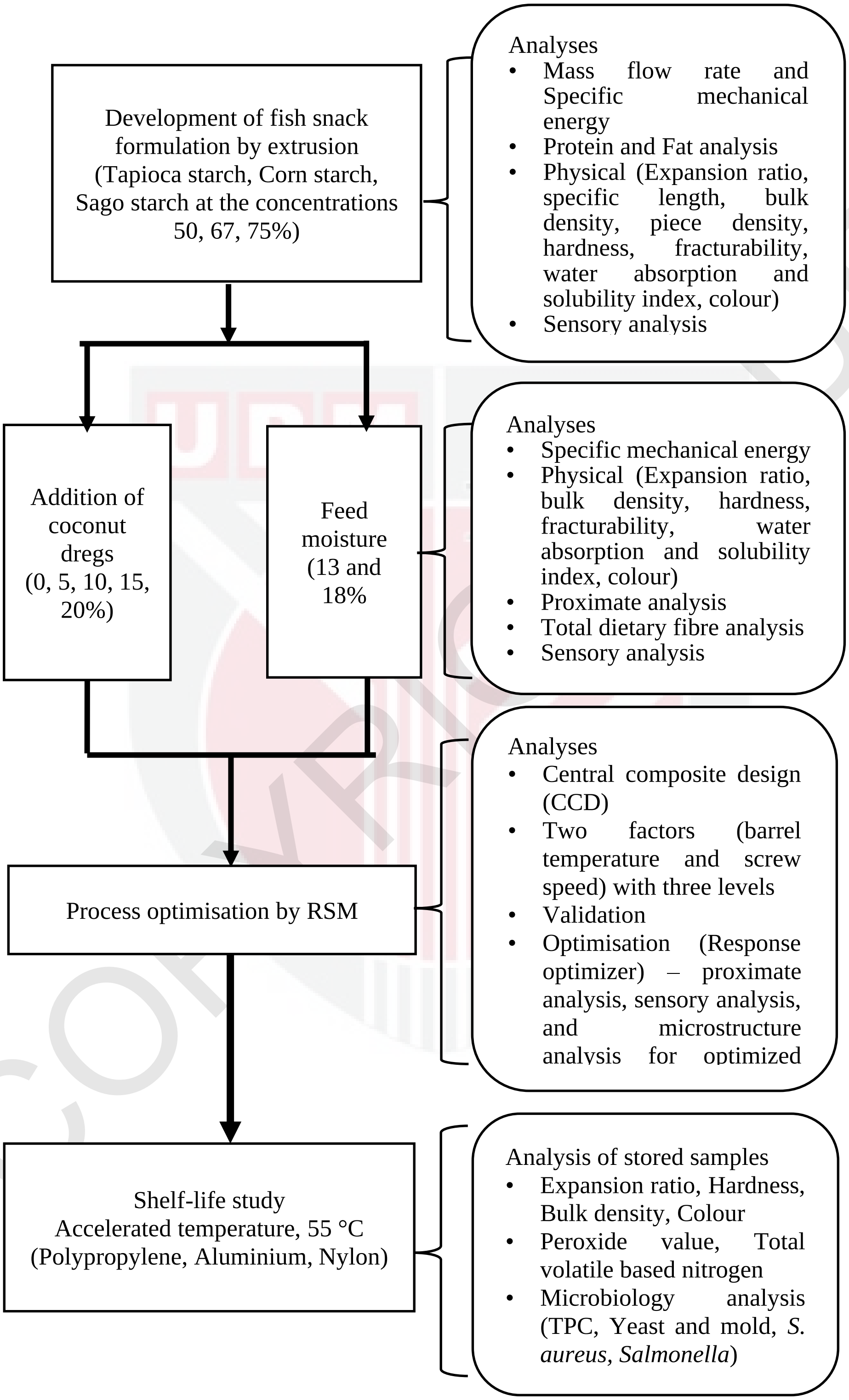

Figure 1.2: Flow diagram of overall experiment.

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