PHYSICOCHEMICAL PROPERTIES OF MODIFIED CASSAVA FLOUR FORTIFIED WITH MUNG BEAN PROTEIN ISOLATE AND ITS UTILIZATION IN BREADMAKING

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FACULTY OF FOOD SCIENCE AND NUTRITION
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ABSTRACT

Cassava (*Manihot esculenta Crantz*) is one of the most important staple crops worldwide. However, cassava flour has major drawback of its poor protein content. Studies have shown that the additions of other ingredients like legumes flour and protein concentrate could improve the physicochemical properties of cassava flour. Hence, this study was designed to investigate the physicochemical properties of modified cassava flour (MCF) fortified with protein isolate (PI) from mung bean and fermented mung bean and the effect of the PI substitution on physicochemical properties of bread. Amylolytic enzymes were used to modify the starch content and functional attributes of MCF, which was then fortified with PI at different percentage (10%, 20%, 30%) and were labelled as T1 - T6. The ratio of MCF and Mung bean PI (MPI), were 90:10 (T1), 80:20 (T2) and 70:30 (T3) respectively, and ratio of MCF and fermented mung bean PI (FMPI) similar to ratio of MCF + MPI and labelled as T4 - T6. Fermentation of mung bean was carried out by using 1% of *Aspergillus oryzae* under solid state fermentation and protein isolates (PI) was extracted using alkaline extraction-isoelectric precipitation. The analyses for physical, chemical and functional properties of PI and flour blends were performed and native cassava flour was used as control. Morphological characterization using scanning electron microscopy revealed that the MCF had rough surface and open cracked granules due to enzymatic treatment. The study showed that FMPI contained significantly ($p < 0.05$) higher protein (89.48%) and hydrophobic amino acids as compared to MPI. Apart from the nutritional properties, FMPI also possessed better functional properties than MPI like oil absorption capacity (3.30 ml/g), foaming capacity (30% at pH 4) and *In-Vitro* protein digestibility (95.08%) that plays an important role in food formulation and processing. Increasing MPI or FMPI in MCF flour blends resulted in significant ($p < 0.05$) increase in protein content (8.12 – 26.96%) with T6 shown the highest protein content. Sample T6 also possessed better functional properties like oil absorption capacity and pasting properties compared to the control flour. The study showed that nutritional values of MPI and FMPI fortified breads were better in nutritional values compared to the control bread; where the fortified breads had significantly ($p < 0.05$) lower moisture (27.28 – 29.39%) and carbohydrate (38.68 – 55.50%) content. Meanwhile, the crude protein of the fortified breads was significantly ($p < 0.05$) higher than the control bread. The texture profile analysis of breads showed that sample F4 had significantly ($p < 0.05$) higher cohesiveness and springiness and significantly ($p < 0.05$) lower specific density, firmness and chewiness compared to control bread. The estimated glycemic index of breads was ranged from 51.18 - 64.57. The overall results indicated that the addition of PI in MCF improved the physicochemical properties of the flour and the quality of bread.

ABSTRAK

SIFAT-SIFAT FIZIKOKIMIA TEPUNG UBI KAYU TERUBAHSUAI DIPERKUAT DENGAN PROTEIN ISOLAT DARIPADA KACANG HIJAU DAN PENGGUNAANNYA DALAM PEMBUATAN ROTI

Ubi kayu (Manihot esculenta Crantz) merupakan salah satu tanaman ruji yang penting di seluruh dunia. Walau bagaimanapun, tepung ubi kayu mempunyai kelemahan utama kerana kandungan proteinnya yang rendah. Kajian-kajian lepas menunjukkan penambahan bahan-bahan lain seperti tepung kekacang dan protein isolat dapat memperbaiki sifat-sifat fizikokimia tepung ubi kayu. Oleh itu, kajian ini direka untuk mengkaji sifat-sifat fizikokimia tepung ubi kayu terubahsuai yang diperkuat dengan protein isolat daripada kacang hijau dan kacang hijau terfermentasi. Enzim-enzim amilolitik telah digunakan untuk mengubahsuai kandungan kanji dan sifat-sifat kefungsian tepung ubi kayu, yang kemudiannya diperkuat dengan protein isolat pada peratusan yang berbeza (10%, 20%, 30%) dan dilabelkan sebagai T1 - T6. Nisbah MCF dan protein isolat kacang hijau (MPI) masing-masing adalah 90:10 (T1), 80:20 (T2) dan 70:30 (T3), dan nisbah MCF dan protein isolat kacang hijau terfermentasi sama seperti nisbah MCF + MPI dan dilabelkan sebagai T4 - T6. Fermentasi kacang hijau telah dilakukan dengan menggunakan 1% kultur Aspergillus oryzae di bawah fermentasi keadaan pepejal dan protein isolat (PI) telah diekstrak dengan menggunakan pengekstrakan alkali-pemendakan isoelektrik. Analisis-analisis bagi ciri fizikal, kimia dan kefungsian PI dan tepung telah dijalankan dan tepung ubi kayu digunakan sebagai tepung kawalan. Pencirian morfologi menggunakan mikroskopi pengimbasan elektron mendapati bahawa tepung ubi kayu terubahsuai mempunyai granul dengan permukaan yang kasar dan retakan yang terbuka disebabkan oleh rawatan enzimatik. Kajian ini menunjukkan FMPI mempunyai kandungan protein (89.48%) dan asid amino hidrofobik yang lebih tinggi dengan signifikan ($p < 0.05$) berbanding MPI. Selain kandungan nutrisi yang bagus, FMPI juga mempunyai ciri-ciri kefungsian yang lebih baik daripada MPI seperti kapasiti penyerapan minyak (3.30 ml/g), kapasiti pembentukan buih (30% pada pH 4) dan kebolehadaman protein secara In-Vitro (95.08%) yang memainkan peranan penting dalam pemformulasian dan pemprosesan makanan. Peningkatan kandungan MPI atau FMPI dalam adunan tepung MCF mengakibatkan peningkatan bagi kandungan protein (8.12 - 26.96%) secara signifikan ($p < 0.05$) dengan sampel T6 mempunyai kandungan protein tertinggi. Sampel T6 juga mempunyai ciri-ciri kefungsian yang lebih baik seperti kapasiti penyerapan minyak dan sifat pempesan berbanding tepung kawalan. Kajian ini menunjukkan bahawa nilai nutrisi roti diperkuat dengan MPI dan FMPI adalah lebih bagus berbanding dengan roti kawalan; di mana roti diperkuat dengan MPI dan FMPI mempunyai kandungan kelembapan (27.28 - 29.39%) dan karbohidrat (38.68 - 55.50%) yang lebih rendah secara signifikan ($p < 0.05$). Manakala, kandungan protein kasar bagi roti-roti yang diperkuatkan adalah lebih tinggi secara signifikan ($p < 0.05$) berbanding roti kawalan. Hasil analisis profil tekstur pada roti-roti menunjukkan sampel F4 mempunyai nilai keanjalan dan kepaduan yang lebih tinggi secara signifikan ($p < 0.05$) dan mempunyai nilai ketumpatan spesifik, kekerasan dan kekenyalan yang lebih rendah secara signifikan ($p < 0.05$). Indeks glisemik anggaran yang diperoleh adalah antara 51.18 - 64.57. Secara keseluruhan, keputusan yang diperoleh menunjukkan bahawa penambahan PI dalam MCF telah menambahbaik sifat-sifat fizikokimia tepung dan kualiti roti.

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AA	-	Amino acids
AM	-	Amylase
AMG	-	Amyloglucosidase
BL	-	Baking loss
Ca	-	Calcium
CF	-	Cassava flour
Cu	-	Copper
EA	-	Emulsion capacity
EAAs	-	Essential amino acids
ES	-	Emulsion stability
FC	-	Foaming capacity
Fe	-	Iron
FMPI	-	Fermented mung bean protein isolate
FS	-	Foaming stability
GI	-	Glycemic index
HCl	-	Hydrochloric acid
HI	-	Hydrolysis index
IVPD	-	In-Vitro protein dgestibility
K	-	Potassium
MCF	-	Modified cassava flour
Mg	-	Magnesium
MPI	-	Mung bean protein isolate
Na	-	Sodium
NaOH	-	Sodium hydroxide
OAC	-	Oil absorption capacity
OAI	-	Oil absorption index
P	-	Phosphorus
PI	-	Protein isolate
PS	-	Protein solubility
RS	-	Resistant starch
RVA	-	Rapid viscosity analyzer
RVU	-	Rapid viscosity unit
SD	-	Specific density
SEM	-	Scanning electron microscopy

SP	-	Swelling power
SV	-	Specific volume
TCA	-	Trichloroacetic acid
TTA	-	Titratable acidity
TPA	-	Texture profile analysis
WAC	-	Water absorption capacity
WAI	-	Water absorption index
Zn	-	Zinc

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Wheat flour is the main component of most production of confectionary products, cake and bread worldwide. The suitability of wheat in the production of confectionary products is as a result of good intrinsic viscoelastic properties of the gluten in wheat (Ibitoye *et al.*, 2013). It also contains desirable nutritional contents like 78.10% carbohydrates, 14.7% protein, 2.10% fat, 2.10% minerals, and significant amounts of vitamins such as thiamine and vitamin B (Okpala & Egwu, 2015). Wheat only can be cultivate in developed country such as Europe and China (FAO, 2017). Unfortunately, many tropical countries like Malaysia cannot grow wheat because of the climate and have to import wheat to sustain the demand of wheat-based diets. Wheat ranked third of staple food worldwide after rice and maize. According to United states department of Agriculture (2017) Malaysia imported 1.7 million tons of wheat flour in 2015-2016 and Australia is the main exporter of wheat to Malaysia (USDA, 2017). Wheat price imported to Malaysia expected to increase due to depreciation of Malaysian currency. Increases demand for quality bread and bakery goods with rising living standards among Malaysian urbanites, wheat imports to Malaysia expected to grow rapidly.

In the bid to lower the import of wheat to Malaysia, promoting composite flour may helpful in production of aerated products such as cake, bread and biscuit. Noorfarahzilah *et al.*, (2014) defined composite flour as a blend of flours, starches and other ingredients with wheat flour or wholly non- wheat blends of flour, starches and other ingredients for bakery and pastry products. Composite flour is considered advantageous in developing countries as it encourages the use of locally grown crops as flour (Hugo *et al.*, 2000). Satin’s report (as cited in Noorfarahzilah *et al.* 2014) mentioned, the Food and Health Organization in 1964 had initiated the concept of composite technology by targeted to reduce the cost of support for temperate countries by encouraging the use of indigenous local crops such as cassava, yam, maize and others in partial substitution of wheat flour.

Cassava root (*Manihot esculenta*) is a tropical root crop, widely cultivated in 102 countries of the world (FAO, 2005). Cassava roots play an important role as staple food for more than 500 million people in the world. In Malaysia, cassava root is third rank of tuber crops after white potato and sweet potato (Tan & Zaharah, 2015). Cassava roots are good sources of carbohydrates. Cassava roots have high starch content about 60-80% and it is therefore an excellent source of dietary carbohydrate (Akingbala *et al.*, 2011). According to Tan and Khatijah (2000), cassava is planted in Malaysia mainly for starch processing. However, an alternative uses of cassava show increasing in demand from production of snack foods such as oil-fried crisp and crackers.

High quality cassava flour has been identified as a local alternative to substitute wheat flour partially in composite flours (Akingbala *et al.*, 2011). Incorporating of high protein containing crops such as cowpea and soybean as a mean of protein enrichment can improve protein content of cassava (Muoki *et al.,* 2012). In fact, cassava- based composite flour have been developed using several combination of legumes and beans such as cassava- cowpea (Jisha *et al.,* 2008), cassava- soybean (Bankole *et al.*, 2013; Tharise *et al.*, 2014).

Gluten-free products were initially designed for people who have celiac disease. Celiac disease is a gluten protein disorder where intestinal digestive function unable to tolerate gluten found in food. So far, the only effective treatment for celiac disease is to use a diet free of proteins with adverse effects on the intestinal mucosa (Holtmeier & Caspary, 2006). Today, there is an increasing number of people interested in wheat-free foods motivated by health concerns but also by the desire to avoid wheat in the diet (Nachay, 2010). In order to develop gluten-free breads for celiac patients, a number of alternative flour types to wheat flour such as corn, cassava and rice been used (Demirkesen *et al.,* 2010). Gluten-free products especially from starch-based formulations usually have low nutritional value. Therefore, several studies have been done on the possibility to enrich gluten-free bread with certain nutrients, such as protein and minerals (Korus *et al.*, 2012; Marco & Rosell, 2008a). Among components which can be used to increase protein content in gluten free-bread formulations are from legume flours, protein isolates and protein concentrate (Crockett *et al.,* 2011; Marco & Rosell, 2008b; Ribotta *et al.*, 2004).

Legumes are considered as poor man's meat. This phrase described as legumes play an important role in providing nutrient (protein) in human especially for low income groups of people in developing country (Tharanathan & Mahadevamma, 2003). Due to the high cost of proteins from animal origin and their inaccessibility by the poor people, the uses of legumes are significance because the price of legumes are cheaper and legumes are rich of protein for dietary sources (Tharanathan & Mahadevamma, 2003).

Legumes are used in a variety of food preparations such as combination with cereals, soups, extruded products, ready-to eat snacks and have been used in the preparation and development of bakery products (Miñarro *et al.,* 2012). Mung bean (*Vigna radiata*) is a genus from famili of Fabaceae. Like other legumes, mung bean is an excellent source of high quality proteins, fiber and several other nutrients. Interest of protein in legumes are increase due to nutritional value content and also good functional properties that play an important role in food formulation and processing (Boye *et al.*, 2010; Dakia *et al.,* 2007). Several studies have been reported about protein isolate from legumes, including faba bean, chickpea, red bean, cowpea, kidney bean and mung bean (Boye *et al.*, 2010; Brishti *et al.*, 2017; Kudre *et al.*, 2013; Shevkani *et al.*, 2015; Tang & Ma, 2009).

Main purpose of protein isolation is separating the protein from other components in such a form that they are able to retain their functionality (Udensi & Okoronkwo, 2006). Protein isolation widely has been study over decades for various purposes especially in food research and development. Protein isolate possessed higher crude protein contents, had improved nutritional and functional properties, effectively used in the formulation of foods and were relatively free of anti-nutrients compared with the original flours (Giami & Isichei, 1999). Production of purified vegetable protein is gaining commercial demands due to the consumer preferences for vegetable sources of food and cosmetic ingredients (Kaur & Singh, 2007). At present, there is an increasing interest on the characterization of protein from mung bean as it has been shown to perform many desirable functions in processed foods, such as water absorption capacity, oil absorption capacity, foaming capacity and stability and emulsification capacity and stability (Brishti *et al.*, 2017; Yi-shen *et al.,* 2018). These findings demonstrated the properties of protein isolate from mung bean can improve the functionality of food processing applications; for example, high water absorption capacity in mung bean protein isolate may render food products brittle and dry to prolong food shelf life (Boye *et al.*, 2010).

1.2 Problem statements

Cassava root (*Manihot esculenta*) ranked fourth as most important food source of carbohydrate in the tropics after rice, maize, and sugar cane. Despite sometimes being stigmatized as a "third world crop", cassava has been the focus of research over decades in a few key areas due to the importance of cassava as a food of the poor and its potential as a commodity in the wider economy (Blagbrough *et al.*, 2010). The cassava crop possesses an outstanding ability to grow in the unfavourable environments of poor soil fertility and even able to grow during drought thus this increases its importance (Nassar, 2001). However, some draw backs limit cassava root utilization including the presence of toxic cyanogenic glycosides and low protein content. Cassava roots have a relatively low content of protein compared to other staple food such as maize and wheat. Cassava roots also are generally lack in important micronutrients such as vitamin A (carotenoid), iron and zinc leading to deficiency of these essential components on cassava-based diets population. Supplementation via capsules or the fortifications of basic foodstuffs are the conventional strategies to encounter these deficiencies.

Cassava as one of the popular tuber crop have been used as substitute of wheat flour in bread making (Giami *et al.*, 2004). Several studies have been made by using cassava as composite flour in making of bread, cake and biscuit (Eddy *et al.*, 2007; Eduardo *et al.*, 2013; Eriksson *et al.*, 2014b; Jensen *et al.*, 2015; Khalil *et al.*, 2000; Oluwamukomi *et al.*, 2011). However, several problems are still linked with the use of cassava flour either partial or total substitution of wheat flour in bread making. Generally, increasing amount of cassava flour in bread making reduces the quality of bread due to the reduction of viscoelastic properties from gluten protein of composite flour dough (Khalil *et al.*, 2000). Besides that, difference in the composition of the starch fraction in cassava compared to wheat flour and limited amylase activity in cassava contributed to poorer baking quality (Aryee *et al.,* 2006; Mejía-Agüero *et al.*, 2012).

Despite having high starch content, cassava flour has limited variations in starch structure and properties. Amylose and amylopectin play crucial compositional and functional roles in cassava starch, influencing properties such as crystallinity, gelatinization, retrogradation, gelling and pasting. Cassava root contained longer chain of amylopectin with about 5–6% branches and amylopectin structure-property correlation analysis showed that the proportion of longer chains resulted high in pasting viscosity and breakdown viscosity (Charles *et al.,* 2005). These are properties not suitable in production of bread and cake as they caused firm and compact product (Defloor *et al.*, 1995). Previous studies have shown that the amylolytic enzymes shorten the external side chains of amylopectin and result in a lower pasting viscosity (Marc *et al.,* 2002; Martínez *et al.,* 2015). Thus, enzymatic modification of cassava flour can diversify the structure and functionality to suit in food applications.

Mung bean is an excellent source of vitamins, mineral and protein with essential amino acid profile comparable with soybean and kidney bean. However, anti-nutritional factors and impairment of flavor limit the incorporation of legumes like mung bean in food applications. Higher percentage of legumes flour used in bread making also increase the beany flavor (Maforimbo *et al.*, 2006). Mung bean has been utilized mainly as whole seeds and sprouts. However, the utilization of mung bean in isolates or concentrates form, has gained increasing attention (Li *et al.*, 2010). Protein isolates exhibited higher protein content, satisfactory functional properties and lower anti-nutritional factors compared to flour basis. Fortification of cassava flour with protein isolate might attribute to better physicochemical properties and lead to favorable opportunity in bread making.

Application of fermentation in processing of some cereals and legumes used in complementary food had shown to increase on consumer acceptance, nutritional composition, functional properties, reduce their anti-nutrients, and increase the safety of those products (Onwurafor *et al.*, 2014). The chemical composition and functional properties of protein isolate from other fermented legume and nut had been studied such as soy bean (Hong *et al.*, 2004) and peanut (Yu *et al.,* 2007). Previous study shown an increase of nutritional properties and increase the potential of functional properties of fermented protein isolate compared to native protein isolate.

1.3 Significance of study

In the last decade, there are studies on physicochemical properties of composite flour from cassava flour and legumes (Bankole *et al.,* 2013; Melini *et al.*, 2017; Miñarro *et al.*, 2012). Those researches demonstrated better nutritional quality in composite flour. However, there are limitations in the quality and organoleptic acceptability of food that produces from that composite flour. This is important to study the effect of legumes protein in cassava flour to explore the potential physicochemical properties of flour blends. However, in the last decades, studies on physicochemical of cassava flour fortified with legume protein isolate are limited. Since cassava roots are common tuber and mung beans are easy to obtain in Malaysia, the potential of the nutritional, physical and functional properties of cassava flour fortified with protein isolate from Mung bean should be further explored to utilize the application of flour blends in food systems especially in bread, biscuit and cake. Several studies recorded potential application of protein isolate in wheat flour (Ugwuona & Suwaba, 2013), maize flour (Ziobro *et al.,* 2013) and rice flour (Shevkani *et al.*, 2015) in bakery product. However, no information regarding the effect of incorporation of mung bean protein isolates with cassava flour on the physicochemical properties of flour blends and quality characteristics of bread are available till date. Thus, this research is consider as a good opportunity to explore new potential of incorporation of mung bean protein isolate and cassava flour in order to optimize the physicochemical properties and the potential use in bread making.