CHARACTERISATION OF BARIO RICE FLOUR AND ITS POTENTIAL APPLICATION IN THE DEVELOPMENT OF GLUTEN-FREE BREAD

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FACULTY OF FOOD SCIENCE AND NUTRITION
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ABSTRACT

Gluten-free grains have been intensively studied as alternatives to wheat flour. Bario rice (*Oryza sativa* L.), an indigenous crop from Sarawak, Malaysia, is noted for its excellent aroma and taste. These cultivars were obtained from Bario Highland, Sarawak (3°44′6″N 115°28′45″E). In this research, Bario rice was ground to produce rice flour which was characterised by its nutritional, phytochemical and antioxidant properties, physical and functional properties, and the best rice flour was utilised in the development of gluten-free bread with the utilisation of rice flour between 70% to 100% complemented with potato starch. Four Bario rice varieties were analysed against the control sample (TQR rice) consisting of non-pigmented and pigmented rice, namely Bario *Adan Halus* (white) and Bario *Tuan* (brown), Bario *Celum* (black), also Bario *Merah Sederhana* (red), respectively. The results revealed that pigmented Bario rice flour contained significantly higher ($p<0.05$) ash, crude fat, and crude fibre content than non-pigmented. The crude protein content, for all rice flour samples, was in the range of 6.89% to 9.43%. Bario *Tuan* and Bario *Merah Sederhana* showed the lowest ($p<0.05$) carbohydrate content among all. The mineral content of Bario rice flour was significantly greater ($p<0.05$) in magnesium, potassium, and zinc content compared to the control. All Bario rice flours had high amylose content (26.67% - 36.52%), positively impacting loaf volume. Pigmented Bario rice flour exhibited greater phytochemical characteristics based on total phenolic content and total flavonoid content and also indicated higher ($p<0.05$) antioxidant properties in 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity compared to non-pigmented. The swelling capacity was significantly ($p<0.05$) high in non-pigmented rice flour. The foaming capacity of Bario *Tuan* (5.59%) was comparable with the control sample (5.63%), which this parameter generally depends on the solubility of the protein. The least gelation concentration ranged from 10% to 20%, and Bario *Merah Sederhana* exhibited the lowest value, 10% which implies a better gelling capacity. The final gelatinisation temperature of Bario *Tuan* (84.45 °C) and Bario *Merah Sederhana* (84.38 °C) were higher among all samples, and gelatinisation temperature has a directly proportional relationship to the specific volume of bread. Bario *Merah Sederhana* was chosen to be utilised in gluten-free bread development, against wheat bread. The ash and crude fibre content were generally higher in gluten-free bread, in contrast, lower in carbohydrate content compared to wheat bread. The texture profile analysis of gluten-free dough and bread appeared to be softer in F4 (70% rice flour with 30% potato starch). The sensory evaluation showed the F4 received the highest overall acceptability among gluten-free bread. In conclusion, due to its physical and functional, nutritional, phytochemicals and antioxidants, Bario rice flour, especially the pigmented types, has shown potential as a gluten-free flour alternative in the production of gluten-free bread. Besides, the addition of potato starch to rice flour increased the texture and general acceptance of gluten-free bread especially by 30%. As a recommendation, future studies might investigate the amino acid profiles of Bario rice varieties which could provide further insight into its functional behaviour in product development.

ABSTRAK

CIRI-CIRI TEPUNG BERAS BARIO DAN POTENSI PEMBANGUNAN DALAM ROTI BEBAS GLUTEN

Bijirin bebas gluten telah dikaji secara intensif sebagai alternatif kepada tepung gandum. Beras Bario (Oryza sativa L.), tanaman asli dari Sarawak, Malaysia, terkenal dengan aroma dan rasa yang sangat unik. Kultivar ini diperoleh dari Bario Highland, Sarawak (3°44'6"U 115°28'45"T). Dalam kajian ini, beras Bario dikisar untuk menghasilkan tepung beras yang dicirikan oleh sifat pemakanan, fitokimia dan antioksidan, sifat fizikal dan berfungsi, seterusnya tepung beras terbaik digunakan dalam pembangunan roti bebas gluten dengan penggunaan tepung beras antara 70% hingga 100% dilengkapi dengan kanji ubi kentang. Empat varieti beras Bario dianalisis dan dibandingkan dengan sampel kawalan (beras TQR) yang terdiri daripada beras tidak berpigmen dan berpigmen iaitu Bario Adan Halus (putih), Bario Tuan (perang), Bario Celum (hitam), Bario Merah Sederhana (merah), masing-masing. Keputusan menunjukkan bahawa tepung beras Bario berpigmen mengandungi lebih tinggi ($p<0.05$) kandungan abu, lemak kasar, dan kandungan serat kasar berbanding tidak berpigmen. Kandungan protein kasar, untuk semua sampel tepung beras, adala 6.89% hingga 9.43%. Bario Tuan dan Bario Merah Sederhana menunjukkan kandungan karbohidrat yang paling rendah ($p<0.05$) antara kesemua sampel. Kandungan mineral tepung beras Bario adalah jauh lebih besar ($p<0.05$) terutamanya magnesium, kalium, dan zink berbanding dengan sampel kawalan. Semua tepung beras Bario mempunyai kandungan amilosa yang tinggi (26.67% - 36.52%), memberi kesan positif kepada isipadu roti. Tepung beras Bario berpigmen menunjukkan ciri fitokimia yang lebih tinggi berdasarkan jumlah kandungan fenolik dan jumlah kandungan flavonoid serta menunjukkan sifat antioksidan yang lebih tinggi ($p<0.05$) dalam aktiviti antioksidan 2,2-diphenyl-1-picrylhydrazyl (DPPH) berbandingtidak berpigmen. Kapasiti pembengkakan menunjukkan perbezaan ketara yang tinggi ($p<0.05$) dalam tepung beras tidak berpigmen. Kapasiti pembusaan Bario Tuan (5.59%) adalah setanding dengan sampel kawalan (5.63%), yang mana parameter ini secara amnya bergantung kepada keterlarutan protein. Nilai ujian kepekatan gelasi paling terendah adalah antara 10% hingga 20%, dan Bario Merah Sederhana menunjukkan nilai paling rendah, 10% yang menunjukkan kapasiti pembentukan gel terbaik. Suhu gelatinisasi akhir Bario Tuan (84.45 °C) dan Bario Merah Sederhana (84.38 °C) adalah lebih tinggi berbanding semua sampel, dan suhu gelatinisasi akhir mempunyai hubungan yang berkadar terus dengan isipadu spesifik roti. Bario Merah Sederhana dipilih untuk digunakan dalam pembangunan roti bebas gluten, roti gandum sebagai sampel kawalan. Kandungan abu dan serat kasar umumnya lebih tinggi dalam roti bebas gluten, sebaliknya, kandungan karbohidrat lebih rendah berbanding roti gandum. Analisis profil tekstur doh dan roti bebas gluten lebih lembut pada F4 (70% tepung beras dengan 30% kanji ubi kentang). Penilaian deria menunjukkan F4 menerima penerimaan keseluruhan tertinggi dalam kalangan roti bebas gluten. Kesimpulannya, berdasarkan sifat fizikal dan berfungsi, sifat pemakanan, fitokimia dan antioksidan, tepung beras Bario, terutamanya jenis berpigmen, telah menunjukkan potensi sebagai alternatif tepung bebas gluten dalam penghasilan roti bebas gluten. Selain itu, penambahan kanji ubi kentang kepada tepung beras meningkatkan tekstur dan penerimaan umum roti bebas gluten. Sebagai cadangan, kajian masa depan dalam profil asid amino varieti beras Bario adalah dicadangkan untuk dianalisis untuk mendapatkan pemahaman lebih mendalam tentang sifat berfungsi tepung beras Bario dalam pembangunan produk.

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LIST OF ABBREVIATIONS

USD	-	United Stated Dollar
MOA	-	Ministry of Agriculture
NRV	-	Nutrient References Value
GI	-	Glycemic Index
HPMC	-	Hydroxypropylmethylcellulose
HAT	-	Hydrogen Atom Transfer
SET	-	Single Electron Transfer
CMC	-	Carboxymethylcellulose
MC	-	Methylcellulose
TPC	-	Total phenolic content
TFC	-	Total flavonoid content
AOAC	-	Association of Official Analytical Chemists
ICPMS	-	Inductively Coupled Plasma Mass Spectrometry
GOPOD	-	Glucose Peroxide
DMSO	-	Dimethyl Sulphoxide
Con A	-	Concavalin A
DPPH	-	2,2-Diphenyl-1-picrylhydrazyl
GAE	-	Gallic Acid Equivalent
QE	-	Quercetin Equivalent
IC_{50}	-	Half maximal inhibitory concentration
FSMP	-	Faculty of Food Science and Nutrition
CIE	-	Commission on Illumination
SPSS	-	Statistical Packages for Social Sciences
ANOVA	-	One-way Analysis of Variance
USDA	-	United States Department of Agriculture
WSI	-	Water Solubility Index
EC	-	Emulsion Capacity
LGC	-	Least Gelation Concentration
PS	-	Potato Starch
RF	-	Rice Flour
TPA	-	Texture Profile Analyser

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Rice (*Oryza sativa* L.) is a significantly consumed cereal after wheat and a crucial staple diet that supports approximately half of the world's population. Rice contributes 80% of the overall calorie intake in a regular Asian diet since it is typically consumed as a whole grain after cooking (Kim & Shin, 2014; Chaudhari *et al.,* 2018). According to Omar *et al.* (2019), the world's five biggest rice manufacturers are also the world's five biggest rice consumers: China, India, Indonesia, Bangladesh, and Vietnam. Nevertheless, the same authors reported that most region's top exporters are countries such as Thailand, Vietnam, and Cambodia, which contributed to 24.5%, 12.9%, and 1.3% of the world's total rice export, respectively whereas, countries such as Indonesia, Malaysia and Philippines are net importers. Apart from that, instead of consuming rice as cooked grains, rice can also be further processed into rice flour and utilised in food industries as well as food services.

Over the past years, the application of gluten-free grains has been studied extensively to substitute wheat flour to develop gluten-free products (Cappa *et al.,* 2016; Gomes *et al.,* 2016; Bolarinwa *et al.,* 2018). Rice flour is one of the essential raw materials for gluten-free bread due to its global availability and cost-effectiveness (Yano *et al.,* 2017; Feizollahi *et al.,* 2018). Besides, it possesses the properties of being easily digested and absorbed by the human body. Moreover, it is well-known for its hypoallergenic properties, colourless, and a natural flavour, which rarely imparts negative results to the end products (Feizollahi *et al.,* 2018; Wu *et al.,* 2019). However, current trends in the application of rice flour emphasise the utilisation of pigmented rice flour rather than white rice flour (Kraithong *et al.*,

2019; Tangsrianugul *et al.*, 2019; Devi & Badwaik, 2022). The utilisation of pigmented rice flour is prudent owing to its beneficial effects on human health (Devi & Badwaik, 2022). Rice flour is widely used in food products in Malaysia, not only in the noodle production industry but also in the preparation of traditional *kuih muih*. In general, gluten-free products are usually developed as a valuable alternative for people with gluten-related disorders. In reality, the only successful treatment to avoid gluten-related disorders is to remove any gluten sources from their daily diet (Diez-Sampedro *et al.,* 2019). Gluten-related disorders can be described as a detrimental response to gluten protein facilitated by the immune system (Dunne *et al.,* 2020). In general, there are two familiar diseases related to exposure to gluten-containing products, known as celiac disease and IgE-mediated wheat allergy (Cabrera-Chávez *et al.,* 2016).

In recent years, the interest in gluten-free products has risen dramatically among researchers and consumers (Cappelli *et al.*, 2020). According to Region and Segment Forecasts (2020), food manufacturers encountered a tremendous upsurge between 2016 to 2019, in which the sale pattern of gluten-free items increased from USD 5.15 billion to 21.61 billion, respectively estimated to grow at 9.2% from 2020 to 2027. In general, the increment of demand for a gluten-free diet is contributed by gluten-disorder patients and people without celiac disease diagnosis, especially women (Littlejohns *et al.,* 2021). In addition, the gluten-free followers perceived that gluten restriction drives a healthy life, which can help in weight reduction prevention of diseases such as irritable bowel syndrome and inflammation (Lebwohl *et al.,* 2015). On the other hand, the benefits of antioxidants and phytochemicals also play an essential role in food. Antioxidants can prevent diseases such as cancer, inflammatory disorders, and atherosclerosis as well as delay ageing (Thorat *et al.,* 2013). The high nutritional content and antioxidant capabilities have led to a recent surge in interest in consuming pigmented rice (Umesha *et al.*, 2015; Klunklin & Savage, 2018) across Europe and the United States as well as Asian countries.

Bario rice is a native crop in Malaysia that can become essential in the future, known for its excellent aroma, taste, and soft texture (Nicholas *et al.,* 2014). Bario rice represents its roots, Bario highland in Sarawak (Teo, 2006). Bario rice is grown using the conventional method and is devoid of artificial fertilisers (Thomas *et al.,* 2014a). There are four varieties of Bario rice, Bario *Adan Halus,* Bario *Tuan*, Bario *Celum* and Bario *Merah Sederhana*. It has been remarked that Bario *Adan Halus* is generally classified as white rice; in contrast, Bario *Tuan* is brownish, Bario *Merah Sederhana,* and Bario *Celum* are red and black, respectively (Nicholas *et al.,* 2014). In general, due to the excellent eating quality of Bario rice, these varieties are potentially used in the development of gluten-free products. Moreover, pigmented Bario rice has been revealed to exhibit higher total phenolic content than non-pigmented Bario rice (Sharma & Lee, 2016). However, the speciality of these varieties remains poorly understood, which in turn limits their long-term marketability (Khazanah Research Institute, 2018).

Currently, there is limited scientific research on the nutritional content as well as antioxidant and phytochemical characteristics of these cultivars. In addition, there has been no prior research on the functional properties also their potential application in any food product, which is one of the most important variables for understanding the behaviour of flour for future product development. Therefore, the objectives of this research are to analyse the characteristics of four Bario rice flour consisting of Bario *Adan Halus*, Bario *Tuan*, Bario *Celum*, and Bario *Merah Sederhana* to provide information on their chemical properties specifically nutritional profiles, phytochemicals and antioxidants, physical and functional properties as well as the potential application of Bario rice flour in the development of gluten-free rice bread.

1.2 Problem Statement

Gluten-free products are more expensive than their counterparts because almost all gluten-free products in the current market are imported from overseas. The price varies depending on the currency rates of the Malaysian Ringgit (Fauad *et al.*, 2020). In conjunction with this issue, Malaysia should enhance the manufacture of

gluten-free food products, particularly those made using local resources. As a result, the import rate of gluten-free items will decrease, resulting in a reduction in the cost of gluten-free products. Nevertheless, Bario rice varieties are potential local raw ingredients known for their remarkable eating quality. Therefore, this research has chosen Bario rice as the main ingredient in gluten-free bread due to its distinctiveness.

Furthermore, gluten-related disorders had risen over the past few years, especially from 2012 to 2017, which subsequently increased the demand for gluten-free products (Naqash *et al.,* 2017; Xhakollari *et al.,* 2019). In Malaysia, the gluten-related disorder is generally unknown due to being underdiagnosed, and it could be a much more significant problem in Malaysia than previously thought (Yap *et al.,* 2015). Generally, rice flour is beneficial due to its broad application in product development, substituting wheat flour. Typically, rice flour is used as raw material to produce bakery products (bread, biscuits, and cakes) and noodles (rice vermicelli, *kway teow* and *laksa* noodles) (Rosniyana, 2013). In respect to that, the substitution of wheat flour with rice flour will benefit consumers with gluten intolerance disorders. Bario rice varieties can be seen as one of the potential local ingredients contributing to gluten-free products. Thus, due to the good nutritional background and eating quality of Bario rice, these varieties have a high possibility to be utilised in rice-based food products.

Besides, the exclusion and restriction of gluten from daily intake have been dramatically increased due to consumers' awareness of healthier food options and healthy lifestyles (Lerner *et al.*, 2019). For instance, most consumers believed that a gluten-free diet was generally good for health (Croall *et al.,* 2019; Woomer & Adedeji, 2020). Whereas in Malaysia, consumers preference tends to choose rice with high quality and also rice-based food products, in correlation to the increased household income as well as live standards owing to awareness of the importance of healthy food (MOA, 2011), which will deliver better chances for Bario rice varieties to be incorporated in food products. Hence, to fulfil the consumer's demand, a study on the raw materials should be conducted to know the functional and nutritional profiles used as the reference for future product development. Besides, functional and nutritional characteristics are important as a medium for

marketing to provide information for health-conscious consumers (Henrita *et al.,* 2015).

In addition, the interest in food products produced with locally-based ingredients has been increasing (Mudau *et al.,* 2021). The reason is that majority of consumers believe that purchasing local food or food incorporated with local produces may support the local farming community and small business, capable of being an essential component of the region's promotion as well as improving the development of local economies (Żakowska-Biemans *et al.,* 2017). However, the variation of products incorporated with local raw materials is still limited in the market. Hence, due to Bario rice varieties being perceived as healthy and possessing uniqueness in their eating quality, these crops potentially be incorporated into gluten-free products. As a consequence, this will increase the market exposure and recognition of Bario rice for its speciality.

1.3 Importance of Study

Essentially, this research is vital to explore potential local produce like Bario rice varieties that can be incorporated into gluten-free bread development. In respect to that, utilising rice in the development of gluten-free bread will help diversify the rice-based food products in the current market. Nevertheless, this research also potentially contributes to a more comprehensive understanding of the distinctiveness characteristics of Bario rice varieties, not only in the aspects of nutritional values, phytochemical and antioxidant properties but as well as functional properties of Bario rice flour and its potential utilisation in the development of gluten-free bread. In conjunction, scientific knowledge provided at the end of this research might benefit future references in product development or even other research fields.

Aside from that, instead of contributing to the development of gluten-free products with local produce, the demand for gluten-free food for people with gluten-related disorders and health-conscious consumers can be fulfilled. In this case, the sales pattern of gluten-free products can be expanded, especially products that involve the utilisation of local produce as ingredients. Finally, the most crucial part is the market exposure of Bario rice will grow, consequently raising economic income, especially in the agriculture sector in Sarawak, and local farmers have chances to generate higher revenue. Thus, by completing this research, the characterisation of Bario rice flour and the potential application of Bario rice flour in the development of gluten-free bread can be actualised, which will help to diversify rice-based products in the market, contribute scientific research on Bario rice varieties, fulfilled demand of targeted consumers, and rise the market exposure of these crops.

1.4 Objectives

This research project investigates four Bario rice flour made from Bario *Adan Halus*, Bario *Tuan*, Bario *Merah Sederhana* and Bario *Celum.* The objectives of this research are as follows:

1. To investigate the chemical properties (nutritional compositions and, phytochemicals and antioxidant properties) of Bario rice flour.

2. To determine the physical and functional properties of Bario rice flour.

3. To investigate the potential application of Bario rice flour in the development of gluten-free rice bread.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice

Rice belongs to the family of Poaceae (Gramineae), the tribe of Oryzeae, and belongs to the *Oryza* genus (Chauhan *et al.,* 2017). However, the *Oryza* genus can be divided into four species complexes: *sativa* complex*, officinalis* complex, *meyeriana* complex and *ridley* complex (Khush, 2005). Under the *sativa* complex, there are two cultivated species, namely *O. sativa,* also known as the Asian rice and *O. glaberrima* steud, grown worldwide and in a few African countries, respectively (Chauhan *et al.,* 2017). Apart from that, the other species under *Oryza* complexes are all wild types. In general, wild types gained less focus from domestication due to the significant drawback, mainly on lodging and grain shattering (Callaway, 2014). Table 2.1 summarises the taxonomy of rice.

Table 2.1: Taxonomy of rice

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Poales
Family	Gramineae or Poaceae
Tribe	Oryzeae
Genus	*Oryza*
Species	*Sativa*

Source: Panesar & Kaur (2016)

Asian countries are known for the dominant rice production worldwide (Gadal *et al.,* 2019). In general, three geographical subregions in Asia involved in the production of rice, specifically East Asia (China, Japan, and Korea), Southeast Asia (Burma, Indonesia, Laos, Kampuchea, Malaysia, Philippines, Thailand, and Vietnam), and South Asia (Bangladesh, Sri Lanka, India, Nepal, and Pakistan)