THE EFFECT OF RICE BRAN OIL AND ITS OIL FRACTION AS SHORTENING SUBSTITUTE IN BREAD PRODUCTION

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THE EFFECT OF RICE BRAN OIL AND ITS OIL FRACTION AS SHORTENING SUBSTITUTE IN BREAD PRODUCTION

by

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LIST OF SYMBOLS

%	percentage
°	degree
ω	angular frequency
γ	strain
σ	constant stress
λ_i	retardation time
μ_{Co}	zero shear viscosity
$>$	more than
$<$	less than
δ	phase angle

LIST OF ABBREVIATIONS

A	absorbance
a*	redness
AACC	American Association for Clinical Chemistry
AOAC	Association of Official Agricultural Chemists
ANOVA	analysis of variance
b*	yellowness
BF	RBOF containing bread
BO	RBO containing bread
BS	CS containing bread
C	celsius
cm	centimeter
CS	commercial shortening
DA	diacetyl
DF	RBOF containing dough
DG	deoxyglucosone
DO	RBO containing dough
DPPH	2,2-diphenyl-1-picrylhydrazyl
DS	CS containing dough
DSC	differential scanning calorimetry
DT	development time
DTG	derivate thermogravimetry
FAME	fatty acid methyl ester
FDA	United State Food and Drug Administration
FFA	free fatty acids
FRAP	ferric reducing ability of plasma
FU	farinograph unit
G	glucosone
g	gram

G*	complex modulus
G’	storage modulus
G”	loss modulus
GC	gas chromatography
GRAS	generally recognized as safe
GO	glyoxal
HCl	hydrochloric acid
HDL	high-density lipoprotein
HPLC	high performance liquid chromatography
hr	hour
Hz	Hertz
J	compliance
J_c	creep compliance
J_{im}	viscoelastic compliance
J_{io}	instantaneous compliance
J_M	maximum compliance
J_R	recovery compliance
kg	kilogram
KOH	potassium hydroxide
kV	kilovolts
L*	lightness
LDL	low-density lipoprotein
LVR	linear viscoelastic region
M	molar
min	minutes
mL	milliliter
mm	millimeter
MS	mass spectrometry
MUFA	monounsaturated fatty acid
m/z	mass-to-charge ratio

N	Newton
NaOH	sodium hydroxide
nm	nanometre
Pa	pascal
PUFA	polysaturated fatty acid
RB	rice bran
RBO	rice bran oil
RBOF	rice bran oil fraction
RC	recovery percentage
rpm	revolution per minute
s	second
SAOS	small amplitude oscillatory shear
SEM	scanning electron microscope
SFA	saturated fatty acid
SFE	supercritical fluid extraction
ST	stability
t	time
TFA	trans fatty acid
TGA	thermogravimetric analysis
TI	tolerance index
TPA	texture profile analysis
UV-vis	ultraviolet-visible
V	volume
W	weight
WA	water absorption
µL	microliter
µm	micrometer

KESAN MINYAK BRAN BERAS DAN PECAHAN MINYAKNYA DALAM PENGHASILAN ROTI

ABSTRAK

Minyak dedak beras (RBO) adalah salah satu produk akhir daripada proses ekstrak dedak *Oryza sativa* (beras) (RB). Ia mempunyai komposisi asid lemak yang seimbang dan sebatian bioaktif yang amat banyak. Melalui proses pecahan dengan pelarut, ia juga boleh menghasilkan pecahan minyaknya (RBOF). Matlamat kajian ini adalah untuk menyiasat and mengaji kebolehlaksanaan RBO dan RBOF sebagai penggantian lelemak dalam penghasilan roti. Lelemak komersial (CS) adalah salah satu bahan penting dalam penghasilan produk bakeri. Walau bagaimanapun, ia mempunyai lemak tepu (SFA) dan lemak trans (TFA) yang tinggi, menyebabkan penyakit kardiovaskular yang berisiko tinggi. Dalam fasa pertama, sifat kimia, tekstur dan terma CS, RBO dan RBOF telah dikaji dan dibanding. RBO and RBOF mempunyai γ-oryzanol dan fitosterol yang lebih tinggi berbanding dengan CS. Sementara itu, terdapat tiada ketara dalam kebolehsebaran antara CS dan RBOF. Kajian sifat terma menunjukkan bahawa takat lebur RBOF yang rendah berbanding dengan CS kerana RBOF mempunyai lemak lepu yang lebih rendah daripada CS. Dalam fasa kedua, sifat reologi, tekstur, dan struktur mikro telah dikaji antara doh DS, DO dan DF. Antaranya, DO telah menunjukkan penyerapan air dan index toleransi yang tinggi dengan masa pembangunan doh dan kestabilan berbanding dengan DS dan DF. Semua sampel doh mempumyai G’ yang tinggi daripada G”. Ini menunjukkan sifat reologi dominan doh seperti pepejal. Doh DS dan DF menunjukkan G’ dan G” yang tinggi berbanding dengan DO. Selain itu, DO menunjukkan pematuhan rayapan

maksimum yang lebih tinggi dengan rintangan perubahan yang lebih rendah. Selain itu, DS mempunyai struktur mikro yang paling padat dan keras. Keputusan ini adalah selaras dengan data kajian sifat reologi dan tekstur. Seterusnya, kajian fasa ketiga adalah untuk menyiasat sifat fizikal, tekstur, dan struktur mikro roti BS, BO dan BF. Analisi tekstur dan imej memaparkan BF yang mempunyai kekerasan yang lebih tinggi dengan susunan liang roma yang tidak sekata. Analisa proksimat dan antioksida menunjukkan bahawa BO mempunyai lemak kasar yang lebih rendah dan aktiviti antioksida yang lebih tinggi berbanding dengan BS dan BF. Kesimpulannya, kemasukan RBO dan pecahan minyaknya boleh dilaksanakan untuk menambah baik formulasi roti dengan kombinasi bahan lain. Analisa deria dicadangkan untuk kajian pada masa depan.

THE EFFECT OF RICE BRAN OIL AND ITS OIL FRACTION AS SHORTENING SUBSTITUTE IN BREAD PRODUCTION

ABSTRACT

Rice bran oil (RBO) is one of the end products from *Oryza sativa* (rice) bran (RB) extraction. It has a balanced fatty acid composition an abundant amount of beneficial bioactive compounds. It can also form its semisolid fraction (RBOF) through solvent fractionation. The study is aimed to study the feasibility of RBO and RBOF as shortening replacer in bread production. Commercial shortening (CS) is one of the important ingredients in bakery products, but it contains high amounts of saturated fat (SFA) and trans-fat (TFA), leading to a high risk of cardiovascular disease. In the first phase of this study, the chemical, textural, and thermal properties of CS, RBO, and RBOF were studied and compared. RBO and RBOF contain significantly higher γ-oryzanol and phytosterol compared to CS, while there was no significant difference between CS and RBOF on the spread ability. The differential scanning calorimeter (DSC) showed a significantly lower melting point of RBOF than CS due to significantly lower SFA content in RBOF. In the second phase study, the rheological, textural, and microstructural properties were determined among dough DS, DO and DF respectively. DO showed significantly higher water absorption and tolerance index with lower development time and stability compared to DS and DF. The G' of all dough samples were significantly higher than G", indicating their predominant solid-like behaviour. DS and DF showed higher G' and G" compared to DO. For the creep recovery test, DO showed significantly higher maximum creep compliance with lower deformation resistance. On the other hands, the most compact microstructure and higher hardness of DS were in accordance with the rheological and textural test data. Next, the third phase study was done to study the physical, textural, and microstructural properties of bread BS, BO, and BF respectively. The texture analysis and image analysis displayed high hardness with uneven pore arrangement of BF, indicating the weaker ability of RBOF on the gluten shortening and lubrication. On the other hand, the proximate analysis and antioxidant analysis showed that BO had significantly lower crude fat with higher antioxidant activity compared to BS and BF. In the conclusion, the inclusion of RBO and its fraction is feasible and possible with the improvement of the bread formulation and combination with other ingredients. Sensory analysis is recommended for further studies.

CHAPTER 1

INTRODUCTION

1.1 Research backgrounds

Rice bran oil (RBO) is one of the by-products from the extraction of *Oryza sativa* (rice) bran. RBO is considered one of the superior edible oils, compared to other oils because of its ideal fatty acid composition. RBO contains approximately 18-24% saturated fatty acid (SFA), 40-43% monounsaturated fatty acid (MUFA), and 31-35% polyunsaturated fatty acid (PUFA) (Balachandran, et al., 2008; Khatoon and Gopalakrishna, 2004). Besides, RBO contains 4.2% unsaponifiable contents (Ghosh, 2007) which is higher than other oils, such as, mustard seed oil (0.56-1.01%) (Konukan, et al., 2019; Singh, 2018), sunflower oil (0.81%) (Konukan, et al., 2019), peanut oil (0.98-1.52%) (Zahran and Tawfeuk, 2019), rapeseed oil (0.97%) (Konukan, et al., 2019), olive oil (1.50%) (Textron, 2011) and others. The important unsaponifiable matters in RBO include γ-oryzanol, phytosterols, tocopherols, squalene and fatty alcohols (Ghosh, 2007; Sahu, et al., 2018). These unsaponifiable matters in RBO contribute to the hypocholesterolemic properties, reducing low-density lipoprotein and total serum cholesterol in the human body (Lai, et al., 2019; Sahu, et al., 2018). Besides, the antioxidant properties of rice bran were also reported in previous studies (Mingyai, et al., 2018; Nagendra Prasad, et al., 2011; Wang, et al., 2002). Other than protective effects, its smoke point (254 °) is high enough to make RBO suitable for cooking and deep frying (Mariod, et al., 2014).

Oil fractionation is a processing technique to vary the oil and fat properties, other than interesterification and hydrogenation. The fractionation process has two stages. Firstly, oils and fat are partially crystallized, undergoing gradual cooling to a specific temperature. Then, the solid (stearin) and liquid (olein) fractions were filtered or centrifuged (Kellens, et al., 2007). Oil is fractionated to become a few fractions of solid or semisolid state with various melting points and solid fat content. Based on the procedures and separation methods, there are three types of fractionations in industry, which are detergent fractionation, dry fractionation, and solvent fractionation (Hasibuan, et al., 2018). Detergent fractionation is a cooling crystallization by batch or continuously with the aid of surfactants and fractions separation (Hasibuan, et al., 2018). Dry fractionation is a thermomechanical separation process while solvent fractionation is partial crystallization due to the partial solubility into the specific solvent (Jääskeläinen, et al., 2017; Zaliha, et al., 2004). Fractionation of rice bran oil was also studied and reported in previous studies (Bakota, et al., 2013; Lee, et al., 2007; Yu, et al., 2006). Rice bran oil was reported to be fractionated with the aid of acetone under a temperature below 0 °C. A study by Bakota, et al. (2014) showed that rice bran oil fraction, which was a product from solvent fractionation of RBO, had been successfully incorporated into baked goods with high acceptability in the sensory test. There are also other forms of rice bran oil fractions reported to be used as an additive in cakes, doughnuts, cookies, muffins and piecrusts (Shaik, et al., 2018).

Bread is one of the most important staple products that is widely consumed in many countries, including Malaysia. Bakery products have undergone positive growth in the Malaysian market. This can be observed through the increasing consumption of bakery products at meal times instead of rice and noodles (TheStar, 2017). Hirschmann (2020) reported that in Malaysia, manufactured breads, cakes and other baked goods had high sales value of approximately RM3.03 billion in 2019. A survey from Puratos (2020) found the evolution of Malaysian consumers on their bakery product preferences. During the corona crisis, citizens are expected to seek healthier and more sustainable products, such as bread with high micronutrients, more grains, and high fibre content with less sugar, improving their diets.

Shortening is one of the important ingredients in bread making. Shortening is a kind of solid fat that is made from the hydrogenation or solidification of vegetable oil (Marcus, 2013). The function of shortening is to coat the protein strands and slow down the process of gluten formation (Modernist Cuisine, 2018). The coating plays a role as a barrier, preventing the gluten proteins from sticking to each other. This will effectively reduce the formation of long chains of protein strands. With these shortened gluten strands, bakers can shape the enriched dough easily. In addition, the clipping of gluten strands can result in the development of soft and tender crumb (Masibay, 2008). This is due to the lubrication of gluten strands that allows internal expansion when the bread is proved and baked. Besides, shortening is used to modify and obtain the desired crust texture (Kaur, et al., 2012). Usually, some monoglycerides are added in shortenings, whether it is in distilled form or added along with diglycerides. Besides, some surfactants may be added in shortenings that are used for commercial baking, for example, propylene glycol fatty acid esters, sorbitol fatty acid esters, glycerol lactic acid esters and others (Penfield and Campbell, 1990).

1.2 Problem statements

Nevertheless, shortening does not contain any bioactive compounds but 100% fat. As aforementioned, shortening is made by partially hydrogenated vegetable oil that contains a high amount of saturated fat and trans fatty acid. Both saturated fat (SFA) and trans-fat (TFA) decrease good high-density lipoproteins (HDL) cholesterol but raise the bad low-density lipoproteins (LDL) cholesterol, insulin and triglycerides levels (Dhaka, et al., 2011). High bad cholesterol is associated with a high risk of high blood pressure, diabetes and cardiovascular disease that is including stroke, coronary heart disease, peripheral vascular disease, and others (Nazario, 2021). There is still no Malaysian law on the limitation of the trans-fat amount in food since it is not present in the Malaysian Food Composition Database (Khoo, 2017). Nevertheless, the Ministry of Health had announced that any foodstuffs that contain no more than 1.5 g trans-fat per 100 g lipid are considered low trans-fat food (Khoo, 2017). According to the United States Department of Agriculture, a food intake that is less than 30% kcal total fat and 10% of calories from saturated fat is desirable and recommended for daily consumption (Lin, et al., 1996). Moreover, the United State Food and Drug Administration (FDA) proclaimed that the generally recognized as safe (GRAS) status of the partially hydrogenated oil was removed (U.S. Food and Drug Administration, 2015).

In Malaysia, rice bran is considered as underutilized as the lipids in crude rice bran are easily decomposed due to lipase activity (Shafie and Norhaizan, 2017). This causes rice bran products to be less interesting to consumers. As mentioned, rice bran oil spread can be used as an alternate shortening in bakery products. In addition, the processed food industry needs to find out alternative zero-TFA fat sources to reduce TFA in the food supply. There are some studies regarding the use of rice bran oil and its derivatives as alternate shortening. Kaur, et al. (2012) replaced shortening partially with 50% rice bran oil (RBO) in bread making. Bakota, et al. (2014) used their developed rice bran oil fraction (RBOF) and produced bread with high sensory acceptability. The previous study by Eng, et al. (2021) concluded the high possibility and feasibility of RBOF as an alternate shortening. On the other hand, the studies regarding the incorporation of RBO in bread product are mostly focused on its sensorial properties. There is less information on the rheology and structure of RBO containing dough and bread.

1.3 Aim and objectives

The aim of this study is to maximize the utilization of rice bran oil and investigate the effect of RBO and its oil fraction as a shortening substitute in bread production. The objectives of the study are to:

- To characterize the commercial shortening (CS), rice bran oil (RBO), and rice bran oil fraction (RBOF) in terms of chemical, textural and thermal properties prior to their inclusion in bakery products.
- To analyze the rheological, textural, and microstructural properties of dough incorporated with RBO and RBOF as CS substitute.
- To analyze the textural, antioxidative and microstructural properties of bread incorporated with RBO and RBOF as CS substitute.

1.4 Significance and scope of research

As more people are concerning about a healthy lifestyle, there is a trend to include nutritious raw material in the food product. The findings deepen the current understanding of the rheology, textural, microstructural and nutritional properties of bread made with healthy oil and oil fraction and provide a possibility for the food innovation.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice

As one of the most significant staple crops, *Oryza sativae* L. which is rice, sustains the people for more than 7,000 years, other than wheat (Li, et al., 2021). With its 21% caloric supply, rice feeds more than half of the world's people. Rice contains no gluten and known allergens in which rice and its components are frequently used as ingredients in the production of gluten-free food products (Li, et al., 2021). The rice composition is shown in **Figure 2.1**.

Rice grains contain approximately 75-80% carbohydrates in the form of starch, 7% of protein and 12% water (Verma and Srivastav, 2017). On the other hand, rice husk is inedible and mostly used for industrial purposes such as fuel, concrete blocks, ceramic, refractory bricks, cements and others (Mistry, 2015). The complete rice milling process is shown in **Figure 2.2**.

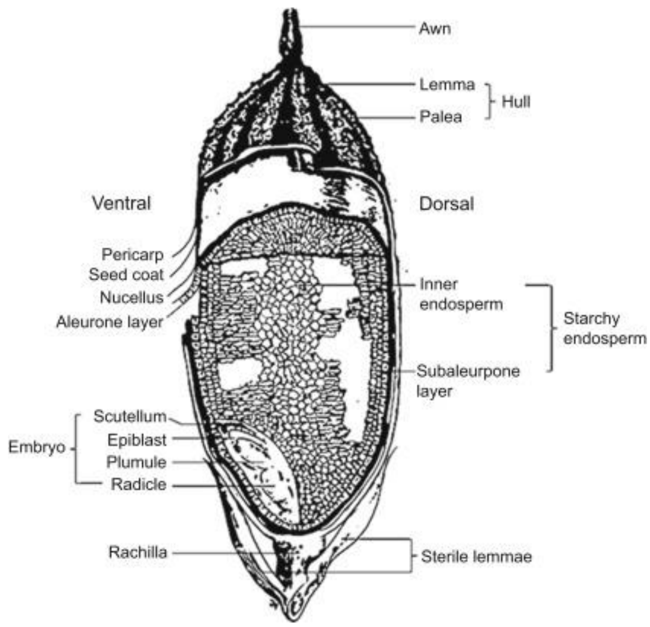

Figure 2.1: The composition of *Oryza sativa* (rice) grain. The rice bran consists of pericarp, seed coat, nucellus and aleurone layer (Juliano and Tuaño, 2019)

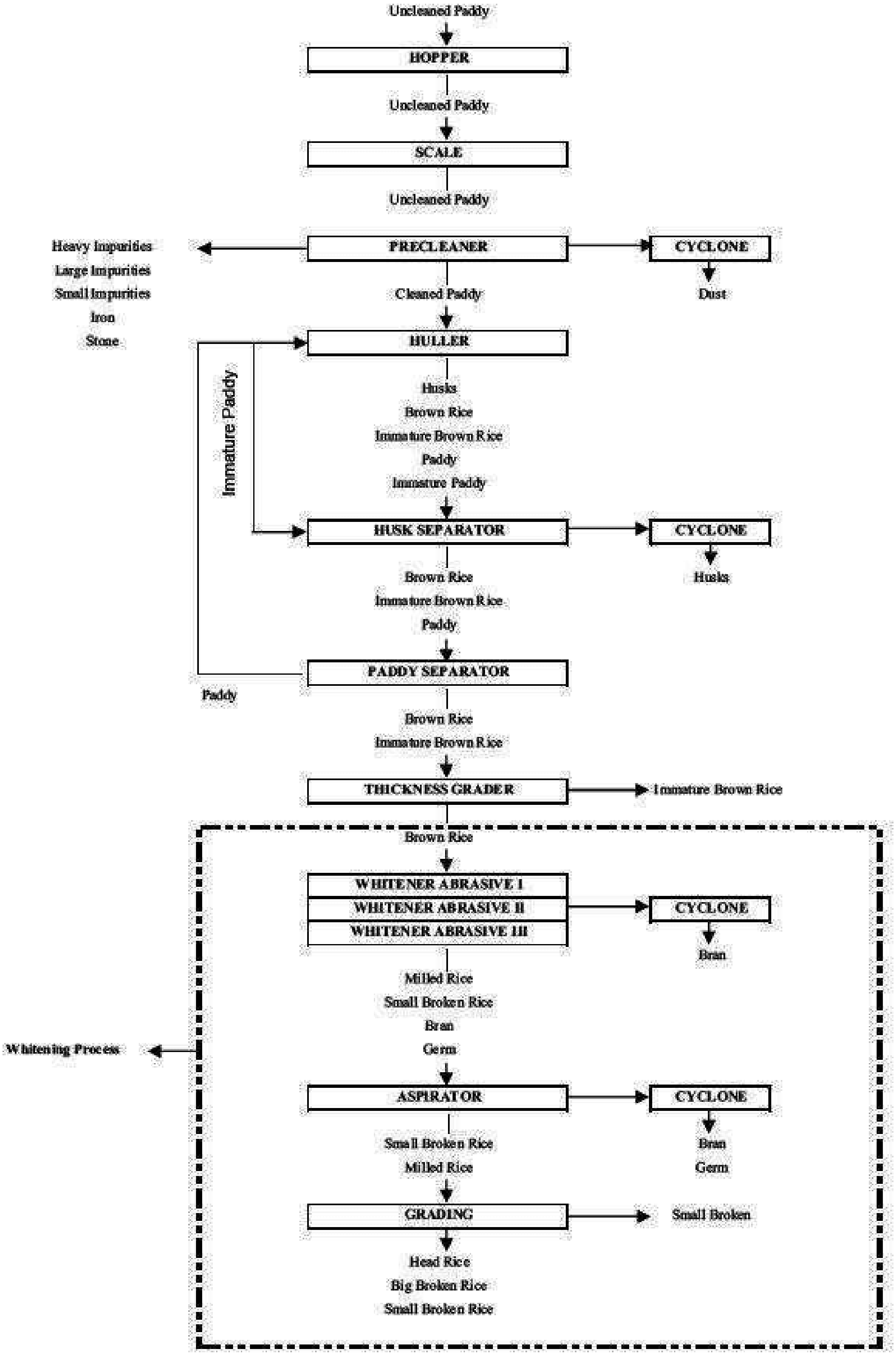

Figure 2.2: Flow chart of the complete rice milling process. Adapted from Sarkar, et al. (2018).

2.1.1 Rice bran (RB)

As aforementioned, rice bran (RB) is the hard outer layer part of rice that is removed as fine grain during the de-husking and milling processes. The RB consists of four parts, which are aleurone, germ, pericarp and sub-aleurone layer. It is a rich source of bioactive compounds, such as γ-oryzanol, phytosterol, tocopherols, tocotrienols, squalene and others. Besides, it consists of 34-52% carbohydrate which is in the form of starch, hemicellulose, cellulose, β-glucan, 18-23% lipids, 14-16% protein, some dietary fibres, and others (Bodie, et al., 2019).

Despite its high nutritional properties, the underutilization of RB is due to its enzymatic activity. During RB removal from the endosperm, the lipase enzyme hydrolyses the lipids, causing the formation of glycerol and free fatty acids (FFA), which results in rancidity and makes it unsuitable for human consumption. About 5-7% of FFA is formed every day while a total of 70% of FFA will be formed in RB during storage for a month. Extracted rice bran oil that contains more than 10% FFA is acknowledged as unfit for consumption. Only RB with FFA less than 5% produces high-grade rice bran oil (Lavanya, et al., 2019). Therefore, RB is mainly used as animal feed, fertilizer, or fuel broiler (Nagendra Prasad, et al., 2011). In order to reduce FFA in RB, a few physical or chemical reactions were conducted, which is called as stabilization method. There are a few types of stabilization methods which are documented in **Table 2.1**.

Table 2.1: Studies on rice bran stabilization methods in recent 10 years.

References	Stabilization method(s)	Findings
(Dhingra, et al., 2012)	Ohmic heating	The treated rice brans have free fatty acid less than 5% compared to raw bran which is 42% after being stored for 75 days .
(Thanonkaew, et al., 2012)	Hot air heating, microwave heating, roasting and steaming	Hot air heating and microwave heating are the most effective stabilization method as they reduce the free fatty acid, peroxide value, and acid value.
(Bagchi, et al., 2014)	Microwave heating	Microwave heating is the most effective treatment with 900 W and 2.5 min.
(Yilmaz, et al., 2014)	Infrared radiation	Rice brans that are stabilized at 600 W IR power for 5 min have a free fatty value of less than 5% but significantly reduced tocopherol content.
(Patil, et al., 2016)	Microwave heating	Rice bran that is treated by microwave heating can be stored for three months with promised oil quality.
(Rafe, et al., 2017)	Extrusion	Extrusion improves the colour and enhances some nutritional compounds such as dietary fibre. However, it also reduces some compounds such as niacin, riboflavin, folic acid, and others.
(Irakli, et al., 2018)	Infrared radiation	Rice brans are stabilized effectively at 140 °C for 15min with a significant increase of phenolic contents and a decrease of vitamin E content.

(Irakli, et al., 2020)	Dry heating, infrared radiation, microwave heating	All treatments are found to reduce antinutrients effectively. Among the treatments, infrared radiation showed the greatest inactivation of lipase activity.
(Liao, et al., 2020)	Hot air-assisted radio frequency heating	The stabilization method does not affect the tocol content in rice bran but has better antioxidant activity than rice bran treated with extrusion.
(Asad, et al., 2021)	Microwave heating	Microwave treatments reduce the free fatty acid value that fits human consumption. In this paper, optimization of the treatment is also conducted.

2.1.2 Rice bran oil (RBO)

One of the common and promising uses of rice bran is extracting rice bran oil from rice bran. There are three types of fatty acids that are dominant in total fatty acids in rice bran oil. These are 12%-18% palmitic acid, 40-50% oleic acid and 30-35% linoleic acid (Ramezanzadeh, et al., 2000). Rice bran oil is considered one of the edible oils that is superior compared to others, due to its ideal fatty acid composition (Tao, Rao and Liuzzo, 1993). The oil extracted from hydrolysed rice bran would contain a high amount of free fatty acid. Rice bran oil that contains more than 10% free fatty acid is not suitable for human consumption (Tao, Rao and Liuzzo, 1993). The unsaponifiable content of rice bran oil is abundant in antioxidants and micronutrients, such as oryzanols, tocopherols, tocotrienols, phytosterol, polyphenols and squalene (Ghosh, 2007).

Due to the antioxidants that existed in the oil, it has better shelf life compared to other edible oils. Wang, Hicks and Moreau (2002) reported that rice bran oil showed excellent antioxidant and antipolymerization properties in their study. Rice bran oil has a high smoke point of 254°C. With a mild flavour, rice bran oil is claimed to be a suitable edible oil for deep frying and stir-frying (Mariod, et al., 2014). Food cooked by using rice bran (hexane ave lower overall calories because it has a lower viscosity, and less oil is absorbed during cooking. Apart from reduced calories, less oil absorbed also leads to food with a lighter taste. This will improve the palatability and flavour of the food (Nagendra Prasad, et al., 2011). Besides, food cooked using rice bran oil would cause a lower possibility of allergenic reaction compared to other vegetable oils (Ghosh, 2007).

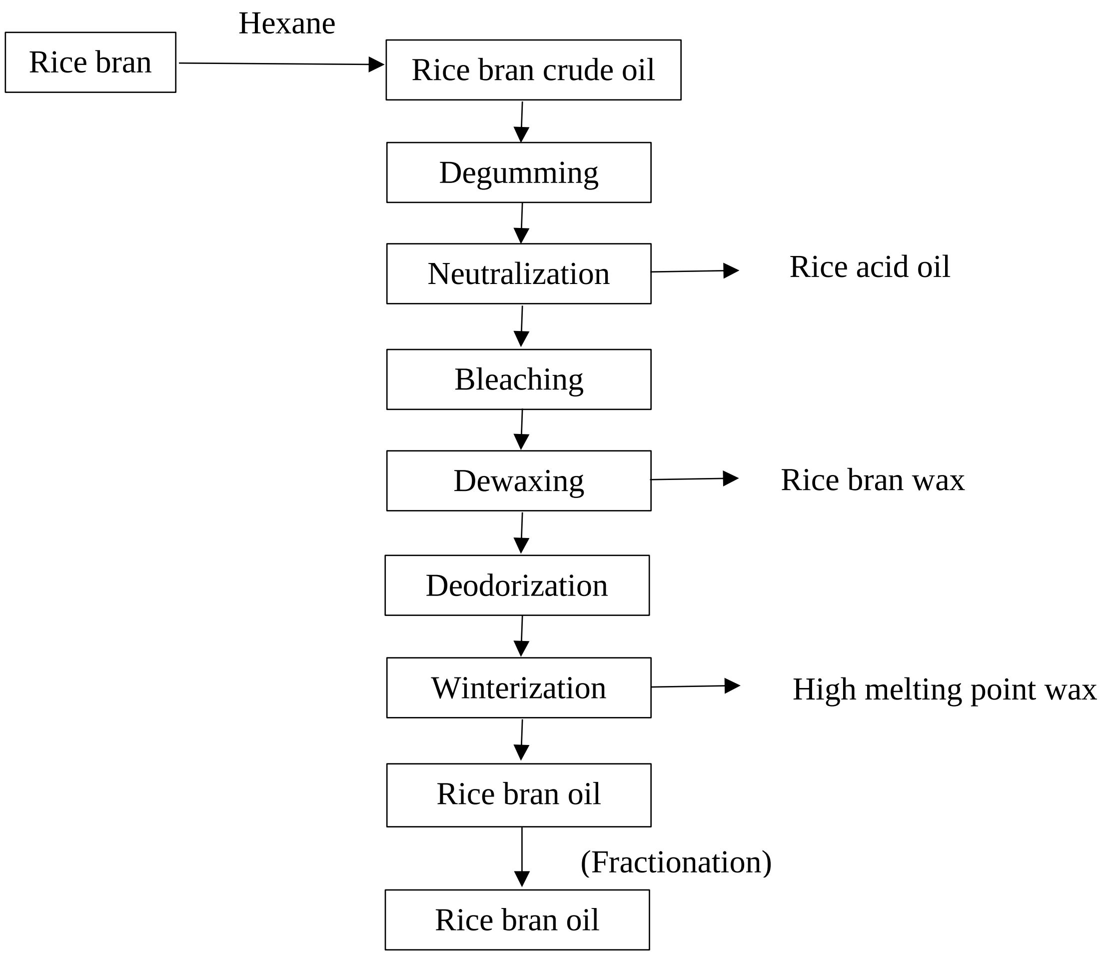

Figure 2.3: Flow chart of rice bran oil production.

2.1.2(a) γ-Oryzanol

The γ-oryzanol was found out that it is a fraction that contains ferulic acid esters of triterpene alcohol and phytosterols (Wheeler and Garleb, 1991; Patel and Naik, 2004; Lai, et al., 2019). Xu and Godber (1999) identified 10 components of γ-oryzanol through reversed-phased HPLC coupled with MS. These 10 components were Δ^7-stigmastenyl ferulate, Δ^7-campestenyl ferulate, Δ^7-sitostenyl ferulate, cycloartenyl ferulate, 24-methylenecycloartenyl ferulate, campesteryl ferulate, stigmasteryl ferulate, sitostanyl ferulate, sitosteryl ferulate, and compestanyl ferulate (Xu and Godber, 1999; Lai, et al., 2019). Among these components, cycloartenyl ferulate, 24-methylenecycloartenyl ferulate and campesteryl ferulate are three dominant γ-oryzanol components, which account for 80 % γ-oryzanol (Rogers, et al., 1993; Patel and Naik, 2004). Same as phytosterols, oryzanol is reported to have good stabilization effects when oil is used for frying (Wang, Hicks and Moreau, 2002).

2.1.2(b) Phytosterols

Phytosterols or plant sterols have similar structures and functions to cholesterol in animals. In terms of structure, both phytosterols and cholesterols have a four-ring steroid nucleus, a 5,6-double bonds and 3ß-hydroxyl group (Fernandes and Cabral, 2007). Regardless of these similarities, cholesterols contain side chain with 8 carbon atoms, while phytosterols contain a side chain with 9 or 10 carbon atoms (Fernandes and Cabral, 2007). A double bond may be located in the alkyl side chain. In term of functionality, phytosterols and cholesterols are important components to stabilize phospholipid bilayer in cell membranes (Fernandes and Cabral, 2007). In addition, phytosterols are precursors of vital biomolecules such as vitamins and hormones (Kim, Chung and Lim, 2014). Researchers have reported more than 100 types of phytosterols found in plant species. Among these phytosterols, the more common types are campesterol, ß-sitosterol and stigmasterol (Fernandes and Cabral, 2007). Phytosterols in rice bran oil can inhibit the absorption of cholesterol in small intestine, resulting to decreasing in plasma cholesterol concentration (de Deckere and Korver, 1996). Meanwhile, phytosterols and their derivatives may have the potential to be effective antioxidant (Wang, Hicks and Moreau, 2002).

2.1.2(c) Tocols

Tocopherols and tocotrienols have an antioxidant property that can inhibit lipid peroxidation occurring in biological membranes. A study by Winkler-Moser, et al. (2013) showed that tocopherols were responsible for protection of steryl ferulates during cooking and the enhancement of antioxidant and antipolymerization activity. Besides, they act to lower blood cholesterol and modulate some degenerative diseases, such as cardiovascular diseases and cancer (Kim, Chung and Lim, 2014).

2.1.3 Extraction method of RBO

2.1.3(a) Solvent extraction

Commercial extraction normally uses hexane as a solvent to extract oil from rice bran (Soares, et al., 2018). After rice bran is stabilized, hexane is mixed with rice bran directly at 20 °C with a solvent to bran ratio of 2:1 (w/w) (Nagendra Prasad, et al., 2011). On the other hand, the extraction can also be done by mixing preheated hexane with stabilized rice bran with solvent to bran ratio of 3:1 (w/w), followed by immersion in a water bath at temperatures up to 40 °C or 60 °C within a predetermined period and vacuum evaporation of hexane from the miscella (Hu, et al., 1996; Nagendra Prasad, et al., 2011). Apart from that, alcohols, such as isopropanol and ethanol had been suggested by Hu, et al. (1996) to be a better choice than hexane, in terms of oil yield and recovery of vitamin E and oryzanol. Short-chain alcohols have greater polarity than hexane and this makes them able to extract more nonglyceride molecules. Oils that are extracted by alcohols contain more unsaponifiable and phosphatide compounds (Hu, et al., 1996). Rice bran oil that is rich in vitamin B and E can be extracted by ethanol while rice bran oil that is rich in vitamin B alone can be extracted by using isopropanol. A study by Xu and Godber (2000) proposed that mixture of solvents, such as hexane and isopropanol can extract oil more efficiently compared to a single organic solvent.

However, the use of solvent for the extraction of oil has several disadvantages. Hexane can cause air pollution. When hexane is released into the atmosphere, it can react with other air pollutants, resulting in the production of ozone and photochemical oxidant (Hanmoungjai, Pyle and Niranjan, 2002). Besides, hexane is toxic to humans and animals, even at a low concentration (Balachandran, et al., 2008; Gíl-Chávez, et al., 2013). It volatiles easily and its vapours may cause explosions if the hexane vapours are not monitored and controlled during industrial oil extraction operations (Sparks, et al., 2006). Rice bran oil requires further refining process after solvent extraction. The solvent needs to be evaporated after extraction since rice bran oil is used as cooking oil or for other food applications (Gíl-Chávez, et al., 2013). Although the solvent is eventually eliminated, there are still some traces of solvent left in the extracts (Herrero, et al., 2010; Soares, et al., 2018). Typically, extraction using organic solvent will be conducted at high temperature for a longer period. This will easily lead to the degradation and volatilization of thermally sensitive compounds (Gíl-Chávez, et al., 2013; Soares, et al., 2018). In addition, solvent-extracted crude RBO contains about 5 to 25 % free fatty acid, 2 to 4 % waxes, and 1 to 2 % phospholipid gums and pigments. This will eventually cause crude RBO difficult to be refined (Arumughan, Skhariya and Arora, 2004; Balachandran, et al., 2008).

2.1.3(b) Enzymatic extraction

In order to reduce the usage of solvents, some alternative ways have been developed. Enzymatic extraction has been used for extraction of the oils from many types of oil-containing materials, such as mustard seed (Sengupta and Bhattacharyya, 1996), soybean (Domínguez, Nunez and Lema, 1993) and sunflower (Domínguez, Nunez and Lema, 1993; Sineiro, et al., 1998). Enzymatic extraction does not require dangerous solvent and produce hazardous by-product (Hanmoungjai, Pyle and Niranjan, 2001). Besides, the extraction temperature is lower compared to typical solvent extraction (Hanmoungjai, Pyle and Niranjan, 2001). Due to its mild operating conditions, enzymatic aqueous extraction of oil from rice bran has been studied and reported. Therefore, few studies had focused on enzymatic aqueous extraction to compare solvent and aqueous extraction. When the rice bran was treated with enzymes such as pectinase and cellulase followed by extraction using hexane, the yield of oil was higher (Sengupta and Bhattacharyya, 1996). A study by Sengupta and Bhattacharyya (1996) extract oil and protein from rice bran and reported that most of the quality parameters of oil recovered from the process were similar with solvent extracted oil and commercial rice bran oil, but the recovered oil had higher acidity and lower number of coloured substances. Hanmoungjai, Pyle and Niranjan (2001) reported a similar result and the peroxide value of recovered oil was higher.

2.1.3(c) Other types of extraction

Besides the liquid extraction, other extraction processes were also proposed, such as supercritical fluid extraction (SFE), microwave-assisted extraction (Zigoneanu, et al., 2008) and ultrasonication followed by three-phase partitioning (Sharma and Gupta, 2004). Although the process of ultrasonication followed by three-phase partitionings had successfully extracted oil from rice bran, almond and apricot, the process required the use of t-butanol and ammonium sulphate.

2.2 Dough and bread

Bread is one of the oldest prepared foods and its making can be dated back to the Neolithic era (Serna-Saldivar and Abril, 2011). The first discovery of bread making was done by scientists from a dig that is suspected to have been 14,000 years old in Black Desert, Jordan (Briggs, 2018). During the stone age, the bread makers were believed to take wild wheat and barley-made flour and mix it with wild plant roots that were pounded to a dry pulp. Water was then added, and the mixture was baked around the fire. Until now, same as rice, bread is still one of the most widely edible foods that supply protein, vitamins, calories and minerals for around 6.8 billion people on Earth. On average, a typical human will gain about 22-24% of the protein and calories from wheat (Serna-Saldivar and Abril, 2011). Different varieties of bread may have different sizes, colour, weights, hardness, cell structure and others (Bolarinwa, et al., 2019). Typically, dough is baked to make bread. Most of them undergo the leavening step, but they may be also unleavened. However, they are also can be fried, steamed, or baked in uncoiled pan (Alemu, 2015). People serve bread in several forms, for example, it can be either used as one of the ingredients in food preparation or consumed as a snack (Bolarinwa, et al., 2019). The common ingredients used in bread production are flour, fat, egg, sugar, salt, and leavening agents, for example, baking soda and yeast. Yet, some ingredients such as raisins, seeds, nuts, or onions may be found in bread products (Alemu, 2015). In order to produce bread with good quality, the dough should be extensible enough for its expansion and relaxation during rising (Baking Industry Research Trust, 2019). A good dough should also have good elasticity as it has sufficient strength to hold the carbon dioxide gases when rising and enough stability to hold the cell structure and shape (Baking Industry Research Trust, 2019).

2.2.1 Ingredients used in the bread production

2.2.1(a) Wheat flour

Bread is produced from wheat flour. Wheat flour determines the overall bread quality, affecting the dough's strength and its technological behaviour directly, and different phase conditions of bread-making indirectly. The quality of bread is also considered in terms of texture, visual, consistency and dimensions of the loaf (Alemu, 2015). A typical wheat flour contains about 75% starch (Yang, et al., 2022). In the food industry, starch is mostly used as a binder, filler, stabilizer, and thickener in some food products, for example baby foods, bakery products, custard powders, ice creams, processed meats and sausages, pie fillings, soups, sauces and gravies and others (Rapaille and Vanhemelrijck, 1992; Zhao, et al., 2022). **Figure 2.4** shows the chemical structure of starch. During breadmaking, gelatinization happens when the starches burst and the inner part releases, forming a thick gel after they are heated and absorb water (Schirmer, et al., 2014). Besides, α-amylase and β-amylase cooperate and hydrolyze the starch into sugar that can feed the yeast to produce carbon dioxide gas (de Souza and Magalhães, 2010; Schirmer, et al., 2014).

Another important molecule found in wheat flour is gluten. Gluten is a type of protein found in grains, such as barley, rye, spelt and wheat, and their related products that are abundant in people's diet (Bjarnadottir, 2022). Gluten is composed of glutenin and gliadin. Gluten is an important protein in bread making as glutens form a network in bread that leads to gas retention and good textural properties (Baking Industry Research Trust, 2019). Gliadin protein is more compact which contributes to the fluidity of dough while glutenin provides the strength for the dough.

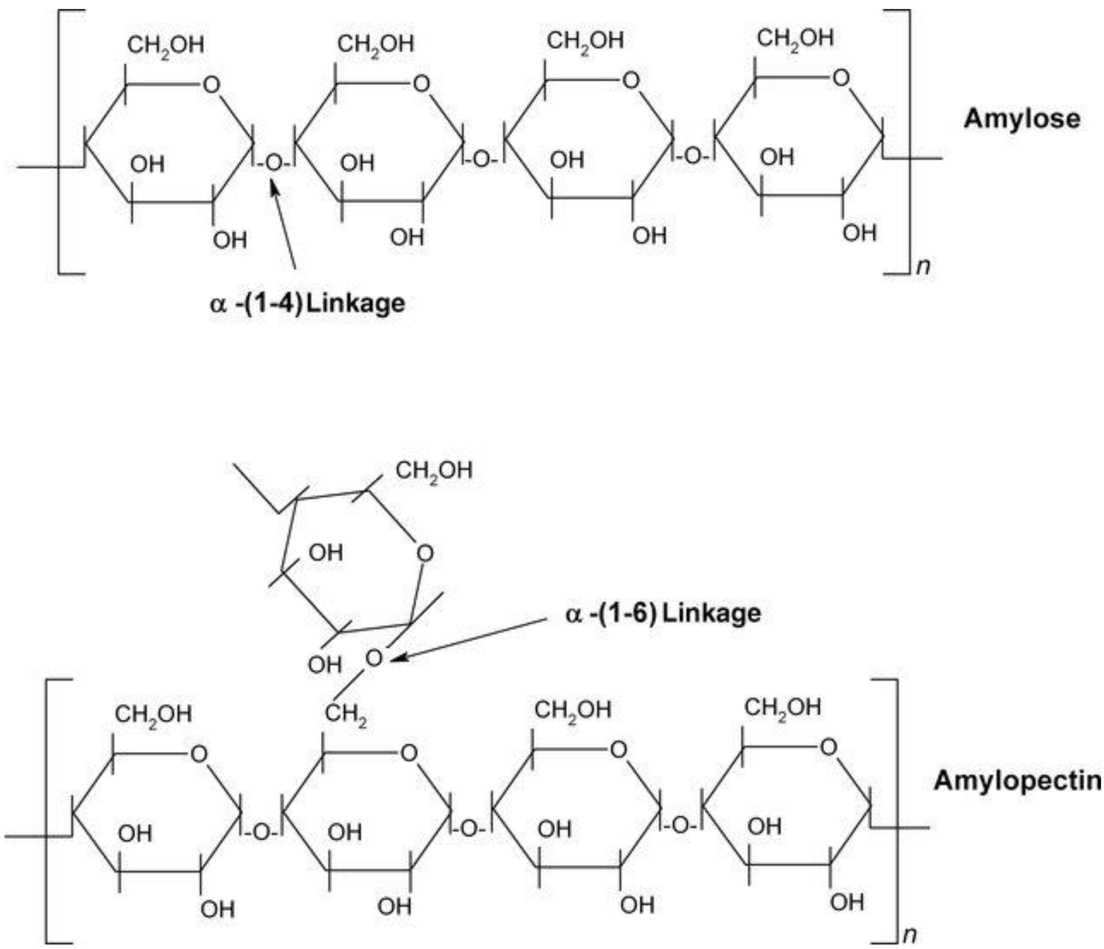

Figure 2.4: Chemical structure of starch with its monomer (amylose and amylopectin) units. Adapted from Visakh, et al. (2012)

People that suffer from celiac disease cannot consume common gluten-containing products as those persons will undergo inflammation, causing damage to their intestines when they are exposed to glutens (Lebwohl and Rubio-Tapia, 2021). Other than celiac disease, people that have gluten intolerance and wheat allergy are also not encouraged to consume related food products. However, for people that do not have celiac disease or gluten intolerance, long-term consumption of gluten does not lead to the risk of chronic diseases such as coronary diseases (Lebwohl, et al., 2017). In contrast, the avoidance of gluten in diet may end up in the insufficient consumption of beneficial grains. Therefore, the development of gluten-free food products is particularly concerning, such as gluten-free baked products, cookies, noodles, and pasta. However, the typical white wheat flour shows lower nutritional value due to the nutrient’s reduction as the nutrients are mostly concentrated in the bran and endosperm which will be removed during the milling process (Mironeasa, et al., 2018). Therefore, refined wheat flour is proved to possess few phytochemicals, fibres, vitamins, and minerals compared to raw grain or whole grain flour (Vignola, et al., 2016; Mironeasa, et al., 2018).

2.2.1(b) Fat

Shortening is fat that is used in bakery products for modification of physical and chemical properties of batters and doughs and lubrication of internal surface as it shortens the gluten network and reduces the formation of a tough matrix in bakery products (Kaur, et al., 2012). Through the action, the dough and batters can be expanded more and leading to greater volume and good texture (Kaur, et al., 2012). However, shortening contains 100% fat, instead of any carbohydrate, protein or other nutrients. Apart from this, shortening is made from the hydrogenation of palm oil that will produce a trans fatty acid, which will increase the risk of getting cardiovascular disease, inflammation, diabetes and other diseases (Leech, 2019).

According to Toan and Van (2019), some trends are being interest in the baking industry. For example, decreasing the fat and oil amount with the replacement of low-fat raw material and replacing unhealthy plastic fat with healthier liquid vegetable oil. Instead of animal fat that contains high saturated fat, polyunsaturated vegetable oil is preferably used in food products. These kinds of trends occur as people nowadays are putting more attention to their health. Apart from good taste, functional and beneficial foods are also becoming significant in people’s daily diets.

The involvement of oil and oil fraction affected the dough and bread in the aspects of rheology, structure, physical properties, and others. **Table 2.2** documented the previous studies involving different kind of oil and oil fractions in bakery products.

Table 2.2: Previous related studies on the incorporation of oil in bakery products.

References	Findings
(Kaur, et al., 2012)	The authors proposed that the replacement of shortening by 50% rice bran is suitable in bread making.
(Bakota, et al., 2014)	The acceptability ratings for RBOF in baked products were also determined and were consistently above 85% and in one case, 100% of panellists rated the experimental sample as acceptable.
(Osuna, et al., 2017)	The replacement of bovine fat by canola oil and olive oil improved the fatty acid profile that made changes in colour, texture, and technological characteristics of bread together with the flour mixes.
(Debonne, et al., 2018)	Wheat germ oil showed the highest overall resemblance with the control concerning the dough water absorption and kneading properties and showed promising shelf-life extending properties in wheat bread without affecting the activity of the baker's yeast.
(Kaur, et al., 2019)	The eggless muffin incorporated with soybean oil showed a lower G' and G" value compared with other eggless muffin incorporated with canola oil, cottonseed oil, sunflower oil and rice bran oil, respectively. The authors concluded that the soybean oil with appropriate ω6:ω3 ratio is suitable for the development of eggless muffin.
(Katyal, et al., 2019)	Groundnut oil (GO) containing dough showed elastic behaviour resulted in increased G'. The tan δ decreases with the increase of GO while increases with the decrease of hydrogenated fat (HF).
(Zhang, et al., 2020)	Peanut oil caused positive effect on dough viscosity and elasticity ratio, springiness and recovery capacity that was proposed to be potential improver for Chinese steamed bread instead of commercial shortening.
(Zhang, et al., 2022)	This study investigated and proposed the mechanism on how the addition of peanut oil within 2.0 to 4.0% facilitated the dough viscoelasticity and steamed bread quality.

2.2.1(c) Leavening agent

Leavening agent is one of the important ingredients in providing aeration and leavening in bakery products that will greatly influence the structure and texture of the final product (Joye, 2021). In most of productions, two types of leavening agents are frequently used, which are the biological agent and chemical agent. Regarding biological agents, yeast is the most used biological agent as it consumes fermentable sugar and releases carbon dioxide gas as part of its metabolism (Joye, 2021). Besides, it produces other significant by-products, such as ethanol, acids, small-thiol containing molecules and others, providing flavour and quality (Joye, 2021). There are a few types of bakers' yeasts. The most used bakers' yeast is active dry yeast (Alfaro, 2020). It needs to be activated using warm water around 40 °C prior to being used. The too high temperature will kill the yeast while the too low temperature cannot activate the yeast completely, reducing its effectiveness on leavening (Alfaro, 2020).

Regarding the chemical agents, there are two types of chemical agents, which are baking soda and baking powder. Baking soda, which is sodium carbonate or carbonate of soda, is a kind of white powder with a pH of 8 or 9 (Alfaro, 2020). The chemical structure of sodium carbonate is shown in **Figure 2.5**. Baking soda is activated immediately once it reacts with buttermilk, yogurt, lemon juice or some acidic salt of a weak mineral acid such as potassium acid tartrate ($KHC_4H_4O_6$), monocalcium phosphate monohydrate [$(CaH_4PO_4)_2 \cdot H_2O$], sodium acid pyrophosphate ($Na_2H_2P_2O_7$), and others in order to generate carbon dioxide gas (Hazelton, et al., 2004). The reaction equation between baking soda and acid is shown below:

$$HX + NaHCO_3 \rightarrow NaX + H_2O + CO_2 \qquad \textbf{(2.1)}$$

Where HX: acid salt;

$NaHCO_3$: sodium bicarbonate (baking soda);

NaX: neutral salt;

H_2O: water;

CO_2: carbon dioxide gas.

On the other hand, baking powder is a kind of powder including baking soda and acidic ingredients. It will be only activated when the water is added, and baking powder is moistened (Alfaro, 2020).

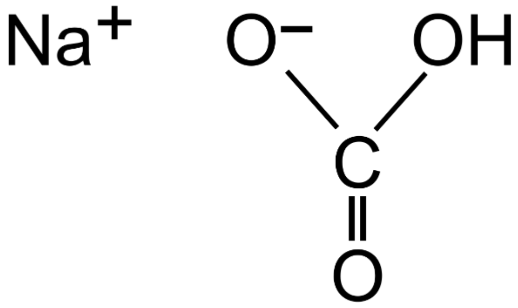

Figure 2.5: Sodium bicarbonate. Adapted from Helmenstine (2019).

2.2.1(d) Salt, sugar, and dough improver

Salt provides improvement of gluten bonding, not only the flavour (Caren, 2018). This is because the repulsion between the gluten proteins inhibits the initiation of gluten interaction. The chloride ions in salt will assist the proteins to overcome the repulsion and promote gluten interaction and bonding.

Dough improver is mainly used to improve the dough stability in terms of anti-stirring properties, heating expansion and uniformity of the dough internal structure (Gioia, et al., 2017). Besides, dough improver helps to increase the bread volume by enhancing the gluten protein binding and maintaining more generated gas during the yeast fermentation (Gioia, et al., 2017). The amylase found in dough improver plays a role in hydrolysing the starch into dextrin, increasing the rate of Maillard reaction, and deepening the bread surface colour. During the stirring process, it makes the dough stable so that the dough is not over stir, improving the gluten network structure.

2.2.2 Breadmaking

There are five types of breadmaking methods listed below (Cauvain, 2016):

a) Straight dough bulk fermentation. The mixing of all ingredients is carried out with low speed and energy input prior to the dough resting for several hours.
b) Sponge and dough. The mixing of a few ingredients is carried out, followed by fermentation and the addition of the rest of the ingredients for final mixing.
c) Rapid processing. The fermentation process is skipped. Instead, the bakers use higher mixing speed and energy input.
d) Mechanical dough development. Energy is measured to facilitate the development of the dough. After mixing, the dough is moved without further delay to be divided into parts.
e) Sourdough. Dough fermentation using wild yeast and *Lactobacilli*.

2.2.2(a) Mixing

During bread making, water is added to start the hydration process of wheat flour. Glutenin and gliadin form gluten that will swell and form a continuous network (Caren, 2018). The interaction and formation of the gluten network are shown in **Figure 2.6**. Mixing will speed up the hydration process and the gluten will link up with each other through disulphide bonds, forming the continuous network that leads to the formation of bread structure (Baking Industry Research Trust, 2019). The bonding in gluten protein leads to the formation of elastic films in dough and this contributes to the structure formation and gaseous trapping (Gavin, 2019). When the mixing continues and the dough is gradually formed, the elongation of protein chains makes them organize into webbing that possesses elasticity and extensibility.

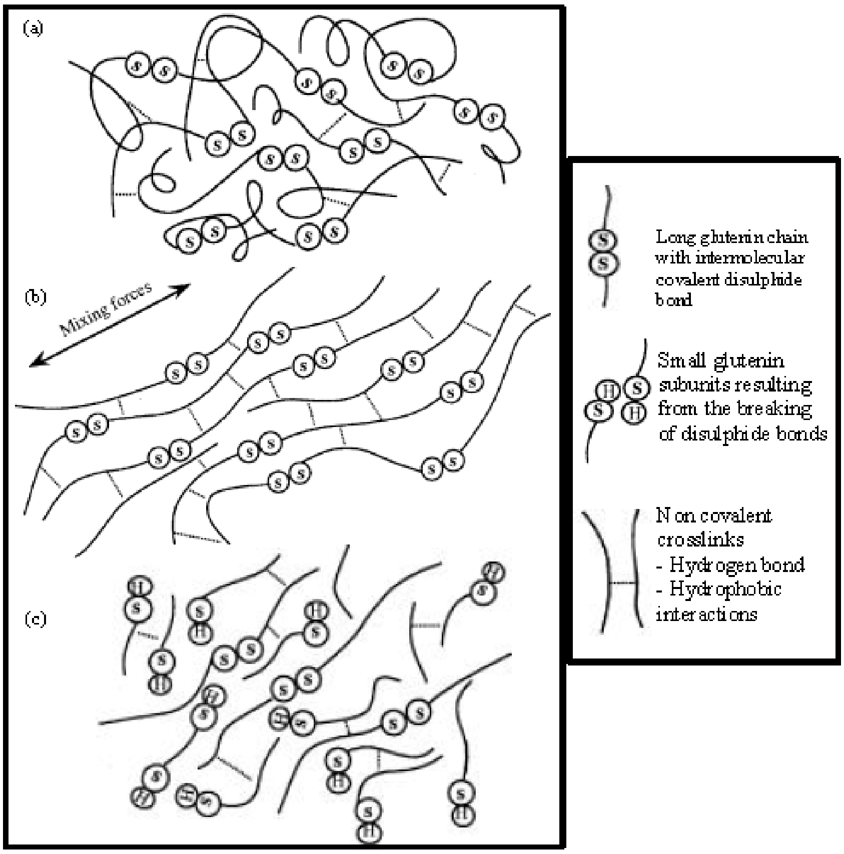

Figure 2.6: Gluten interaction during a) the beginning of mixing; b) optimum development; c) overmixing. Adapted from Letang, et al. (1999).

2.2.2(b) Fermentation

During fermentation, yeast consumes sugar and produces carbon dioxide gases. Gases are trapped within the food matrix which ends up in the leavening of bakery products. The gluten network is continuously developed to modify the bread rheology so that its ability to expand is improved to resist the increased gas pressure when the gases are continuously generated (Cauvain, 2016).

2.2.2(c) Baking

When the dough is heated, the water evaporates faster at the bread surface than at the interior. High temperature kills the yeast, and the starch takes up the water