

Processing of Foods, Vegetables and Fruits: *Recent advances*



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Editors: Ching Lik Hii, Sachin Vinayak Jangam, Sze Pheng Ong, Pau Loke Show and Arun Sadashiv Mujumdar

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PREFACE

This e-book is an edited and updated collection of selected keynote papers addressed in the 3rd International Symposium on Processing of Foods, Vegetables and Fruits (ISPDFVF) held in The University of Nottingham Kuala Lumpur Teaching Centre, Malaysia from 11-13 August, 2014. This symposium was jointly organized by The University of Nottingham, Malaysia Campus and The Transport Process Research (TPR) Group of Prof. A.S. Mujumdar (McGill University). It was initiated based on an idea proposed by Prof. Arun S. Mujumdar, who was with NUS, Singapore at the time.

This e-book aims to present the latest R&D works carried out by researchers, especially from South East Asia (Thailand, Philippines and Malaysia). The topics range from edible food products such as Bird's nest, cassava, sweetener, rice bran oils, and the development of rubber packaging materials for foods to drying equipment.

This e-book can be freely downloaded from Prof. Mujumdar's website (<http://www.arunmujumdar.com/>) and the readers are also encouraged to access the other e-books from this website. These e-books aim to serve the needs of academics, students, industrial practitioners and libraries at no cost. This is a major professional contribution to the global community since the access is free and worldwide.

We take this opportunity to thank the contributing authors for their time and effort to complete this e-book. We also would like to thank the Advisory Board and Local Organizing Committee of ISPDFVF for successful organization of the symposium for the third time since 2011.

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Chapter 1

1,5-Anhydroglucitol (AG) as a New Functional Sweetener and Versatile Food Material

Y. Konishi

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1.1 INTRODUCTION

Humans of all races, sexes, and cultures inherently enjoy sweet blissful flavors. Sucrose is historically the most popular and important sweetener. However, excess intake of sucrose is associated with a high incidence of lifestyle-related diseases such as hypertension, diabetes, and obesity. Therefore, alternative sweeteners with good taste and low or reduced caloric contents have been developed (O'Brien Nabors, 2012).

The polyol 1,5-anhydroglucitol (AG; 1-deoxyglucose) (**Figure 1**) is chemically stable and has been found at trace concentrations in various plant and animal tissues and in microorganisms. In animals, AG is produced from glycogen via a two-step reaction. First, 1,5-anhydrofructose (AF) (**Figure 1**) is formed from non-reducing glucose residues of glycogen by α -1,4-glucan lyase [EC 4.2.2.13]. Subsequently, AF is reduced to AG by NADPH-dependent AF reductase [EC 1.1.1.263] in an alternative glycogenolytic pathway to amyolytic and phosphorolytic pathways (Yu et al., 1993; Yu and Fiskesund, 2006; Yu, 2008). Plasma AG concentrations are maintained in the range of 116–250 mM (Akanuma et al., 1988; Stickle and Turk, 1997) in healthy human subjects. However, the concentrations are significantly decreased in patients with diabetes along with an increase in urinary excretion of AG because of inhibition of tubular re-absorption of glucose and AG (Akanuma et al., 1988). A few studies have reported on the functions of AG. AG has been shown to inhibit glycogenolysis by inhibiting phosphorylase in rat hepatocytes (Carabaza et al., 1992) and to stimulate insulin release in insulinoma cell lines (Yamanouchi et al., 2003). In plants, starch degradation pathways via AF have been suggested to occur in germinating amaranth seeds and ripening bananas, and in both cases, AG levels increased with decreasing starch levels (Konishi et al., 2000).

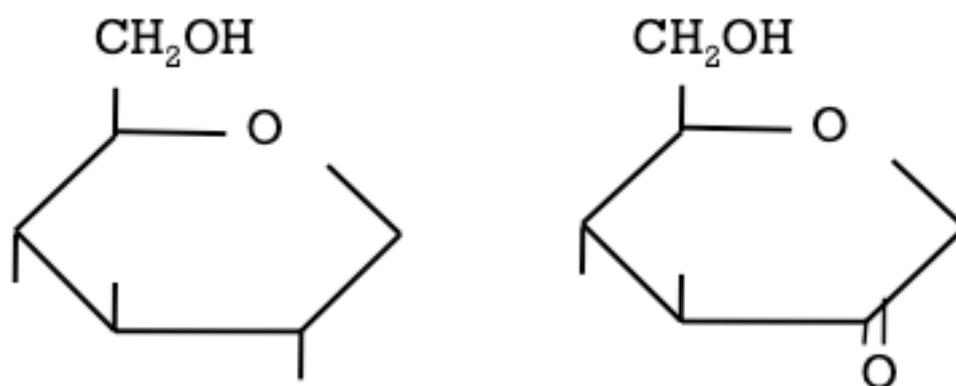


Figure 1. Structure of 1,5-anhydro-D-glucitol (left) and 1,5-anhydro-D-fructose (keto form) (right)

Because AG is not readily available in large quantities, it has not been studied widely in food science or food processing. AG has been chemically synthesized from glucose (Ness et al., 1950). In the present study, we purified large quantities of AG from *Polygalae radix* (*Polygala tenuifolia* root), which is

a traditional Chinese herbal medicine (Kawasaki et al., 2000), and demonstrated its potential as a functional sweetener. In addition, we have described a method for the enzymatic conversion of AG to AF. AF is known to have multifunctional properties including anti-oxidative, anti-browning, anti-microbial, and anti-tumor activities (Fiskesund et al., 2010).

1.2 Purification of AG

Polygalae radix (*Polygala tenuifolia* root), which was purchased from a local Chinese herbal medicine pharmacy, was ground to powder by hand, and the defatted sample was homogenized in 5 volumes of chilled 5% trichloroacetic acid (TCA) using an Ultra Turrax homogenizer, followed by centrifugation at 10,000 rpm for 20 min. The supernatant was then mixed with activated charcoal powder (6 g/ml) for 30 min. After removal of charcoal with a glass filter, the filtrate was allowed to condense to one-sixth of the original volume by boiling for 2–3 h, and the pH increased from 1.2 to 3, probably because of the partial decomposition of TCA. The sample was then loaded onto an AG 1-X8 column (1.32 × 29 cm) linked to an AG 50W-X8 column (0.75 × 25 cm) and eluted using water as the mobile phase. The eluted AG fraction, measured using a Lana 1,5-AG assay kit from Nihon Kayaku (Tokyo, Japan), was freeze-dried. The mean yield of AG was 5.2% (3 experiments). The purity was 96%, which was coincided with that of a standard AG (Wako Pure Chemical Industries, Osaka, Japan), on an HPLC column packed with a cation (Na⁺) exchange resin (MCI Gel CK08S, 8 × 500 mm, Mitsubishi Chemical Co., Japan).

The structure of purified AG was examined using FAB-MS, ¹³C-NMR, and ¹H-NMR spectra. FAB mass spectra were recorded in the negative-ion mode using glycerol as the matrix, and the highest peak was detected at m/z 163.09 [M-H]⁻ using Jeol JMS-700T. ¹³C-NMR and ¹H-NMR spectra were obtained in DMSO-d₆ at 40°C using a Varian UNITY plus 500 spectrometer. The ¹³C-NMR (125 MHz) spectrum showed 6 signals in the field with a chemical shift of δ 61.5–81.5, suggesting that all carbons were linked to oxygen atoms. The ¹H-NMR (500 MHz) spectrum indicated the presence of oxymethines and oxymethylenes. Furthermore, optical rotations were determined in water using JASCO P-1030. The specific optical rotation $[\alpha]_D^{25}$ was observed at +66° in water (c = 0.33) at 25°C and was consistent with the measured value of standard AG (Wako Pure Chemical Industries). These spectral data confirmed that the purified AG was 1,5-anhydro-D-glucitol.

1.3 CHARACTERISTICS AND SWEETNESS OF AG

1.3.1 Panels for sensory evaluation of AG

For sensory evaluation, 10 students aged 22–24 years were recruited from Osaka City University as untrained panelists who could distinguish between the flavors of sucrose (0.5%), NaCl (0.14%), tartrate (0.007%), caffeine (0.025%), and monosodium glutamate (0.06%) at near threshold concentrations. The sweetness and characteristics of AG were evaluated in comparison with sucrose.

1.3.2 Sweetness of AG

The sweetness of AG solution (5.0%) was compared with that of sucrose solution (2.0%–4.0%) and was found to be $58 \pm 2.7\%$ of sucrose at 20°C. The panellists remarked that the sweetness of AG disappeared more rapidly than that of sucrose and left a slightly bitter aftertaste (**Figure 2**).

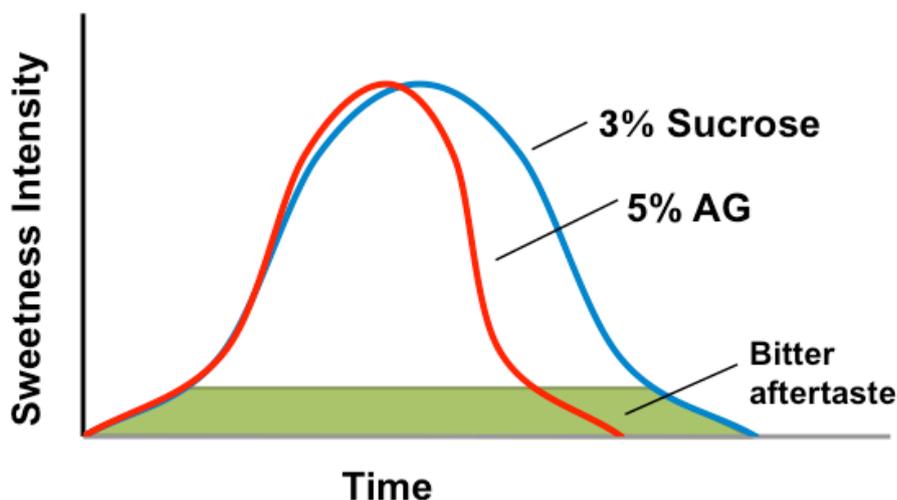


Figure 2. Taste profile of AG

1.4 SOME PHYSICOCHEMICAL PROPERTIES OF AG

1.4.1 Maillard reaction

No Maillard reaction occurred, because AG is not a reducing sugar (see Fig. 1).

1.4.2 Hygroscopicity

Hygroscopicity, the tendency for materials to absorb moisture from their surroundings, is an important property of food ingredients. We observed that AG did not begin to absorb moisture until the atmospheric relative humidity exceeds 80% at 25°C for 28 days.

1.4.3 Water activity (A_w)

Water activity is also one of the most important factors for food preservation. The A_w of AG was measured using the method of Landrock and Proctor (1951). In brief, 1-ml aliquots of the sugar solutions (40% w/v) were weighed and placed in sealed containers. After adjusting the A_w using saturated salt solutions [KCl (A_w : 0.842), C_6H_5COONa (0.880), KNO_3 (0.924), K_2SO_4 (0.970), and $K_2Cr_2O_7$ (0.980)], the containers were stored for 35 days at 25°C. Differences from initial weights against varying A_w were plotted. The A_w of sugar was determined as the intersection between the plotted curve and the vertical axis of zero.

The A_w of AG was 0.912 (Figure 3), which was lower than that of glucose (0.920), fructose (0.962), sorbitol (0.925), and sucrose (0.962). Application of AG with low A_w for a food additive is now under investigation.

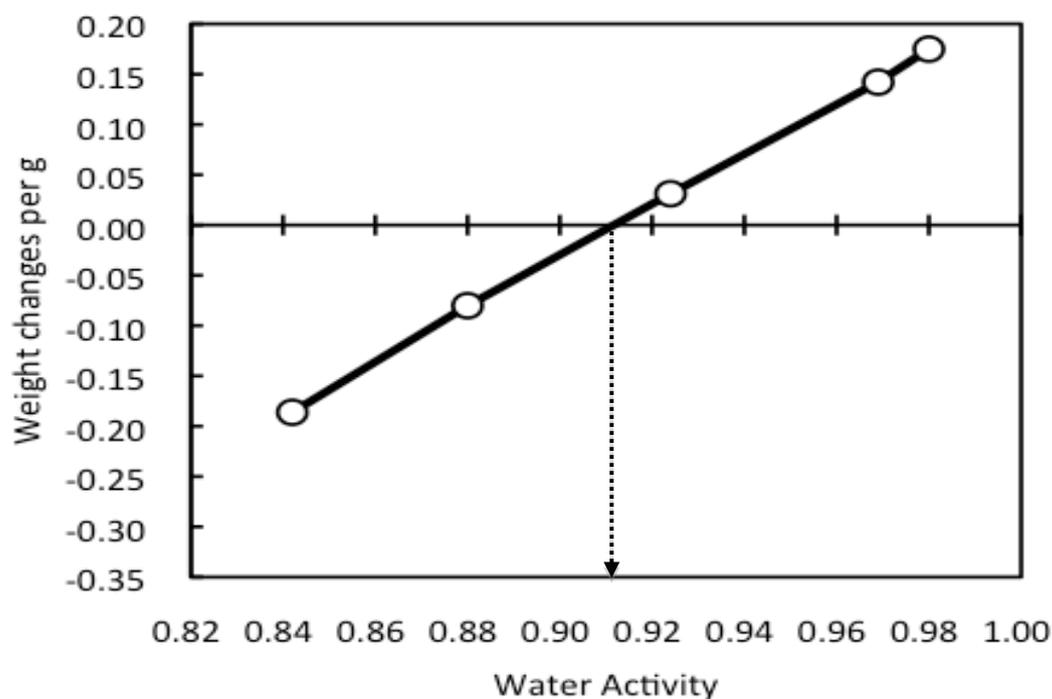


Figure 3. Measurement of A_w of AG

1.5 DISTRIBUTION AND FUNCTION OF AG IN RATS

1.5.1 Tissue distribution of AG after a single oral administration

Five-week-old male Wistar rats (approximately 80 g in BW; Clea Japan, Tokyo) were let fasting for 24 h before experimentation. To determine the pharmacokinetics of AG, rats were orally administered AG (375 mg/kg BW) and fed laboratory chow *ad libitum* for 72 h. Urine was collected at 0–9, 9–24, 24–48, and 48–72 h. Plasma and urine AG concentrations were determined using Lana 1,5-AG assay kits. Subsequently, AG concentrations in liver, kidney, skeletal muscle, and brain tissues were determined after extracting the tissues with 4% TCA using a mini column packed with anion and cation exchange resins, as described previously (Yabuuchi et al., 1989).

Figure 4 shows the changes in plasma AG levels in rats after a single administration of AG (375 mg/kg BW). The half-life of AG in plasma was approximately 12 h, which is shorter than the half-life of 3 days when AG was given at 5 mg/kg BW (Pikänen and Pikänen, 1984). This difference is probably due to the difference in dosage. Plasma AG levels rapidly decreased over 9 h (Figure 4), and 58% of the administered AG was recovered in urine (Figure 5).

AG levels in tissues (kidney, brain, liver, and skeletal muscle) rapidly increased within 3 h and then gradually decreased until 72 h, with a half-life of 22–24 h (Figure 6). AG levels in these tissues were significantly higher than those in the tissues of control animals, suggesting that the turnover of AG is slow. These data suggest that a high dose of AG (375 mg/kg BW) is rapidly cleared from plasma into urine, reflecting differing AG compartmentalization from that of the steady state. Further pharmacokinetic evaluations of various AG doses are required to confirm this.

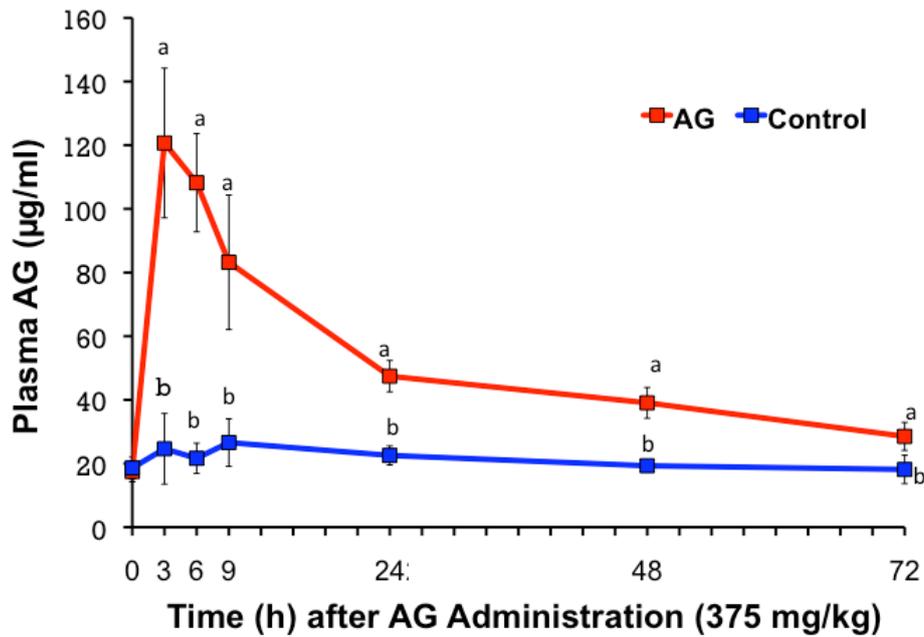


Figure 4. Changes in plasma AG levels after a single administration of AG. Fasted rats were orally given AG (375 mg/kg BW) (■) or water (control, ■) and fed a laboratory chow *ad libitum*. a vs b, $P < 0.05$.

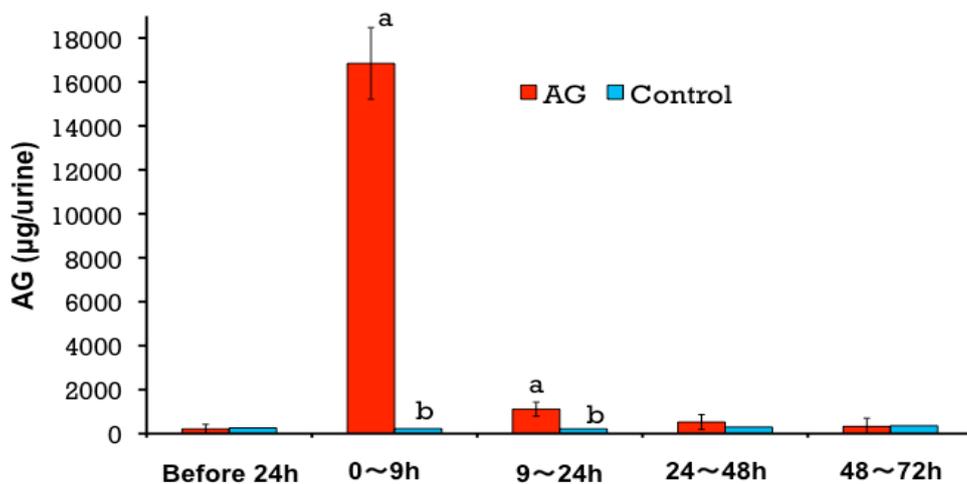


Figure 5. Urinary excretion of AG

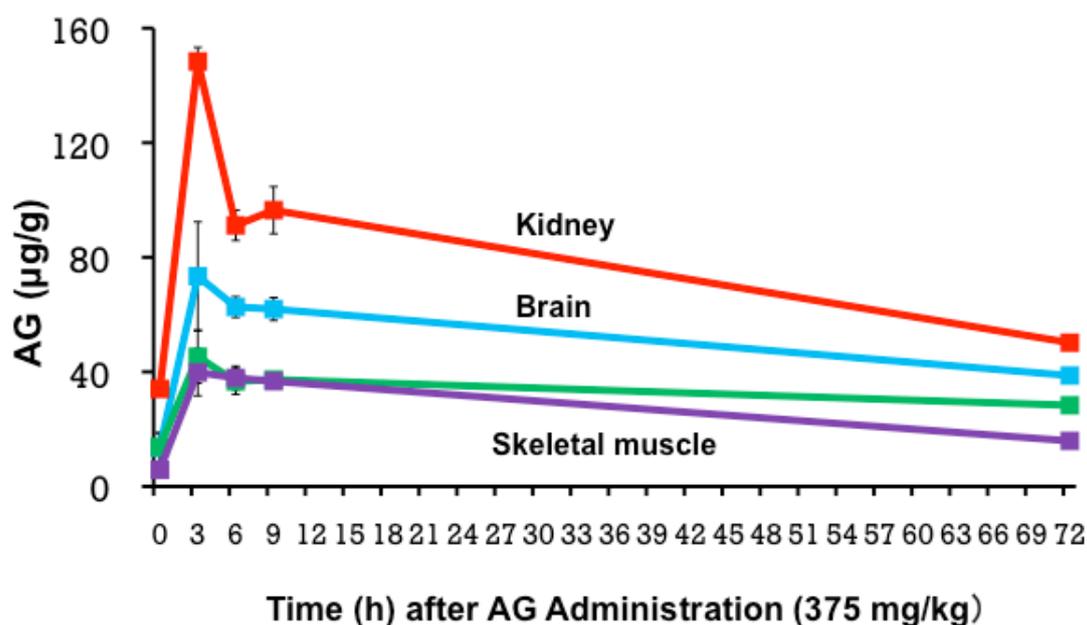


Figure 6. Changes in AG concentrations of various tissues of rats after a single administration of AG.

Fasted rats were orally given AG (375 mg/kg BW) and then fed a laboratory chow *ad libitum*.

1.5.2 Prevention of increase in postprandial blood glucose level

To examine the effects of AG on postprandial blood glucose levels, fasted rats (80 g BW) were orally administered glucose (200 mg i.e. 2500 mg/kg BW) with and without AG (30, 60, 120 mg i.e. 375, 750, and 1500 mg/kg BW, respectively) using a stomach tube. Tail veins were cut with a razor and blood was collected from the wounds. Blood glucose levels were measured using a glucose sensor (Medisafe mini GR-102, Terumo Co., Ltd., Tokyo, Japan). Blood was also collected using heparinized capillary tubes and centrifuged to obtain plasma. All experiments were performed according to the guidelines for animal experimentation at Osaka City University.

Single doses of AG at 375, 750, and 1500 mg/kg BW to rats did not affect blood glucose levels (data not shown). However, as shown in **Figure 7**, the increase in blood glucose levels following a single dose of glucose at 2500 mg/kg BW was prevented by combined dose of AG in a dose-dependent manner.

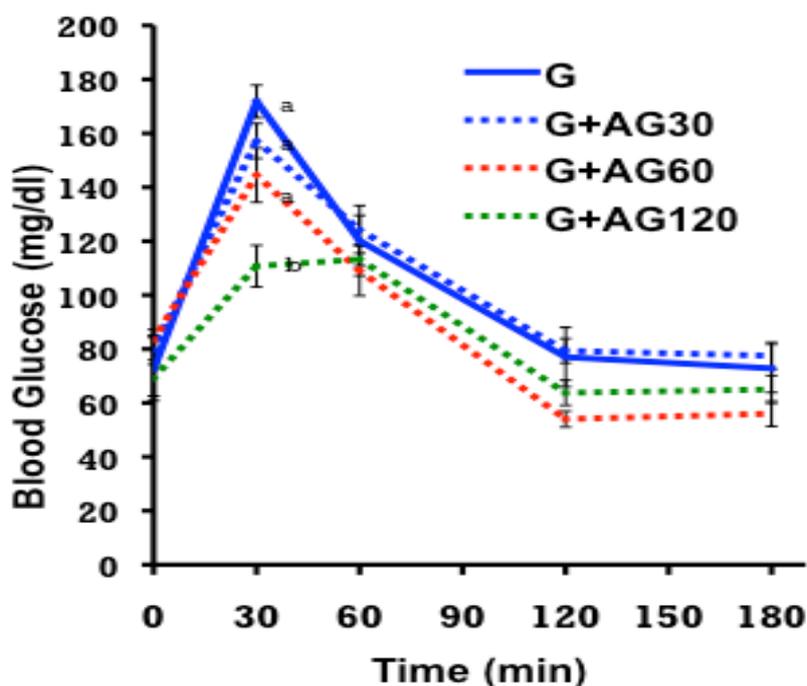


Figure 7. Effects of AG on postprandial blood glucose levels in rats.

Fasted rats were given glucose (2500 mg/kg BW) (blue solid line) with AG [375 mg (blue dotted line), 750 mg (red dotted line), 1500 mg (green dotted line)/kg BW]. a vs b, $P < 0.05$

These results suggest that the intestinal absorption of glucose is competitively inhibited by AG given that glucose and AG share the same intestinal transport system (Crane et al., 1960).

Kato et al. (2013) also observed AG-mediated suppression of postprandial hyperglycemia in normal mice, and reported that long-term feeding of AG significantly decreased blood glucose levels in db/db diabetic mice. Thus, AG has the potential to be used as a functional sweetener that prevents diabetes or obesity when consumed routinely.

1.6 ENZYMATIC CONVERSION OF AG TO AF

Standard AF was provided by Nihon Denpun Kogyo Co., Kagoshima, Japan, and pyranose oxidase (PROD) was supplied by Ikeda Tohka Industries, Fukuyama, Japan. Catalase was purchased from Sigma-Aldorich Japan. A reaction mixture (250 ml) containing 20 mM AG, PROD (1 U/ml), catalase (900 U/ml), and 38 mM citrate–phosphate buffer (pH 4.0–7.0) was incubated at 37°C for 1–18 h. After termination of the reaction by heating, the AF product was analyzed using an HPLC column packed with Na^+ exchange resin (MCI Gel CK08S) equipped with an RI 704 detector (GL Sciences, Osaka, Japan). Water was used as the mobile phase at a flow rate of 0.5 ml/min at 40°C. Under these conditions, the retention times of AG and AF were 23 and 25 min, respectively.

The optimum conditions for enzymatic conversion of AG to AF were 1 U/ml PROD at pH 4.0 for 18 h, which led to an AF yield of 88%. Doubling the amount of enzyme did not improve the yield (**Table 1**).

Table 1 Conversion rate of AF (%) from AG

PROD (U/ml)	Incubation time		
	1h	3h	18h
0.5	13.9%	26.2%	69.3%
1.0	18.3%	35.9%	87.6%
1.5	29.0%	57.4%	87.1%

Preparation of AF using AG and PROD is simpler than that using α -1,4-glucan lyase, which mimics *in vivo* metabolism (Fujisue et al., 1999; Yu et al., 2004). However, technical problems with this method include the requirement of an immobilized enzyme system, which may represent a challenge for increasing the efficiency of conversion of AG to AF. AF has anti-oxidative, anti-browning, and anti-microbial activities, which may facilitate food reservation. Furthermore, AF has potential medical applications as an anti-cariogenic, anti-inflammatory, anti-cancer (Fiskesund et al., 2010), and anti-obesity agent (Kojima-Yuasa et al., 2012). AF has been shown to have low or no toxicity in rats because AF was metabolized to AG and excreted in urine (Yu et al., 2004).

1.7 CONCLUSION

In this work, 1,5-anhydroglucitol (AG) was successfully produced at laboratory scale from *Polygalae radix* using a simple method. Study indicates that AG is a promising functional sweetener that may help in preventing lifestyle-related diseases. In addition, AG could be used as a source of the multifunctional sugar AF (1,5-anhydrofructose).

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Chapter 2

Cassava Grates and Flour Processing System

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2.1 INTRODUCTION

Fresh or dried cassava grates are the main ingredients used in many local food products such as *pitsi-pitsi*, cassava cakes, choco-rolls, *suman*, *puto*, chippy, *cacharon*. For cassava flour, studies have shown that it could fully or partially substitute wheat flour in many baked food products as shown in **Table 1** (Tan and Orias, 2008).

Table 1. Acceptable level of substitution of cassava flour as wheat flour substitute in food products

Food products	Acceptable substitution level (%)	References
Paborita, Cheese crackers, Coconut cookies, Doughnut,	50	Palomar <i>et al.</i> (1981)
Polvoron	100	
Pan de sal	20	Fementerera <i>et al.</i> (1984)
Fried cheese stick	50	
Cinnamon roll, Muffins, Cassava shrimp sticks	50	Lauzon <i>et al.</i> (1985)
Chiffon cake, Butter cake, Cacharon	100	
Hot rolls	20	
Loaf bread	10	
Soy sauce	100	Diamante & Data (1985)

PhilRootcrops and the Department of Food Science and Technology of the Visayas State University have developed food products from cassava grates and cassava flour to produce puffed products like *cacharon*, chippy and *cabcab*. These products, especially the chippy and *cabcab* are continuously produced locally. The ready to fry chippy or *cabcab* are sold at P160 – P200/kg. Once cooked, they can be sold as high as P400/kg.

Table 2 shows that the total wheat flour importation in 2012 reached 3,645,000 MT. A 10% substitution of this wheat flour with cassava flour would mean a reduction of wheat flour importation of 364,500 MT and the utilization of the same amount of locally produced cassava flour, which can be translated into substantial foreign exchange savings.

Table 2. Wheat flour importation of the Philippines

YEAR	IMPORT	UNITS OF MEASURE	GROWTH RATE
2010	3224	(1000 MT)	0.84%
2011	4075	(1000 MT)	26.40%
2012	3645	(1000 MT)	-10.55%

Sources: Corpuz, V., and Verzani, W. (2013).

Cassava production in the country in 2006 reached 1,756,859 MT with a total area of 204,578 hectares (BAS, 2007). In Central Visayas where Bohol is located, the total production in the region in 2006 was 69,566 MT. Most of the cassava produced in Central Visayas is in Bohol because of the presence of Philstarch that manufacture cassava starch.

Cassava is easily grown even in marginal areas with low yield performance. But cassava can easily yield more than 30 tons/ha with proper management practices as observed in commercial farms. The national average yield in 2006 has been officially reported at only 8.59 tons/ha as this reflects the predominantly semi-subsistence type of farming.

As shown, there is already an existing demand for cassava grates and flour, and the raw materials which are the fresh cassava tubers are readily available. Also, the technology of producing cassava grates and flour already exists. However, there is a need to further improve the system to be able to meet the demand of the cooperator in Bohol, in terms of performance, capacity and efficiency. This project therefore set-up and established a cassava grates and flour processing system in Bohol based from the existing technology.

Tan and Orias (2008) reported the technology in producing dried grates and cassava flour. The dried grates processing technology included the following machines: 1) cassava washer for washing the peeled cassava tubers, 2) cassava grater for grating the peeled tubers, 3) cassava grates spinner for removing the large amount moisture from the grates before drying, 4) cassava grates pulverizer for pulverizing the lump of grates produced after spinning, 5) rotary drum dryer for drying the cassava grates. The product produced from the dried grates processing technology should have moisture content of less than 10% for safe storage, and ready for milling to flour. The flour milling process includes a single machine which is the cassava flour mill. The capacity of the whole dried cassava grates and flour processing system is limited by the capacity of the rotary dryer of only about 10 kg/h.

Although the technologies in the production of cassava grates and flour are already developed, improvements were needed before the actual transfer of the machines to the site in Bohol, which were the following: 1) Improving the processing system fit for commercial application; 2) properly matching the individual machines (i.e. the grater, spinner, pulverizer, dryer and flour mill) included in the system in terms of capacity, and as required by the end-user.

The objectives of this project are: i) to improve the existing cassava grates and flour processing system to meet the required output capacity of the cooperator, ii) to pilot test the improved cassava flour and grates processing systems at commercial scale in Bohol.

2.2 DEVELOPMENT OF THE CASSAVA GRATES AND FLOUR PROCESSING SYSTEM

2.2.1 Fabrication of the machines

Machines included in the system were fabricated based on the existing cassava grates and flour processing system. For this project, the machines were classified into two based on the level of work to be done on the machines: 1) Machine/ Equipment that needed scaling-up, which is the rotary drum dryer for grates; and 2) All the other machines which needed no scaling up but were fabricated based from the existing machines

2.2.1.1 Scaled-Up Rotary Drum Dryer for Grates

The existing rotary drum dryer for cassava grates had only drying capacity of about 9 kg/h making the drying process the limiting capacity of the whole processing system. Therefore a new rotary drum dryer design was fabricated. The new design basically was a scaled-up version of the existing rotary dryer. Its capacity was designed to reach 25 kg/h. The fabricated dryer was evaluated by PhilRootcrops and by the Agricultural Machinery Testing and Evaluation Center (AMTEC). After the evaluation and further improvement, the dryer was brought to the project site in Tagbilaran City, Bohol for piloting together with the other machines included in the cassava grates and flour processing system.

2.2.1.2 Other machines

Other machines included in the cassava grates and flour processing systems besides the dryer are the following: 1) Cassava root washer, 2) Cassava grater, 3) Cassava grates spinner; 4) Cassava grates pulverizer, 5) Cassava flour mill. The prototypes of these machines already exist. For this project, another set of these machines were fabricated, and brought to the site in Bohol after thorough evaluation by PhilRootcrops and by AMTEC.

2.2.2 Transport and Establishment of the Machines at the Pilot Site

All the machines were brought to the site in Tagbilaran City, Bohol. These were set up in the temporary processing site, since the permanent location has yet to be finalized.

2.3 THE CASSAVA GRATES AND FLOUR PROCESSING SYSTEM

2.3.1 Scaled-up Dryer

Figure 1 is the existing rotary drum dryer for grates. Tan and Orias (2008) and Tan *et al.* (2007) reported that this dryer is a rotary drum-type dryer with 3 layers of cylinders. Its heat source is the set of 3 electric heaters capable of providing a maximum drying air temperature of more than 100°C. The dryer is installed with a variable speed motor, and a blower. It has a capacity of about 9 kg/h. The grates to be dried have to pass through the dryer twice to obtain the desired moisture content of about 5% wb.

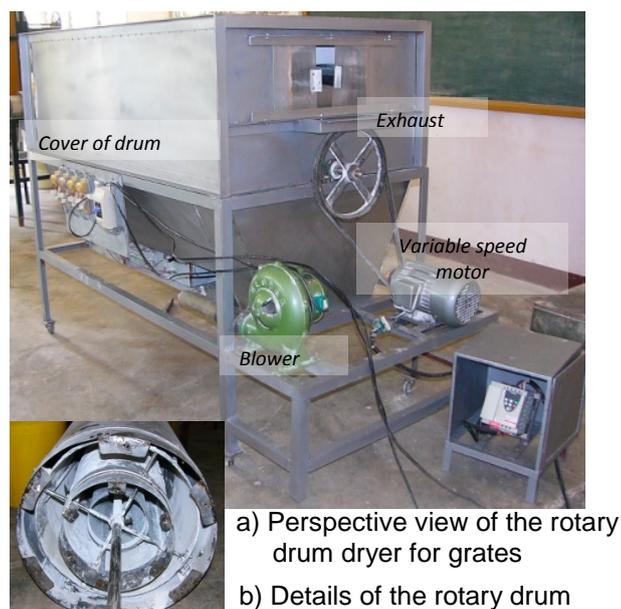


Figure 1. The rotary drum dryer for drying the cassava grates

Based on this rotary dryer, another design was made and fabricated to have a capacity of about 25 kg/h. **Figure 2** shows the newly fabricated rotary drum dryer. It had 4 sets of drums with 3 cylindrical layers per drum. The dryer was made of all stainless steel materials. Its heat source was a diesel-fuelled furnace with heat exchanger. The furnace consisted of 3 burners with pressurized diesel fuel tank. A blower drove the heated air to the individual drum sets inside the drying chambers.

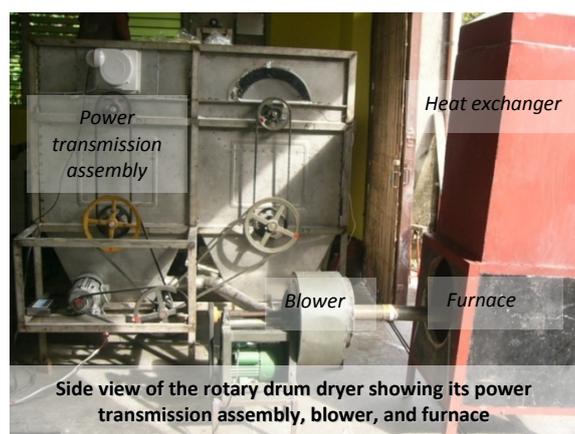


Figure 2. The newly fabricated rotary drum dryer

The Rotary Drum Dryer was tested using cassava grates with an average moisture content of 49.0%. With loading rate set at 30 kg/h and an average drying air temperature of 100.3 °C, the continuous-flow type dryer had a drying rate of 22.8 kg/h of dried cassava grates with final moisture content of

3.92%. The dryer had heating system efficiency of 89.1%, and furnace fuel consumption was 1.02 L/h of diesel. The total power consumption of the dryer motor was 0.58 kW, while the total power consumption of the blower was 0.85 kW. Two persons were needed to operate the dryer namely to load the materials to be dried and to monitor the furnace.

2.3.2 Fabricated Machines

1. Cassava Root Washer

The cassava root washer is as shown in **Figure 3** was designed for cleaning and washing the peeled cassava roots. Its main part was the rotating cylinder where the roots were loaded. The cylinder was made of expanded stainless steel sheet. During operation, the rotating cylinder was partly soaked with water in the water basin to wash the roots during operation. The roots were loaded on one end of the cylinder and then moved by gravity through inside the rotating cylinder until discharged on the other end. The basin was fitted with a drain hole at the bottom of the basin to remove water by gravity after each operation. The machine capacity was about 700 kg/h with 128 L of water loaded to the basin. It had also a machine efficiency of 91.10% showing that there were losses of about 9% when washing the peeled roots, due to the abrasive action of the expanded steel plate with the roots during operation.



Figure 3. The cassava root washer

A closer inspection of the peeled roots after washing showed that some parts of the peeled roots were removed especially those at the edges of the roots. The roots should not stay longer than necessary in the rotating cylinder to prevent more losses of the peeled roots during operation. The machine was able to clean the dirt from the peeled roots. Two or three persons were needed to operate the machine – one or two persons for loading the materials and one person for replacing the container once the container is filled with the roots.

2. Cassava Grater

The newly fabricated grating machine (**Figure 4**) was a circumferential-type grater, which had the punctured surface of the tapered cylinder as its blade. It was driven by a 1-hp electric motor. During operation, roots were loaded to the inlet hopper individually and pressed by the other roots to the rotating grater blade. The roots could either be loaded without pressing against the rotating blade or the individual roots could be pressed against the blade to facilitate faster grating. The grated roots exited through the outlet hopper below the grater blades.



Figure 4. The cassava grater during operation

The cassava grater had an average input capacity of 500 kg/h of fresh cassava and an output capacity of 470.0 kg/h of grated cassava at grater's shaft speed of 732 rpm with grates recovery of 94.0%. The power consumption of the grater was 0.39 kW. During operation, the noise emitted by the machine at the feeder's ear level was 88 db(A). Two persons were needed to operate the machine, one for feeding the cassava into the machine and the other for collecting the cassava grates.

3. Cassava Grates Spinner

The fabricated spinner (**Figure 5**) is used to remove water from fresh cassava grates. To prevent lumping of the grates during drying, it is necessary that moisture content of the grates be reduced to about 45%wb (from more than 65%wb) before drying. Alosnos (2007) reported that the bulk density of the grates produced using the presser was not significantly different from the bulk density of the grates produced using the spinner. This indicates that the pressure exerted to the grates by the manual presser and the mechanized spinner was not significantly different. Pono (2004) also showed that the spinner was able to reduce the moisture content of the grates to the desired level (i.e., MC level where no lumps are formed by the grates when pulverized). There was a decrease in moisture content from 48.14% to 44.75% as the rotation speed was increased from 1750 to 2800 rpm. A decrease in moisture content was observed from 48.48 to 44.40% when spinning time was increased from 2.5 to 7.5 minutes. However, there was an

increase in moisture content from 45.50% to 47.17% as the load was increased from 10-18 kilograms. Maximum loading capacity of the spinner was found to be 289 kg/h. At a loading capacity of 12 kg/batch, the spinner had an average Input capacity of 120.0 kg/h of grated cassava at spinner's shaft speed of 2229 rpm with final moisture content of 44.1%. The power consumption of the spinner was 1.03 kW. One person was needed to operate the machine.



Figure 5. Interior view of the cassava grates spinner, also showing the lump of grates inside straw bags formed after spinning

4. The Cassava Grates Pulverizer/Siever

The newly fabricated pulverizer/siever machine (**Figure 6**) does not only pulverize the lump of grates after spinning, but also separates the grates from the coarse materials before drying. Its operating mechanism includes the rotating paddle enclosed in a stationary cylindrical perforated steel plate and a screen (mesh #10). The lump of grates are pulverized in the loading hopper by the short screw conveyor, and then brought inside to the paddle then to the screen. The grates that passed through the screen are the desired grates ready for drying. When tested using cassava grates that passed through the spinner with average moisture content of 44.1%, it had an average input capacity of 673.3 kg/h of grated cassava and an output capacity of 497.5 kg/h of pulverized cassava grates at pulverizer's shaft speed of 452 rpm. The machine had product recovery of 98.5% and a power consumption of 0.14 kW. During operation, the noise emitted by the machine at the feeder's ear level was 87 db(A). One person was needed to operate the machine.



Figure 6. Perspective and interior views of the cassava grates pulverizer/siever

5. Cassava Flour Mill

The Cassava Flour Mill (**Figure 7**) was a centrifugal type flour mill. It was powered by a 2.24 kW electric motor which drove directly the milling shaft through a belt-pulley transmission system. The machine consisted of the hopper, milling chamber and cyclone. The inlet hopper was where the materials to be milled were fed. It was made of stainless steel sheet and installed above the milling chamber. The milling chamber consisted of a radial centrifugal fan, and double layer screens. The size (D x W) of the centrifugal fan was 445 mm x 100 mm and had five blades. The double layer screens were installed inside the milling chamber, around the fan. The inner layer screen had perforations of Mesh 80 while the outer layer screen which served as support to the inner layer screen had perforations of 6 mm.



Figure 7. The perspective view of the cassava flour mill

Connected to the milling chamber was a cyclone separator where the milled product was collected. The Cassava Flour Mill had an input capacity of 71.4 kg/h while the milling capacity was 70.0 kg/h with milling recovery of 98.0%. Laboratory analysis of samples taken from the output of milling unit showed that the fineness modulus of the ground product was 0.009 with an average particle size of diameter of 0.105 mm. Its power consumption was 2.11 kW.

2.3.3 The Cassava Grates and Flour Pilot Plant in Bohol

The pilot plant started operation producing dried cassava grates to supply the demand of the cooperator's food products. **Table 3** shows the record of their operations, where it processed 2,257 kg fresh cassava roots into dried grates in a period of 11 days. It is noted that the average dried grates recovery of the pilot plant in processing the dried grates is only 23.05% which is way below the average dried grates recovery of 27% recorded at PhilRootcrops. This could be due to the varieties and sizes of the roots processed. When computed, the losses after peeling the roots was very high at 38.32%, which at PhilRootcrops averaged only at 20%. The smaller the roots, the higher amount of peels and wastes are produced than larger roots.

Table 3. Initial production of the cassava grates and flour processing system at Bohol in 2012

Day	Processed Cassava, kg						Dried Grates Recovery, %
	Fresh Roots	Peeled Roots	Grated Roots	Spinned Grates	Sifted Grates	Dried Grates	
1	219	126	126	97.0	93.0	48.5	22.1
2	152	102	102	89.0	84.0	49.0	32.2
3	212	129	129	90.0	85.0	42.0	19.8
4	239	173	173	123.5	118.5	52.5	22.0
5	209	124	124	89.0	83.0	43.5	20.8
6	136	90	90	75.0	69.0	42.5	31.3
7	221	131	131	87.5	82.0	46.5	21.0
8	210	129	129	85.5	81.0	44.0	20.9
9	213	138	95	85.0	45.0	49.0	23.0
10	146	97	97	88.0	84.0	46.7	32.0
11	300	153	153	120.0	90.0	56.0	18.7
Total	2257	1392	1349	1029.5	914.5	520.2	23.1

The dried grates are being used to replace fresh grates in many food products. The cooperator tried to use the dried grates in their cassava cake business with the plan of expanding to other places outside Bohol. It was, however, found out that the sales of the cassava cake went down after using the dried grates for about a month. With this observation, there is a need to conduct rehydration studies of the dried grates to determine the factors that contribute to the difference in quality of the dried grates from that of the fresh grates in the cassava cake product. Furthermore, actual shelf life of the grates

at different storage conditions needs to be determined. There is a need to lengthen the storage life of the grates beyond 6 months as an industry practice. Also, the material-in-process characteristics and the cooling characteristics of the dried grates need to be established.

2.4 CONCLUSIONS

All the machines that comprise the cassava grates and flour processing system were fabricated according to the desired specifications. The fabricated machines functioned and performed as expected – built with all stainless steel materials, build stronger, and built with all the exposed moving parts covered.

The rotary drum dryer for cassava grates was scaled and attained a drying capacity of 27 kg/h in the first two sets of drums with a final moisture content of 8.64%, and 22.8 kg/h in the 3rd and 4th sets of drums with a final moisture content of 3.92%, which was near the desired capacity of 25 kg/h.

The cassava grates and flour processing set-up already operated in the site in Tagbilaran City, Bohol, but still needs further improvement both in the product and the process.

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Chapter 3

Processing, Nutrition and Health Aspects of Cold-Pressed Rice Bran Oil

R. Singanusong

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3.1 INTRODUCTION

Rice bran oil (RBO), or also known as heart oil or world's healthiest oil is rich sources of commercially important bioactive phytochemicals with antioxidant activities and potential health benefits, especially in food, nutritional, pharmaceutical and cosmetic applications (Chen and Bergman, 2005; Danielski et al., 2005; Da Silva et al., 2005; Lerma-Garcia et al., 2009; Van Hoed et al., 2010; Singanusong and Noitup, 2012). In addition, the antioxidants of RBO have a potential use as additives to improve the storage stability of foods (Nanua et al., 2000; Kim and Godber, 2001; Chen and Bergman, 2005). Furthermore, RBO has a very good balance in its fatty acid composition (Ghosh, 2007) and does not produce any allergenic reactions when digested (Crevel et al., 2000). Hence, it is one of the three healthiest edible oils recommended by World Health Organization.

γ -Oryzanol, tocotrienols, tocopherols, phytosterols, polyphenols and squalene are valuable bioactive components commonly found in RBO. These bioactive unsaponifiable compounds can be found more intensively in CPRBO due to its production process. CPRBO has received more attention and become more profitable in Thailand, Malaysia and Japan as food supplement, main ingredient for nutrition, cosmetics, pharmacy and for extraction of those bioactive components. CPRBO is produced by mechanical pressing the oil from freshly milled fine rice bran without any chemicals and successive steps of refining, except membrane filtration whereas refined RBO involves using chemical and heat treatments in order to improve quality of the oil. Without severe conditions, CPRBO retains much higher concentrations of such minor compounds than those refined RBO (Singanusong and Noitup, 2012), free of chemicals and is considered a safer method (Thanonkaew et al., 2012). Furthermore, mechanical or cold pressing is simple and safe (Singh and Bargale, 2000), technically less expensive and less labour-intensive than the conventional solvent extraction method (Thanonkaew et al., 2012) that is normally used for production of commercially edible refined RBO.

Singanusong and Noitup (2012) reported that γ -oryzanol content of CPRBO (11,550 ppm) was much higher (6.03x) than that of refined RBO (1,915 ppm) and the γ -oryzanol content of liquid non-dairy creamer from CPRBO was 3,955 ppm, 6 times greater than that of liquid non-dairy creamer from refined RBO (638.57 ppm). Loyda et al. (2012) determined the γ -oryzanol content in freshly milled fine Pathumthani1 rice bran and CPRBO during the production process. They reported that the γ -oryzanol content was as high as 50,450 ppm in the oil of freshly milled rice bran but continuously decreased in every step of production process until reaching 16,950 ppm, the lowest, at the last step, indicating 66% loss of γ -oryzanol content during the production process. Further research and development on production process of CPRBO is therefore needed in order to minimize loss of this valuable antioxidative compound.

3.2 RICE BRAN

Rice bran, the outer layer of brown rice and one of the major by-products in the rice milling process accounting for approximately 8-10% of milled rice (Shih et al., 1999; Lilitchan et al., 2008) is the only material for the production of CPRBO. Hence, using good quality rice bran would result in good quality

CPRBO. Rice bran generally contains high lipid content ranging from 18 to 22%, mainly unsaturated fatty acids (Orthofer, 1996; McCaskill and Zhang, 1999) and active enzymes including lipase, lipoxygenase and peroxidase. The high unsaturated fatty acids content and active enzymes cause drastic quality degradation and shelf-life reduction of rice bran (Ramarathnam et al., 1989; Orthofer, 2005) mainly through lipid peroxidation.

Prevention of lipid peroxidation or rancidity of rice bran can be achieved by two effective methods namely extraction of oil soon after milling or stabilization (Ju and Vali, 2005). The former may not always be practical, particularly for the commercial scale due to distance and transportation of rice bran from rice mill to the factory. Various methods of stabilization of rice bran have been attempted by various researchers, for example, microwave heating (Lakkahula et al., 2004; Zigoneanu et al., 2008), ohmic heating (Ramezanzadeh et al., 2000), steaming (Juliano, 1985), extrusion (Zhu and Yao, 2006), refrigeration and pH lowering (Amarasinghe et al., 2009).

Thanonkaew et al. (2012) investigated the impact of Thailand's domestic heating on cold pressing yield, quality and antioxidant properties of CPRBO. They reported that hot air and microwave heating were the most effective methods for stabilization of rice bran since it provided high oil yield, lower acid, free fatty acid and peroxide values and higher contents of bioactive compounds such as phenolic compounds, flavonoids and γ -oryzanol. However, for small and medium scale, hot air heating could be more applicable than microwave heating in term of economical and stabilizing efficiency.

However, only few researches have been studied on CPRBO. This is mainly due to its low yield and limitation on efficiency of the pressing machine. This present work reports data on pressing of oil from rice bran soon after milling process or within 24 h since the study was conducted at local small and medium enterprises (SME) of CPRBO producers.

3.3 PRODUCTION OF CPRBO

3.3.1 Collection of rice bran

Without stabilization process, the most suitable rice bran for oil pressing is fine rice bran freshly milled within 24 h. The oil content of fine rice bran varies between 10-25%, consistent with the value of 15-20% reported by Juliano and Hicks (1996). The concentrations can vary substantially according to various factors such as rice variety, growing conditions, stages of harvest, storage time and conditions of paddy before milling and milling conditions. Rice bran collected from different rice mills showed different physical and chemical properties as detailed in [Table 1](#) and [Table 2](#).

Table 1. Physical properties of rice bran samples

Sample	Type	Colour	Foreign matters
1	Fine	Light brown	Broken rice grains and rice husks
2	Fine	Brown	Milled rice grains, broken rice grains and rice husks
3	Fine	Light yellow	No foreign matters
4	Fine	Cream-light yellow	No foreign matters
5	Fine	Cream-light yellow	No foreign matters

Table 2. Chemical properties of rice bran samples

Sample	Content \pm SD				
	pH	Moisture (%)	Total lipid (%)	Free fatty acid (g/100 g oil)	γ -Oryzanol (ppm)
1	6.77b \pm 0.00	7.98c \pm 0.01	10.27c \pm 0.50	3.60c \pm 0.10	1,590.50a \pm 0.01
2	6.16d \pm 0.01	7.83c \pm 0.15	24.59a \pm 0.50	9.30a \pm 0.20	1,574.00b \pm 0.01
3	6.54c \pm 0.01	8.87a \pm 0.08	17.95b \pm 0.06	9.23a \pm 0.30	1,258.10c \pm 0.01
4	6.76b \pm 0.00	8.88a \pm 0.18	19.20b \pm 0.00	5.01b \pm 0.30	1,079.10e \pm 0.01
5	6.86a \pm 0.01	8.22b \pm 0.03	20.75ab \pm 0.57	5.50b \pm 0.40	1,225.40d \pm 0.01

Means with the same letters in the column are not significantly different ($p>0.05$).

3.3.2 Sieving of rice bran

Although fine rice bran is used for oil pressing, it is recommended to sieve before pressing due mainly to remove any foreign matters present in rice bran such as rice grains, rice husks and stones. These foreign matters can negatively affect the oil quality and the machine's pressing efficiency or in worst case, it can damage the screw expeller. Sieving can be achieved by sifting rice bran through a sieving machine. Any foreign matters can then be separated from the rice bran. Example of a local made sieving machine for SME used in Thailand is shown in **Figure 1**. This sieving machine is cheap and low energy consuming but low efficiency and takes long time for sieving. However, a commercial sieving machine is also available. The latter is less time consuming, has higher sieving efficiency but more expensive than the former and is illustrated in **Figure 2**.



Figure 1. Local made sieving machine



Figure 2. Commercial sieving machine

In this present work, rice bran was sieved using a laboratory sieving machine with different mesh screens. **Table 3** shows that rice bran obtained at each mesh size consisted of different components and its content by weight.

Table 3. Rice bran constituents and content by weight at different mesh size

Mesh Size (μm)	Picture	Component and content by weight
1400		- Rice husk - Broken rice grain - 17.36%
710		- Fine rice husk - 14.56%
500		- Fine rice husk - Rice bran - 40.15%
300		- Rice bran - 24.25%
150		- Rice bran - 3.68%

Moreover, as can be seen from **Figure 3**, sieved rice bran from different mesh sizes showed different lipid content, those with smaller pore size (bigger mesh No.) had higher lipid content than those with larger pore size (smaller mesh No.). This is because at the bigger mesh No. there is no other foreign matter present in the rice bran while those with smaller mesh No. contained rice husk and broken rice grain. Rice husk and broken rice grain contain less lipid content than rice bran, contributing lower lipid content in rice bran that remained at the smaller mesh No.

This information indicates the importance of sieving of rice bran before pressing for oil due to removal of any foreign matters that may adversely affect the quality of the oil, pressing efficiency and life time of the cold pressing machine.

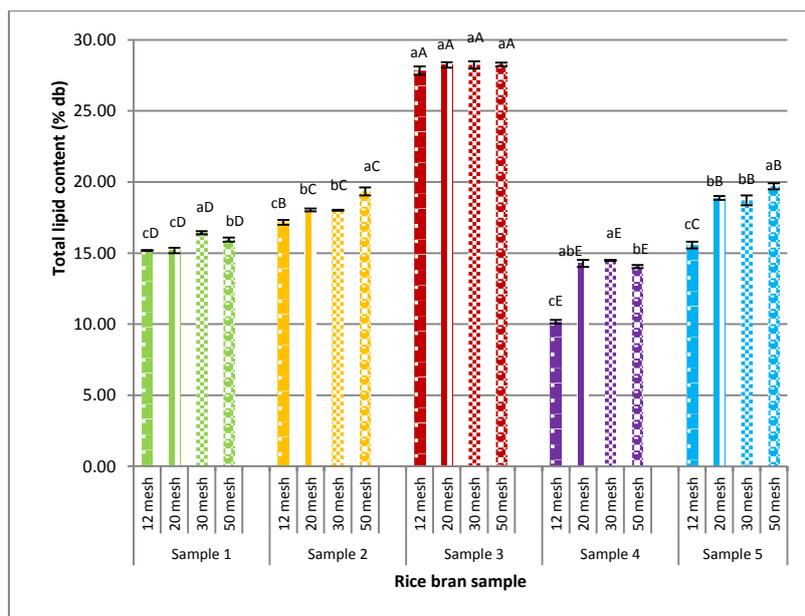


Figure 3. Total lipid content of rice bran samples with different pore sizes

Means with the same small letters in the sample are not significantly different ($p>0.05$).

Means with the same capital letters in the same mesh number are not significantly different ($p>0.05$).

3.3.3 Pressing of rice bran

A 2 HP screw press manufactured in Thailand as shown in **Figure 4** was used in this work.



Figure 4. The 2 HP screw press

The screw press consists of 6 main components, namely feed hopper, driving motor, power transmission and gear reduction unit, maddock screw type, barrel and frame. The pressing method is described as follow. Sieved freshly milled fine rice bran was fed into a hopper. The screw rotates towards the discharge end of the barrel conveying the rice bran along and to the end of the barrel. While the pressed rice bran cake is discharged through the circular aperture between the tapered end of the screw and the barrel, the pressed CPRBO drains through perforations in the barrel's bottom

(Sayasoonthorn et al., 2012) directly to a 1-mm in diameter strainer situated at the top of the container (**Figure 5**) before further use in filtration process.



Figure 5. CPRBO drained through perforations in the barrel's bottom to a strainer and container

To date, the pressing efficiency (50-55%) of the screw press manufactured in Thailand is relatively low. Using 2 HP pressing machine, 60 kg of rice bran can be pressed in an hour and 100 kg of rice bran yields 3-4 kg of CPRBO. For 7 HP pressing machine, 100 kg of rice bran can be pressed in an hour and 100 kg of rice bran yields 5-6 kg of CPRBO, leaving behind the oil in the cake or defatted rice bran. This shortcoming of the press machine implies further research and development on pressing efficiency is needed. Srikantha (1980) reported that mechanical press was relatively inefficient, leaving 8-14% of the available oil in the cake which is consistent to this present work. Minimum loss of oil and high pressing yield are the most potential areas of immediate actions for CPRBO industry in Thailand.

3.3.4 Filtration of CPRBO

After pressing, the CPRBO was left overnight in the container for precipitation of any fine rice bran that suspended in the oil. It was then filtered through Whatman No. 91 filter paper (10 μm) (**Figure 6**) and 10 plates of each 1 μm , 0.70 μm and 0.25 μm filter presses (**Figure 7**), respectively in order to remove any fine rice bran particles and to obtain clear CPRBO.



Figure 6. Filtration of CPRBO through Whatman No. 91 filter paper



Figure 7. Filtration of CPRBO through membrane filter press with different particle sizes

3.3.5 Sterilization of CPRBO

Finally, the filtered CPRBO was pumped into a 5-m long tube curling around a 50-Watt UV lamp for sterilization (**Figure 8**) before packaging.



Figure 8. Sterilization of CPRBO by UV lamp

3.3.6 Packaging of CPRBO

Generally, CPRBO can be packed in various types of packages, depending on format of sales or customer requirements. It can be packed in the opaque white or amber PVC plastic bottle, clear or amber glass bottle, PET plastic bag, soft gels or capsules with secondary packages. However, the package is very important as it affects the quality and shelf-life of CPRBO. Ideally, good package is those that can protect against migration of moisture, air, gas and light into the product due to prolong shelf-life of the product. Example of some packages of CPRBO is illustrated in **Figure 9**.



Figure 9. Packaging examples for CPRBO products

3.4 NUTRITION AND HEALTH ASPECTS OF CPRBO

Over the past few years, increased interest in CPRBO has been observed mainly due to its high nutritive and antioxidative properties and beneficial to health. CPRBO contained 11,550 ppm γ -oryzanol content (Table 4), lower than the value reported by Loyda et al. (2012) which ranged between 16,950-24,100 ppm and those of 20,300-23,000 ppm reported by Thanonkaew et al. (2012). However, the level found in this study was much higher than that from crude RBO (9,800 ppm) reported by Xu and Godber (1999) but similar (12,200 ppm) to those reported by Pestana et al. (2008). Variations in γ -oryzanol content may be due to the origin of the rice (Arab et al., 2011). γ -Oryzanol, ferulate ester of triterpene alcohol, can be found naturally only in RBO, and it has the potential to reduce LDL cholesterol (Lichenstein et al., 1994; Rong et al., 1997; Gerhardt and Gallo, 1998), protect from chronic disease caused by high cholesterol levels (Seetharamasah and Chandrasekhara, 1989), inhibit platelet aggregation (Kaimal, 1999), inhibit linoleic acid and cholesterol oxidation (Xu and Godber, 2001; Xu et al., 2001) prevent coronary artery disease (Imsanguan et al., 2008), protect the skin from the sun burning and prevent wrinkles of the skin (Graf, 1992).

Table 4. Chemical composition of CPRBO

Composition	Content
Moisture content (%)	0.0296
γ -oryzanol (ppm)	11,550
Vitamin E (α -tocopherol) (mg/100 g)	30.82
Antioxidant activity (DPPH) (%)	94.24
Trans fatty acid (g)	0
Fatty acid profile (g/100 g)	
Palmitic acid (C16:0)	21.00
Stearic acid (C18:0)	2.50
Total saturated fatty acids	23.50
Oleic acid (C18:1)	44.30
Total monounsaturated fatty acids	44.30
Linoleic acid (C18:2)	30.80
Linolenic acid (C18:3)	1.40
Total polyunsaturated fatty acids	32.20
Total unsaturated fatty acids	76.50
PMS ratio	1.4:1.9:1

Sources: Adapted from Singanusong and Jiamyangyuen (2012); Singanusong and Noitup (2012); Katsri et al. (2014)

Antioxidant activity of CPRBO in this study was particularly high (94.24% DPPH scavenging effect, Table 4). γ -Oryzanol (11,550 ppm) and α -tocopherol

(30.82 mg/100) reported in this work corresponded to this high antioxidant activity. Raghuram and Rukmini (1995); Sugano and Tsuji (1996); Sugano et al. (1999); Devi and Arumughan (2007) and Lerma-García et al. (2009) reported that RBO is rich in natural antioxidants or phytochemicals such as γ -oryzanol, tocotrienols, tocopherols, phytosterols, polyphenols and squalene. Therefore, CPRBO may also contain all of these antioxidants and contributing to the high antioxidant activity. These antioxidants have been reported to be very efficient in reducing low density lipoprotein and total serum cholesterol (Arab et al., 2011).

Five types of fatty acids in CPRBO in descending order were oleic, linoleic, palmitic, stearic and linolenic acids, respectively (Table 4) and all these fatty acids are long-chain fatty acids (16:0-18:0). This finding is consistent to that of Katsri et al. (2012); Singanusong and Jiamyangyuen (2012) and Singanusong and Noitup (2012). CPRBO predominantly contained 44.30% monounsaturated fatty acid (M) following by 32.20% polyunsaturated fatty acid (P) and 23.50% saturated fatty acid (S), respectively (Table 4) with its PMS ratio of 1.4:1.9:1 which is considered as a balanced oil (Ghosh, 2007). The PMS ratio of CPRBO is almost similar to those (1:1-1.5:1) recommended by World Health Organization, Food and Agriculture Organization and American Heart Association. Furthermore, these organizations also recommended RBO as appropriate edible oil for consumption.

Apart from having a balanced PMS ratio, CPRBO contains high MUFA which has important roles in reducing risk of coronary artery diseases by acting as antioxidants (Chen and Bergman 2005; Da Silva et al., 2005; Danielski et al., 2005; Lerma-Garcia et al., 2009; Van Hoed et al., 2010), reducing LDL cholesterol and increasing HDL cholesterol level (Lichtenstein et al., 1994), protecting against LDL cholesterol synthesis, inhibition of oxidation of LDL cholesterol, inhibition of platelet aggregation (Bucci et al., 2003) and preventing coronary artery disease (Imsanguan et al., 2008).

Unlike edible soybean oil, rapeseed oil, sunflower oil and corn oil in China that found the total trans fatty acid of 1.15, 1.37, 1.41 and 2.01 g/100 g, respectively (Hou et al., 2012), CPRBO had no trans fatty acid (Table 4). The trans fatty acid has been reported to be a contributor to heart disease and cancer (Fernandez et al., 2007; Hu et al., 2001; Ascherio et al., 1999; Mensink and Katan, 1990). A 2% increase in trans fatty acid intake has been calculated to increase the risk of coronary heart disease by 23% (Mozaffarian et al., 2006). Therefore, CPRBO is safe with regard to coronary heart disease caused by trans fatty acid.

3.5 VALUE ADDED CPRBO

Thailand in the past, rice bran was primarily used as animal feed because of its high protein and fiber content and a rapid development of rancidity. At present, advance in food science and technology had brought rice bran into a wider range of utilizations such as it is processed into cooking oil, CPRBO, food supplements, ingredient for health foods, cosmetics and medicines. However, only a small portion of rice bran was used for such applications and the rest is still underutilized or used for animal feed.

Processing of rice bran to RBO, particularly CPRBO generates a great value added due to the differences in selling price. It can be seen from **Table 5** that the selling price of rice bran is 5-10 Baht/kg while the selling price of refined RBO is 50-200 Baht/L (depending entirely on the γ -oryzanol content) which is 10-20 times greater than that of rice bran. On the other hand, the selling price of CPRBO is 500-20,000 Baht/kg (depending on format of packaging and sales) which is 100-2,000 times greater than that of rice bran, indicating the value added of processing of rice bran to RBO.

Table 5. Selling price of rice bran, RBO and defatted rice bran in Thailand

Product	Price (Baht/kg)
Rice paddy	15-20
Brown rice	50-100
Milled white rice	20-40
Fine rice bran	5-10
Refined RBO	50-200
CPRBO	500-20,000
Defatted rice bran	5-10

3.6 CONCLUSION

It can be concluded that CPRBO contains high concentration of bioactive compounds that benefits human health. CPRBO is a highly nutritive oil for food supplements, medicinal, pharmaceutical and cosmetic applications. However, production of CPRBO is still limited to SMEs and pressing machine efficiency needs further improvement. More applications of CPRBO in food and non-food industries should be promoted in the market.

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Chapter 4

Review on Challenges and Future Drying Trend in Edible Bird's Nest Industry in Malaysia

S.H. Gan, C.L. Law and S.P. Ong

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4.1 INTRODUCTION

Edible bird's nest (known in Chinese as “Yan Wo”, Indonesia as “Sarang Walet” and Japanese as “Enso”) is produced from saliva of swiftlets. Swiftlets characteristically build a ‘bracket shaped’ nest, in the form of a hanging half-cup in which the extraneous nest material is held together by ‘nest cement’ (Medway, 1966). Majority of edible bird's nests traded worldwide come from two heavily exploited species, the White-nest swiftlet (*Aerodramus fuciphogus*) and the Black-nest swiftlet (*Aerodramus maximus*). Edible bird's nests are classified according to the location where the nests are built, edibility, external features, colour, quality and cleanliness and can be basically be classified into two main categories, which are cave edible bird's nest and farm edible bird's nest.

Edible bird's nest is considered as a highly valued traditional medicine dating as far back as the Tang (618-907 AD) and Sung (960-1279 AD) dynasties (Koon and Cranbrook, 2002). A number of claimed benefits of edible bird's nest includes dissolving phlegm, relieving gastric troubles, alleviating asthma, suppressing cough, curing tuberculosis, strengthening immune system, speeding recovery illness and surgery (Konga et al., 1987; Ma and Liu, 2012).

To date, certain populations of swiftlets have recorded huge declines and prone to extinction. With high demand and market value of edible bird's nests, there are more and more opportunities for possible adulteration of edible bird's nest using less expensive materials and other unscrupulous methods (Sims, 1961). Recently, edible bird's nests were discounted in the China market owing to quality related issues. High level of nitrite found in edible bird's nests has raised public concern, doubting whether these bird's nests are truly “edible” and safe.

Moreover, different drying techniques will affect the colour, shape, nutrients and flavour of edible bird's nest. In the past, studies on the effect of drying on edible bird's nest quality were mostly conducted using conventional hot air dryers and by blowing. None of the studies attempted to apply advance drying technology to improve the quality of edible bird's nest. Various studies have reported the use of low temperature drying and intermittent infrared drying for food and crop products (Ginzburg, 1969; Beary, 1988; Chua and Chou, 2005; Law et al., 2011). Studies have concluded that low temperature and intermittent drying give better product quality with lesser energy consumption as compared to hot air drying (Law et al., 2011; Putranto et al., 2011).

The objectives of this chapter are to discuss, analyze and evaluate the recent challenges and advance drying technology available for edible bird's nest industry with energy and quality as standpoints. Limitations and research gaps are identified and direction of future research on drying is recommended.

4.2 CHALLENGES IN EDIBLE BIRD'S NEST INDUSTRY

4.2.1 Reproduction Capability of Swiftlets

Normally, most of the male swiftlet will build the nests made mostly from the saliva secreted by the swiftlet's sublingual glands. During nesting and breeding season, the reproduction of nests will be increased due to the swiftlet's sublingual salivary glands increase their weight from 2.5 mg to 160 mg and reach their maximum secretory activity (Medway, 1962). The white nest is made almost entirely from saliva (Sims, 1961) while the black nest contains about 10% feathers with 8% of the protein content in the nest. The swiftlets will attach the nest to the vertical walls of inland or seaside caves (Kang et al., 1991). In order to support the mother and the nestlings, the weight of the nest must be 1-2 times the body weight of the swiftlets. The swiftlets normally take about 35 days to finish construct a nest (Marcone, 2005). The dry mass of nest, the period of swiftlets spend in building the nest, and the fat and protein content of hardened saliva are the main factors affecting the grades of edible bird's nest (Ma and Liu, 2012).

The population of swiftlets is more restricted to the tropical and sub-tropical regions extending from the western Indian Ocean (i.e. Seychelles Islands) through southern continental Asia, Indonesia, Palawan in the Philippines, New Guinea, northern Australia and the islands in the south-west of Pacific (Chantler and Driessens, 1995). But, edible bird's nests are mainly from South-east Asian countries, such as Sumatra, Java, Kalimantan and the Lesser Sunda Islands in Indonesia, Thailand, Sabah and Sarawak in Malaysia, Vietnam (Nguyen, 1990; 1994) and Myanmar (Chantler and Driessens, 1995). In the past few years, China is importing huge amounts of edible bird's nest from countries such as Malaysia and Indonesia.

Due to high demands and market price of edible bird's nest, the swiftlets populations has been reducing and has led the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) to consider adding the swiftlets and its nests to lists of endangered species. Hong Kong is the largest consumer of edible bird's nest in the world with ethnic Chinese of North America being the second largest market (Lau and Melville, 1994). Due to high nutritional and medicinal values of the nests and the dangers that may be faced during collection by the collectors, the edible bird's nest is the world's most expensive animal product costing between 2,000-10,000 USD per kilogram (Hobbs, 2004; Koon and Cranbrook, 2002; Sankaran, 2001). Indonesia is the biggest supplier of edible bird's nests and then followed by Thailand, Vietnam, Malaysia, Burma, Southern Indian and Sri Lanka. Koon and Cranbrook (2002) has argued that the species of swiftlet and its nests may extinct in five to ten years if harvesting is continued at its current rate (Koon and Cranbrook, 2002).

To date, harvesting pressure has increased and may be continuous throughout the year, with the eggs and chicks damaged/killed at the time of collection. Consequently, some populations of swiftlets have marked huge declines (Hobbs, 2004). Lau and Melville (1994) have investigated that a

decline of about 48% in the population of black-nest swiftlets at Niah between 1935 and 1987, and in Baram, a decline of about 43% in the population of edible bird's nest over 17 years (Lau and Melville, 1994). With an attempt to recover the swiftlets populations, in April 1989 the Sarawak government announced a three-year ban on nest-collecting at Niah Cave that contain the largest swiftlet colony in the world. Due to lack of enforcement, this ban seems not to work efficiently. Nevertheless, the black-nest prices in Sarawak have increased from RM140/kg in 1987 to RM4800/kg in 1991; in turn, there were more illegal collectors whom were new and inexperienced than before the ban. As a result, the number of fatal accidents has increased where eight collectors died in the first week of January 1991. Due to all these incidents, the ban was withdrawn on 21 March 1992 and harvesting is now allowed by collectors that are licensed by the Sarawak Forestry Department in accordance to a schedule determined by the Department of Preservation of Birds (Lau and Melville, 1994).

Undoubtedly, the nest harvesting is normally carried out by local people according to a complex system of ownership and management (Hobbs, 2004; Koon and Cranbrook, 2002). The unique technique of harvesting the edible bird's nest is required by the collectors and will be influenced by the cave site and height above the ground or water bed (Leh, 2001). The collectors might need to use temporary scaffoldings made from bamboo or iron/wood if the bird's nest is high on the cave walls (Marcone, 2005).

4.2.1.1 Practice of Swiftlet Farming

To overcome the dangerous nest harvesting process, nest collectors in Indonesia have developed houses for swiftlets farming. The eggs of white-nest swiftlets are placed in the nest colonies, once mature the white-nest swiftlets will return to the farm and form a colony. This practice has been done by many nest collectors in Indonesia (60%), Thailand (20%), Malaysia (10%) and Vietnam (7%). The main export market of edible bird's nests are Hong Kong (50%), China (10%), Taiwan (8%), Vietnam (7%), Macau (6%) and Malaysia (4%), with estimated consumption of 160 tonnes for 2006 (Kuan and Lee, 2005).

The largest market demand is from China and Hong Kong. The current wholesale price of cleaned and dried edible bird's nest is about USD 1731.96 per kg, and the retail price is 2.5 – 8 times that amount. The market for bottled bird's nest is also huge. The demand in China alone is estimated to be about 140 million bottles per year, equivalent to about 140 tonnes of edible bird's nest per year (Jordan, 2004). The growth is exponential in view of the strong growing economy of the Chinese market alone. The swiftlet farming industry is a new emerging industry in Malaysia and has achieved its critical mass five years ago.

However, the edible bird's nest industry in Malaysia has a much longer history and is made up primarily of cottage style operations with main source of edible bird's nests obtained from cave nests. As the swiftlet farming industry continues to expand and grow, more edible bird's nests are sourced

from purpose-built swiftlet farms. However, researchers from the World Wide Fund for Nature believed that swiftlets farming has produced far less bird's nest as compared to natural cave (Jeanine, 1997). Hence, this is one of the main factors that affects the production of bird's nest.

Swiftlet farming industry in Malaysia has been growing over the last eight years. Before 1998, there were about 900 swiftlet farms throughout the country. By the end of 2006, the number of swiftlet farms was close to 36,000 units, with an average annualized growth rate of 35% per year in the last five years (Merican, 2007). The overall value of edible bird's nest market in Malaysia is still very much determined by the quality of raw bird's nests.

4.2.2 Adulteration of Edible Bird's Nest

Scientific investigation on the medicinal and nutritional properties of edible bird's nest is still limited, especially given the fact that these properties appear to vary with the harvesting period and location. This has provided opportunities for adulteration of edible bird's nest during processing using less expensive materials such as karaya gum, red seaweed, fried pork skin, egg white, gelatine, soybean, rice, starch, agar, fish vesicae and *Tremella* fungus. Other methods also involved such as staining, bleaching and mixing cheaper edible bird's nest into the more expensive ones (Norhayati et al., 2010).

Edible bird's nest is usually white but those found in caves are dull brownish or orange red in colours. In the market, red blood nests are much more expensive than white edible bird's nests and claimed to have better health benefits. It was thought that the red edible bird's nests are swiftlet's blood mixed with saliva or due to the special type of food that swiftlet consumed. Others believe that the caves contain specific minerals and iron which turn it to red (Shaw et al., 2013). However, edible bird's nest that are too red could be tainted with dye additives which is hazardous to health.

To solve the problem, a new, accurate and fast detection technologies should be developed. The difference between chemically treated processed edible bird's nests can be easily discovered by the gloss of the cement strands after soaking in water for 30 minutes. Chemical additives may lead to nest that is too glossy. The adulterated edible bird's nest will have wrinkle surface and arranged uniformly with slight medicated smell after soaking in water for 30 minutes (Wu et al., 2007). Besides, under an optical microscope, genuine bird's nest is semi-transparent and has many fine textures while adulterated bird's nest with *Tremella* fungus is non-transparent and has very coarse textures (Ma and Liu, 2012; Wu et al., 2007; Yang et al., 2013).

Recently, Yang et al. (2013) has designed a holistic and scientific testing method for the authentication and quality assurance of edible bird's nest. The analytical system involves a concerted approach by applying the gas chromatography-mass spectrometry (GC-MS) fingerprint of oligosaccharides, the environmental scanning electron microscopy (ESEM) of microstructure, and the dot blotting of epidermal growth factor (EGF) in edible bird's nest (Yang et al., 2013). Results of Yang et al. (2013) study showed that genuine

edible bird's nest had the presence of five monoses and EGF, whereas the counterfeit edible bird's nest did not. Moreover, sialic acid and epidermal growth factor are established as unique indicators for the grades of edible bird's nest. A unique three-dimensional, crater-like microstructure was also observed in authentic edible bird's nest, but not in the fake products. It was concluded that the holistic approach, including chemical, physical and biochemical studies of edible bird's nest is a reliable and scientific method for the verification of genuine and high quality of edible bird's nest (Yang et al., 2013).

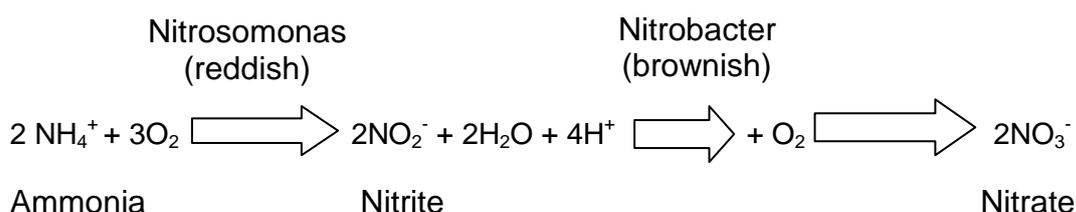
4.2.3 Nitrite Content in Edible Bird's Nest

4.2.3.1 What is Nitrite?

In 2011, surveillance conducted by China Mainland authority and local studies found that nitrite was present in various bird's nests, especially in blood-red bird's nest, available at the market (Centre for Food Safety Hong Kong, 2011). Basically, nitrate (NO_3^-) and nitrite (NO_2^-) are naturally occurring ions that are ubiquitous in the environment. Both are products of the oxidation of nitrogen by microorganisms in plants, soil or water and to a lesser extent, by electrical discharges such as lightning. Nitrate is the more stable form of oxidized nitrogen but can be reduced by microbial action to nitrite, which is moderately reactive chemically. Aromatic amino acids such as tyrosine, phenylalanine and tryptophan in edible bird's nest possess phenyl rings that could react with nitric acid/nitrous acid, which derived from hydrochloric acid and sodium nitrate reaction or bacterial fermentation of bird droppings, to form yellow edible bird's nest through formation of aryl-C-N and NO_2 side group.

Paydar et al. (2013) hypothesized that sources of nitrite and nitrate could have been derived from ammonia through anaerobic fermentation by nitrobacteria. Nitrobacteria are a genus of mostly rod-shaped, gram-negative and chemoautotrophic bacteria. It is mostly found in soil, freshwater and on building surfaces, especially in areas that contains high levels of nitrogen compounds. Grundmann et al. (2000) stated that Nitrobacteria seem to grow optimally at 38°C and at pH 7.9, while Holt et al. (1993) stated that Nitrobacteria seem to grow optimally at 28°C and at pH 7.6 - 7.8 and will die at temperature exceeding 49°C or below 0°C .

In generally, nitrite and nitrate are produced through nitrification. Nitrification is the conversion of ammonia to nitrate performed primarily by soil-living bacteria and other nitrifying bacteria (reaction as shown below).



Nitrobacteria uses energy from oxidation of nitrite to nitrate to fulfill their energy needs. Nitrobacteria fix carbon dioxide via "Calvin cycle" for their carbon requirements. According to Fritz Industries (2012), nitrifying bacteria are photosensitive, especially to blue and ultraviolet light. After they have colonized a surface of this light, edible bird's nest will not contain high contents of nitrite and nitrate.

4.2.3.2 Effects of Nitrite

The high level of nitrite found in edible bird's nests has raised public concern, casting doubt whether these EBNs are truly "edible". In 2011, a few sellers attempted to turn white bird's nest into blood-red bird's nest to persuade customers who believe the blood-red bird's nests are more nutritious and higher in grade. Unfortunately, adulterated blood-red bird's nest contains much higher nitrite and nitrate content as compared to white bird's nest (AQSIQ, 2011).

Nitrite has been used as a food preservative and antibotulinal agent in the food industry to protect against microorganisms growth. However, its level is strictly controlled to prevent food toxicity since nitrite can react with secondary amines in food products or in the digestive system to form nitrosoamines, a class of carcinogenic compounds (DSM, 2011). Also, nitrate can readily be converted into nitrite by microbial reduction and about 20% of nitrate may be converted to nitrite in the mouth by the action of saliva and bacteria and more will be converted in the stomach (Kamaruddin, 2012). Another side effect is nitrite can oxidize hemoglobin in blood and make it unable to carry oxygen to the body tissues, thus the patients may develop blue or purple colouration and the condition is called methaemoglobinaemia. Excessive amount of nitrite could also expose pregnant mothers to high risk of having pre-mature baby due to the lack of oxygen in the blood (DSM, 2011).

Nitrate which always present as sodium nitrate, the more stable form but tend to be oxidized into nitrite in the existence of bacterial activity and oxygen. Thus, both nitrate and nitrite must be monitored to ensure the quality and safety of meat products and other food products as well. Hence, World Health Organization (WHO) has issued the allowable daily intake (ADI) of nitrate and nitrite to a maximum of 3.7mg and 0.07mg per kg body weight respectively. For example, the allowable daily intake for a 60kg adult is 222mg for nitrate and 4.2mg for nitrite per day.

In August 2011, the Chinese government imposed a ban on EBN products imported from overseas, due to the high level of detected nitrite in blood red bird's nest. The contamination issue has made a great impact to the edible bird's industry and many farmers, traders and exporters are affected. This issue has also given a great impact to Malaysia edible bird's nest export. WHO Standard of Nitrite restriction control at 30 ppm nitrite level is based on the MS 2334:2010 Edible Bird's Nest (EBN) Specification and is also in line with the Food Regulations 1985. Nitrite is naturally formed via oxidation to form sodium nitrite, NaNO_2 . Thus, it is impossible to keep edible bird's nest nitrite close to 0 ppm (AQSIQ, 2011).

4.2.3.3 Methods to Reduce Nitrite and Nitrate Content in Edible Bird's Nest

Paydar et al. (2013) found that microenvironment of the EBNs (swiftlet house or the caves) plays a crucial role in the prevalence of nitrite and nitrate. Foaming white EBNs with nitrite enriched bird droppings could turn EBN into brownish yellow, but not red as derived from sodium nitrite in acidic conditions. The swiftlet house bird droppings has a lower nitrite and nitrate contents compared to cave guanos that contain bird or bat droppings mixed with other organic materials rich in nitrite and nitrate. Paydar et al. (2013) study's data provided evidence that EBNs from the caves generally contained higher nitrite and nitrate levels compared to those from swiftlet houses. This is because most operators clean their swiftlet houses frequently by removing the bird soil.

In addition, swiftlet houses usually have better ventilation compared to the caves, which do not favor the fermentation process. In contrast, the caves, where the EBNs are harvested, have a pool of guano underneath it with strong ammonia odour. Thus, frequent cleaning of the bird droppings is recommended to ensure ample ventilation and prompt harvesting of the bird nests once it is formed, are feasible ways to control the nitrite and nitrate content in EBNs (Paydar et al., 2013).

Chan (2013) claimed that the highest nitrite content found in edible bird's nest was 6,400 ppm, and that red edible bird's nest contained the highest, followed by yellow and white edible bird's nest. By proteomic analysis, A protein was isolated from red edible bird's nest, which was identified as nitrate reductase deriving from microbes, and this enzyme converted nitrate to nitrite within edible bird's nest. Addition of specific inhibitor of nitrate reductase successfully abolished formation of nitrite in edible bird's nest.

Chan (2013) also investigated the efficiency of traditional cooking method of edible bird's nest for nitrite removal. Up to 98% of nitrite was removed in the first step of soaking for a minimum of 1 hour (farm bird's nest) and 6 hours (cave bird's nest). Water used for soaking edible bird's nests should also be replaced once or twice during the soaking process. Further removal of nitrite from edible bird's nest after cooking was observed.

4.2.4 Colour Changes in Edible Bird's Nest

Another major issue concerning the processing of edible bird's nest is severe colour change in cleaned dry bird's nest. After cleaning and drying, the bird's nest turns yellowish which is undesirable to consumers. The industry (but not all) normally overcome this problem by applying bleaching agents which are chemical based and hazardous to health. Improper processing condition such as high temperature and long duration processing causes severe colour change to the bird's nest (Law et al., 2011). Hence, there is a need to introduce a suitable processing method to avoid the browning

reactions and therefore eliminate the current issue of edible bird's nest bleaching.

Quality of edible bird's nest in terms of colour, taste, odour and texture are used in quality control during processing. However, the first quality judgement made by a consumer on edible bird's nest at point of sale is on its appearance (Elcin and Belma, 2009). Colour is, perhaps the most important appearance attribute because abnormal colours, especially those associated with deterioration in eating quality or with spoilage, cause the product to be rejected by the consumer.

Colour change of edible bird's nest during processing takes place due to the physiochemical reactions, which occur inside the edible bird's nest. These reactions could be due to Maillard condensation of amino components and oxidation of ascorbic acid (Elcin and Belma, 2009). The final colour measurements of the dried product can be used as quality indicators to evaluate deterioration caused by thermal processing since colour measurement is simpler and faster than a complete physiochemical analysis.

4.2.5 Conventional Drying Practice in Edible Bird's Nest Industry

Postharvest processing of agricultural, herbal, food products and bio-materials typically requires drying in order to lower the water activity to a level that is safe for storage (Arun and Law, 2010; Fabiano et al., 2010). Likewise, in the edible bird's nest industry, drying is also required to reduce the water activity for similar aforementioned purposes. As edible bird's nest contains active ingredients such as glycoproteins and epidermal growth factor (EGF) that are sensitive to heat (Konga et al., 1987), processing of edible bird's nest at too high temperature is detrimental to the retention of these bio-active ingredients (Law et al., 2008).

Excessive degradation of quality attributes, such as colour, nutrients and flavour may occur due to prolonged exposure to elevated drying temperature (Zhang et al., 2005). Typical drying time of edible bird's nest using this method is 4-12 hours depending on the operating temperature (Law et al., 2011). In the case of hot air drying, volume reduction is usually accompanied by wrinkles and deformation which indicates the collapse of bird's nest. Considerable reduction in volume and decrease in porosity can be observed during hot air drying when the solid matrix of the bird's nest can no longer support its own weight and this increases the brittleness of the bird's nest (Jankovie, 1993; Krokida and Maroulis, 1997; Ratti, 1994).

To date, freeze drying is a popular drying method in the edible bird's nest industry and known to produce high quality products compared to other methods (Ratti, 2001). The process effectively retains the original characteristics of the bird's nest, including nutrients, texture, colour, taste, and shape (Ratti, 2001; Ismail, 2013). However, freeze drying is known as the most expensive process which could increase the capital cost in the during processing.

According to Flink (1977b) and Judge et al. (1981), the energy cost breakdown for freezing is 4%, vacuum is 26%, sublimation is 45% and condensation is 25% in freeze drying. New improvements are needed to improve the heat transfer characteristic in order to help sublimation, to shorten drying time to reduce vacuum usage and avoid the use of condensers (Flink, 1977b; Judge et al., 1981).

4.3 STEPS TO ENSURE SUSTAINABLE EDIBLE BIRD'S NEST INDUSTRY

The demand for edible bird's nests from Hong Kong and mainland China is growing although more restrictions have been set by the Chinese government for importation of edible bird's nests recently. However, a standardized benchmark and quality assurance from the relevant local authorities should be enforced to ensure the safety of Malaysia's edible bird's nests (Lim, 2011).

The assurance in maintaining and safeguarding the Malaysian edible bird's nest industry is guided by the Malaysian Standards. To date, the Department of Standard Malaysia has developed five standards for use by the EBN sector, which are MS 2334: 2011 Edible Bird's Nest Specification, MS 2333: 2010 Good Manufacturing Practice (GMP) for Processing Raw Unclean and Raw Clean Edible Bird's Nest, MS 2273: 2009 Good Animal Husbandry Practices on Edible Bird's Nest Ranching and Its Premises, MS 2503: 2012 Good Animal Husbandry Practices on Edible Cave Bird's Nest Ranching and Good Manufacturing Practice (GMP) for Edible Bird's Nest Product. The government is putting emphasis on GAHP compliance for the premises and GMP Golden Rules for processing (Fadzilah, 2013).

The most challenging procedure in processing edible bird's nest is drying. The drying process for raw-unclean EBN should be carried out in controlled drying environment at temperature not higher than 60°C to avoid loss of important bioactivities or bio-molecules in EBNs (Department of Veterinary Services, 2009). Extensive soaking, washing and drying during processing may result in loss of bio-molecules which are water soluble and heat-sensitive. Hence, implementation of quantitative standardization method is required to ensure the quality of edible bird's nest products.

4.4 FUTURE DRYING TREND IN EDIBLE BIRD'S NEST INDUSTRY

4.4.1 Low Temperature Drying

Law et al. (2011) investigated the potential of dehumidified air drying at temperature of 26°C and 27% RH in enhancing the kinetics and minimizing the colour change of cleaned edible bird's nest. Comparisons were made against conventional hot air drying at temperatures of 50°C-90°C and 2-24% RH. Results showed that the edible bird's nest sample under low temperature drying took about 9 hours to achieve is equilibrium moisture content and total colour change was the lowest among other drying methods. It was also observed that colour kinetics of the sample was sensitive to temperature

particularly in the first five hours of drying. Hence, low temperature is recommended especially at the initial stage of drying.

Low temperature processing has been reported to produce good quality dehydrated ganoderma (Chin and Law, 2010), dehydrated salak slices (Ong and Law, 2011), dehydrated ciku cubes (Chong and Law, 2010) and dehydrated chempedak (Chong et al., 2008) in terms of bio-active ingredients retention and colour change. Low temperature dehumidified air is therefore a suitable drying medium for edible bird's nest that contains heat sensitive bio-active ingredients (Law et al., 2011).

4.4.2 Intermittent Infrared Drying

Infrared drying is based upon the same principle as conventional drying (Beary, 1988) and Ginzburg (1969) has suggested that infrared (IR) radiation, operating under an intermittent mode may be applied to dry heat sensitive biomaterials and the effectiveness of drying can also be increased using combined radiant-convective method. In many cases, the intermittent radiation treatment can decrease the drying duration effectively and hence the amount of energy required. The quality of the dried products are also better, particularly in drying of heat sensitive materials (Chua and Chou, 2005; Ginzburg, 1969).

Paakkonen et al. (1999) has shown that intermittent infrared drying could improve the quality of herbs, while Dontigny et al. (1992) has demonstrated that intermittent infrared drying of graphite slurry could increase drying rate significantly. Zbicinski et al. (1992) has investigated that convective air drying with infrared and intermittent infrared radiation drying coupled with convective air drying is suitable for heat sensitive materials. Other researchers such as Dostie et al. (1989) and Carroll and Churchill (1986) have also reported shorter drying time with improved product quality for intermittent infrared drying. Now, intermittent drying is one of the technical solutions to this problem as the intermittency applied during intermittent drying allows sufficient time for moisture to transfer from the center to the surface during the tempering period. In turn, quality degradation, heat damage to the surface and wastage of heat energy can be minimized effectively (Chandan and Mohammad, 2014). Putranto et al. (2011) claimed that intermittent drying is able to decrease the effective drying time and drying air utilization, thus reduces energy consumption.

4.5 CONCLUSION

The major issue concerning the processing of edible bird's nest is the high nitrite content in edible bird's nest due to high drying temperature and long processing duration. Undesirable colour change (yellowness) is usually observed in the dried bird's nest and in some cases hazardous bleaching agent is used to modify product appearance. Hence, there is a need to introduce a suitable drying method to process edible bird's nest at temperature below the critical point that triggers the undesirable colour change and able to accomplish processing at the shortest period possible

while maintaining the quality and safety of the edible bird's nest below 30ppm nitrite content.

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Chapter 5

Measurement on the Effect of Microwave Energy in Drying -Technical Review

T.C. Tham, C.L. Hii, S.P. Ong, N.L. Chin, A.C. Luqman and C.L. Law

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5.1 INTRODUCTION

Contemporary drying method of wet natural rubber granules is usually dominated by hot air drying as a result of fuel combustion ranging from diesel, coal or heavy oil. Existing industrial hot air dryer dries crumb rubber at temperature around 100 °C to 130 °C for about 3 to 4 hours which is unfavorable in term of energy consumption especially during energy crisis. In fact, majority of the industrial rubber dryer operates deep bed system with bed temperature above 100 °C to maximize the product throughput (John.C., 2005; Sekhar, 1971; Tirawanichakul, 2011; Yushan, 1985). In terms of rubber quality, hot air drying yields adverse effects to the property of natural rubber due to the severe temperature treatment that affects final product quality.

Though, hot air drying method is reliable but yet less efficient in heat and mass transfer because thermal energy is conducted from the outer surface to the core of the natural rubber, whereas the moisture diffused from opposite direction and resulted in the built up of dried layer from the outer surface. This has often results in longer drying hours during falling rate period and incomplete drying especially for rubber which has poor thermal conductivity. Alternatively, few advanced drying methods have been explored and employed by researchers for natural rubber drying particularly with microwave energy. This paper presented the application of microwave energy on the drying of wet rubber crumb and its effects toward the drying kinetics, product quality and productivity.

5.2 CONVENTIONAL DRYING METHOD

Natural rubber granules or crumb rubber is a semi processed granulated rubber originated from latex tapped from a tree known as *Hevea Brasiliensis*. The crumb was obtained after cleaning, size reduction by mechanical means through combination of pelletizing, shredding and creping to form granulated rubber. It is often treated as rubber that has been reduced to a particle size of 3/8 inch or less (TNRCC, 1999) and exhibits in discrete form when wet but return to its natural tack during the drying process.

A typical industrial drying system for rubber comprises both mechanical dewatering and drying. During initial dewatering, mechanical force is used to remove water content down to approximate 85% dry rubber content (DRC) while further drying at temperature above 100 °C will produce DRC of at least 99.2% for treated rubber as regulated by Standard Malaysia Rubber (SMR) introduced since 1965 (Anonymous, 2008). The strict restriction of volatile matters is mainly to preclude mould growth which lead to malodour issues at volatile level greater than 0.6%. In most driers, heat maybe supplied either by force air circulation with heated air generated from fuel combustion or indirectly by the use of heat exchanger. Two types of dryer namely box and trolley dryers are extensively used for crumb rubber drying in either batch, semi continuous or continuous mode. These dryers consist of a tunnel through which boxes are pushed or dragged through along with hor air circulation (John.C., 2005). The crumb rubber is arranged in deep bed layers of approximates 30 to 50 cm thick to allow the passage of hot air in the inter-granular spaces (Naon, 1995). Air flow, humidity and temperature play important roles in every aspect of drying. In this context, high humidity is

crucial in the early stage of drying to preclude the development of non-porous skin surface which is undesirable for heat and mass transfer (Rahman, 1985).

Kongchana (2007) suggested hot air drying strategy i.e. 40 minutes at 130 °C followed by 180 minutes of drying at 110 °C produced good visual characteristic, shorter drying time, better product quality as compared to other drying strategies. Yu Shan et al (1981), on the other hand had successfully introduced the conveyor dryer for continuously drying of standard rubber. In contrast with conventional trolley dryer, this invention claims to reduce drying time from 4 to 8 hours. Also, the experiment concluded that a combination of drying temperature of 125°C and air velocity of 5.3 to 5.4 m³/s were optimum for thick rubber drying without too much oxidation or over cure issue (Wei Y.S., 1989).

Unlike other materials, natural rubber is heat sensitive and possesses certain degree of complexity in terms of drying and prone to quality deterioration under high heat. According to Loke's (1974) on the effect of heating during and after drying on Plasticity Retention Index (PRI) for processed rubber; it was demonstrated that the increase in temperature with 40 °C difference (60°C and 100°C, respectively) during drying of raw natural rubber did not necessarily weaken PRI, but the same treatment when applied to the rubber after drying would reduce PRI remarkably. Indeed, existing industrial dryers for rubber processing are design for operation at high temperature, yet, it is worth mentioning that literatures on rubber drying at low temperature is still rare and further research is vital in order to discover an innovative drying method for rubber.

Conventional drying method is also time consuming which in turn leads to high operating cost and high energy consumption. Utomo (2010) disclosed that the drying energy involved in the production of low grade rubber crumb SIR 20 was 1.17MJ/kg of dry rubber (98.06% from total fuel energy consumption) whilst high grade rubber SIR 3 was about 97.9% of total fuel energy i.e. 1.98 MJ/kg of dry rubber.

5.3 MICROWAVE HEATING

5.3.1 Introduction

Hot air drying accelerates the formation of dried skin on the outer layer of rubber through heat conduction. This impedes moisture migration since melting occurs concurrently inside the rubber. Another alternative drying technique for rubber drying would be electrical volumetric heating using microwave heating. Microwave energy is able to remove moisture more efficient with short processing time under low temperature condition (Cuccurullo, 2012).

5.3.2 Fundamental of heating with microwave

Microwave is a form of electromagnetic radiation in the frequency range of 3,000 MHz to 30,000MHz with a common used frequency at 2450MHz (Nave,

2012). The dielectric heating process involves the transformation of electrical energy into thermal heat either to increase the temperature of wet product (for drying purpose) or to a critical level for melting purposes. Different from hot air drying, microwave heating does not solely relies on the conduction of heat through solid such as rubber but rather through self-excited molecular movement particularly for polar materials i.e. water due to the absorption of microwave energy. In other word, microwave heating depends on the interaction between polar groups in molecules of non-conductive materials and the alternating electric field of oscillating magnetic field. During heating, polarity orientation of water changes in line with variation of external electromagnetic field. This generates internal resonance effects and creates collisions between water molecules which in turn transformed to thermal energy (Chen, 2012). Again, the cooling effect on the surface of materials due to rapid water evaporation resulted in pressure gradients which facilitate moisture diffusion and heat transfer. Obviously, microwave heating has greatly enhances the migration of volatile matter during drying which is much better than conventional hot air drying.

Basically, the evaluation of absorption efficiency by dielectric material when it is placed in a high frequency electric field which also known as local volumetric heat generation in the product can be represented by:

$$P = \sigma |E|^2 = 2\pi f \epsilon_0 \epsilon' (\tan \delta) |E|^2 \quad (1)$$

where P , refer to microwave energy absorbed (W/m^3) per unit volume of the sample at any instant of time; σ is effective conductivity; f is frequency; ϵ_0 is permittivity of free space (8.86×10^{-12} F/m); ϵ' is relative dielectric constant; $|E|$ is the magnitude of internal electric field; $\tan \delta$ is the loss tangent coefficient. The above equation has clearly shown the relationship between microwave energy absorption which is proportional to the frequency of the applied electric field, dielectric loss factor as well as to the square of local electric field (Rattanadecho, 2007). Therefore, the substance can be heated excellently in a microwave field if the substance possess higher loss factor, for instance water and all aqueous substance.

Besides, Wang et al.(2010) recommended initial moisture content of rubber i.e. 10%~20% for microwave drying particularly to enhance the drying rate as well as quality control. Also, the whole process of microwave drying for natural rubber is divided into acceleration, deceleration and constant drying which are comparable to others general drying curves (Bousquet, 2000).

5.3.3 Applications of microwave heating in rubber processing and its effect on rubber quality

Microwave energy offers shorter drying times whilst preserving the quality and nutrients of biomaterials make it an attractive and alternative source of thermal energy (Bushra, 1992; Izli & Isik, 2014). A case study of natural rubber drying tested with continuous microwave belt drier concluded better microstructure arrangement under Scanning Electron Microscope (SEM) (Rattanadecho, 2007). In the experimental setup, microwave powers were varied from 2.4kW to 11.2kW by means of compressed air cooled magnetrons of 800W each. The experiment proved that reduction in drying time with

increasing number of magnetron was mainly due to increase in intervals of heating. Indeed, the paper was also considering adverse effect of microwave drying known as thermal runaway. This phenomenon occurs when minor deviation of microwave power causes the temperature to increase rapidly to the melting point of substances which results in quality deterioration. As such, thermal runaway prevention and control are essential in the development and selection of microwave as a drying option.

Similarly, Chen et al (2012) explored the advantage of microwave drying of rubber with 93.6% reduction of drying time to 13.47 min with microwave drying (Figure 1) and product qualities were found better than those dried by hot air. Results showed that Plasticity Index (P_0) and Plasticity Retention Index (PRI) of rubber dried with microwave is superior to that of hot air drying with PRI measured at 88.2 and 79.8, respectively (Table 1). The short drying time by microwave energy is therefore capable to prevent the degradation of natural rubber molecular chain by oxidation and hence achieve higher thermal oxidative aging resistance of natural rubber.

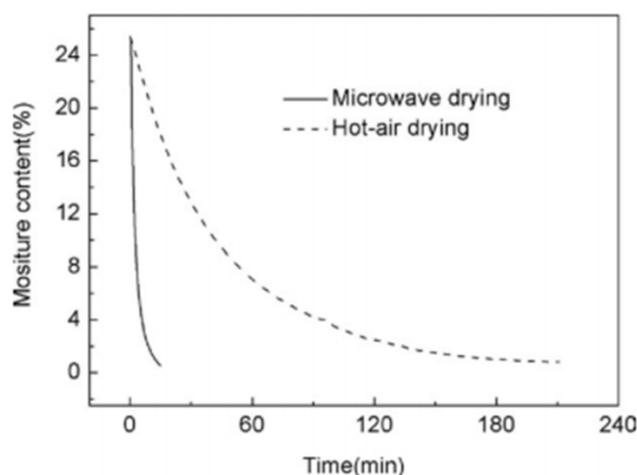


Figure 1 Drying kinetics of rubber by different drying techniques (Chen, 2012)

Table 1 Properties of natural rubber samples obtained by different drying methods(Chen, 2012)

Samples	Natural rubber	
	P_0	PRI
Hot air drying	32.2	79.80
Microwave drying	44.2	88.20

On the other hand, Lin et al.(2008) showed that microwave drying of natural rubber had an average dehydration efficiency of 9.22%, which is 4.7 times greater than the far infrared and 17.7 times higher than fuel.

5.3.4 Advantages and the limitations of microwave drying in rubber processing

Chua (2001) commented on advantages of microwave drying including enhanced heat and mass transfer, increased drying rates without over heated surface temperatures and thus better product quality. Besides the fact that

microwave drying is hygiene and non-toxic, the waste heat is minimized and recovered. For example, the design of microwave system is built as such to maximize the absorption of energy by dielectric material as well as to absorb any microwave energy that bounces back from the coated wall which in turn prevent heat loss. Others than rubber drying, studies by Tulasidas (1995) on microwave drying of raisin found samples were superior in quality as compared to hot air dried samples in terms of colour, darkness, stickiness and uniformity. Likewise, desirable colour, shrinkage and rehydration properties are observed for carrot dried using microwave (J. Wang, Xi, Y.S., 2005)..

Although electrical volumetric drying offers advantages in terms of drying efficiency and product quality, however, the limitations of microwave processing is equally important during selection to achieve balance between cost and quality. Among the limitations include non-uniformity in drying due to uneven distribution of electromagnetic energy in the drying chamber or microwave cavity and non-uniform moisture distribution in the drying material. Apart from that, Studies have recommended three features to improve heating uniformity in microwave drying (Li, Wang, & Kudra, 2011); i) by improving the uniformity of electromagnetic field in microwave cavity by complicating the electromagnetic field pattern in microwave cavity in term of nodes and anti-nodes; ii) by improving the uniformity of microwave energy absorption by randomly displacing the material or disordered movement of the particulate material in space during drying; iii) by combining with other drying method. Item (i) could be resolved by the application of mode stirrer or various microwave sources with different frequency on the microwave system (Bows, 1999; Plaza-Gonzalez, 2005). Additionally, in the effort to overcome uneven distribution of electromagnetic field, a microwave vacuum rotary drum dryer was used to move and mix the chili particulates (Kaensup, 2002). Meanwhile, a continuous vacuum microwave dryer was designed with spiral movement on the conveying system to improve the uniformity of microwave heating on apple slices (Han, 2006).

Rahman (1985) concluded that microwave drying of crumb rubber is not cost effective yet too capital intensive from economical points of view. In fact, existing application of microwave drying is also limited i.e. in rubber curing or vulcanization (Ishii, 1995), which infers that microwave drying has not been used extensively at industrial scale even though microwave drying of rubber have been reported in literatures. Unlike hot air drying where the product temperature never exceeds the hot air temperature; microwave drying is hard to control and often lead to excessive temperature increment along the corner or edges of the products especially during final stage of drying (Chandrasekaran, 2013). As such, Datta et al (2005), Sumnu et al (2005), Chou and Chua (2001) demonstrated that microwave drying combined with other drying techniques such as forced air and infrared radiation could enhance the process efficiency and reduce cost. Indeed, microwave drying associated with mechanical dewatering or osmotic dehydration could also reduce energy consumption in drying (Kudra, 2009).

In an optimization study conducted by Setiady et al.(2007), raw potatoes were dried by microwave-vacuum system at 60°C for 150 minutes without scorching. Regier et al.(2005) extended microwave study by comparing the carotenoid retention and concluded that microwave vacuum outperformed

others in comparison to freeze drying and convection drying. Correspondingly, Sharma and Prasad (2006) conducted microwave/convective drying of garlic and obtained drying time reduction of 80% with superior quality dried garlic when combining microwave energy at 0.4 W/g with hot air at 60°C to 70 °C. Apparently, this shows promising results which likely to promote wider industrial acceptance in near future.

5.4 CONCLUSION

This paper mainly discusses the potential application of microwave energy in crumb rubber drying and the effects on product quality. Unlike hot air drying which is less energy efficient, microwave drying is capable to enhance moisture diffusion within the rubber and improve heat and mass transfer. Meanwhile, studies reported on microwave-dried rubber showed superior quality compared to hot air drying. Even though initial capital cost for microwave drying system is high, yet, researchers had suggested integration with other drying technique for instance hot air and vacuum drying to enhance drying efficiency and reduce operating cost.

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Chapter 6

Review of Food Toxicological Issues Associated with Rubber Products

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6.1 INTRODUCTION

Rubber has evolved into an important commodity for Malaysia since early 20th century (Drabble, 2001). It is the sap, a milky colloidal substance found beneath the bark of rubber tree (*Hevea brasiliensis*) that has made great contribution to the world. Rubber tree is originated from Brazil and its seedlings were then exported to Sri Lanka, Singapore and other Asian countries in the 19th century (Kinnaman, 1997). Based on the production statistics from year 2014, Malaysia's natural rubber production is 6.86% of world production which includes both rubber latex and dry rubber (DOS, 2014). **Table 1** shows the world production statistics for natural rubber and also for Malaysia. **Figure 1 - 5** show the rubber tree and several types of rubber products produced during farm processing.

Table 1. World production of NR from 2010 to 2014 ('000 tonnes)

Country	Malaysia	World	Percentage of Malaysia Production compared to World Production (%)
2010	939.24	10,393	9.04
2011	996.21	11,230	8.87
2012	922.79	11,603	7.95
2013	826.42	12,042	6.86
2014 (Jan – Jun)	342.29	5,334	6.42

Source: Department of Statistics Malaysia (2014)



Figure 1. Rubber tree (*Hevea brasiliensis*)



Figure 2. *Rubber slab after maturation*



Figure 3. *Cleaning process of rubber slab*

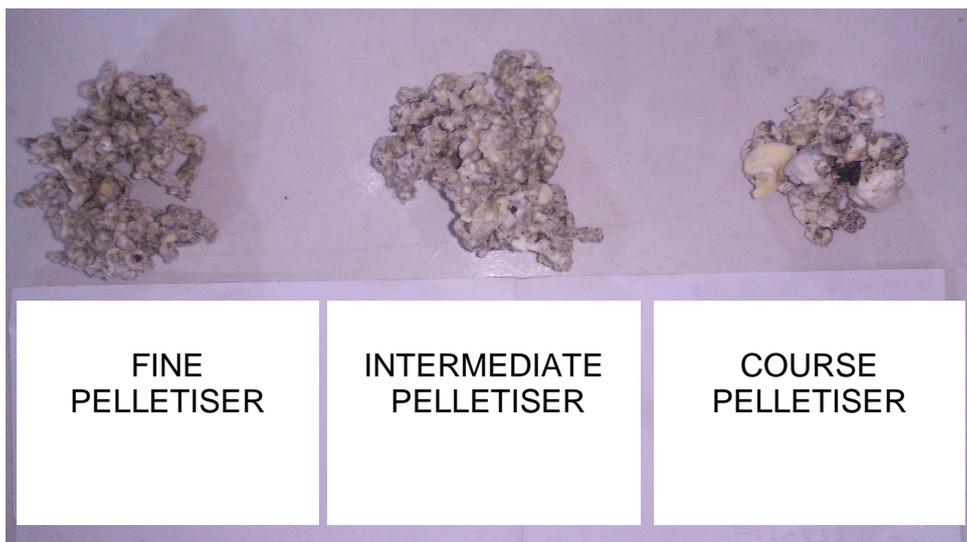


Figure 4. Rubber pellets



Figure 5. Technical specified rubber (TSR 20)

The early usage of rubber was restricted to shoes and then it was further popularized when Charles Goodyear vulcanized rubber into modified rubber (Steven, 2006). Rubber is also widely used in various manufacturing industry i.e. in food processing (Faille, 2009). Examples of applications in food industry are such as conveyor belts, seals, hosing and gaskets. Current main rubber products are commonly produced from both concentrated latex and solid rubber. Crumb rubbers (a type of solid rubber) are commonly sold in various grades after drying as shown in [Table 2](#) (Utomo, 2010).

Table 2. Summary of technical specified rubber (TSR)

Parameters	Grades					
	TSR-CV	TSR-L	TSR-5	TSR-10	TSR-20	TSR-50
Dirt content, %wt (Max)	0.05	0.05	0.05	0.10	0.20	0.50
Ash content, %wt (Max)	0.60	0.60	0.50	0.75	1.00	1.50
Nitrogen content, %wt (Max)	0.60	0.60	0.50	0.60	0.60	0.60
Volatile matter, %wt (Max)	0.80	0.80	0.80	0.80	0.80	0.80
Initial Wallace plasticity, Po (Min)	-	30	30	30	30	30
Plasticity Retention Index, PRI (Min)	60	60	60	50	40	30
Colour, Lovibond units (Max)	-	6	-	-	-	-
Money viscosity	60±5	-	-	-	-	-

In the past, many studies had been carried out to investigate potential food contamination issues due to rubber products (Chmielewski, 2003, Xianming, 2009, Sokunrotanak, 2013). Increasing number of reports showed that vulcanization and cross linking processes of rubber might produce chemical constituents that can be easily migrated into food products. Determination of N-nitrosamine content in milk after two hours contact time with rubber nipple showed that there were 8% to 13% increase in the nitrosamine levels (Havery, 1982). The nitrosamine level increased in the rubber nipple after sterilization process indicated the presence of amine precursors.

Fajen (1979) had revealed that N-Nitrosamine that exists in rubber products has carcinogen properties. There are approximately 300 different N-Nitrosamine are carcinogenic (Stephen, 1997). In the case of coloured rubber products, the pigments could contaminate the food materials when in contact with acidic, alcoholic, mineral oil or fatty products in foods (Sidwell, 2000). The migration of chemical substance from rubber products through liquid are more rapid compared to dry food (Sheftel, 2000).

This chapter aims to provide an overview of potential toxicological problems associated with rubber products and also to find out potential food safety issue when rubber products come into contact with food products during and after processing.

6.2 RUBBER MANUFACTURING PROCESS

Rubber manufacturing usually comprised of raw materials handling, compounding, milling, extruding, calendaring and vulcanizing process. The

original form of natural rubber do not usually contain high amount of contaminants but the inclusion of chemical additives in rubber compounding (to crosslink and vulcanize) cause the contaminants to exist in rubber products. However, natural latex sap itself contained some non-isoprene component i.e. proteins, which often lead to allergic reaction upon contact.

6.2.1 Handling of Raw Rubber

The natural latex sap will be treated with ammonia in order to maintain its alkalinity. If high ammonia latex is required, the chemical would be added during the concentrating process. Ammonia itself is a precursor to nitrogenous compounds. The ammonia that is added to raw rubber would retain in the end products and might affect the smell or taste upon contact with food.

6.2.2 Milling and Compounding Process

Rubber milling or compounding is the process of mixing various chemicals into solid rubber or concentrated latex, respectively, as part of the rubber products preparation process. The raw polymer and a variety of compounding chemical additives are usually introduced into a mixer (**Table 3**). Other materials such as fillers, antioxidants, retarders, reinforcing agents and pigments can also be included to improve rubber properties (Fishbein, 1983).

Table 3. Summary of Compounding Chemicals

No.	Types of Chemical	Chemical formula	Function
1	Sulphur / Sulfur Donor	S	Vulcanizing agent
2	Zinc Oxide	ZnO	Activator
3	Hexamethylenetetramine (Hexamine/ Urotropine)	C ₆ H ₁₂ N ₄	Accelerator or catalyst
4	Zinc Dibutyl Dithio Carbamate	C ₁₈ H ₃₆ N ₂ S ₄ Zn	Accelerator or catalyst
5	Zinc Diethyl Dithio Carbamate	C ₁₀ H ₂₀ N ₂ S ₄ Zn	Accelerator or catalyst
6	Zinc 2-Mercaptobenzothiazole	C ₁₄ H ₈ N ₂ S ₄ Zn	Accelerator or catalyst
7	Tetraethylthiuram Disulfides	C ₁₀ H ₂₀ N ₂ S ₄	Accelerator or catalyst
8	Titanium Oxide	TiO ₂	Pigment
9	Calcium Carbonate	CaCO ₃	Reinforcement agent/filler
10	Potassium Hydroxide	KOH	pH Stabilizer
11	Ammonia	NH ₃	Antimicrobial agent
12	Sodium 4-Dodecylbenzenesulfonic	CH ₃ (CH ₂) ₁₁ C ₆ H ₄ S O ₃ Na	Latex stabilizer
13	Paraffin Wax	C ₃₁ H ₆₄	Softener

The main component in compounding formulation is sulphur, which functions as a cross-linker and zinc oxide as activators. The addition of sulphur and zinc oxide are to promote the vulcanization processes and thus strengthen the rubber products. Due to the long curing time in rubber processing, the addition of accelerator such as, urotropine, dithiocarbamates, benzothiazoles, guanidines or thiurams is required. The accelerator is used in combination with sulphur and zinc oxide to speed up rubber maturation time. Nearly all rubber compounds contain vulcanizing agents, activator and accelerators. When heat is applied to rubber products, chemical reactions take place throughout the manufacturing process. In example, during heating, thermal decomposition of urotropine would lead to the release of ammonia and formaldehyde (Dreyfors, 1989). The released vapor might contaminate the food that come into contact with rubber at high temperature. Based on the determination of thiuram and carbamate derivatives released from rubber gloves into synthetic sweat, it is assured that chemical migration is possible (Knudsen, 1993). The curing or vulcanizing process might give rise to new and more volatile chemicals.

The use of derivatives of secondary amines will cause nitrosating to occur as well, as they are the precursors of N-Nitrosamine (Spiegelhalder, 1982). Besides, some bacteria are able to catalyze N-nitrosation reaction and that might further increase the N-nitrosamine content in rubber products (Michael, 1991). The nitrosatability of commercial sulfenamide accelerator was compared with the new derivative of safer amino components and results showed reduction in nitrosamine level (Wacker, 1987). Based on the research finding, the derivative without secondary aliphatic amine functional group is the safe amines. By eliminating nitrosating agents and all nitrogen oxide sources, N-nitrosamine concentration will be lowered in rubber products (Oury, 1997). The use of branched and cyclic N,N-dialkyldithiocarbamyl accelerators would produce a similar quality rubber products with non-detectable N-nitrosamine (Robert, 1996). Nitrosamines content is usually detected using gas chromatography (GC) analyzer with the use of thermal energy analyzer. In order to measure the migration of nitrosamines and nitrosatable substances, the test can be conducted based on a standardized protocol (EN 12868).

During processing of rubber products, there will be some addition of plasticizer to improve the durability of natural rubber products. However, this would lead to phthalates contamination upon long contact time with food products. Direct contact of rubber products with fatty food is prone to phthalates exposure as phthalates are considered as organic lipophilic compound (Fierens, 2012). Consumption of phthalates has reported causing liver carcinogen (Cao, 2010) and reproductive system disruption (Cherif Lahimer, 2013).

6.2.3 Reinforcing Agents and Its Relation with Contaminants

Besides the aforementioned chemicals to elevate rubber properties, fillers are currently being used at high percentage in natural rubber products such as carbon black and amorphous silica. The reinforcing fillers added into

rubber products are usually relatively inert and stable materials. However, for edible oil and milk products, the filler content has to be lower than 10% according to FDA legislation (Regulatory, 2013). The chemical formula and CAS No. of the reinforcing fillers are as shown in the **Table 4**.

Table 4. Summary of reinforcing fillers

Reinforcing Fillers	Chemical formula	CAS No.
Amorphous Silica	SiO ₂	7631-86-9
Carbon Black	C	1333-86-4

From literature, carbon black itself is also a nitrosating agent which could increase the nitrosamine content in rubber products. This is due to the absorption of nitrogen oxides during carbon black formation. However, nitrosation by carbon black depends on the surface area and state-of-art technology that is used to produce the materials. The study by Dwight (1994) showed that the choice of carbon black grade and origin of suppliers would affect the differences in nitrosamine level up to 93%. Carbon black is not only a nitrosatable substance, but it is also a potential contamination of polycyclic aromatic hydrocarbons (PAHs). The PAH in carbon black and various rubber products could be measured and identified by spectrophotometric method (Charles, 1968). Sheftel (2000) mentioned that trace quantities of toxic substance are likely to migrate from elastomers to foodstuffs, but the possibility of acute toxicity is relatively low. Cumulative toxic effects might happen due to repeating ingestion of the toxic substance and lead to public health hazard. Thitiworn (2010) reported that high concentration of PAHs is produced during the smoking process to turn natural rubber sap into ribbed smoked sheet (RSS). This means that PAH might be deposited on the RSS products and this rubber sheets are usually used to produce many types of rubber products including food contact materials. However, the investigations on the extraction and migration behaviour of PAHs from cured rubber showed that the potential release of PAHs cannot be identified (Stephan, 2009).

6.2.4 Releasing Agents and Its relation with contaminants

Besides, some releasing agents such as calcium carbonate, potassium stearate or silicone based lubricants are used to reduce the tackiness of rubber products for packaging and storage purposes. **Table 5** shows lists of releasing agents and their respective chemical formula.

Table 5. Summary of releasing agents

Releasing Agents	Chemical formula	CAS No.
Calcium Carbonate	CaCO ₃	1317-65-3
Potassium Stearate	CH ₃ (CH ₂) ₁₆ COOK	593-29-3
Polydimethylsiloxane	CH ₃ [Si(CH ₃) ₂ O] _n Si(CH ₃) ₃	63148-62-9

These chemicals are not bonded chemically to rubber and chemical migration might occurs and contaminate food products upon contact (Grob, 1991). In the example of glove manufacturing, the releasing agents would be

coated at the outer surface of rubber gloves. The chemicals on the outer surface of gloves which would be used to handle food and vegetables would possibly transfer the contaminants to food surface. The decomposition of releasing agent, like potassium stearate, will lead to formation of nitrogen oxides, which is the nitrosating agents.

6.3 RUBBER AND FOOD RELATED TOXICOLOGICAL ISSUES

Food grade rubber products are generally produced from synthetic rubbers such as nitrile rubber, ethylene-propylene rubber, fluorocarbon rubber, silicone rubber, thermoplastic elastomers and also natural rubber. Natural rubber products are normally used in conveyor belt and rubber hose in food industry. Mixed synthetic-natural rubber products are produced by using base polymer of natural rubber (NR) and styrene-butadiene rubber (SBR). Usually, synthetic rubbers are used for oily or chemically active food applications while natural rubbers are used for non-reactive materials. In either case, the compounding materials used is prone to chemical migration in food products (Forrest, 2007).

Forrest (2006) has summarized key findings for the extraction test on rubber compound which showed that natural rubber products could not meet the FDA requirements for fatty and acidic foods. The natural rubber compound is only suitable to be used for applications in contact with aqueous foods. Amine, N-nitrosamine and formaldehyde are found in the specific constituent migration test on natural rubber compound. The effects of ageing, sanitising and cleaning agents on rubber compound showed that surface hardening could reduce the migrating species but chemical sanitation resulted in the formation of chlorinated curative residues. Kromhout (1994) reported 56% of the variation in solvent exposure in rubber manufacturing process involved direct utilization of solvent. The acidic cleaning agent used in rubber products generates more N-nitrosamines due to reaction with thiurams or dithiocarbamates accelerators (Forrest, 2006). Thus, the selection of solvent for regular cleaning should be selected from non-acidic solvents.

6.4 CONCLUSION

The unique properties of rubber lead to it being used in varieties of products although it is facing competition from the synthetic rubbers commercially. Due to the inherent properties of rubber and the processing requirements, compounding process is generally practiced by the industry to improve the final properties. Possible migration of N-nitrosamine and polycyclic aromatic hydrocarbon should be minimized with proper selection of chemicals. The selection of rubber products to be used in food manufacturing should be considered based on the rubber-food surface contact condition.

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Chapter 7

Trends in Drying and Extracting Bioactive Components from Herbs

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7.1 INTRODUCTION

For centuries, natural herbs are being studied for their medicinal purposes throughout human history. The benefits of herbs are wide which include healing of different diseases, maintaining and restoring body balance. They have been proven to be more favourable than modern synthetic drugs which could contain possibility side effects when consumed. Moreover, modern medicines are more expensive compare to herbal medicine and the herbs can be grown and collected easily with little or no cost. The World Health Organization estimated that 80% of the world populations depended on herbal medicine for their primary health care. Malaysia's herbs have the potential to produce medicine which reported to possess high antioxidant activity, anti-AIDS, and anticancer properties (Liza *et al.*, 2010).

Bioactive compounds are produced as secondary metabolites in plants other than the primary biosynthetic compounds such as amino acids, proteins, carbohydrates and lipids. The types of bioactive components that can be found in herbs include glycosides (e.g. saponins and anthraquinone glycosides), resins and phenol compounds (e.g. flavonoids, tannins and quinones). Natural antioxidant compounds are much effective than the synthetic compounds, which contain toxicity and carcinogenicity. These compounds help to defense human bodies by deterring the formation of free radicals chain reaction. Hence, formation of hydroperoxides and attacks of free radicals to the human body's cells are prevented (Karakaya and Kavas, 1999). Moreover, antioxidant compounds are responsible for preventing diseases such as cancer, cardiovascular disease, Alzheimer's disease and muscular degeneration (Wootton-Beard and Ryan, 2011). Selected feedstocks must contain huge amount of bioactive compounds such as phenolic compounds which exhibit antioxidant activity.

The post-harvesting process of medicinal herbs is crucial in industrial production which affects the quantity and quality of the bioactive components (Khorshidi *et al.*, 2009). However, the 87inimize87g process of extraction of bioactive compounds from Malaysia's herbs has not been done to develop a cost and productivity efficient process. According to research, the process flow that is commonly applied to produce antioxidant for medicine is pretreatment by drying, extraction and then concentration. It is worth noting that detailed research on knowledge-based process synthesis is the key for designing an effective process in terms of economic and production. This is because the improvement of process design will help to 87inimize the productivity and 87inimize the operation cost for polyphenolic compounds production (Cerón *et al.*, 2014). Therefore, the research gaps of process synthesis for pharmaceutical industry need to be fulfilled.

There are three approaches of conceptual designs for the production of bioactive compounds described by Fermeglia (2008). One of the methodologies is to perform process analysis, whereby existing process is studied to find alternative conditions that improve the process or to justify the effectiveness of the design. Next is process synthesis whereby different process configurations are compared in order to identify the best pathway.

The third possibility is process design and simulation which helps in determining the optimal operation conditions for different technologies.

Process intensification (PI) described by Ponce-Ortega *et al.*, (2012), is an approach to develop novel methods in comparing the traditional ones to improve the existing processes which leads to a substantial production. Some examples in achieving PI is by reducing equipment size, minimizing waste generation, energy consumption, and increasing production efficiency (Lutze *et al.*, 2011).

7.2 TECHNOLOGIES APPLIED FOR THE PRODUCTION OF BIOACTIVE COMPOUNDS

7.2.1 Pretreatment Technologies (Drying)

Drying is the process of reducing water content by means of heat and mass transfer, which helps to preserve the product from decomposition, reduce weight for transportation and save storage space (Karimi *et al.*, 2012). The application of drying for pre-treatment of extraction had been reported to give a better productivity of antioxidant (Cerón *et al.*, 2014). In the study, drying process is important as the tissue of dried raw materials will be more brittle which allows a rapid cell wall breakdown for grinding and homogenizing steps in extraction. However, the drying process should provide the highest retention of valuable compounds which are present in the raw material and the changes in chemical composition should enhance or create new products representing higher bioactive potential than their precursors.

The advanced state of the art technologies are ultrasonic, microwave, heat pump, vacuum and hybrid system. From **Table 1**, the shortest drying time for dehydration process until an optimum moisture content is microwave vacuum drying (MVD) followed by air drying (AD) which also known as convective air drying (CAD). Ultrasonic drying researched by Rodriguez *et al.* (2013) is able to obtain moisture content of 18% dry basis (d.b.) while using freeze drying (FD), microwave vacuum drying (MVD) and air drying (AD) are 6.8%, 9.0% and 12.7%, respectively, as investigated by Schulze *et al.* (2014). For high-quality products and cost saving, it is important to avoid drying technologies with high temperature and long drying time. FD appears to have high appraisal in maintaining the quality however it is a time-consuming process with high operation cost as it requires high energy cost. Even though AD has the lowest operating cost but it is unable to preserve much bioactive compounds.

Microwave assisted drying technologies has been reported to have shorter drying time compared to convective hot air drying. The application of convective microwave vacuum drying (C/MVD) reduces the drying time as there is a rapid removal of internal moisture due to large vapour pressure difference between the outer and inner part of the targeted substances (Chong *et al.*, 2013, Chong *et al.*, 2014). The molecules with permanent dipolar moment rotate rapidly with the change of electric field that gives rise to friction force and collision of free ions due to the electric field generating heat. As water molecules are polar, it is suitable to use microwave energy for

dehydration process compared to the conventional hot air drying. This is because hot air drying has lower drying rate that results in longer drying time. The product surface has to be dried first and subsequently mass transfer occurs from the inner part of the substance being dried to the external surface. However, the dried outer layer is a poor conductor of heat throughout the drying process. Whereas microwave drying can penetrate the dry outer layer and heat the substances throughout the high moisture area (Nijhuis *et al.*, 1998). MVD is a technology that combines microwave heating with vacuum drying. This hybrid system is able to dry thermal sensitive products in vacuum conditions at lower liquid vapour temperature in the absence of oxygen. MVD also promotes efficient removal of water and reduces structural change by preventing shrinkage and induces porous tissues (Schulze *et al.*, 2014).

Table 1. State of the Art Drying Methods and Conditions

Source	Part Used	Drying Methods	Drying Condition (m/s, °C, hr)	Additional Information	References
<i>Thymus vulgaris L.</i>	Leaves	Ultrasonic assisted dryer (UD)	1-3, 40–80,-	Power ultrasound (0, 6.2, 12.3, 18.5 kW m ⁻³), Final moisture content 18% (d.b.)	(Rodriguez <i>et al.</i> , 2013)
<i>Artemisia Absinthium</i>	Leaves	Air drying (AD)	0.906, 47.38, 10.35	Drying rate 0.35 g water/(g h), Moisture content 0.15 g/g	(Karimi <i>et al.</i> , 2012)
Apple slices	Fruit	Microwave vacuum drying (MVD), Freeze drying (FD), and AD	MVD: N/A, 50, 30(min) FD: N/A,-,72 AD: -,50,14	MVD: 500W then to 1000W end 80W,20-100hPa Moisture content 6.8% FD, 9.0% MVD and 12.7% AD	(Schulze <i>et al.</i> , 2014)
Apple Cubes	Fruit	Heat Pump (HP), Convective Vacuum Microwave	HP: 4, 35,- VM: 1.4, 11.54±20, - HP/VM: 1,	Final moisture content for HP, VM, HP/VM 0.121, 0.051,0.029	(Chong <i>et al.</i> , 2014)

Source	Part Used	Drying Methods	Drying Condition (m/s, °C, hr)	Additional Information	References
		(VM), and combination of both (HP/VM)	2, -		
Cherries	Fruit	Vacuum drying	-,46–74,8.5-43.5	Pressure 17–583 mbar, a_w value 0.646	(Šumić <i>et al.</i> , 2013)

7.2.2 Extraction Technologies

The process of extracting essential components is intricate due to the low boiling point of the components that are easily disintegrate when expose to high temperature. Studies are carried out in preserving and gleaning high amount of bioactive constituents from herbs. There are 2 types of extraction that are being applied comprehensively in the industry which are conventional extraction (CE) using different solvents and supercritical fluid extraction (SFE). It is noticeable that extraction of bioactive compounds from medicinal herbs is mainly SFE for higher yield. The supercritical fluid used is CO₂ and the co-solvent is organic solvent. CO₂ is an excellent solvent for extraction of bioactive compounds as it inert, non-flammable, non-toxic, cheap, low critical temperature of 31.26 °C and pressure of 7.38 MPa where it is effective for extraction of heat sensitive components. It is also approved as a harmless substance in food and pharmaceutical products (Kumoro and Hasan, 2007). Supercritical CO₂ has higher diffusivity than other fluids which enhance mass transfer for higher extraction rate (Chen *et al.*, 2011). The significant disadvantage of SFE is high economic cost and therefore optimisation is critical to improve the cost effectiveness of the process. Study shows that, the addition of solvent to SFE process is economically viable. This is because bioactive compounds such as flavonoids and tannins are polar compounds. Hence, co-solvent (modifier) is added to increase the polarity of CO₂ which increases the extraction yield. Organic solvents (e.g. ethanol, methanol, hexane, dichloromethane, and acetate) are used in the process of SFE.

Liza *et al.* (2010) investigated the extraction of flavonoid bioactive compounds from *Strobilanthes Crispus* with the use of supercritical CO₂ and ethanol mixture which reported to have low toxicity. The solvation power of supercritical fluid can be manipulated by changing pressure and temperature in order to achieve high selectivity. The author also proved that pressure of SCF is the most important factor that affects the yield of bioactive compound followed by the extraction time and temperature. Therefore, it is advisable to determine the pressure and extraction time before temperature.

Solvent extraction is a traditional method in obtaining bioactive compound from plants. It deals with transporting solute (bioactive compounds)

from one phase (raw materials) into another one (solvent) until it reaches the final equilibrium distribution. In industry point of view, this method is a mature technology with little risk and able to cope with most of the challenges in extraction. However, the usage of solvent brings environmental concerns as the effluent maybe harmful and hazardous. Therefore, selection of solvent is important to minimise this unwanted effect. A method of selection is by knowing the properties of the solvents, which are mostly organic compounds with lipophilic and hydrophobic properties. This is because the solute has an opposite properties (hydrophobic and lipophilic) than the organic solvent and it allows the transfer from aqueous solution into the organic phase. This phenomenon relates to the solubility of the solvent and solute. Researchers are also putting effort in dealing with regenerating the extractant to achieve a sustainability development (Rydberg, 2004).

In recent years, good solvents are being researched and developed in terms of environmental impact and cost reduction. The solvents that have these potentials in extraction process are ionic liquid (IL) and deep eutectic solvent (DES). They have unique properties and are formed when 2 or more solid crystalline compounds are mixed together. The application of these solvents is mainly in purifying biodiesel by removing unwanted substances instead of pharmaceutical industry due to the toxicity of these solvents. However, natural product components such as sugars, organic acids, amino acid, choline or urea have been discovered as excellent solvents in extraction of bioactive compounds. They are known as natural deep eutectic solvents (NaDES). Natural products are ideal source of ILs and DES because of their enormous chemical diversity, high solubilisation power of both polar and nonpolar compounds, biodegradable properties and pharmaceutically acceptable toxicity profile. It has low melting point of $<100^{\circ}\text{C}$. NaDES have an advantage over IL because it is easier to prepare with high purity at low cost (Dai *et al.*, 2013). The author elucidate that NaDES is an efficient extraction solvent for phenolic metabolites from *Carthamus tinctorius*. The characteristic of this solvent is more beneficial in extraction process compared to the DES and IL as it has adjustable viscosity, sustainable and exist as liquid state even below 0°C . However, this solvent inherits high viscosity properties which will hinder the extraction efficiency. Few methods were suggested to increase the yield by mixing water with the NaDES, increase the operating temperature or increase the transport rate by mechanical agitation. The report stated that the yield of phenolic compounds extracted using the NaDES as solvent is 14% higher than water and ethanol. To date, there is limited publication on extraction of herbs using NaDES as solvents. It is considered a new technology and much research is needed before it can be used in industrial scale. This solvent is a step forward in extraction processes because the physicochemical properties of this solvent can be adjusted to dissolve many kinds of solutes and hence increases the selectivity of extractions. The atom economy Of NADES is 100% as it does not react chemically (Paiva *et al.*, 2014). Therefore, the recovery of solvent is possible and it can reduce production cost.

7.2.3 Concentration Technologies

Concentration process is necessary after extraction because diluted extracts may not be a profitable product. The concentration process is necessary when the extracted product does not reach bioactive compounds or dry products requirements. The traditional technologies for concentration process are such as steam and vacuum distillation that require high temperature and energy consumption. Bioactive compounds are heat-sensitive and steam evaporation is not recommended in process design. The loss of low molecular weight compounds occurs as it will be removed together with the solvent (Yang and Wang, 1999).

The conventional method in concentrating natural compounds is by membrane technologies i.e. micro, ultra and nano-filtration. Literatures that successfully applied these technologies are such as concentration of *Sideritis* *ssp.* L. and propolis by cross-flow nanofiltration for large scale application (Tylkowski *et al.*, 2011), fermented grape pomace investigated using ultra- and nano-filtration (Díaz-Reinoso *et al.*, 2009). Díaz-Reinoso also studied the two ultrafiltration membrane configurations (5 and 10 kDa) for polyphenolic compounds from *Castanea sativa* leaves and concluded that the compounds are retained after processing. Membrane technologies are able to minimize the loss and degradation of the polyphenolic compounds under room temperature operating condition and absence of phase transition. Thus, this lead to lower consumption of energy and operating cost. However, the mechanism of this technology is dependent on the membrane–solvent interaction to transport organic compounds through the membrane where membrane rejection will occur (Matta *et al.*, 2004; Silva *et al.*, 2005). For solid–liquid extraction, water–organic mixtures are used as solvents. The membrane is hydrophobic in nature and the molecular weight cut-off (MWCO) factors affect the separation of bioactive compounds and solvents. Therefore, pure solvents are recommended for organic solvent resistant membranes. Tylkowski *et al.* (2011) reported that MWCO has to be >400 Da in order to separate low molecular weight bioactive compounds such as phenols and flavonoids.

The working principle of membrane processes is that the driving force acts as a mechanical pressure that only allows smaller or equal size molecules to pass through according to the pore size of the membrane. The advantages of this technology are such as preservation of product quality, low operating temperatures and energy consumption, low processing cost, and easy solvent recovery. However, the downside is the high power consumption due to the usage of pressure force and frequent fouling of the membrane that cause high maintenance cost. Membrane fouling is the major challenge where flux declines with time and cause low production rate. Fouling is the plugging of pores by deposition of substances such as debris, soluble macromolecules, and discrete solute at the inlet side of membrane. Yazdanshenas *et al.* (2007) reported an industrial cross-flow ultrafiltration plant that was used to investigate membrane flux decline in the production of apple juice. The research proved that fouling and concentration polarization are indeed the controlling factor to permeation.

Vacuum distillation (VD) is the most conventional concentration methods for antioxidant extracts. Chumsri *et al* (2008) reported optimum conditions for the concentration of roselle extract evaluated at vacuum and atmospheric evaporation. VD uses lower amount of oxygen, temperature and time compared to atmospheric pressure distillation. This is because vacuum condition increases the volatility of the substance and more evaporation occurs. Thus, it enhances the production rate and minimizes oxidation losses. The disadvantage of this technology is high energy consumption compared to membrane concentration as it requires vacuum pressure and higher temperature to operate.

7.3 OPTIMISATION METHODOLOGY

7.3.1 Overview

Fig. 1 shows the conceptual synthesis and design of bioactive compounds production from medicinal herbs. Different processing pathways are able to be determined using this structure. The structure is distributed into four stages, viz. process synthesis, process simulation, optimisation and fuzzy optimisation. For the first stage, the potential process configurations are decided based on three different technologies stages, which are pre-treatment, extraction and concentration. Each technology is reviewed and considered using knowledge-based strategy as described in previous section. One of the selected options is hybrid system (C/MVD) for pre-treatment because it is more economic viable compared to freeze-drying without compromising much on production yield. For each technologies stage, two best options are chosen by taking into consideration production cost and quality. In this stage, few process configurations are designed with the chosen technologies. Next, this designed process is simulated to obtain the process yield and operating cost. This data is crucial for the following stage in synthesizing the best process configuration using maximum production yield and minimum operating cost. Finally, fuzzy optimisation can be used to obtain the most satisfied process alternatives as it considers the primarily techno-economic criteria. This sequential procedure is able to help in comparing and designing various process alternatives to reach the same objective.

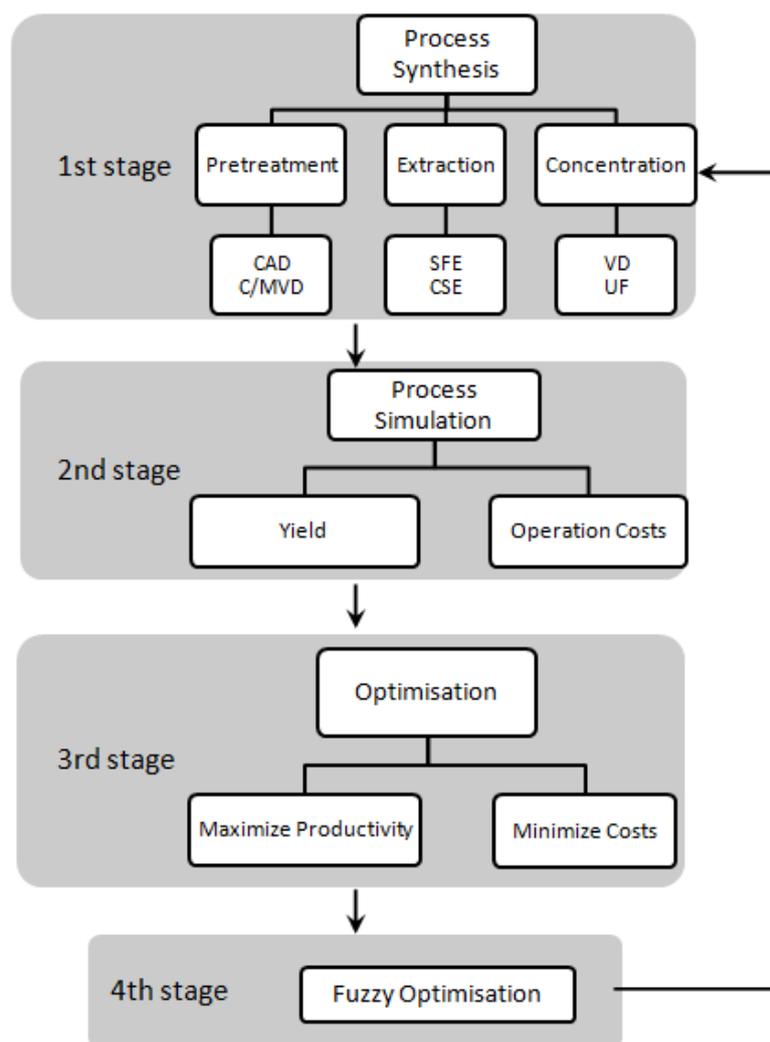


Figure 1. Optimisation methodology for Process Synthesis

7.3.2 Process Synthesis

Process synthesis is the step to design conceptual process flow diagram that leads to higher production yield and lower operating cost. Different technologies are integrated and formed various process configurations that can be analyzed rigorously. The potential process configurations for the production of bioactive compounds from medicinal herbs are designed with the chosen technologies as follow:

CAD-SFE-UF,	CAD-SFE-VD,
CAD-CSE-UF,	CAD-CSE-VD,
C/MVD-SFE-UF,	C/MVD-SFE-VD,
C/MVD-CSE-UF,	C/MVD-CSE-VD.

Where CAD: Convective Air Drying, SFE: Supercritical Extraction, UF: Ultrafiltration, C/MVD: Convective Microwave Vacuum Drying, VD: Vacuum Distillation, CSE: Conventional Solvent Extraction

The operating conditions for each technology are tabulated in Table 2. The parameters are set for the next stage of process simulation.

Table 2. Operating Conditions for Each Technology

Technologies	Parameter	Value	Ref.
<u>PRETREATMENT:</u>	Pressure, kPa	4-6	(Wojdyło <i>et al.</i> , 2009)
• Combined microwave vacuum drying	Microwave power, W	480	
• Convective air drying	Air Velocity, m/s	0.8	(Jałoszyński <i>et al.</i> , 2008)
	Temperature, °C	50	
<u>EXTRACTION:</u>	Ethanol Concentration, %	60	
• Supercritical Fluid extraction	Temperature, °C	45	(Lenucci <i>et al.</i> , 2010)
• Conventional Solvent Extraction	Pressure, MPa	30	
	NaDES Concentration, %	75	(Dai <i>et al.</i> , 2013)
	Temperature	40	
<u>CONCENTRATION:</u>	Transmembrane pressure, kPa	240	(Vladislavljević <i>et al.</i> , 2003)
• Ultrafiltration Membrane	Temperature, °C	25	
• Vacuum Distillation	Temperature, °C	45	(Cerón <i>et al.</i> , 2014)

7.3.3 Process Simulation

The design process for production of bioactive compounds is simulated using PROII 9.1 (Invensys Software, USA). The procedure to perform simulation of production of bioactive compounds is as follow. Firstly, the input components and desired products need to be defined in the simulation for conventional and non-conventional components. The input stream of the solid feedstock only considers the main components of the herbs such as lignin, cellulose, sugars (glucose), protein (lysine), and the biological molecules such as ferulic acid, vanillic acid and gallic acid. Next, is to select the suitable physicochemical properties for the simulation, which is considered as the most important step (Carlson, 1996). NRTL model is chosen because of its ability to represent vapour-liquid extraction and liquid-liquid extraction phase behaviour for polar and electrolyte compounds. After that, the unit operations are chosen and subject to the operating conditions set accordingly in the simulation environment. At this stage, the process flow diagram can be run and checked if the system is converging. Mass and energy balance can be obtained from the simulation run for evaluation.

Figure 2 shows example of a completed process simulation of CAD-SFE-VD configurations. The yield of polyphenolic compounds from this configuration is about 24.0%. **Figure 3** shows the setting of CAD using the PROII simulator. A solid dryer is used to depict the convective air drying with

moisture content set to 0.2837 kg H₂O/ kg of DM (wet basis). Another set of process configuration is C/MVD-SFE-UF, which is simulated and the overall product yield is about 26.0%. This configuration shows 2.0% higher than the previous configuration. Therefore, the C/MVD is a potential drying technology to obtain higher yield compared to CAD drying technology. However, in economic point of view, C/MVD will cause higher operating cost as higher amount of electricity is needed to convert microwave energy to electrical energy. Hence, optimisation has to be done in order to determine the most adequate process configuration.

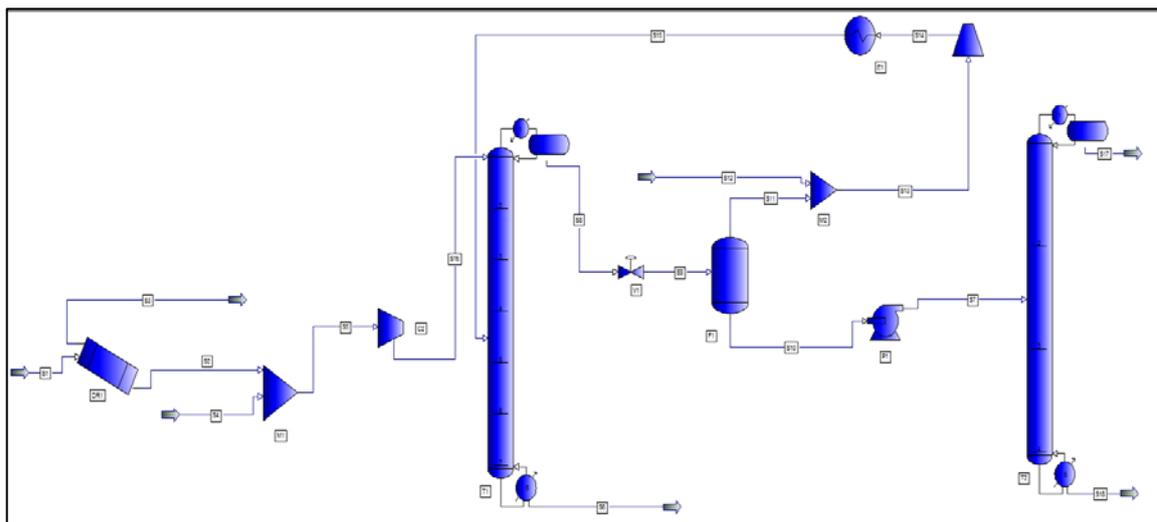


Figure 2. Process Simulation of CAD-SFE-VD

 A screenshot of the 'PRO/II - Solids Dryer' dialog box. The window has a menu bar with 'UOM', 'Define', 'Range', 'Help', 'Overview', 'Status', and 'Notes'. Below the menu bar, there are fields for 'Unit: DR1' and 'Description:'. The 'First Specification' section has a dropdown menu set to 'Pressure' with a value of '101.30 kPa' and a 'Thermodynamic System' dropdown set to 'Default (NRTL01)'. The 'Second Specification' section has two radio buttons: 'Unit Specification' (unselected) and 'Product Specification' (selected). Under 'Product Specification', it shows 'Stream S2 (Bottoms) Moisture Content on a Weight Basis = 0.28370 within the default tolerance'. At the bottom, there are 'Temperature Estimate:' and 'Pressure Estimate:' fields, both currently empty, and 'OK' and 'Cancel' buttons.

Figure 3. Setting of Operating Condition for CAD

7.3.4 Optimisation

Process modelling and simulation is crucial for the assessment of each option to determine the optimum configuration. Knowledge-based process

synthesis is a strategy for designing an effective process as stated by Sánchez and Cardona (2012) for ethanol fuel production from sugarcane. This study adopted the technique to optimise the production of antioxidant from herbs for minimising the cost of manufacturing. According to Ceron *et al.* (2014), different technologies showed direct effects on the production cost. From their studies, a superstructure of the whole optimisation process is constructed in **Figure 2**. Evaluation on different processes including pre-treatment (CVM and C/MVD), extraction (SFE and CE) and concentration (VD and UF) are required. Mathematical models based on solvent mass integration and overall material and energy balance is adopted which will be tested by simulation to obtain the production rate and energy consumption rate (Eq.1-9). **Figure 4** shows the overall superstructure presented for optimisation. The abbreviations j , l and n represent pre-treatment, extraction and concentration, respectively. The input flow rate of raw materials is denoted as F_i , flow rate from pre-treatment to extraction is F_k , from extraction to concentration process is F_m and the output of final product is F_p .

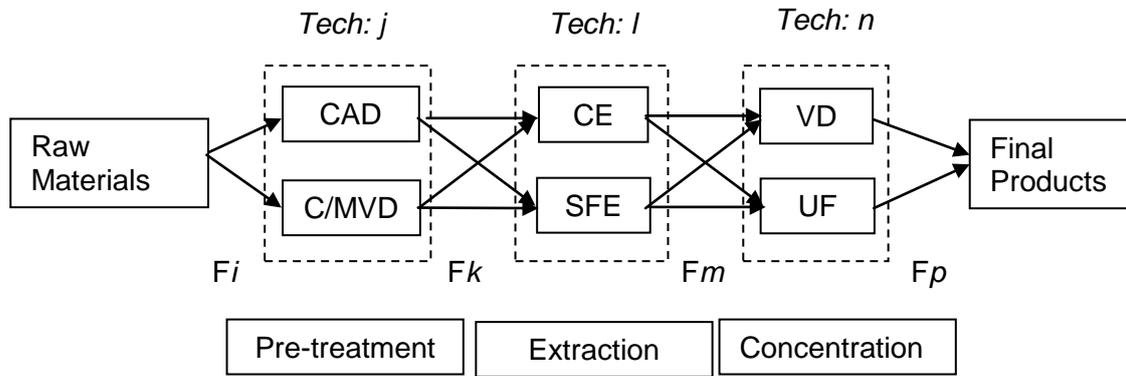


Figure 4. Superstructure for Optimisation of Process

Optimisation steps to obtain the highest production efficiency include reduction utilities usage, increase phytochemical yield, reduce the usage of extraction solvents and reduce processing time. The mathematical models for optimising different processes can be obtained from papers by Cerón *et al.*, (2014) and Ng *et al.*, (2012). Mass balance is generated using PROII 9.1. The model for feedstock flow rate entering the pretreatment is as follow:

$$F_i = \sum_j F_j \quad (1)$$

where F_i denotes input flow rate, F_j is output flow rate of technology j (pre-treatment). The equation for dried materials, k is the output from technology j with flow rate F_k and yield, Y_{jk} :

$$F_k = \sum_j F_j Y_{jk} \quad \forall k \quad (2)$$

The equation of dried materials k is given as ratio of the extraction technology to flow rate of F_{kl} :

$$F_k = \sum_l F_{kl} \quad \forall k \quad (3)$$

The extracts m at the flow rate F_m from the extraction process l at the conversion Y_{klm} is described in Eq.4. The model for technology n which has the input spilt from the extracts m has a flow rate F_{mn} stated in Eq.5. Eq.6 shows the model of yield Y_{mnp} and extracts production p that is concentrated at the flow rate F_p from concentration technology.

$$F_m = \sum_l \sum_k F_{kl} Y_{klm} \quad \forall m \quad (4)$$

$$F_m = \sum_n F_{mn} \quad \forall m \quad (5)$$

$$F_p = \sum_n \sum_m F_{mn} Y_{mnp} \quad \forall p \quad (6)$$

The recovery of solvent used in the extraction process is modeled as below:

$$F_{solv}^{fresh} = F_{solv}^{feed} - F_{solv}^{recycle} \quad (7)$$

The energy consumption in each process is important by taking into account of the energy required to produce 1 kg of polyphenolic compounds. PRO II 9.1 is able to simulate the energy demand. The simulated data will be used for calculation of socio-economic situation to obtain production costs that mirror the reality of the country. The total cost of energy consumed in different processes are to be determined by the following equation:

$$Energy\ Cost_h = C^{hot} E_g^{hot} + C^{cold} E_g^{cold} + C^{Elec} E_g^{Elec} \quad (8)$$

$$Total\ Energy\ Cost = \sum Energy\ Cost_h \quad (9)$$

where h denotes the different technologies presented at above (e.g. j , l and n), C is the cost and E_g stand for the energy output of the specific technologies (Ceron *et al.*, 2014).

7.3.5 Fuzzy Optimisation

Fuzzy optimization is used to balance out techno-economic analysis (e.g. production yield and economic). It is more robust compare to crisp optimisation (Tay *et al.*, 2011). The results from previous process flow are not easily satisfied as the two objectives contrast with each other whereby high efficiency equipment will increase the operating cost. Hence, optimisation is needed to find a balance between these parameters. In the optimisation model, λ represents the multiple objective functions. The range of λ is between 0 and 1. Hence, λ will be near to 1 when final product flow rate, F_p comes close to the upper limit and vice versa. On the other hand, the operating cost (OC) which is the constraint has the inverse response compare to F_p which is the goal of the study. Therefore, upper and lower limit has to be predetermined to reach a satisfaction degree for the fuzzy goals to be maximized (Ceron *et al.*, 2014; Sánchez and Cardona, 2012). The fuzzy optimisation equations are as follow:

$$\frac{OC^U - OC}{OC^U - OC^L} \geq \lambda \quad (10)$$

$$\frac{F_p - F_p^L}{F_p^U - F_p^L} \geq \lambda, (0 \leq \lambda \leq 1) \quad (11)$$

Where OCU and OCL are the upper and lower limit of operating cost (OC), F_p^U and F_p^L is the upper and lower limit of each process flow rate respectively where λ is the interdependence variable. This generic model can be applied to different case studies (Tay *et al.*, 2011).

7.4 CONCLUSION

Process synthesis and simulation are able to predict suitable process configurations for large scale productions of bioactive compounds and at the same time minimise production costs. Various process configurations are taken into account by integrating different processes viz., pre-treatment, extraction and concentration. The newly developed technologies are analysed based on experimental results and optimised data are used to perform simulation. In this chapter, an advanced hybrid method is applied in the drying stage and newly discovered solvent is chosen for the extraction stage. Best process configuration will be judged based on economic evaluation. It was found that high-end technologies generates the best yield but the total production cost is slightly higher. However, it is still possible to be selected as the best option in large scale processing. Therefore, fuzzy optimisation is needed to balance out the techno-economic analysis.

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NOMENCLATURE

AD	Air Drying
CAD	Convective Air Drying
C/MVD	Convective Microwave Vacuum Drying
CSE	Conventional Solvent Extraction
C	Cost
DES	Deep Eutectic Solvent
Eg	Energy Output of Specific Technologies
FD	Freeze-Drying
F_p	Final Product Flow Rate
F_p^L	Lower Limit of Each Process Flow Rate
F_p^U	Upper Limit of Each Process Flow Rate
F	Flow Rate
HP	Heat Pump
h	Different Technologies Presented at Above (e.g. j , l and n)
IL	Ionic Liquid
j	Pre-treatment

k	Output from technology j
l	Extraction
n	Concentration
MWCO	Molecular Weight cut-off
MVD	Microwave Vacuum Drying
OC	Operating Cost
OCU	Upper Limit of Operating Cost
OCL	Lower Limit of Operating Cost
SFE	Supercritical Fluid Extraction
UF	Ultrafiltration
VD	Vacuum Distillation
Y	Yield
λ	Multiple Objective Functions

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Chapter 8

Coulomb Force-Assisted Heat Pump Drying

S. K. Chin, B. K. Chung and Y.H. Lee

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8.1 INTRODUCTION

Drying process is required in almost all material processing industries, ranging from agriculture to pharmaceuticals. In most cases, it is the most energy-hungry unit operation of the whole material processing system. Drying technology deals not only with removal of liquid to produce solid product but also with the post drying properties of the dried material. Hence, many advanced drying technologies have been developed in the past decades to address the energy efficiency aspect without affecting the quality of the dried product. Some drying technologies may provide high drying rate but give rise to degradation of the product quality (in terms of nutrients, flavour, mechanical strength, volume and surface structures, colour, heat conductivity and specific heat capacity) due to heating, while others have moderate drying rate but offer better quality control that is desirable for certain products. These advanced drying technologies include Inert Particles Drying (Kutsakova, 2007), Impinging Stream Drying (Wang and Mujumdar, 2007), Superheated Steam Drying (Haque and Sargent, 2008), Vac Jet Drying (Maekawa, 1994), Contact-Sorption Drying (Ye et al., 2007), Ultrasound-assisted Drying (Carcel et al., 2002), Pulse Combustion Drying (Kudra, 2008), Heat Pump Drying (Sakar et al., 2006), Atmospheric Freeze-Drying (Alves-Filho et al., 2007), Refractance Window Drying (Nindo and Tang, 2007), Microwave-Vacuum Drying (Cui et al., 2005), and Radio Frequency-Assisted Heat Pump Drying (Marshall and Metaxas, 1999). These technologies could be expensive to implement in terms of equipment cost and operating cost.

Most of the drying technologies rely on moisture diffusion driven by mass concentration gradient between the core and the surface of the material being dried. Moisture diffusion is a slow process, and it essentially limits the drying rate. Moisture transfer mechanism can be enhanced using methods such as microwave heating and ultrasound assistance. However, there is a limit on the amount of drying enhancement achievable. For example, the applied microwave power must not be too high such that it overheats and destroys the desired properties of the material due to volumetric heating. A major breakthrough will be made if an efficient method to move water droplets from the core to the material surface is found.

Hybrid drying techniques involving heat pump combined with other methods such as radio frequency / microwave heating, infrared heating, solar heating, hot air, have been studied by a number of researchers with the purpose to enhance the drying rates and moisture diffusivity of the drying materials during heat pump dryer. The recent researches are mainly focusing on experimental determination of their suitability for certain types of material (food, agriculture, industrial, and pharmaceutical products) in terms of energy efficiency, drying rate, and properties of dried products. There are a few models to explain the moisture transfer mechanism but some refinements of the theory are yet to be made in order to match experimental results satisfactorily.

Coulomb force-assisted-heat pump (CF-HP) drying has been proposed as an alternative drying technology to enhance the moisture diffusivity, without the need to heat up the drying material. Not only it will save energy, the

desired properties of the dried material can be preserved since there is little heating. In the configuration of CF-HP dryer shown in **Figure 1**, a high voltage plate (15 kV, 50 Hz) is incorporated in the heat pump dryer in order to enhance the removal rate of bound moisture, which in turn counteract the long drying time required by heat pump dryer due to mild temperature drying. The working principle of the microwave drying shows that water dipoles are capable to re-orient themselves in the rapid reversal of electromagnetic field (the frequency of electromagnetic field can be ranged from 300 MHz to 3000 MHz) (Bradshaw et al., 2011). As CF-HP drying was conducted at low frequency; water molecules could be polarized almost instantly and heat generation due to rapid re-orientation of water molecules could be minimized. Owing to the bipolar property of moisture content inside the drying samples, a positive net force (Coulomb force) can be induced when the samples are placed near to the high voltage (15 kV), but low frequency (50 Hz) plate. The generated force enhances moisture diffusion in the samples which consequently dried by convective air flow produced by heat pump system at mild drying conditions. The quality of the drying material can be preserved as this CF-HP drying operates at mild drying temperature, low relative humidity and relatively high drying rate. The operating conditions of CF-HP dryer are listed in **Table 1**.

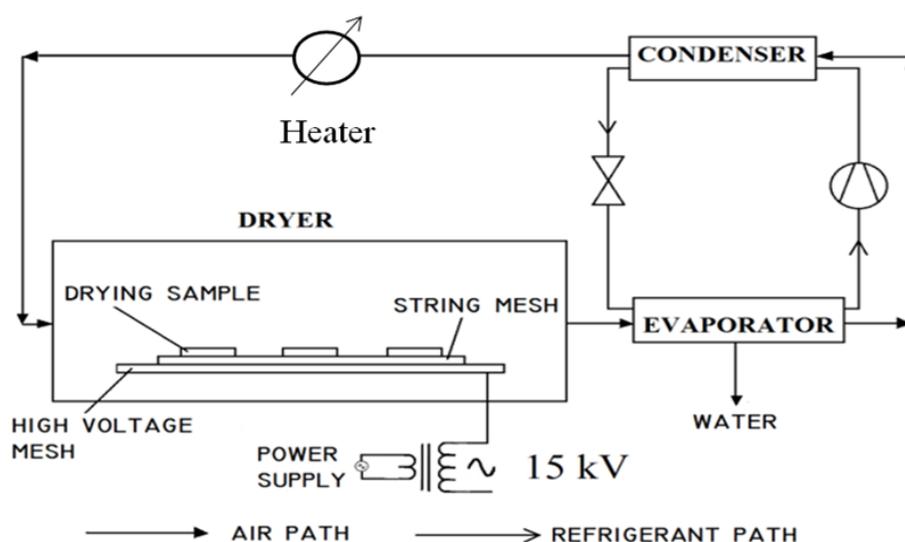


Figure 1. Schematic diagram of the Coulomb-force-assisted heat pump (CF-HP) dryer

Table 1. Operating conditions in the coulomb-force-assisted heat pump dryer

Treatment	Label	V_{air} (ms^{-1})	T_{air} ($^{\circ}\text{C}$)	RH_{air} (%)
Heat pump	HP	1.1	22	34
Coulomb force-assisted-heat pump	CF-HP	1.1	22	34
Coulomb force-heater-assisted-heat pump	CF-HT-HP	1.1	32	24

8.2 Major Findings

8.2.1 Drying characteristics

The drying performance of coulomb-force-assisted heat pump dryer during drying of lemon fruit slices is illustrated in **Figure 2** and **Figure 3**. Heat pump drying at low relative humidity condition enhances the moisture evaporation of the lemon slices although it was conducted at mild drying temperature. Nevertheless, when moisture content of the sample further decreases during drying, drying temperature starts to dominate the drying process. Heat pump drying integrated with Coulomb force significantly enhanced the drying rate of lemon slices and shortened the total drying time when towards the end of drying. The induced Coulomb force in hybrid heat pump drying could overcome the adhesion force between the bound moisture and the interior surface of the lemon pulp to enhance the moisture transportation through the semi-permeable membrane. As a result, there was an improvement in terms of drying rate for CF-HP and CF-HT-HP drying of lemon slices as compared to heat pump drying alone which in turn shortened the total drying time.

As the drying rates of lemon slices were prevailed by internal moisture diffusion, the effective moisture diffusivity of the drying process for coulomb-force-assisted drying of lemon slices was investigated. The effective diffusivity values for the HP, CF-HP, and CF-HT-HP dried lemon slices were found in the range of 0.4 to $3.4 \times 10^{-9} \text{ m}^2\text{min}^{-1}$, 0.6 to $3.8 \times 10^{-9} \text{ m}^2\text{min}^{-1}$, and 0.6 to $4.6 \times 10^{-9} \text{ m}^2 \text{ min}^{-1}$, respectively. The profile of effective diffusivity of all drying methods was similar to Region I and Region II of the diffusion model which was proposed by Luikov (1970), which indicated that vapour phase diffusion dominated the mechanism of internal diffusion. As the temperature of the lemon slices rose at early stage of drying process, the water vapour pressure inside the lemon slices increased. This resulted in rapid transportation of moisture content through the semi-permeable membrane and pressure induced opening of pores and thus, enhanced the moisture diffusivity of lemon slices (Darvishi et al., 2012). Simultaneously, the induced Coulomb force enhanced the moisture diffusion by pulling out the moisture content towards the surface of lemon slices for vapour evaporation process (Kudra and Mujumdar, 2009). Nevertheless, rapid declined in effective diffusivity was observed for all drying methods when moisture content dropped from $3.0 \text{ g H}_2\text{O} / \text{g dry weight}$ to EMC. This could be due to the shrinkage effect as the collapse of interior structure of lemon slices mitigated the moisture diffusion and only small amount of moisture was able to diffuse out to the surface when the moisture content of the slices was near to EMC (Chin and Law, 2013).

The total drying time required for both CF-HP and CF-HT-HP dried lemon slices was found to be 24.4% and 34.4% shorter than those dried by using HP drying, as shown in **Table 2**. Mild temperature drying of heat pump system prolongs the total drying time especially at the late phase of drying process, due to slow internal diffusion of bound moisture in the lemon slices which limits the drying rates. Unlike other porous drying materials, the moisture content was encapsulated in the semi-permeable membrane of lemon pulp.

Thus, diffusivity of moisture to the surface of the lemon slices was totally depends on moisture transportation through the semi-permeable membrane (Geankoplis, 2003). Similar behaviour of drying process was reported by Chen et al., (2005). Hence, the assistance of auxiliary heater as well as Coulomb force intensified the drying rates by drawing out the vapour within the lemon slices which in turn expedited the diffusion of moisture to the product surface. This shortened the total drying time required by coulomb-force-assisted heat pump drying as compared to the heat pump drying alone. The average drying rate, average effective moisture diffusivity and total drying time of lemon slices during heat pump and coulomb-force-assisted heat pump drying were illustrated in **Table 2**.

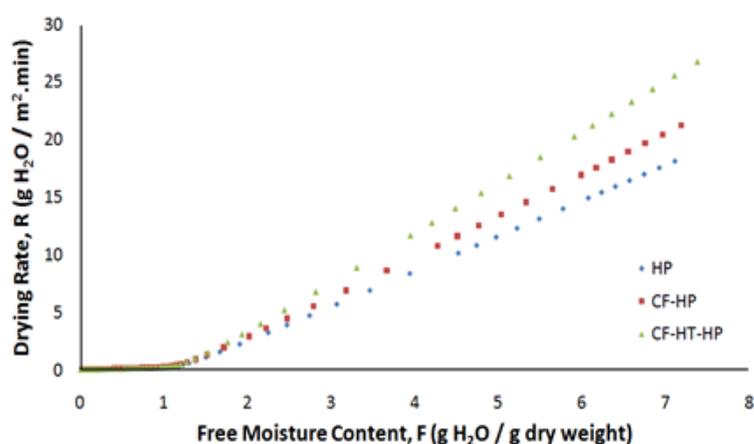


Figure 2. Graph of drying rate against free moisture content of lemon slices during heat pump and coulomb-force-assisted heat pump drying

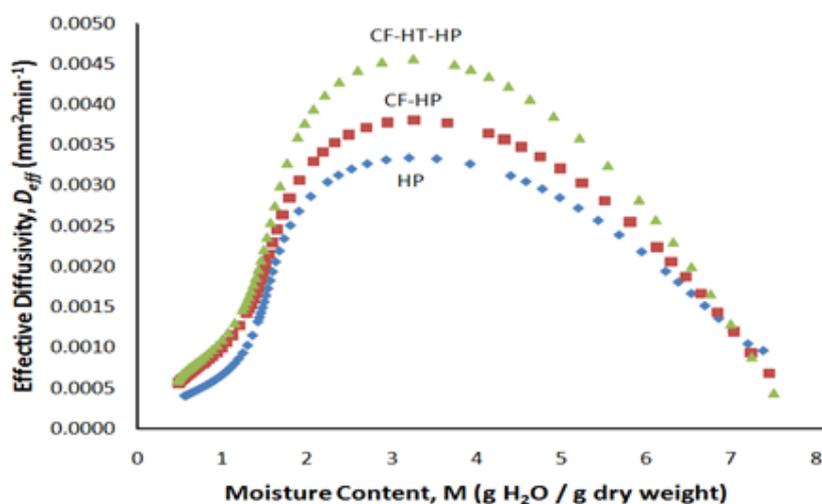


Figure 3. Variation of effective moisture diffusivity with moisture content of lemon slices during heat pump and coulomb-force-assisted heat pump drying

Table 2. Average drying rate and effective moisture diffusivity of lemon slices during heat pump and coulomb-force-assisted heat pump drying

Treatment	Duration of drying	Average R (g H ₂ O / m ² .min)	Average D_{eff} ($\times 10^{-9}$ m ² min ⁻¹)	Total drying time (min)	Total time reduced (%) [*]
HP	Until EMC [#]	3.2	1.77	9000	-
CF-HP	Until EMC	3.5	1.95	6800	24.4
CF-HT-HP	Until EMC	4.0	2.24	5900	34.4

[#]EMC = equilibrium moisture content; ^{*}As relative to HP drying.

8.2.2 Quality attributes

Ascorbic acid (or typically known as vitamin C) is a heat sensitive bioactive ingredient in most of the agricultural products. Hence, mild temperature drying methods such as heat pump could be considered for drying of agricultural products in order to preserve high amount of ascorbic acid in the dried products (Kaya et al., 2010). During HP, CF-HP and CF-HT-HP drying of lemon slices, mild drying temperature with relatively high drying rate could minimize the deterioration of ascorbic acid through thermal degradation as well as enzymatic oxidation. Among the dried samples, CF-HP dried lemon slices contained the highest amount of Vitamin C as according to **Table 3**. Integration of Coulomb force in heat pump drying (CF-HP) enhanced the drying rates and shortened the total drying time required as compared to HP drying, which in turn mitigates the enzymatic oxidation of ascorbic acid and retains high amount of ascorbic acid in CF-HP dried lemon slices. However, the used of auxiliary heater in CF-HT-HP drying method deteriorated the ascorbic acid content of lemon slices to 5.77 mg Ascorbic Acid/ g dry weight. The significant degradation of ascorbic acid in CF-HT-HP dried samples indicates the prominent effect of elevated drying temperature on the thermal degradation of ascorbic acid, as compared to the effect of drying duration.

In contrast to the results shown in ascorbic acid analysis, CF-HT-HP dried slices retained the highest amount of TPC compared to HP and CF-HP dried samples as shown in **Table 3**. High retention of TPC in lemon slices dried at elevated temperature might be due to the availability of precursors of phenolic molecules by non-enzymatic inter-conversion between phenolic molecules (Vega-Galvez et al., 2009; Moraes et al., 2013). Furthermore, fast drying rate of the lemon slices during CF-HT-HP drying also prevents the degradation of TPC through volatilization, oxidation and heat destruction process (Darvishi et al., 2014). Among the heat pump and hybrid heat pump dried slices, the TPC in CF-HP and CF-HT-HP was insignificantly difference, but the amount was significantly higher than those in HP dried slices. This could be due to shorter total drying time required by hybrid heat pump drying as compared to heat pump drying of lemon slices. The results show that drying duration significantly affects the total TPC of lemon slices as compared to drying temperatures. **Figure 4** shows the physical appearances of the dried lemon slices.



a. Fresh lemon slices



b. CF-HP dried lemon slices



c. CF-HT-HP dried lemon slices

Figure 4. Dried lemon slices (before and after drying)

Table 3. Ascorbic acid (AA) and total phenolic content (TPC) of heat pump and coulomb-force-assisted heat pump dried lemon slices

Treatment	Ascorbic acid content (mg AA [#] / g dry weight)	% AA as relative to HP dried slices	Total phenolic content (mg GA [#] / g dry weight)	% TPC as relative to HP dried slices
HP	6.01±0.57 ^{ab*}	-	9.24±0.50 ^{b*}	-
CF-HP	6.74±0.40 ^a	12.15	10.14±0.37 ^a	9.74
CF-HT-HP	5.77±0.42 ^b	-4.0	10.44±0.38 ^a	13.0

#AA = Ascorbic acid; GA = Gallic acid. *Different superscript letters indicates there is a significant difference ($p < 0.05$) in ascorbic acid / total phenolic content.

8.3 CONCLUSION

The advantages of Coulomb-force-assisted heat pump (CF-HP) drying in agricultural products were justified by experimental results. This drying technique intensified the drying rates, enhanced the moisture diffusivity and consequently shortened the total drying duration as required by heat pump drying method. With respect to product quality, it appears that CF-HP drying is a highly recommended drying method for drying of foods and agricultural products for the preservation of antioxidants such as ascorbic acid and TPC. Moreover, the discolouring and shrinkage effect of the dried products also envisaged to be greatly reduced due to mild temperature drying at relatively fast drying rates. However, further investigation should be conducted to verify the above hypothesis, as well as to include the economic prospects (e.g. equipment cost and energy usage) of this drying method. Besides foods and agricultural products, the potential of this drying method can be extended for drying of materials with poor heat-transfer characteristics, such as ceramics and glass fibres, which are difficult to dry with convection heating.

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Chapter 9

Fermentation in Food Processing

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9.1 INTRODUCTION

Food fermentation represents one of the oldest known uses of biotechnology in food processing and preservation (Campbell-Platt, 1994). Microorganisms such as bacteria, yeast, mould are used in the production of traditional fermented food which can be dated back many centuries, with early evidences of the alcoholic fermentations of barley to beer, grapes to wine (Campbell-Platt 1994) and the production of yoghurt and cheese (Fleet, 2007).

Fermented foods have contributed to approximately one-third of the human diet worldwide, with dairy, cereal and beverage products dominating around the globe (Campbell-Platt, 1994). Some examples of fermented foods are Indonesian tempe, Malaysian kicap and belachan, Japanese miso, Korean kimchi, cassava, sauerkraut, yogurt, vinegar, wine, cider, beer, Chinese soy sause, hum-choy (Steinkraus, 2002) and Puer tea (Abe, 2008).

9.2 DEFINITION OF FERMENTED FOOD

According to Campbell-Platt (1994), the production of fermented foods involves microbial or enzymatic actions which cause desirable biochemical changes and significant modification to the food. This definition was further supported by Steinkraus (2002). Enzymes particularly amylases, proteases or lipases from edible microorganisms stimulate the hydrolysis of polysaccharides, proteins and lipids to simpler nontoxic products which are pleasant and appealing in flavour, aroma and texture (Steinkraus, 2002).

However, the ecosystem of fermentation is very complex. Factors such as added salt, particle sizes, temperature as well as oxygen levels may also have important impact on the chemistry that occurs during fermentation (Mcfeeters, 2004).

9.3 FERMENTING ORGANISMS

Microorganisms that involved in the fermentation process play a key role in determining types of compounds or products formed. Compounds that are formed during fermentation include organic acids, alcohol, aldehydes and ketones (Campbell-Platt, 1994).

Microorganisms that are industrially important and commonly used in food fermentations include the following.

9.3.1 Bacteria: *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Lactococcus*, *Bacillus*.

Lactic acid bacteria are the most common bacteria used in fermentation. They have been demonstrated to possess antimicrobial activity with majority of them produce bacteriocins such as nisin (Chojnacka). Lactic acid bacteria are effective in inhibiting the development of other non-desirable organisms that may decompose or spoil the food (Evans et al., 2013). This could indirectly reduce the use of chemical preservatives in fermented products (Chojnacka).

Some lactic acid bacteria have stabilizing and viscosity forming properties, which may avoid the addition of synthetic stabilizers and emulsifiers in fermented products (Chojnacka). This was in agreement with Leroy et al. (2002) that exopolysaccharides produced by lactic acid bacteria are sugar polymers that enable the improvement of the rheological and textural properties of the fermented products.

9.3.2 Yeast: *Saccharomyces*, *Candida*, *Torulopsis*.

Yeasts are frequently minority companions of lactic acid bacteria. They are commonly used in the production of carbon dioxide in beer and breadmaking or ethanol in alcoholic beverages (Chojnacka).

9.3.3 Mould: *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*.

Moulds are used to produce enzymes that are able to degrade polymeric components such as cell wall polysaccharides, proteins, lipids, which is significant for texture, flavour and nutritional value (Chojnacka).

9.4 CLASSIFICATION OF FERMENTED FOODS

Overall, food fermentation can be classified in a few different ways (Dirar, 1993), namely by categories (Yokotsuka, 1982), by classes (Campbell-Platt, 1987), by commodity (Kuboye, 1985; Odunfa, 1988) or by functional basis among Sundanese (Dirar, 1993).

Steinkraus (1997) classified fermentation based on the major products that are formed during the fermentation process, such as lactic acid fermentation, alcoholic fermentation, acetic acid fermentation, alkaline fermentation and amino acid fermentation (Steinkraus, 1997). Some examples of food fermentations are as below:

9.4.1 Lactic acid fermentation

Lactic acid fermentation is commonly used to prepare food traditionally with the fact that the techniques are generally simple, effective, inexpensive, yield highly acceptable and diversified flavours (Steinkraus, 1992).

During lactic acid fermentation, the fermentable sugars are converted to lactic acid with the aide of lactic acid bacteria (Steinkraus, 2002). Example of the lactic acid fermented foods are maize porridge (Chelule et al., 2010), kimchi and sauerkraut (Joshi et al., 2011).

Foods that have been undergone lactic acid fermentations become resistant to microbial spoilage and toxin development. This finding was supported by Todorov and LeBlanc (2014). Lactic acid bacteria was found to be able to produce various antimicrobial substances including bacteriocins, lactic acid, hydrogen peroxide, fatty acids, diacetyl and other low molecular weight compounds during fermentation (Todorov and LeBlanc, 2014).

Besides conferring preservative and detoxifying effects on food, lactic acid fermentations also modify flavour, improve palatability, quality, protein solubility, micronutrient availability and nutritive value of food products (Karovičová, 2003).

Lactic acid bacteria are mainly used in fermentation practice among the developing countries. Specifically, lactic acid fermented porridge called “pap” is the traditional weaning complementary food for infants (Battcock, M. and Azam-Ali, S., 1998) and staple breakfast for adults as it is nutritionally enhanced and digestibility improved when fermented (Steinkraus, 2002). On top of that, the energy density of fermented porridge will be elevated after being acidified during lactic acid fermentation, therefore indirectly helping the consumer to meet the energy requirements efficiently (Battcock, M. and Azam-Ali, S., 1998). By consuming regularly, lactic acid fermented foods are believed to be able to improve the immune system and protect the body against pathogenic infections (Chelule et al., 2010).

9.4.2 Alcoholic fermentation

Alcoholic fermentation is one of the most ancient and important techniques, used mainly to produce alcoholic beverages (Chojnacka). The products are generally safe, for example beers, fruit wines, Chinese lao-chao and Indonesian tape ketan (Steinkraus, 2002).

Under anaerobic condition, fermentable sugars from substrates are converted into alcohol and carbon dioxide by yeasts (Fleet, 1992; Sevda and Rodrigues, 2011). It has been experimentally proven that nearly equal weights of ethanol and carbon dioxide are produced during fermentation (Steinkraus, 2002).

The most widely used yeast strain as starter in alcoholic production process is *Saccharomyces cerevisiae*. Besides allowing rapid and reliable fermentations, it can also reduce the risk of sluggish or stuck fermentations and prevent microbial contaminations (Sevda and Rodrigues, 2011).

In some cases, the alcoholic fermentation also involves the use of yeast-like moulds such as *Amylomyces rouxii* and mould-like yeasts such as *Endomycopsis* and sometimes bacteria such as *Zymomonas mobilis* (Steinkraus, 2002).

There are a few key factors in determining the successful fermentative processes of alcoholic production, such as pH values, sugar contents, nitrogen contents, initial sugar concentrations, fermentation temperatures, SO₂ concentrations and specific yeast strains (Sevda and Rodrigues, 2011). For instance, the fermentation time and types of starter culture used during fermentation may affect the alcohol content in tape ketan (Law et al., 2011).

9.4.3 Acetic acid fermentation

In contrast to alcoholic fermentation, in acetic acid fermentations, acetic acid bacteria belonging to genus *Acetobacter* can oxidize the ethanol to acetic acid in the presence of oxygen (Fleet, 1992). Examples of the fermented acetic acid beverage are vinegar and kombucha (Steinkraus, 2002).

Vinegar is produced by a second fermentation of beer, cider, or wine. The quality of vinegar is very much dependent on the acetic acid bacteria species being used during the oxidation process. Vinegar has been used as a condiment and food preservative. Apart from this, it was claimed to serve as the basis for simple remedies for human and animals (Mas et al., 2014).

Kombucha possesses various health-promoting qualities. However, the heavy consumption of kombucha can lead to indigestion and even death (Steinkraus, 2002).

According to Steinkraus (2002), acetic acid exhibits stronger preservative properties than ethanol. It is also more inhibitory compare to lactic acid and can inhibit the growth of bacteria, yeasts and moulds (Caplice and Fitzgerald, 1999).

Acetic acid is produced aerobically by two-step vinegar process. The first step involves the production of ethanol from a carbohydrate source by yeasts. The second step is the oxidation of ethanol to acetic acid with the aide of acetic acid bacteria. During secondary fermentation, small quantities of volatile substances that formed are varies from vinegar to vinegar depending on the starting materials (Raspo and Goranovič).

Fermentation is usually stopped at a minimum residual ethanol level to avoid overoxidation of acetic acid to water and carbon dioxide (Raspo and Goranovič). Generally, the final quality of vinegars depends on the selection of appropriate starter cultures to lead the process. However, other factors including the quality of the starting material, the production method and the aging process might also important in determining the quality of the vinegars (Mas et al., 2014).

9.4.4 Alkaline fermentation

Foods that are fermented through alkaline fermentations are generally safe, nutritious and as a source of low-cost nitrogen. Alkaline fermentations are commonly used in fermenting protein rich foods such as soybeans and legumes. Examples of the alkaline fermented products include ogiri, ugba, kawal, owoh, pidan (Wang and Fung, 1996), Nigerian dawadawa, Japanese natoo, Thai thua-nao and Indian kenima (Streinkraus, 2002).

Proteolytic microorganisms such as *Bacillus subtilis* are the dominant species that are involved in the alkaline fermentation process. Protease produced by the bacteria hydrolyzes the protein of the raw material to peptides and amino acids (Streinkraus, 2002). Thereby the nutritional value and the bioavailability of the alkaline fermented foods are increased (Steinkraus, 2002). Meanwhile, the release of ammonia during fermentation increases the pH and causing strong ammoniacal smell to the final products (Wang and Fung, 1996).

Research showed that the combination of free ammonia, high pH and rapid growth of essential microbes at high temperatures can inhibit the growth of other

microbes that might spoil the product. Hence, food that undergone this process are quite stable and well-preserved (Streinkraus, 2002).

9.4.5 Amino acid fermentation

Being the most important source of energy, amino acids have been the cornerstone of the metabolic activities and physiological processes in all living organisms (Shakoori et al., 2012).

Fermentation is widely used in the industrial production of most amino acids (Wang et al., 2012). Example of amino acids that have been produced by fermentation processes include L-glutamic acid, valine and L-methionine (Shakoori et al., 2012).

Amino acids secreting bacteria such as *Escherichia coli* and *Corynebacterium glutamicum* are used widely for the commercial production of many types of amino acids (Shakoori et al., 2012). For example, besides being the most effective glutamic acid producer (Kinoshita, 1999), serine and lysine that have found novel applications in food and pharmaceutical industries are also produced by *Corynebacterium glutamicum* industrially (Jo et al., 2006).

The importance of amino acids in food and pharmaceutical industries has drawn the attention of many scientists to discover the effective way to produce amino acids on commercial scale at low cost. It was found that amino acids can be produced cost effectively by using isolated bacterial strains from natural sources (Shakoori et al., 2012). In recent years, advanced techniques that focus on the improvement of amino acid producing strains for desired phenotypes through mutagenesis and genetic engineering has also been exploited extensively (Parekh et al., 2000; Pasha et al., 2011).

In addition to the selection of bacterial strains, there are a few others determining factors that are crucial in the fermentative production of amino acids. Parameters such as temperature, pH, different constituents of fermentation media, water quantity, incubation time and aeration were proven to have a great impact on the growth of amino acid producing bacterium that lead towards the biosynthesis of amino acid (Aida, 1986; Shakoori et al., 2012).

9.5 BENEFITS OF FERMENTED FOODS

Fermentation plays a few vital roles in food processing, where different types of food are fermented and served as a staple diet for human.

9.5.1 Low cost preservation

Fermentation is known to modify food composition and soften food texture that result in minimal energy required in preservation or cooking process (Chelule et al., 2010). Fermentation process is characterized by the limited need for energy input, allowing microbial fermentations to proceed without external heat sources (Achi, 2005). Most fermented products such as pickled vegetables, sauerkraut, kimchi

generally require little, if any, heat during fermentative process and can be consumed directly without cooking (Steinkraus, 2002).

Hence, fermentation has emerged to be the most economical preservation method (Kohajdová and Karovičová, 2007) to increase the shelf-life (Joshi et al., 2011) and safety ((Holzapfel, 2002; Rolle and Satin, 2002) of the food products (Kohajdová and Karovičová, 2007).

It was believed that a range of metabolites that is produced during fermentation can suppress the growth and survival of undesirable microflora in food. This low-cost technique may be useful to reduce the bacterial contamination and thus reducing the prevalence of diarrheal diseases (Kohajdová and Karovičová, 2007).

In certain area, preserved food through fermentation technique is a necessity (Kohajdová and Karovičová, 2007). Fermentation itself is largely independent of weather (Marshall, E. and Mejia, D., 2012) or environment conditions (Roy et al., 2004). This was in agreement with several authors. Foods that are preserved through fermentation technique could help to balance the fluctuation in food availability in Southeast Asia during the season of monsoonal circulation (Law et al., 2011). In the tropics, highly perishable foods may be preserved as fermented products. Those fermented foods able to provide vitamins, particularly during long cold months in the northern parts of East Asia (Aidoo, 1986).

9.5.2 Physical and flavour enhancement

Fermented foods enrich human diet by providing vast quantities of nutritious food in a wide diversity of flavours, aromas and textures (Steinkraus, 1994). Some moulds and yeasts can secrete enzyme to hydrolyze unpalatable complex carbohydrate and protein into simpler structure of sugar and amino acids (Law et al., 2011). Similarly, Beuchat (1987) reported when during fermentation, moulds converts starches to simpler sugars, whereas yeast converts fermentable sugars to ethanol and other flavour components.

These special organoleptic properties contribute to the palatability of the fermented foods and thus they are generally more popular and preferable than the unfermented foods (Blandino et al., 2003). Survey showed that the rural folk in most developing countries prefer fermented over the unfermented products due to the pleasant flavour, aroma, colour and texture (Chelule et al., 2010).

A few studies have been carried out to study on the flavour changes during fermentation. Marsili and Miller (2000) studied the flavour impact compound in fermented pickled cucumber brines. In 2002, Czerny and Schieberle observed a number of changes in odorants when whole meal wheat flour was fermented by a commercial sourdough starter culture.

One of the characteristic odour compounds generated in the baking process of sourdough bread is 2-acetyl pyrroline (Schieberle, 1995). Ornithine, as the precursor of 2-acetyl pyrroline during baking, is synthesized by lactic acid bacteria. It was observed that the variation in the amount of ornithine found during sourdough

fermentation may be due to the type of starter culture used in fermentation (De Angelis et al., 2002; Thiele and Vogel, 2002).

McFeeters (2004) reviewed the influence of lactic acid bacteria on flavour changes in food fermentation. In many cases, the most obvious changes in a lactic acid fermentation is the production of acid and lowering pH that results in an increase in sourness and a decrease in sweetness due to metabolism of sugars. Lowering the pH in lactic acid fermentations may reduce or completely inactivate enzymes activity in food that involved in the generation of either flavour components or flavour precursor compounds. Finally, the role of metabolizing the flavour components or flavour precursor compounds will be taken over by fermenting microorganisms (McFeeters, 2004).

Apart from that, fermentation can be used to create a meat-like flavours and odours which is important for cultures or in the area where meat is scarce. It was reported that the traditional fermented seeds of *Parkia biglobosa*, also known as Dadawa, can be used as a nutritious flavouring additive and may serve as meat substitute (Odunfa, 1985; Achi, 2005). It has also been documented that over the years, Sudanese has developed fermented products to substitute meat in their diets, namely fermented legume leaves called “*kawal*”, fermented sesame presscake called “*sigda*” and fermented sorrel seeds called “*furundu*” (Battcock, M. and Azam-Ali, S., 1998).

Fermentation may also play a role in altering the rheological characteristics of food such as dough. Sourdough is the mixture of flour and water that is then fermented with lactic acid bacteria (Hammes and Gänzle, 1998). It is employed during the production of breads, cake and cracker. It was demonstrated that the addition of sourdough during bread production may cause major changes in the structure of the dough (Clarke et. al., 2002).

9.5.3 Nutritional value

Apart from contributing certain desirable physical and flavour characteristics (Joshi et al., 2000), fermentation also enable the enrichment of human dietary with vitamins, essential amino acids, protein and essential fatty acids (Steinkraus, 2002). Scientific evidences indicated that fermentation may magnify specific nutrient of foods, influencing the bioavailability and activity of the chemical constituents (Selhub et al., 2014).

During fermentation, both digestibility and nutrient profile of a particular food may be improved. The breakdown of the non-digestible material or macronutrients after being predigested by the enzymatic activity of microbes may lead to the improved availability of trace elements and mineral (Kalantzopoulos, 1997). Furthermore, Svanberg and Lorri (1997) suggested that the degradation of anti-nutritional factors and synthesis of promoters for absorption during fermentation may also help to increase the bioavailability of nutrients. Nout and Ngoddy (1997) reported that in some cases, lactic acid fermentation enhances the protein solubility and the bioavailability of limiting amino acids by approximately 50%.

Several researches have been carried out to study the nutritional value of fermented product. In West Africa, a popular fermented porridge called *ogi*, can be a complementary food for young children due to its highly nutritious content after being fermented (Omemu et al., 2007). Vergara-Salinas et al. (2013) discovered that the fermented grape pomace generates more total antioxidants compared to its unfermented counterpart. Research showed that riboflavin, pyridoxine and thiamine elevates during palm sap fermentation. Therefore, palm toddies are regarded by Steinkraus (1994) as one of the low-cost sources of vitamins B among the poor communities.

Being one of the oldest and most popular fermented cereal foods of turkey, the composition and nutritive value of Turkish tarhana was studied by Daglioglu (2000) and reviewed by Georgala (2013). It was observed that its nutritional value increased by fermentation (Georgala, 2013). It is a good source of total minerals, vitamins, organic acids, free amino acids and protein (Daglioglu, 2000; Georgala, 2013). Due to its high nutritional contents, tarhana is healthy for children, the elderly and medical patients (Georgala, 2013). Likewise, Greek trahanas is one of the most popular fermented milk-cereal products of Greece. It is a very nutritive food, enriched with proteins, minerals and therefore is used largely for feeding people (Georgala, 2013).

Antioxidant properties of rice wine such as t-resveratrol and phenolic acids were evaluated by Woraratphoka et al. (2007). Furthermore, the presence of ethyl α -D-glucoside, D-glucose glycerol, amino acids and organic acids in rice wine are suggested to be a potential effective skin anti-aging agent (Seo et al., 2009).

The nutritional content of cassava or rice in tape ketan after fermentation was reviewed by Steinkraus (1996). It was found that the content of thiamine in tapai is comparable to the unpolished rice. *Beri-beri* is a disease brought on by a thiamine deficiency. Southeast Asian consumers usually consume polished rice as their main diet and are therefore at higher risk of getting *beri-beri*. However study showed that this problem can be prevented by consuming thiamine-rich tapai (Steinkraus 1996).

Soybean is highly nutritious with the presence of isoflavones, protein, iron, unsaturated fatty acids and niacin (Jinapong et al., 2008; Kim et al., 2006). However, the beany flavour and its consumption that may lead to digestive problems associated with the presence of indigestible oligosaccharides, lead to the limited widespread consumption of soymilk (Blagden and Gilliland, 2005; Yang and Zhang, 2009). These problems can be solved and its acceptability can be improved through the application of fermentation in soybean products (Hati et al., 2013). Researchers found that fermentation improves the bioavailability of isoflavones, assists in digestion of protein, provides more soluble calcium, improves intestinal health and supports immune system (Ismail and Hayes, 2005; Kuo et al., 2006).

The antioxidant activity of fermented soyfoods was observed to be remarkably stronger than unfermented steamed soybeans (Berghofer et al., 1998; Esaki et al., 1994; Murakami et al., 1984; Sheih et al., 2000). Similar result was reported by Wang et al. (2006) that fermented soymilk has higher antioxidant activity than unfermented soymilk. In Africa, soymilk which is used in the fermentative production of

yoghurt, can serve as a simple, safe and affordable food to combat malnutrition (Ashaye et al., 2001).

9.5.4 Detoxification

Foods are susceptible to various types of contamination either naturally or through infestation by microbes. Certain moulds produce secondary toxic metabolites or known as mycotoxins (Sweeney and Dobson, 1998). Most of them are heat-stable and cannot be removed by cooking. Whereas the use of chemical method such as alkaline ammonia treatment to degrade toxins from contaminated food, is found to be potentially detrimental to health or may diminish the nutrient content of food.

On the contrary, detoxification of mycotoxins in food through lactic acid fermentation is more favourable. It is a milder method which retains the nutritional value and flavor of decontaminated food (Bata and Lásztity, 1999). It is believed that through toxin binding effect, lactic acid fermentation irreversibly degrades mycotoxins without leaving any toxic residues (El-Nezami et al., 2002; Haskard et al., 2001; Turbic et al., 2002).

Oluwafemi and Ikeowa (2005) reported that the decrease of pH by *S. cerevisiae* during the process of fermenting maize to beer is important in detoxifying aflatoxin B. They also observed that in fermenting maize to ogi, the content of aflatoxin B1 was reduced remarkably after 3 days of fermentation (Oluwafemi and Ikeowa, 2005).

Milk is a well-known nutrient-rich food for human, but often gets contaminated by harmful bacteria and easily gets spoilt if left unpreserved at ambient temperature. It may also contain residual toxins such as aflatoxin M1 from the food consumed by the cows prior to milking (Katz, 2001). These contamination and toxicity can be solved by fermentation process. It was found that yoghurt, a fermented milk product possesses the ability to eliminate pathogens and regulate the immune system (Ashaye et al., 2001; Katz, 2001). *Amasi*, traditional fermented milk, has been demonstrated to experimentally restrain the growth of *Escherichia coli* and *Salmonella enteridis* that usually contaminate raw milk (Mufandaedza et al., 2006).

Meat, as one of the richest food sources of protein (Lücke, 2000) gets contaminated easily by pathogenic microorganisms present in animals prior to being slaughtered. A few authors suggested that one of the favourable methods used to attain the qualities of safety, stability, storage of the meat is fermentation (Hugas and Monfort, 1997; Hugas et al., 2003; Jones et al., 2008). Research revealed that fermentation of meat products using selected lactic acid bacteria strains strongly restrained the pathogenic bacterial growth, with the organoleptic properties of the products remained intact (Metaxopoulos et al., 2002).

Research demonstrated that *Lanhouin*, a fermented fish was found to be safer and free of spoilage bacteria such as *Salmonella* after being processed by lactic acid fermentation. Therefore, it could be stored for longer periods. Besides free from the fishy odour and taste, its nutritional quality did not affected by fermentation (Gelman et al., 2000).

Cyanogenic glucoside, a naturally occurring toxin is found present in poisonous plant named cassava. This toxin releases potentially fatal cyanide into the body if cassava is eaten raw or improperly processed (Battcock, M. and Azam-Ali, S., 1998). Cooking is not effective in cyanogens detoxification, as it leaves residual cyanogens in processed cassava, which are equally toxic as their parent compounds in unprocessed cassava (Ravi and Padmaja, 1997). Caplice and Fitzgerald (1999) reported that lactic acid fermentation able to reduce cyanogens effectively following the fermentation of cassava food products.

9.5.5 Medical advantages

Studies discovered that many fermented products have associated medicinal benefits (Marshall, E. and Mejia, D., 2012).

Kefir, a fermented milk product, contains an insoluble polysaccharide with antibacterial and cicatrizing properties that show potential application in medicine (Rodrigues et al., 2005). It has been documented that the camel milk koumiss of Russia, called Chal, has been used in a clinic to treat tuberculosis and other lung ailments (Kosikowski, 1982; Oberman, 1985). Another example of a product fermented from camel's milk is known as garris. It has been used in Sudan to cure leishmaniasis or *kala-azar* (Dirar, 1993).

Fermented foods have the potential to influence brain health. For example, fermented soy products have been associated with lower risk of depression (Nanri et al., 2010; Suzuki et al., 2013). Gea et al. (2012) also discovered that moderate consumption of alcohol particularly red wine can lower the risk of depression.

Puer tea has been consumed by people in China for centuries. It is a unique fermented Chinese tea produced by microbial activities, characterized by its pleasant fragrance and brown colour (Abe, 2008). As Puer tea has recently become a popular functional beverage in Japan, this has attracted the interest of a few scientists from Japan to investigate its fermentation process by microbial and physicochemical analysis (Abe, 2008). Puer tea is believed to have an anti-obesity effect (Abe, 2008). Besides that, its hypolipidemic and antioxidative properties have also been documented by a few authors (Duh et al., 2004; Jie et al., 2006; Kuo et al., 2005; Sano et al., 1986).

According to Kalantzopoulos (1997), fermented products may lower the serum cholesterol concentration by regulating the absorption of dietary and endogenous cholesterol or inhibiting the synthesis of cholesterol in liver. Korean research team discovered an enhanced anti-inflammatory activity in fermented fish oil versus its unfermented counterpart (Han et al., 2012).

Fermented rice and rice bran such as red yeast rice and red mold rice have been shown to prevent or treat disease. They are produced after fermenting rice with *Monascus*. Red yeast rice has been used in the treatment of cholesterol, type II diabetes, cardiovascular disease and for cancer prevention (Kalaivani et al., 2010). Besides being used in China for many centuries for flavour enhancement purpose, red mold rice has also been used in medicinal treatments. These include the prevention of cardiovascular disease, cancer, Alzheimer's disease (Lee and Pan,

2011).

Foods that are hard to digest can lead to digestion problem, diarrhoea and constipation. Therefore, it is important to take predigested foods such as fermented foods which can aid in digestion as well as boost the immune system (Evans et al., 2013). Studies showed that the occurrence of sexually transmitted disease (Reid et al., 2001) and diarrhea (Adebolu et al., 2007) can be reduced by consuming fermented food such as ogi.

9.6 FUTURE STUDY

Fermentation can be used to preserve and process foods at costs within the budget of the average consumer (Steinkraus, 1994). As described by concrete scientific research, fermented products have special organoleptic qualities and some even possess health promoting properties as well as curative properties (Todorov and LeBlanc, 2014). Therefore, fermented foods are widely acceptable, accessible and treasured as staple dietary constituents especially in developing countries (Holzapfel, 2002, Rolle and Satin, 2002).

It was predicted by Steinkraus (1994) that the demand for fermented products is likely to increase in the 21st century when world population reaches 8–12 billion. Majority of the traditional fermentation processes being used are lengthy with little or no microbiological control. To cater the increasing demand for fermented products globally, traditional fermentation process has evolved from 'natural' processes through the use of starter cultures to biotechnological state that involved gene technology (Campbell-Platt, 1994). Achi (2005) also expressed the need for a cooperative research network designed to upgrade the technology for large-scale industrial production of fermented food.

Despite of the need of advanced technology to be applied in the processing of fermented products for large commercial scale, the knowledge about the role of metabolite, chemical interaction, interaction functionality of different microorganisms during fermentation is still insufficient (Law et al., 2011) and merits further investigation.

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Processing of Foods, Vegetables and Fruits: Recent advances

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