International Food Research Journal 17: 527-534 (2010)

Potential use of Malaysian rubber (*Hevea brasiliensis*) seed as food, feed and biofuel

[a]Eka, H. D., [a,]*Tajul Aris, Y. and [b]Wan Nadiah, W. A.

[a]*Food Technology Division, School of Industrial Technology*
[b]*Bioprocess Technology Division, School of Industrial Technology*
Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia

Abstract: Rubber seed as a waste product from rubber plantations contains nutritive values that can be harnessed as food for human, feed for animals or biofuel for energy. Proximate analysis showed moisture content of 3.99%, protein content of 17.41 g/100g, fat content of 68.53 ± 0.04 g/100g and ash content of 3.08 ± 0.01 g/100g. Amino acid in rubber seed is high in Glutamic acid (16.13%) and low in Cysteine (0.78%). Despite its potential as a source of protein, fresh rubber seeds contain a toxic factor, cyanogenetic glucoside (186 mg/kg). FAME analysis indicated that rubber seed oil is high in oleic, linoleic and linolenic acid. The fuel potential of rubber seed (585.41 kJ/kg) is in reasonable agreement with ASTM.

Keywords: Rubber seed, potential use, food, feed, biofuel

Introduction

A global population that will surpass 30 billion by 2030 (FAO, 2002) will use 50% more fuel than it did in 2005 (International Energy Outlook, 2008). Meeting the world's growing need for food, feed and fuel from renewable and sustainable sources forces one to look beyond the traditional into alternative and non-conventional sources. One neglected potential source would be the rubber seeds.

Rubber (*hevea brasiliensis*) tree starts to bear fruits at four years of age. Each fruit contain three or four seeds, which fall to the ground when the fruit ripens and splits. Each tree yields about 800 seeds (1.3 kg) twice a year. A rubber plantation is estimated to be able produce about 800-1200 kg rubber seed per ha per year (Siriwardene and Nugara, 1972), and these are normally regarded as waste.

According to the Association of Natural Rubber Producing Countries, Kuala Lumpur, Malaysia has an estimated acreage of 1,229,940 hectares of rubber plantation in 2007 (Malaysian Rubber Board, 2009) Based on an estimated average of 1000 kg seeds per ha/ yr, the projected annual production of rubber seeds in Malaysia would be 1.2 million metric tons. Despite Malaysia being a major rubber growing country, to date, there is a dearth of information on the chemical composition of the Malaysia rubber seed. According to Bressani *et al* (1983), the rubber seed kernel (hull has been removed) contains 29.6% fat and 11.4% protein. Thus, it is estimated that Malaysia wastes about 355,200,000 kg fat and 136,800,000 kg protein per year. Even in countries such as Vietnam, there are 420,000 ha of rubber trees with density of 500 tree/ha. Based on an estimated production of approximately 300 kg rubber seed /ha, it is then possible to collect nearly 130,000 metric tons rubber seed equivalent to 65,000 metric tons of rubber seed meal without hulls every year from this level of rubber production.

This seed of *Hevea brasiliensis* has been subject to range of chemical composition investigation (Giok *et al*., 1967; Stosic and Kaykay, 1981; Bressani *et al*., 1983; Narahari and Kothandaraman, 1983; Ravindran, 1983; Ukhun and Uwatse, 1988; Njwe *et al*., 1988; Achinewhu, 1998; Oyekunle and Omode, 2008). To the best of our knowledge chemical composition of this seed obtained from Malaysia has not been the subject of much study.

The aim of this study is to determine of the proximate, ash, calcium, iron, magnesium, free fatty acid and HCN contents as well as acid, amino acid and fatty acid profiles of rubber seeds from Malaysia. This investigation will be useful to identify the area of potential application.

*Corresponding author.
Email:eka_dongs@yahoo.com, taris@usm.my
Tel: +604-6532112; Fax: +604-6573678

Material and Methods

Material

The fresh rubber seeds used in this study were collected from a rubber plantation in Sungai Petani, Kedah, Malaysia (5º38'45.99" N and 100º29'19.19" E). In order to ensure the samples used were from the same source throughout the experiment, the fresh seeds were bought in large quantities of about 125 kg at a time. The seeds were stored in a freezer (-10ºC), until further required.

Method

The seeds were defrosted at room temperature for about 1 hour prior to testing, and were then de-hulled, and the kernel milled using a household dry blender (50 Hz). Analyses were carried out according to the methods described by AOAC, *vis-à-vis* protein (method 960.52), moisture (method 934.01), ash (method 923.03), fat (method 963.15) and mineral (method 965.09). Oil was extracted with petroleum ether using a Soxhlet extractor for four hours.

Free fatty acid and acid values (method 940.28), Iodine value (method 993.20), Peroxide value (method 965.33), Saponification number (method 920.160) and HCN (method 915.03; Alkaline titration method), amino acid profile (method 963.22), fatty acid profile (method 963.22) were also determined (AOAC, 1990). Tests were carried out in triplicates. Fuel potential of the rubber seed oil was calculated using the relationship

$$\text{Biofuel potential, } \mathbf{H_\mu} = 47.645 - 4.187\,\mathbf{I} - 38.31\,\mathbf{S}$$

where **I** and **S** are the iodine value and the saponification number of the oil respectively (Batel *et al.,* 2008).

Results and Discussion

Rubber seed as food

The moisture content of rubber seeds as determined in this study was 3.99% (Table 1). Moisture content reported from earlier studies were 3.0% (Ukhun and Uwatse, 1988); 5.8% (Madubuike *et al*, 2006) and 9.0% (Oyekunle and Omode, 2008).

The rubber seeds also showed a protein content of 17.41 g/100 g. This is lower than the value reported by Giok *et al.* (1967) (27 g/100 g) but is higher than that found by Bressani *et al.* , (1983) (11.4 g/100 g). The Recommended Dietary Allowance (RDA) for proteins in human is 56 g for males (from 19 to >70 y), and 46 g for females (from 14 to >70 y) (United States Department of Agriculture, 2009) would thus be met by the sole consumption of about 300 g rubber seeds/day.

Similarly, the fat content (68.53%) of rubber seeds in the present study was in good comparison with 37.30-50.00 % quoted by earlier workers (Ukpebor *et al.*, 2007; Oyekunle and Omode, 2008). The differences in fat contents as well as other proximate values in our study compared to that of previous studies might be due to the difference in the strain of the rubber trees, as well as the soil and climatic conditions of the rubber plantation. Rubber seed oil has traditionally been used as a partial substitute for linseed oil for paint and varnish.

Ash content is an indication of the level of inorganics in the sample (Oyekunle and Omode, 2008). In this study, the ash content was 3.08 g/100 g. Reported ash content in rubber seed meal varied between 3.5-5.0% (Ukhun and Uwatse, 1988; Oyekunle and Omode, 2008). Mineral composition of rubber seed were determined to be 850 $x10^{-3}$, 10 $x10^{-3}$ and 9.29, mg/g for Ca, Fe and Mg respectively (Table 2), in contrast to reported values for Ca and Fe of $2.4x10^{-3}$ mg/g and $1.7x10^{-3}$ mg/g (Oyekunle and Omode, 2008) and 16.2 $x10^{-3}$ mg/g and 50.3 $x10^{-3}$ mg/g (Ukhun and Uwatse, 1988).

Minerals are essential nutrients that the human body needs in small amounts to work properly. In human, Calcium is needed for muscle contraction, blood vessel contraction and expansion, the secretion of hormones and enzymes, and sending messages through the nervous system. Iron (Fe) is a key component of red blood cells and many enzymes whilst Mg is for heart rhythm, muscles and nerve function. For Calcium, the recommended intake is listed as an Adequate Intake (AI), developed by the Institute of Medicine (IOM) of the National Academy of Sciences. AI for males and females 9 years and above is 1000-1300 mg/day (Anon, 2007). In terms of Calcium content, a 100 g serving of rubber seed kernel provides about 85 mg Calcium, which is only 8% of AI.

The Food and Drug Administration (FDA) of United States requires all food labels to include the percent Daily Value (%DV) for iron. DVs are reference numbers developed by the FDA to help consumers determine if a food contains a lot or a little of a specific nutrient. The percent DV indicates the percent of the DV that is provided in one serving. The DV for iron is 18 milligrams (National Institute of Health, 2007). A food providing 5% of the DV or less is a low source while a food that provides 10-19% of the DV is a good source. A food that provides 20% or more of the DV is high in that nutrient. In this light, a serving of 100 g rubber seed kernel has an iron content of 0.24 mg, or equivalent to 1.3% of the DV.

Table 1. Proximate analysis of rubber seed

	Value
Moisture, %	3.99 ± 0.01
Protein, g/100g	17.41 ± 0.01
Fat, g/100g	68.53 ± 0.04
Ash, g/100g	3.08 ± 0.01
Total Carbohydrate, % (by difference)	6.99

Table 2. Various chemical parameters of rubber seed

Mineral: Ca, mg/g	(850 ± 40) x10^{-3}
Fe, mg/g	(10 ± 0) x10^{-3}
Mg, mg/g	9.29 ± 0.15
FFA, %	4.11 ± 0.08
Acid Value, mg KOH/g	8.17 ± 0.15
Iodine Value	28.07 ± 1.23
Peroxide Value, mEq/kg	18.91 ± 0.71
Saponification Value, mg KOH/g oil	13.46 ± 6.51
HCN, mg/Kg	186.00 ± 4.62
Fuel potential, H_{μ} (kJ/kg)	585.41 ± 3.73

Thus, rubber seed is a poor source of both calcium and iron. However, it is important to remember that foods that provide lower percentages of the DV or AI may also contribute to a healthful diet.

Table 2 also shows the value of free fatty acids, FFA (4.11%) and acid value (8.17 mg KOH/g). FFA and acid value of rubber seeds in Nigeria were 1.6 % and 3.2 mg KOH/g respectively (Oyekunle and Omode, 2008). A relatively high acid value makes oil undesirable for nutritional applications. Aigbodion and Bakare (2005) reported that the FFA (21.40%) and acid values (43.62 mg KOH/g) show that rubber seed oil is highly acidic, implying that larger amounts of polyols will be required in the trans-esterification reaction before the polyesterification reaction leading to the formation of alkyds.

The Iodine Value of rubber seeds obtained in study was 28.07. Achinewhu and Akpapunam (1985) reported an Iodine Value of 82.5 in rubber seed oil. The Iodine Value measures the amounts of unsaturated acid esters which contaminated the fractions (Jamieson and Baughman, 1930). The low iodine value of the rubber seed oil exposed to ambient light during storage may be due to the oxidation.

Peroxide Value in this study was 18.90 mEq/kg. Earlier workers reported peroxide values of 12.4 mEq/kg (Oyekunle and Omode, 2008) and 2.5 mEq/kg (Achinewhu and Akpapunam, 1985). The Peroxide value of an oil is used as a measurement of the extent to which rancidity reactions have occurred during storage.

The saponification as reported by Aigbodion and Bakare (2005) of about 203 mg KOH/g shows that the nature of alkali hydrolysis of rubber seed oil is similar to that of common vegetable oils such us as coconut oil. Oyekunle and Omode (2008) also found the saponification value of rubber seed oil was 203.4 mg KOH/g but that measured in this study was only 13.46 ± 6.51 mg KOH/g.

Despite its potential as a protein source, the presence of a toxic factor, cyanogenetic glucoside in rubber seed might be a hindrance to its use as a food source. The presence was confirmed in this study (18.6 mg/100 g). There have also been reports that fresh rubber seeds and its kernel contain about 63.8 to 74.9 mg of hydrogen cyanide (HCN) per 100 g (George *et al.,* 2000), as well as about 200 mg /100 g of seeds (Batel *et al.,* 2008). Storage at room temperature for a minimum period of two months has been shown to be effective in reducing the hydrogen cyanide (HCN) content of rubber seeds (Narahari and Kothandaraman, 1983). In addition, boiled and drained rubber seeds are eaten by Indians in the Amazon Valley of South America (Njwe *et al.,* 1988) without adverse effects.

The determination of amino acid content of the rubber seeds carried out in this study indicated that rubber seed is high in glutamic acid (16.13%) and low in cysteine (0.78 %), both being non-essential amino acids (Table 3).

Essential amino acids are more important to life because the body cannot make these amino acids, and they have to come from food or amino acid supplements. The dietary requirement for protein will be the minimum intake which satisfies metabolic demands and which maintains appropriate body composition and growth rates, after taking into account any inefficiency of digestion and of metabolic consumption. To satisfy the metabolic demand, the dietary protein must contain adequate and digestible amounts of nutritionally indispensable or essential amino acids (FAO/WHO, 1991).

Rubber seed meal and cakes are higher in the essential nutrients than soybean meal and is highly promising as a protein supplement (Table 4). It is also higher than maize for Lysine (4.26, 2.9 mg/16 g Nitrogen respectively) phenylalanine (4.88, 4.6 mg/16 g Nitrogen respectively) and valine (7.08, 5.3 mg/16 g Nitrogen respectively). Rubber seed is also higher in Isoleucine, leucine, threonine and valine than the FAO pattern

Oluyemi *et al*. (1975) indicated that Nigeria rubber seed is rich in the essential amino acids L-Lysine (3.6%) and Methionine (1.4%). Methionine is important in high cassava diets because of its role in the detoxification of hydrogen cyanide (Buitago, 1990).

Amino acids contribute to the taste characteristics of the food, producing MSG-like taste from aspartic and glutamic acid, sweet taste from alanine, glycine and threonine; bitter taste from arginine, histidine, isoleucine, leusine, methionine, phenylalanine, tryptophan and valine; and flat taste from lysine and tyrosine (Yang *et al*., 2001; Hardy, 1985). A taste profile from the amino acid contents of the rubber seeds amino acid indicates that the bitter taste is more dominant (Figure 1) with the scores of 38.82 (bitter), 27.31 (MSG-like), 19.46 (sweet) and 7.14 (flat)

Rubber seed as Feed

In view of their chemical composition and nutritive content, rubber seed kernels can alternatively be considered as potential feed stuff. Since it is well known that protein sources are the main constraint for the improvement of animal production in many tropical regions of the world, the seed with 11-25% crude protein is a potential protein supplement for live stock. Rubber seed is an important by-product of rubber cultivation in many tropical countries, and is often included as a component of supplements fed to ruminants. It has a high content of semidrying oil which may be used in the paint industry, leaving the press cake as a potential source of high-protein food for cattle or sheep, but heat treatment and storage are required to reduce the level of hydrocyanic acid (HCN) (UNIDO, 1987).

Earlier workers used rubber seed as a feed supplements for pig (Pech, 2002), for sheep in Cameroon (UNIDO, 1987; Njwe *et al*, 1988) and as a diet for broilers in Malaysia (Yeong and Syed, 1979). As as been shown in Table 5, rubber seed is higher than maize for lysine, phenylalanine and valine. This shows that rubber seed meal is a good complement for maize in poultry and pig rations (Ensminger and Olentine, 1978). However, it can be seen that rubber seed meal or maize could not provide the total amino acids required by poultry chicken (Tab 5) and may be used only up to a certain percentage of the feed.

Ong and Yeong (1977) reported a reduction in growth when pigs were fed diets containing more than 20% of the meal. Devendra (1983) also considered 20% as optimum. In Sri Lanka, Ravindran (1983) found impaired performance traits with pigs fed more than 10 percent. However, this effect was attributed to a deficiency of lysine and methionine rather than to the cyanogenic glucoside present in the meal. More recently, Fuller (1988) suggested that with correctly treated meal, the diet could contain up to 40 percent. He also concluded that since rubber seeds slowly lose hydrocyanic acid, storage for a minimum of four months, or detoxification by roasting (350°C for 15 minutes), or soaking, in hot water or in a 2.5% ash solution for 12 hours, could solve this problem. These methods would eliminate the anti-nutritional factors and improve palatability.

Buvanendran and Siriwadene (1970) recommended that rubber seed meal was a very useful substitute for coconut meal in broiler and higher in total nutrients compared to maize, which is considered one of the high energy feeds (Stosic and Kaykay, 1981).

Rubber seeds have also been suggested as having good potential as feeds for live stock in Cambodia (Pech, 2002). A study in Sri Lanka found that the inclusion rates of Rubber Seed Meal (RSM) in feeds did not affect the egg production, but egg size, shell thickness, hatchability and chick weight were reduced (Rajaguru, 1971). In Malaysia, it was found that the body weight and feed efficiency of 5-10 weeks were adversely affected when the RSM supplemented with 0.15% crystalline methionine was included in the diet (Yeong and Syed, 1979).

Rubber seed as Biofuel

Based on the extraction process, the oil from rubber seeds was 68.53%. This value is comparable to those of palm oil which stands at 45-50%, indicating that

Table 3. Amino acid profile of rubber seed

NON-ESSENTIAL AMINO ACIDS			ESSENTIAL AMINO ACIDS		
		g/100g protein			g/100g protein
Aspartic acid	(Asp)	11.18 ± 1.57	Threonine	(Thr)	3.72 ± 0.42
Serine	(Ser)	5.89 ± 0.38	Valine	(Val)	7.08 ± 0.27
Glutamic acid	(Glu)	16.13 ± 0.34	Methionine	(Met)	1.37 ± 0.31
Glycine	(Gly)	5.14 ± 0.00	Lysine	(Lys)	4.26 ± 0.41
Histidine	(His)	2.95 ± 0.25	Isoleucine	(Ile)	3.28 ± 0.04
Arginine	(Arg)	12.45 ± 0.19	Leucine	(Leu)	6.81 ± 0.08
Alanine	(Ala)	4.71 ± 0.15	Phenylalanine	(Phe)	4.88 ± 0.46
Proline	(Pro)	6.77 ± 0.02			
Cysteine	(Cys)	0.78 ± 0.79			
Tyrosine	(Tyr)	2.88 ± 0.25			

Table 4. Essential and related amino acids in rubber seed protein in comparison with some other proteins.

Amino Acid, mg/16 g Nitrogen	FAO pattern[a]	Rubber Seed	Whole Egg[a]	Soy Bean[b]	Maize[a]
Isoleucine	2.8	3.28	6.6	4.62	4.7
Leucine	6.6	6.81	8.8	7.72	13.2
Lysine	5.8	4.26	6.6	6.08	2.9
Phenylalanine		4.88	5.8	4.84	4.6
Tyrosine	6.3[c]	2.88	5.0	1.24	6.2
Methionine	2.5[d]	1.37	3.1	1.22	1.9
Threonine	3.4	3.72	5.0	3.76	4.0
Tryptophan	1.1	NP	1.7	3.39	0.6
Valine	3.5	7.08	7.4	4.59	5.3

NP : Not Present
[a] Data from FAO/WHO (1991) report on protein requirements
[b] Bau *et al.* (1994)
[c] Tyrosine + Phenylalanine
[d] Methionine + Cystine

Table 5. Nutrient requirements of Meat-Type Hens for Breeding Purposes as Units per Hen per Day (90 % dr y matter).

Nutrient	Unit	Requirements
Protein	g	19,5
Arginine	mg	1,110
Histidine	mg	205
Isoleucine	mg	850
Leucine	mg	1,250
Lysine	mg	765
Methionine	mg	450
Methionine + cystein	mg	700
Phenylalanine	mg	610
Phenylalanine + tyrosine	mg	1112
Threonine	mg	720
Tryptophan	mg	190
Valine	mg	750

Source: National Research Council, 1994

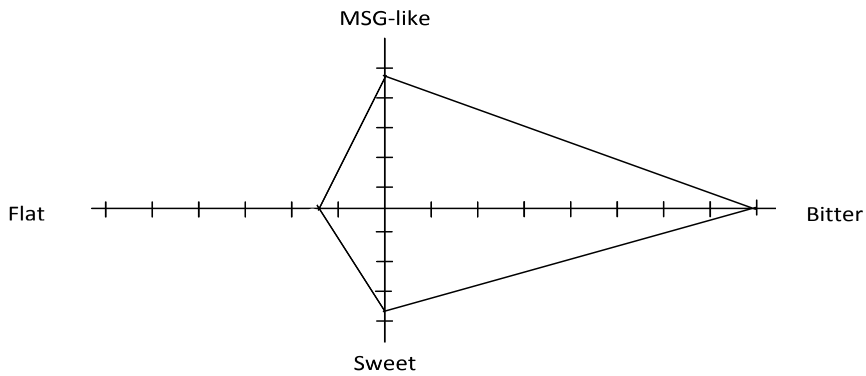

Figure 1. Diagram taste sensory evaluation from acid amino rubber seed

it can be a promising source of biodiesel production (Jibrail *et al.*, 2008). According to its fatty acid composition, rubber seed oil is rich in unsaturated fatty acid (oleic acid, linoleic and linolenic) (Table 6).

Aigbodion and Bakare (2005) reported levels of oleic acid (25.33%), linoleic acid (37.50%) and linolenic acid (14.21%) in rubber seed oil to be in agreement with that found by Achinewhu (1998) who reported the levels of oleic acid, linoleic acid and linolenic acid to be 23.34%, 37.07% and 20.35% respectively. Ramadhas *et al.* (2004) found that oleic acid (24.6%) and linolenic acid (16.3%) in rubber seed oil to be higher than soybean oil (23.26% and 6.31%) and suggested that the type and percentage of fatty acids contained in vegetable oil depends on the plant species and on the growth conditions of the plant.

From this study, the derived fuel potential of rubber seed oil is 585.41 kJ/kg. Almost all vegetable oils can be directly mixed with diesel oil. Ramadhas *et al.* (2005) found rubber seed oil to be a promising alternative fuel source for compression ignition engines. The tested properties of methyl esters of rubber seed oil are found to be in reasonable agreement with ASTM (American Society for Testing and Material) 6751 (36,500 kJ/kg) (Ramadhas *et al.*, 2004). According to Ikwuagwu, *et al.* (2000), the methyl ester of rubber seed oil has relatively closer fuel properties to diesel fuel compared to other oil seeds which include soybean, linseed and sunflower oils.

Conclusion

According to chemical composition of rubber seed, its can be considered as good sources for human food, animal feed and biofuel. Rubber seed from the waste product of rubber plantation can be use as by-product. Amino acid content from rubber seed making it good companion for maize as feed for animal. Storage and heat treatment can reduce HCN content. Fat content is 68.53 % and as semi-drying oil. It has potential industrial application in the production of soap, cosmetics and paint. From the fatty acid content of rubber seed, rubber seed also promises to be valuable oil as a substitute or and additive to diesel and also for compression ignition engines.

Table 6. Fatty acid profile of Rubber seed

Component	Concentration, %
C6:0	NP
C14:0	NP
C16:0	NP
C16:1n7	4.85 ± 2.05
C18:1n7	5.43 ± 0.84
C18:2n6	16.12 ± 5.46
C18:3n6	42.99 ± 13.43
C18:3n4	30.60 ± 17.99
C18:4n3	NP
C18:3n3	NP
C20:3n6	NP
C20:4n3	NP
C20:1n9	NP
C20:4n6	NP
C20:5n3	NP
C22:3n3	NP
C22:6n3	NP

NP = Not Present

Acknowledgment

The authors gratefully acknowledge the financial support from USM Research University Fellowship.

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