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Proximate Analysis of Gluten-Free Milk Boosting Cookies Containing Banana Flowers

Normah Haron* and Nurul Izzaty Mohamad Yusof
Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia
*Correspondence author (e-mail: normahh@iium.edu.my)

The World Health Organization (WHO) suggests that mothers should exclusively breastfeed their babies for the first two years after birth. *Musa paradisiaca* flowers, also known as banana flowers, have traditionally been consumed by mothers in Malaysia for its milk-boosting effect (the galactagogue effect). In this study, banana flower-based lactation cookies were formulated using three gluten-free flours, namely tapioca flour, rice flour and potato starch. Proximate analysis of these cookies were evaluated for their ash, moisture, protein, fat, crude fibre, carbohydrate and caloric content. Analysis was performed according to AOAC standard methods (dry ashing, hot air-oven drying, Kjeldahl nitrogen digestion, and Soxhlet-extraction). The results showed that the cookies made from rice flour had the highest nutritional content in terms of protein, fat and calories (12.29%, 54.14% and 54.03 kcal, respectively). The nutritional content of all three types of cookies showed significant differences ($p<0.05$) except in the case of ash content. A comparison with commercially available lactating cookies (fenugreek-based) showed that the *Musa paradisiaca* flower cookies contained higher levels of fibre, fat, and carbohydrate ($p<0.05$). However, the commercialized cookies had a higher calorie content (180 kcal) compared to our cookies (54.03 kcal). In conclusion, cookies made with *Musa paradisiaca* and rice flour were found to have a higher nutritional content than commercially available cookies and should be further researched for its effects on milk production in lactating mothers.

Key words: Musa paradisiaca flower; galactagogue; milk-boosting; proximate analysis

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The *Musa paradisiaca* flower, or banana flower, is the scientific term that was first introduced by Carl Linnaeus in Species Plantarum (1753) [1]. It is categorised under the *plantae* kingdom of *Magnoliophyta* division and classified as *Liliopsida* of the *Zingiberales* order. The banana family is called *Musaceae*, and its genus is *Musa*, while the species are named *Musa paradisiaca or Musa sapientum* [2]. Sampath Kumar and colleagues [3] found that banana flowers contain important nutrients such as dietary fibre, proteins and unsaturated fatty acids, as well as antioxidants (vitamin E and flavonoids) which can be used against infection by pathogenic bacteria such as *B. cereus*, *B. subtilis* and *E. coli.* Banana flowers help in reducing the blood glucose levels of diabetic patients, treat excessive menses bleeding in women and provide antioxidants for the body. The banana flower is also traditionally believed to induce lactation and has been scientifically proven to have a galactagogue effect [4]. A study on the galactopoietic effect of *Musa paradisiaca* banana flowers on lactating rats has shown an increase in milk production of up to 25% within ten days [4]. However, the banana flower's galactagogue effect has not been well recognized in our local industry compared to fenugreek and other galactagogue herbs. Lower milk production during lactation is currently an issue for new mothers. The Global Strategy on Infant and Young Child Feeding in 2002 recommended that mothers exclusively breastfeed their babies for the first 6 months to a maximum of two years or more [5]. Lack of milk production might affect at least the minimum recommended lactation duration. Mothers are advised to consume high-protein and high-vitamin foods including fish, meat, fruits, legumes, banana flowers, barley drinks, and soy-based foods [6].

A gluten-free product is defined as a food that does not contain ingredients that are made from any type of wheat, rye, barley or any crossbreeds of these grains [7]. For processed grains that undergo gluten-removal, the maximum gluten availability permitted is 20 parts per million (ppm) in these products. Gluten-free products are mainly produced to help people who are not able to consume gluten-containing food such as wheat-based, barley-based or oat-based products [8].

Gluten-containing foods are known to seriously affect patients with celiac disease and gluten sensitivity, also known as non-celiac gluten intolerance [9]. In Malaysia, many patients with

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irritable bowel syndrome should avoid gluten-containing foods since fructants in wheat starch may be the cause of their issues [10]. Therefore, gluten-free food products are important for Malaysians with gluten-related diseases. To date, there is no medical cure for these diseases and sufferers must eliminate foods with gluten from their daily diet throughout their lifetime [11].

There are plenty of commercial products in the form of supplements, juices, biscuits etc. that contain banana flowers for lactating purposes. For commercialization purposes and to widen the market for this product, gluten-free banana flower-based products were developed in this study, and the nutritional content (ash, moisture, protein, carbohydrate, crude fibre, fat and calorie) of these products was determined.

MATERIALS AND METHODS

Sample Preparation

In this study, the flower of *Pisang Berangan* (*Musa paradisiaca*), a Malaysian banana variety belonging to the Musaceae family, was used. The sample and other ingredients were purchased from a local supermarket. The banana flower was kept refrigerated at 4 ºC before being treated with 0.3% citric acid to prevent oxidation. The sample preparation procedure followed the method of Elaveniya and colleagues [12]. After an hour, the banana flower was removed from the acid solution and sealed in a clean plastic container which was stored in the freezer at -80 ºC for 2 to 3 days before it was freeze-dried for up to two weeks. Tthe freeze-dried banana flower later was ground into a powder using a dry mill blender, and stored in an air-tight container.

Preparation of Cookies

The milk-boosting cookies were prepared by modifying the ingredients used in two separate studies with a 1:15 ratio of banana flower to gluten-free flour [12-13]. Three types of gluten-free flour were used, namely tapioca flour, rice flour and potato flour. Other ingredients included margarine, eggs, vanilla extract, corn starch, gluten-free oat flour, fine almond powder, powdered sugar, full cream milk, banana flower powder and chocolate chips. The cookies were prepared in the Nutrition Sciences Food Preparation Laboratory, Kulliyyah of Allied Health Science. The materials were separated into dry and wet ingredients. The dry ingredients were prepared first. All materials were weighed using a digital kitchen balance. The dry ingredients included tapioca flour, corn starch, fine almond powder, gluten-free oat flour, powdered sugar and banana flower powder. All these ingredients were mixed thoroughly and put aside. The wet ingredients were prepared by mixing melted margarine, an egg, a teaspoon of vanilla extract and full cream milk. Both wet and dry ingredients were mixed and kneaded until a dough mixture was formed. Then, the dough was left to rest for 30 minutes. Baking paper was cut to fit the oven baking tray, and the oven was pre-heated at 170 ºC for 10 minutes. After 30 minutes, the dough was weighed and rolled to form a round shape, which was cut into circular pieces for baking. These were placed on the baking tray (24 pieces per tray) and baked in the oven at 170 ºC for 20 minutes. The cookies were left to cool for 20 minutes at room temperature before being stored in a labelled airtight container. The rice flour cookies and potato flour cookies were made following the same procedures as above. Tapioca flour was replaced with 175 g of rice flour or 235 g of potato flour to make the other two types of cookies.

Proximate Analysis

Proximate analysis was conducted for all samples except the commercialized cookies. A comparison with the commercialized cookies was made based on the nutrition label on its packaging. The proximate analysis conducted in this study included ash, moisture, protein, fibre and fat content.

Ash Analysis

Two grams each of the crushed cookies were added into labelled crucibles. Each crucible was covered with a lid and placed in a cold muffle furnace. The furnace was then set to 550 ºC and left overnight. The samples were removed the next day and cooled in a desiccator for approximately 30 minutes or until they reached room temperature. The results were calculated using the formula below:-

$$Ash\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100\%$$

Moisture Analysis

The analysis followed the AOAC method and used a hot air oven [14]. Samples in labelled petri dishes were heated in the oven at 100 ºC overnight. The results were calculated using the formula below:-

$$Moisture\ content = \frac{fresh\ sample\ weight - dry\ sample\ weight}{fresh\ sample\ weight} \times 100\%$$

Protein Analysis

Protein content was determined using the Kjeldahl method, also known as the nitrogen digestion method. The method followed AOAC 950.48 and used a BÜCHI KjelDigester K446/K-449, and KjelMaster K-375. The analysis procedure comprised three major steps: sample digestion, neutralization and distillation, and finally titration. Protein content was calculated using the formulae below:-

$$Nitrogen\ content\ of\ sample\ (\%) = \frac{ml\ acid\ \times\ normality\ of\ standard\ acid}{weight\ of\ sample\ (g)} \times 0.014 \times 100\%$$

$$Crude\ protein\ content\ (\%) = nitrogen\ content\ \times\ 6.25$$

Crude Fibre Analysis

The analytical procedure followed the Gerhardt Analytical System method as the analysis was performed using the Fibretherm FT12. The instrument was set to run the digestion and filtration process in four different phases (washing phase I, acid removal, washing phase II and alkali removal). The whole process was completed in approximately 2 hours. Finally, all the weights were recorded and the following formula was used to determine the crude fibre value.

$$Crude\ fibre\ (\%) = (M3 - M1) - (M4 - M5) \times \frac{100}{M2}$$

M1-Fibre bag

M2-initial sample weight

M3-crucible and dried fibre bag after digestion

M4-crucible and ash

M5-blank value of the empty fibre bag

Crude Fat Analysis

The crude fat analysis was performed using the Soxhlet extraction system [14]. All samples were crushed and weighed into cellulose thimbles (2 g per sample). Approximately 250 ml petroleum ether was added into the Soxhlet system which was run for 4 hours. The extracts were cooled to room temperature before the evaporation process using a rotary evaporator. The rotary evaporator was set at 57 ºC, 335 mbar. The fat was weighed and then calculated using the equation below:

$$Crude\ fat\ (\%) = \frac{weight\ of\ fat}{weight\ of\ sample} \times 100\%$$

Carbohydrate Content

The carbohydrate content was determined by calculating the difference as shown in the formula below:

$$Carbohydrate = 100\% - (\%\ ash\ + \%\ moisture\ + \%\ protein\ + \%\ fat\ + \%\ fibre\ )$$

Total Caloric Content

The total caloric content was determined by calculation, as follows:

$$Calories\ from\ carbohydrates = grams\ of\ carbohydrate\ \times\ 4kcal/g$$
$$Calories\ from\ protein = grams\ of\ protein\ \times\ 4kcal/g$$
$$Calories\ from\ fat = grams\ of\ fat\ \times\ 9kcal/g$$

Table 1. Proximate analysis of cookies based on the type of flour used.

Analysis Sample	Tapioca Flour	Rice Flour	Potato Starch Flour	
	(Mean ± SD)	**(Mean ± SD)**	**(Mean ± SD)**	**p-value**
Ash (%)	1.23 ± 0.12	1.08 ± 0.12	1.23 ± 0.23	0.23
Moisture (%)	2.88 ± 0.08	3.49 ± 0.16	3.90 ± 0.15*	0.00*
Protein (%)	6.70 ± 0.51	12.29 ± 0.28*	6.76 ± 0.49	0.00*
Fibre (%)	7.39 ± 0.43	6.31 ± 0.36	8.58 ± 0.08*	0.00*
Fat (%)	31.40 ± 1.61	54.14 ± 0.02*	34.32 ± 0.48	0.00*
Carbohydrate (%)	48.83 ± 0.92*	21.97 ± 0.34	44.67 ± 0.96	0.00*
Calories (kcal) per 100 g	48.81	54.03	49.66	-

**Significantly different at p<0.05(ANOVA analysis)*

RESULTS AND DISCUSSION

Nutritional Composition of Three Types of Cookies

The proximate composition (ash, moisture, protein, fibre, fat, carbohydrate and caloric content) of the three prepared cookies are summarized in Table 1. The results indicate that the moisture, protein, fibre, fat and carbohydrate contents of all three cookies made from tapioca, rice and potato flours showed significant differences ($p<0.05$). The potato flour cookies had the highest moisture (3.90%) and fibre (8.58%) content in comparison with the other cookies. On the other hand, the rice flour cookies had the highest protein, fat and caloric content, with 12.29%, 54.14 % and 54.03 kcal, respectively ($p<0.05$). However, the highest amount of carbohydrate (48.83%) was observed in the tapioca flour cookies. In addition, it was observed that the potato flour cookies had the second highest amounts of protein (6.76%), fat (34.32%), carbohydrate (44.67%) and calories (49.66 kcal). The rice flour cookies had the second highest moisture content, 3.49%, while the tapioca flour cookies had the second highest fibre content, 7.39%. From these results, the tapioca flour cookies had the lowest amounts of moisture (2.89%), protein (6.70%), fat (31.40%), and calories (48.81 kcal), respectively ($p<0.05$).

The results of the proximate analysis of the potato starch cookies in the present study differed from the results reported in a previous study [15], which revealed that the substitution of sweet potato flour with maize flour significantly ($p < 0.05$) reduced the levels of protein from 6.8% to 4.4%, moisture from 5.3% to 5.0%, crude fibre from 3.4% to 2.5% and fat from 9.8% to 8.5% in the composite flours and cookies. These differences showed that different types of flours used in the recipe formulation affected the nutritional content of the cookies produced as well.

A study by Samuel and his colleagues reported that tapioca flour cookies had much lower values of ash (0.92%), protein (0.52%), fibre (0.66%), and fat (0.15%) compared to the present study [16]. However, the carbohydrate content was higher (95.58%) compared to our study. It was highlighted that tapioca is known to contain the lowest protein to energy ratio of all staple crops, and therefore additional ingredients are required to enrich the tapioca food formulation [16]. This supports our results, which showed that tapioca flour cookies had the lowest nutritional content compared to the other flours used.

A study by Shruti and colleague found that white rice flour contained a higher moisture content of 11.2% but lower ash, protein, and fat contents as compared to the rice flour cookies in this study [17]. However, it was highlighted that there were various formulations used for the cookies. Therefore, the differences in the nutrient content of the flour and the cookies were found to be significant. The cookies made from these mixed flours had a different nutritional composition compared to the tapioca, rice and potato flours due to the formulation which contained other ingredients that also provide a variety of nutritional components. The increase in the ash, moisture, protein, and fibre content of our tapioca and rice flour cookies may be due to the addition of banana flower powder into the formulation. According to a study by Arya and Sinija, the flower of *Musa paradisiaca* contained 3.21% ash, 90.1% moisture, 1.99% protein, and 12.8% fibre [18].

In the selection of the best gluten free cookies in terms of nutritional content, one needs to take into account the needs of the target group, lactating mothers. Women require a much higher nutritional intake during pregnancy and lactation [19], because the nutrients from the mother are channelled to her baby through breast-milk. Thus, a mother needs to monitor her nutrient intake to support her baby's development. The recommended nutrient intake (RNI 2017) states that lactating mothers require a higher amount of protein, about 15 – 20 g more than healthy women (19-59 years old) who need only 55 g protein per day. This is to ensure that the protein is sufficient to produce a good amount of casein for breast-milk. A good supply of casein in mother's breast-milk helps the baby's gut absorb calcium and phosphate. Lactating mothers also require much higher caloric intake of up to 2000-2500 calories per day or 2600-3000 calories per day for feeding a single child or twin children, respectively [19]. However, according to the Centers for Disease Control and Prevention (CDC), the additional caloric intake may differ with age, body mass index (BMI), activity level and whether the mother breastfeeds exclusively or not [6].

Therefore, lactating mothers are encouraged to consume foods that are high in protein and calories. Based on this requirement, rice flour was found to be the most suitable flour for making milk-boosting cookies. Furthermore, rice flour cookies were found to have a higher nutritional content compared to the other cookies in this study. If tapioca or potato flour cookies were selected, the formulation would need to be enriched with more nutritious ingredients. Otherwise, these cookies would only act as a milk booster for gluten-intolerant mothers and not provide sufficient nutrients. Therefore, rice flour cookies were selected as having the best nutritional content for mothers and their children, besides providing a milk-boosting effect and being gluten-free.

Nutritional content of lab-made milk boosting cookies in comparison with a commercially available product

Many milk boosting products are available in the market to help mothers increase their breast-milk production. However, in Malaysia, products that are milk-boosting as well as gluten-free are few in number. The network meta-analysis results of four studies indicated that consumption of fenugreek significantly increased the amount of breast milk produced (11.11, CI 95% 6.77, 15.46) [20]. Fenugreek contains protein (2.55%), fat (0.71%), carbohydrate (6.48%) and fibre (2.7%) and minerals such as iron, magnesium, potassium and calcium [21]. On other hand, blessed thistle, a galactagogue plant, contains sesquiterpene lactones, triterpenoids, lignans, tannins, essential oils, flavonoids, and polyenes. In the present study, one of the commercialized lactation cookies made from fenugreek and blessed thistle was selected to compare with our rice flour cookies containing *Musa paradisiaca* flowers. Table 2 shows a comparison of the nutritional content of both types of cookies. It can be clearly seen that the rice flour *Musa paradisiaca* flower cookies had significantly higher levels of fat, carbohydrate and fibre compared to the commercialized cookies, while the commercial product was only higher in caloric content. The fat used in the recipe affects the texture of the cookies and browns them, which influences flavour and colour. Substituting a different fat in place of butter changes the flavour and texture of the cookies, as other fats (lard, vegetable oil, margarine, etc.) have different melting points [21].

A comparison of animal studies showed that the *Musa paradisiaca* flower efficiently increased milk production in lactating rats by 25% compared to 16.1% for fenugreek [4, 22]. This indicated that *Musa paradisiaca* was a better milk boosting agent than fenugreek. Therefore, it can be concluded that the *Musa paradisiaca* flower cookies are more efficient as a milk booster and have a nutritional content compared to certain commercially available cookies.

CONCLUSION

The present study showed that *Musa paradisiaca* milk boosting cookies had a higher nutritional content compared to commercially available lactation cookies and could be made gluten-free. As the milk-boosting cookies made with *Musa paradisiaca* flowers had a higher nutritional value, it has potential for commercialization as a lactation product. However, further research is recommended including toxicity analyses and bioavailability studies of these cookies. Protein sequencing and profiling of the flours are also needed to confirm the gluten-free status of the cookies.

Table 2. Comparison of proximate analysis results for rice flour cookies and a commercially available product

Analysis	Rice flour cookies	UpSpring cookies
Calories (Kcal) per 100g	54.03	180.00
Fat (%)	54.14	10.00
Carbohydrate (%)	21.97	9.00
Fibre (%)	6.31	4.00

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