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NUTRITIONAL COMPOSITION, TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID CONTENT OF WHEAT BREAD AND QUINOA-WHEAT COMPOSITE BREAD

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ABSTRACT

Objective: Present study was conducted to examine the nutritional composition, total phenolic and total flavonoid content of bread samples prepared with different formulations of wheat bread and quinoa-wheat composite bread. **Method**: The formulations of breads include 100 % wheat bread and composite bread made of 10% quinoa flour and 90% wheat flour. The proximate compositions, total phenolic and total flavonoid content of wheat bread and the quinoa-wheat composite flour bread samples were determined using standard procedures. **Results:** The addition of 10% quinoa flour significantly increased ($p < 0.05$) the ash and protein content in the quinoa-wheat composite bread. There was no significant different observed in both bread for fat content. However, it significantly decreased ($p < 0.05$) the carbohydrate and moisture contents as compared to wheat bread. The total phenolic and total flavonoid content was increased by 10% and 46%, respectively as compared to 100% wheat flour bread.**Conclusion**: Incorporating 10% quinoa flour into wheat bread increased ash, protein, total phenolic and flavonoid contents in comparison to 100% wheat bread. Thus, this study suggests that bread with an inclusion of 10% quinoa could be of great nutritional advantage that could offer health potential benefits.

Keywords: *Quinoa, wheat, bread, nutritional composition*

1. Introduction

In general, wheat is grouped as grains and the main type of wheat that commonly used in bread making is *Triticum aestivum*. Increase in socioeconomic status, urbanization and economic modernization in Asia has caused elevating consumption of wheat among the population. The increasing of wheat consumption is a great index of the extent of diet diversification in Asia and the demand is projected to increase to 322 million tons in 2020 compared to 205 million tons in 1993 [1].

Lately, it has been an urge to increase the intake of wholegrains products instead of wheat bread made from white flour [2]. This is mainly because of the traditional food that are rich in complex carbohydrates, micronutrients, fiber and phytochemicals has been replaced with refined carbohydrates that has caused direct impact on the prevalence of certain chronic disease [3].

Wholegrain products provide considerable nutritional value because of the bran possess health benefits. However, low bread production markets over time and the taste has becomes conflict to consumer [2]. Thus, healthy alternative food such as quinoa was introduced to support the movement of healthy food intake.

Chenopodium quinoa wild also known as quinoa is the family of *Chenopodiaceae* used as common grains in Andean region particularly in South America. Quinoa is considered as gluten-free grain, where it content protein (16.5g), fat (6.3 g), carbohydrate (69.0 g), and crude fiber (3.8 g) in 100 grams of it fresh weight [4].

Quinoa also hgas higher antioxidant potential as compared to wheat, barley, millet, rice and buckwheat [5]. Baked goods has been popularly consumed as the main meals among Malaysian replacing rice and noodles. It has been a positive growth of baked goods in Malaysia since it has becoming a trend and a habit among the public to consume bread as the staple food with coffee. Due to increase awareness on the health benefits, the consumer increasingly seeking fortified bread as their meal.

This study explore the potential of quinoa as a substitute meal or to supplement other cereals in everyday diet. This was achieved by studying the nutrient composition, total phenolic content and total flavonoid between wheat bread and quinoa-wheat composite bread. Finding of this study provide information to the public about the nutrient benefits between these two and assists them to make healthier food choices. Furthermore, it provide information to the food industry to develope a much wider spectrum of foods for population based on different type of crop. Diversification of healthy alternatives foods can help the consumer to overcome many health problems such as overweight, obesity and type 2 diabetes mellitus.

2. Materials and Method

2.1. Procurement of sample

Wheat flour and yeast were procured from TESCO (Malaysia) Stores Sdn. Bhd. and quinoa from BMS Organics shop at IOI Putrajaya Mall, Malaysia. Each individual sample was purchased at the same time.

2.2 Bread preparation

Breads were prepared according to Approved Methods of the American Association of Cereal Chemists International (ACCI) method 10-11.01 [6]. About 420g of flours, 252 g of water and 14 g of yeast were well-mixed and fermented in a preheated oven of 30˚C for four hours. Then, the dough was kneaded for three minutes and rested in a pan for five minutes at room temperatures. Then, it was baked for 218˚C for 25 minutes. There are two breads formulation used in this study; 100 % wheat flour and 10% quinoa flour mixed with 90% wheat flour.

2.3 Chemical analyses of breads

2.3.1 Nutritional analysis

The proximate composition of both 10% quinoa-wheat composite bread and 100% wheat bread were performed according to AOAC methods [7] on dry basis. The moisture (air-oven method); ash (dry-ashing); protein (micro-Kjeldahl method N × 6.25) with; and lipid content (Soxhlet method) were determined. Carbohydrate was determined using Chlegg-Anthrone method.

2.3.2 Sample extraction

Wheat bread and quinoa-wheat composite bread were extracted using the following method with slight modification whereby 99% methanol is used according to Fischer, Wilckens. Jara and Aranda (2013) [8]. Firstly, 5.0 g of dried powder bread was placed it in a conical flask. Then, added with 125 ml of 99% of methanol. The extraction was performed at 200 rpm at 50˚C for 2 hours using a temperature controlled orbital shaker. Lastly, the extract was filtered using Whatman No.1 filter paper.

2.3.3 Total phenolic content

The total phenolic content in the extracts was determined by using Folin-Ciocalteau reagent [9]. Briefly, about 0.5 ml of the extract was added with 0.5 ml of water, 5.0 ml of Folin-Ciocalteau reagent (0.2 N) and 4.0 ml of saturated solution of sodium carbonate, (75g/l). Then, it was mixed thoroughly with all the reagent and solutions added and was incubated for 2 hours at room temperature. Then, the absorbance of the mixture was measured at 765 nm using a spectrophotometer. The phenolic content was compared to a gallic acid standard curve. The total phenolic content of the samples was expressed as milligrams GAE per gram of sample.

2.3.4 Total flavonoid content

Total flavonoid content of wheat bread and quinoa-wheat composite bread were determined according to the colometric assay by [10] with slight modification. Firstly, 1 ml of extracts wad added with 4 ml of distilled water. Then, 0.3 mL of 5 % sodium nitrite solution was added followed by 10% aluminum chloride solution. Next, the test tubes were incubated at room temperature for five minutes and then 2 ml of sodium hydroxide was added into the mixture followed by 2.1 ml distilled water. Then, the mixture was thoroughly vortexed, and the absorbance was determined at 510 nm using spectrophotometer. The total flavonoid content was compared to quercetin standard curve. The results were expressed as mg quercetin equivalent per 100 g dried bread.

2.4 Statistical analysis

The results obtained were expressed as means and standard deviation (SD). The statistical analysis was performed by using IBM SPSS version 21. The difference in the means of nutrient content between wheat bread and quinoa wheat composite bread were analyzed by independent t-test. The significant difference was set at $p < 0.05$.

3. Results

The nutritional analysis including moisture, ash, protein, fat and total available carbohydrate as well as total phenolic and total flavonoid content has been conducted for this study.

3.1. Nutritional composition of the breads

The nutritional composition of wheat bread and quinoa-wheat composite bread is presented in Figure 1. The result showed that the moisture content and total available carbohydrate of wheat bread were significantly higher than quinoa-wheat composite bread. Meanwhile, the ash and protein content of quinoa-wheat composite bread were significantly higher than wheat bread. The fat content was found to be higher in quinoa-wheat composite bread. However, an independent t-test showed that, there were no significant different in both bread.

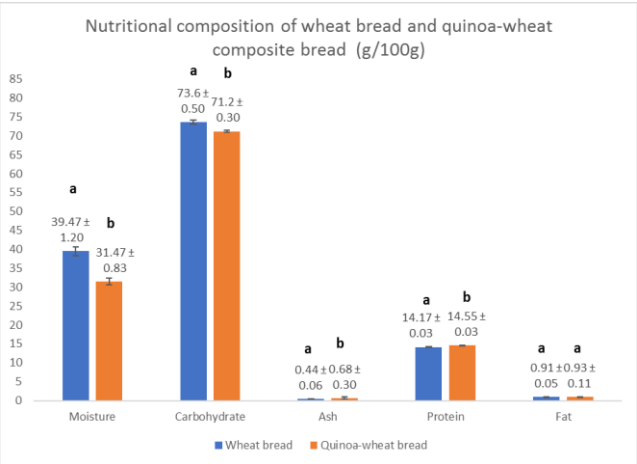

Figure 1: Nutritional Composition of Wheat Bread and Quinoa-Wheat Composite Bread (g/100g).
Means ± standard deviation (n=3) with different letter in the same column were significantly different at p<0.05. The values are expressed on the dry weight basis except for moisture and ash using wet weight.

3.2. Total phenolic content of the breads

The Folin-Ciocalteau assay was used to determine total phenolic content in free and bound phenolic of wheat and quinoa-wheat composite breads. According to Galili and Hovav (2014) [11], Folin-Ciocalteau reagent is a mixture of phosphotungstic acid and phosphomolybdic acid involved in oxidation-reduction reaction. The reduction of the mixture by phenolic compound produces blue solution.

Total phenolic content of free and bound dry weight breads were determined using a standard curve of gallic acid with the concentration ranging from 0 - 0.10 mg/ml. Total phenolic content of wheat bread vs quinoa-wheat composite bread was shown in Figure 2. The results displayed that total phenolic content of quinoa-wheat composite bread was significantly higher than wheat bread.

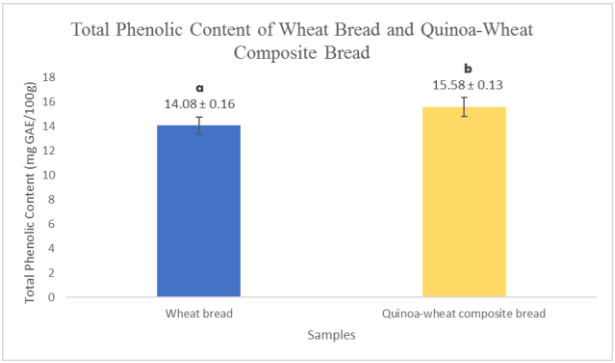

Figure 2: Total phenolic content of wheat bread and quinoa-wheat composite bread.

3.3. Total flavonoid content of the breads

The total Aluminum chloride colorimetric method was used to determine total flavonoid content in wheat and quinoa-wheat composite breads. The principle of aluminum chloride colorimetric method is that aluminum chloride forms complexes with group of flavones and flavonols [12].

Total flavonoid content of dry weight breads was determined using a standard curve of quercetin with the concentration ranging from 0 - 0.10 mg/ml. Total flavonoid content was determined from the standard curve of quercetin. The results of total flavonoid content of quinoa-wheat composite bread displayed (Figure 3) significantly higher in flavonoid content than wheat bread.

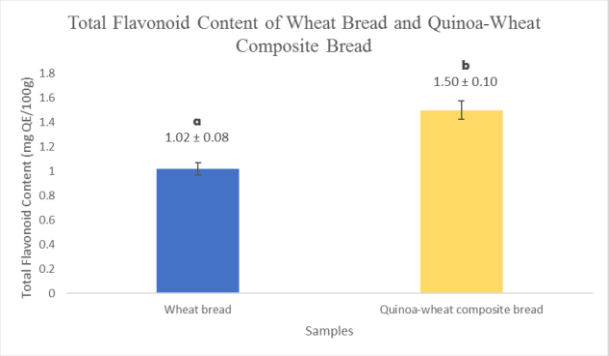

Figure 3: Total flavonoid content of wheat bread and quinoa-wheat composite bread.

4. Discussion

The moisture content of wheat bread was significantly higher than quinoa-wheat composite bread. This is similar to the study report by Stikic et al, 2012 [13], where the study showed the moisture content of wheat bread (13.29g/100g) was significantly higher than bread with addition of 10% purified quinoa seeds (12.84 g/100g). In contrast to this, study reported that moisture content of quinoa and wheat flours were 110.2 g/kg and 121.1 g/kg of dry weight basis. The differences in moisture content between wheat bread and quinoa-wheat composite bread was explained to be from their water absorption index (WAI), water soluble index (WSI) and swelling power (SP) of quinoa flour which was significantly higher than wheat flour as reported in Wang, Opassathavorn and Zhu, 2015 [6]. However, it is important to note that quinoa starch granules are approximately 1 to 3 µm much smaller than wheat [14]. This small size of granules would possibly result in lower moisture content in quinoa flour therefore quinoa-wheat composite flour is significantly lower in moisture content than wheat bread.

Ash content of wheat bread and quinoa-wheat composite bread were 0.44 g/100 g and 0.68 g/100 g of wet weight respectively. The ash content of quinoa-wheat composite bread was significantly higher than wheat bread. Contradict with previous study by Stikic et al. (2012) [13], whereby there was no significant difference in the ash content of wheat bread (2.98 g/100g) and bread with addition of 10% quinoa seeds (2.82g/100g). However, comparing two type of flours, wheat and quinoa flours, Wang, Opassathavorn and Zhu (2015) [6] reported that ash content of quinoa flour (25.4 g/kg) was significantly higher than wheat flour (6.3 g/kg). This suggests that addition 10% of quinoa flour into wheat bread probably could contribute to the difference of ash content compared to 100% wheat flour bread.

Protein content of wheat bread was 14.17 g/100 g of dry weight. Whereas, protein content of quinoa-wheat composite bread was 14.55 g/100 g of dry weight. Previous study by Stikic et al. (2012) [13] revealed that the protein content of bread with 10% addition of purified quinoa seeds (13.29g/100g) was significantly higher than wheat bread (11.89g/100g). Additionally, based on nutrition information panel (NIP) from the packaging, quinoa flour showed higher protein content which was 10.71g/100g compared to wheat flour which was 9g/100g. Wang, Opassathavorn and Zhu (2015) [6] also reported that protein content of quinoa and wheat flours were 140.3 g/kg and 104.7 g/kg of dry weight basis respectively. The study indicated that quinoa flour was significantly higher in protein content rather than wheat flour. The total protein content of quinoa was reported to be higher than the rice, barley, corn, rye and sorghum and is close to wheat (USDA, 2015). This could explained the significant difference of this study. The rationale of adding quinoa flour to wheat flour was mainly because of nutritional advantages especially on its high quality of protein which are the essential amino acids that wheat is lacking off. This could be seen clearly from study of Stikic et al. (2012) [13] whereby the supplementation of wheat flour with 20% quinoa flour enhanced the protein content of resulting bread by 16%, while enriching the contents of lysine, methionine and histamine (essential amino acids) by 26.5, 8.8 and 9.8%, respectively. However, this study would not able to indicate the differences in protein content as previous study. In general, wheat bread and quinoa-wheat composite bread are good sources of protein.

Fat content of wheat bread and quinoa-wheat composite bread were 0.91 g/100g and 0.93 g/100g of dry weight basis respectively. Although, the fat content of quinoa-wheat composite bread was higher, but it was not significantly different from wheat bread ($p>0.05$). The study conducted by Stikic et al. (2012) [13] reported that the oil content of bread with 10% addition of purified quinoa seeds (1.36g/100g) was significantly higher than wheat bread (0.98g/100g). Increase in fat content in quinoa-wheat composite bread should not just imply that it contributed to more fat in the bread. Addition of quinoa flour into wheat bread actually resulting of more good fat content as indicated by Navruz-Varli and Sanlier (2016) [15] whereby quinoa is indeed rich in terms of essential fatty acids such as linoleic (omega-6) and alpha-linolenic acids (omega-3). In comparison of respective flours, quinoa flour (7.15g/100g) showed higher in fat content compared to

wheat flour (1g/100g) based on nutrition information panel (NIP).

Both wheat bread and quinoa-wheat breads consist mostly of carbohydrate which were 73.60g/100g and 71.20g/100g of dry weight basis respectively. The carbohydrate content of wheat bread was significantly higher than quinoa-wheat composite bread ($p<0.05$). Generally, the majority of both breads are starch as they mainly consist of wheat and quinoa flours. This is supported by a study which had reported that starch content of wheat bread (70.25g/100g) was significantly higher than bread with 10% addition of purified quinoa seeds (67.96g/100g) (Stikic et al., 2012). Based on nutrition information panels (NIP) of respective flours, wheat flour (74.0g/100g) showed higher in carbohydrate content compared to quinoa flour (71.4g/100g). Wang, Opassathavorn and Zhu (2015) [6] also indicated that carbohydrate content of wheat flour was 704.7 g/kg and quinoa flour was 659.7 g/kg of dry weight basis. This clearly indicate that wheat flour was significantly higher than quinoa flour in regard to carbohydrate content. Again, this might be understandable with the smaller starch granules of quinoa compared to wheat.

Total phenolic content of wheat bread was 14.08 mg GAE/100 g of dry weight. Whereas, total phenolic content of quinoa-wheat composite bread was 15.58 mg GAE /100 g of dry weight. The results displayed that total phenolic content of quinoa-wheat composite bread was significantly higher than wheat bread ($p<0.05$). In comparison to total phenolic content of wheat bread to previous study, there was huge difference comparing to a study by Alvarez-jubete, Wijngaard, Arendt, & Gallagher (2010) [16]. Total phenolic content of 100% wheat bread was 29.1 mg GAE/100 g dry weight basis. The huge difference in total phenolic content of wheat bread between previous and present studies could be attributed to the different extraction procedures and methodology followed. The study used methanol, 0.16mol/L hydrochloric acid and water at ratios 8:1:1 and followed by acetone to extract the samples while present study used only 99% methanol. Generally, quinoa flours contain high phenolic compounds particularly flavonoid and phenolic acids [17] which contributed to higher phenolic content in quinoa-wheat composite bread. However, very little information has been published concerning the phenolic content of quinoa-wheat composite bread and limited data on total phenolic content of 10% addition of quinoa flour into wheat bread.

According to Chlopicka, Pasko, Gorinstein, Jedryas and Zagrodzki (2012) [18], addition of 15% quinoa flour increased total phenolic content by 10.6% as compared to 100% wheat flour bread. The study reported that total phenolic content of wheat bread which act as control bread was 1.7mg/g, quinoa bread of 15g/100g was 1.88 mg/g and quinoa bread of 30g/100g was 2.54 mg/g of dry weight [18]. However, the result of wheat bread and quinoa-wheat composite bread are not comparable with the previous study as the ingredients of breads are slightly different from present study. The ingredients such as salt and sugar might affect the phenolic content in the breads. Nonetheless, the present study shows the phenolic content increment was about 10.7% with addition of 10% quinoa flour. This would imply that addition of quinoa flour at 10% or above increased phenolic content of the bread.

The results of total flavonoid content showed that wheat bread contained 1.02 mg QE/100g of flavonoid content and quinoa-wheat composite bread contained 1.50 mg QE/100g. Quinoa-wheat composite bread displayed significantly higher in flavonoid content than wheat bread ($p<0.05$). In contrast to previous study by Chlopicka et al. (2012) [18] whereby wheat bread which has been the control was 20.3 ug/g or 2.03 mg/100g of dry weight. Flavonoid content can be seen gradually increased by addition of quinoa flour by 15% (27.5 ug/g or 2.75mg/100g) and 30% (28.7 ug/g or 2.87mg/100g). The results indicated that, there is no significant difference in flavonoid content between 100% wheat bread and addition of 15% quinoa flour, but addition of 30% quinoa flour is significantly higher in flavonoid content than wheat bread and addition of 15% quinoa flour into wheat bread. The discrepancy of the results can be explained by the difference ingredients used for breads and different analysis comparing present to previous study. Nevertheless, researchers have also reported that addition of 15% quinoa flour increased total flavonoid content by 36% as compared to 100% wheat flour bread [18]. Flavonoids are natural phenolic antioxidants mainly from diet and was reported to have anti-inflammatory effects and believed to regulate the metabolism [19]**.** Therefore, the result of this study indicated that quinoa flour is a good source of flavonoid and addition of quinoa flour in wheat bread increased the flavonoid content. This is supported by Repo-Carrasco-Valencia et al. (2010) [20] and Rocchetti et al. (2017) [17] to the fact that quinoa is a very good sources of flavonoids.

5. Conclusion

Addition of 10% quinoa flour into wheat bread had better nutritional properties. The quinoa-composite bread had higher ash, protein and total phenolic and flavonoid contents. 100% wheat bread were comparatively lower in these nutrients. The study indicates that quinoa can be one of the potential health alternative that can be added into wheat bread because of its nutritional advantages.

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