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In vitro bioaccessibility of calcium, iron and zinc from breads and bread spreads

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Abstract

The *in vitro* bioaccessibility of calcium, iron and zinc of breads added with different bread spreads was determined. The mineral contents were assessed by flame atomic absorption spectrophotometer and expressed in fresh weight (mg/100 g). For the mineral bioaccessibility determination, *in vitro* gastrointestinal digestion was applied. Among the bread samples, calcium content of wholemeal bread with chocolate hazelnut spread ranked the highest (159.96±0.869 mg/100 g). For iron, white bread with chocolate hazelnut spread (6.92±0.411 mg/100 g) showed highest iron content while for zinc, white bread with peanut butter was the highest (1.82±0.015 mg/100 g). For calcium bioaccessibility, white bread with orange marmalade ranked the highest (39.33±4.865%) while wholemeal bread with peanut butter (14.70±0.265%) showed the lowest. The application of orange marmalade spread onto wholemeal bread increased the iron bioaccessibility significantly (9.73±1.387%). The acidic properties attributed by organic acids found in orange marmalade may favour both calcium and iron absorption. The zinc bioaccessibility of white bread alone remained the highest (20.63±3.536%) while wholemeal bread added with peanut butter (5.90±1.137%) showed the lowest. Overall, the addition of bread spreads particularly peanut butter and chocolate hazelnut spread had increased mineral contents of the bread samples. However, the presence of mineral enhancers (organic acids) and inhibitors (phytate and polyphenols) played some significant role in influencing the mineral bioaccessibility.

Introduction

Bread made of wheat has long been a staple in the diets of people all over the world which provide good sources of macro and micronutrients such as calcium, iron and zinc (Cauvain, 2007). Breads that are commonly consumed include white bread and wholemeal bread. The major differences of these two breads are their constituents, namely bran, endosperm and germ (Food Agriculture Organization [FAO], 1994). Despite the well-established health benefits of calcium, iron and zinc, the intakes of these particular minerals among Malaysian remain low and thus, subject vulnerable groups (children, pregnant women, elderly) to high prevalence of nutritional deficiencies related health complications (Ramakrishnan, 2002; Loh and Khor, 2010; Cynthia *et al*., 2013).

The role of bread as source of minerals depend on the amount of the mineral available for absorption, which is also known as bioaccessibility. Bioaccessibility has been defined as the fraction of a compound that is released from its matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (i.e., enters the blood stream) (Benito and Miller, 1998). Bioaccessibility includes the entire sequence of events that take place during the digestive transformation of food into material that can be assimilated by the body, the absorption/assimilation into the cells of the intestinal epithelium, and lastly, the presystemic metabolism (both intestinal and hepatic). The presence of other dietary factors that interact through some form of bonding could enhance or inhibit the minerals availability. Phytate, oxalate and polyphenols are the examples of binding agents that can impair minerals absorption whereas organic acids such as ascorbate and citrate may have a favourable effect on the minerals (Gropper and Smith, 2012).

Most of the time, bread is not eaten on their own but will be accompanied by bread spreads such as butters, margarines, jams, jellies and peanut butter which are applied on bread for better flavour and texture. It is believed that the components found in these spreads can somehow influence the mineral

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bioaccessibility of breads (Dewettinck *et al.*, 2008). Currently, there are limited studies regarding the minerals bioaccessibility of breads added with bread spreads. The aim of this research therefore was to determine the bioaccessibility of calcium, iron and zinc added with different bread spreads commonly found.

Materials and Methods

Sample selection and collection

In this study, two types of breads namely white bread and wholemeal bread and three types of bread spreads including peanut butter, chocolate hazelnut, and orange marmalade were used. All these samples were randomly obtained from retailers in Serdang, Selangor. Different amount of spreads including 32 g of peanut butter, 37 g of chocolate hazelnut and 20 g of orange marmalade based on recommended serving size were applied on per 2-slice serving of breads. The samples were then homogenized.

Determination of calcium, iron and zinc

The samples were subjected to dry ashing and the total calcium, iron and zinc contents were assessed by flame atomic absorption spectrophotometer (FAAS) (Analytik Jena, Germany) according to AOAC Official Method 985.35 (2005). Briefly, 10 g bread sample was weighed onto a crucible and ashed overnight at 555°C. The resulting white ash was dissolved in concentrated hydrochloric acid. Lanthanum chloride solution was added to final dilution to make (0.1%, w/v) for calcium determination. Lastly, the mixture was filtered into a 100 ml volumetric flask and subjected to FAAS. Standard stock solution of calcium, iron and zinc were prepared from AAS grade chemicals (Merck, Malaysia) by appropriate dilution.

Determination of calcium, iron and zinc bioaccessibility

Bioaccessibility of calcium, iron and zinc were determined using in vitro dialyzability according to Luten *et al.* (1996) with simulated peptic and pancreatic digestion. Digestive enzymes and bile salts were purchased from Sigma-Aldrich, Malaysia. A pepsin solution was prepared by dissolving 16 g of pepsin (P-7000, from porcine stomach mucosa) in 100 ml of 0.1M HCl. The pancreatin solution contained 4 g of pancreatin (P-1750, from porcine pancreas) and 25 g of bile extract (B-8631, porcine) with 1000 ml of 0.1M $NaHCO_3$.

Peptic digestion

Firstly, 10 g of homogenized bread sample was adjusted to pH 2.0 with 6 M HCl and then 3 ml pepsin solution was added. The mixture was brought up to 100ml with distilled water and incubated at 37°C for 2 hours.

Titratable acidity

A titration was performed in which 20 ml of gastric digest was mixed with 5 ml of pancreatin-bile mixture and the amount of 0.5 M NaOH needed for this mixture to achieve a pH of 7.5 was determined.

Pancreatic digestion

Segment of dialysis tubing dialysis tubing (Ø = 20.4 mm; MMCO of 10k Da; Sigma-Aldrich, Malaysia) containing an amount of $NaHCO_3$, being equivalent to the moles of NaOH needed for the pancreatic digestion (titratable acidity), was made up to 25 ml with distilled water. The dialysis tube was then placed into the conical flask containing 20 ml of gastric digest and incubated for 30 minutes. After attaining a pH of 7.0-7.5, 5 ml of pancreatin-bile mixture was added and the incubation continued for another 2 hours.

The content in the dialysis tube was analysed by FAAS for the calcium, iron and zinc contents. Lastly, the bioaccessibility of the minerals was expressed as the following formula:

$$\text{Bioaccessibility (\%)} = \frac{Y}{Z} \text{ X } 100\%$$

Where

Y= dialysate portion of calcium, iron or zinc (mg mineral 100 g of sample)
Z= total calcium, iron or zinc content (mg mineral/100 g of sample)

Statistical analysis

The data collected were analyzed by using IBM SPSS version 22.0. The contents and bioaccessibility of calcium, iron and zinc were expressed in mean and standard deviation for the triplicate measurements of each sample. The differences of the contents and bioaccessibility of minerals among different samples were analyzed by one way ANOVA with Duncan Test as post hoc test. The significant limit for the analysis was fixed at $p<0.05$.

Results and Discussion

Among the eight samples analysed, the calcium content of wholemeal bread with chocolate hazelnut spread ranked the highest (159.96±0.869 mg/100 g),

Table 1. Total calcium, iron and zinc contents of bread samples

Sample	Calcium (mg/100 g)	Iron (mg/100 g)	Zinc (mg/100 g)
white bread	141.31±3.199[a]	5.28±0.426[a]	1.48±0.070[a,b]
white bread + peanut butter	147.66±6.127[a,b,c]	5.13±0.284[a]	1.82±0.015[b]
white bread + chocolate hazelnut	159.27±8.902[b,c]	6.92±0.411[b]	1.67±0.387[a,b]
white bread + orange marmalade	138.49±14.440[a]	5.14±0.146[a]	1.41±0.023[a]
wholemeal bread	136.20±3.646[a]	4.20±0.614[a]	1.56±0.190[a,b]
wholemeal bread + peanut butter	144.63±9.699[a]	3.85±1.111[a]	1.52±0.155[a,b]
wholemeal bread + chocolate hazelnut	159.96±0.869[c]	5.08±0.666[a]	1.56±0.324[a,b]
wholemeal bread + orange marmalade	145.86±2.357[a,b]	4.11±0.789[a]	1.39±0.015[a]

Values were expressed as mean ± standard deviation for triplicate measurements for each sample (n=3).
Means followed by different alphabet in the same column indicated significant difference (p<0.05).

which showed to be significantly (p<0.05) higher than all bread samples except for white bread with chocolate hazelnut spread and peanut butter (Table 1). Hazelnuts, being good source of minerals are widely used and incorporated into spread production due to their high nutritive value (Shakerardekani *et al.*, 2013). While looking at white bread with chocolate hazelnut spread and peanut butter (159.27±8.902 mg/100 g and 147.66±6.127 mg/100 g) which ranked the second and the third highest of calcium levels, no significant difference (p>0.05) was found with some other bread samples. Although hazelnut and peanut are good source of minerals; however, processing techniques like roasting and dehulling may reduce the mineral composition of the nut spreads (Shakerardekani *et al.*, 2013).

Meanwhile, wholemeal bread with peanut butter showed the least iron content (3.85±1.111 mg/100 g), which was significantly (p<0.05) lower than white bread with chocolate hazelnut spread, which had the highest iron content (6.92±0.411 mg/100 g). According to USDA food composition database (2014), iron content found in chocolate hazelnut spread (4.38 mg/100 g) are around twice as compared to that of peanut butter (1.74 mg/100 g). Besides, the iron content of white bread with chocolate hazelnut spread (6.92±0.411 mg/100 g) was significantly (p<0.05) higher than that of wholemeal bread with the same spread (5.08±0.666 mg/100 g). Usually, white bread rather than wholemeal bread is more commonly fortified with iron in order to restore the levels of this micronutrient to the amounts found naturally in wheat before the milling (Food Standard Agency [FSA], 2008).

For zinc content, white bread with peanut butter showed the highest level (1.82±0.015 mg/100 g) and was significantly higher (p<0.05) than both of the white and wholemeal bread applied with orange marmalade. It is known that zinc is commonly found in nuts and legumes but not in fruits, which contribute lesser amount of zinc in daily diet (National Coordinating Committee on Food and Nutrition [NCCFN], 2005). According to the USDA food composition database (2014), the amount of zinc found in peanut butter itself can reach up to 2.51mg/100g but this could be reduced during processing of peanut butter (Shakerardekani *et al.*, 2013).

Comparison of mineral contents of bread samples with other references

Table 2 shows the comparison of mineral values of bread samples found in this study with Malaysian and USDA Food Composition Database. The calcium content of white and wholemeal bread were comparable with those values based in USDA food composition database but higher than those of the Malaysian database (Tee *et al.*, 1997). In contrast,

Table 2. Comparison of mineral contents of bread samples in current study with Malaysian and USDA Food Composition Database

Sample	Current study (mg/100 g)	Malaysian (mg/100 g)	USDA (mg/100 g)
Calcium			
White bread, wholemeal bread	141.31, 136.20	40, 41	144, 161
White/wholemeal bread + spreads	138.49-159.96	-	182-269
Iron			
White bread, wholemeal bread	5.28, 4.20	3.0, 3.2	3.61, 2.74
White/wholemeal bread + spreads	3.85-6.92	-	2.89-7.99
Zinc			
White bread, wholemeal bread	1.48, 1.56	-	0.74, 1.77
White/wholemeal bread + spreads	1.39-1.82	-	0.78-4.28

the iron contents found in this study were higher than both Malaysian and USDA databases. For zinc, the content of white bread was double than that of USDA database but for wholemeal bread, its content was slightly lower. While comparing other bread samples with spreads, only iron contents were within a close range with that of USDA database. As for the calcium and zinc contents, their values were fairly different.

The variations showed in the comparison may due to the differences in food fortification guidelines across the countries. The minimum amounts for bread fortification claim in Food Acts 1983 and Regulation of Malaysia are at least 150 mg/100 g for calcium and 2.1 mg/100 g for iron (Legal Research Board, 2009). The results obtained in current study complied with the guidelines as the breads used were claim to be fortified.

Bioaccessibility of calcium, iron and zinc

Based on Table 3, the calcium bioaccessibility of white bread with orange marmalade ranked the highest (39.33±4.865%), which was significantly ($p<0.05$) higher than the other bread samples except for white bread (36.10±0.800%). Since the efficiency of calcium absorption is highly pH-dependent, an acidic condition will favour the calcium solubility prior to be absorbed (Leon and Alain, 2000). On the other hand, the calcium bioaccessibility of wholemeal bread with peanut butter was the lowest (14.70±0.265%). This may be explained with the high contents of phytate which form insoluble complexes with calcium and in turn, reduce the absorbability of calcium (Schlemmer *et al.*, 2009). While comparing wholemeal bread with white bread, it was found that calcium absorption were significantly decreased, either with or without the presence of any spread. These findings further support the possible inhibitory effects of phytate in wholemeal bread on calcium absorption (Lopez *et al.*, 2002). A study by Morris and Ellis (1985) among adult men consuming non vegetarian diet found that molar ratios of phytate/calcium exceeding 0.2 may be pose a risk because of low bioavailability of dietary calcium.

The addition of orange marmalade to wholemeal bread had significantly ($p<0.05$) increased the iron absorption with its higher iron bioaccessibility up to 9.73±1.387%. This finding was consistent with the study by Derman *et al.* (1981) where it revealed that iron absorption from cereals was significantly increased with molar ratio of ascorbic acid to iron at 1.1:1.

Although the same effect took place on white bread applied with orange marmalade (8.83±1.185%); however, there was no significant difference ($p>0.05$) when compared with some other bread samples. Over the years, research that focused on the effect of organic acids like ascorbic acid and citric acid with their ability to form soluble iron complex or as binding agents in improving iron absorption are well demonstrated (Packer, 1997). Nevertheless, due to their vulnerability towards photo-degradation, especially while preparing the samples, the enhancing effects of organic acids on iron may be loss (Zhan *et al.*, 2013). Moreover, in spite of having notably higher iron content (6.92±0.411 mg/100 g), white bread with chocolate hazelnut spread could only

Table 3. Bio-accessibility of calcium, iron and zinc of bread samples

Sample	Calcium (%)	Iron (%)	Zinc (%)
white bread	36.10±0.800[a]	6.40±0.265[a]	20.63±3.536[a]
white bread + peanut butter	21.67±2.696[b]	6.90±0.600[a,b]	6.43±1.137[b]
white bread + chocolate hazelnut	30.56±3.983[c]	5.23±0.702[a]	10.47±0.398[b,c]
white bread + orange marmalade	39.33±4.865[a]	8.83±1.185[b,c]	17.83±2.253[a,c]
wholemeal bread	24.97±1.756[b]	6.53±2.155[a,b]	13.73±1.446[a,b,c]
wholemeal bread + peanut butter	14.70±0.265[d]	6.63±1.222[a,b]	5.90±1.127[b]
wholemeal bread + chocolate hazelnut	22.23±0.577[b]	5.67±1.504[a]	9.17±2.360[b,c]
wholemeal bread + orange marmalade	24.67±3.647[b]	9.73±1.387[c]	8.70±1.564[b]

Values were expressed as mean ± standard deviation for triplicate measurements for each sample (n=3).
Means followed by different alphabet in the same column indicated significant difference ($p<0.05$).

achieve iron bioaccessibility of 5.23±0.702%, which was significant lower ($p<0.05$) as compared to both the white and wholemeal bread applied with orange marmalade. This may due to the exceptional high amount of polyphenol contributed by cocoa beans and hazelnuts used in the spread production, which has inversely impacted iron bioaccessibility (Zujko and Witkowska, 2014).

For zinc bioaccessibility, white bread showed a high value of 20.63±3.536%, which was significantly ($p<0.05$) higher than the other bread samples except for white bread with orange marmalade and wholemeal bread. Looking at the effect of orange marmalade, when it came to wholemeal, its zinc bioaccessibility (8.70±1.564%) was significantly ($p<0.05$) lower than that of white bread with added spread (17.83±2.253%). It seems that the organic acids found in orange marmalade may only exert little effect on zinc absorption (Packer, 1997). Furthermore, the enhancing effect of organic acids on zinc might be further lowered by the amount of phytate found in wholemeal bread which might be higher to inhibit zinc absorption. The addition of both peanut butter and chocolate hazelnut spread to white bread had significantly ($p<0.05$) decreased the zinc bioaccessibility. Although both peanut and hazelnut are known to be good sources of zinc; yet, it was noticed that the amount of zinc might not able to overcome the high level of phytate to zinc at a molar ratio in excess of 15:1 (Morris and Ellis, 1989).

Conclusion

In conclusion, bread in plain form or with added orange marmalade spread might allow higher mineral bioaccessibility. Conversely, the mineral bioaccessibility of bread samples applied with peanut butter and chocolate hazelnut spread were low. These findings indicated that total mineral consumed did not always reflect the actual amount of mineral absorbed when the enhancers or inhibitors came into play.

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