International Journal of Food Science and Nutrition
ISSN: 2455-4898, Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 1; Issue 5; August 2016; Page No. 01-05

Effect of heat on antioxidants, its activity and acceptability of biscuit made from germinated pigeon pea flour

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Abstract

The effect of heat on antioxidants, its activity and acceptability of biscuit made from germinated pigeon pea flour was conducted. These biscuits which were produced from different blends and mixtures of germinated pigeon pea and wheat flour were with pigeon pea flour evaluated for total phenol, total flavonoid, 1, 1, Diphenyl-2-Picrylhydrazy (DPPH), reducing power and ferric reducing antioxidant power (FRAP) to evaluate the effect of heat on them. Sensory properties of the biscuits were measured. The study revealed that antioxidant and their activities in germinated pigeon pea flour were much more than that of ungerminated pigeon pea flour. Biscuits from germinated pigeon pea had higher levels of antioxidants and antioxidant activities when compared with products made from the untreated sample. The products also had acceptable sensory quality. The phenolic content and antioxidant activities in the flour decreased when the batters were baked. However, biscuits containing wheat/germinated pigeon pea in this research study still contained considerable phenolic content and exhibited antioxidant activities that are higher than commercially existing biscuits. The biscuits produced from the composite flour based on the antioxidants and antioxidant activities can contribute significantly to the reduction in the risk of developing certain degenerative diseases such as cancer and diabetes.

Keywords: Pigeon pea, Biscuits, Antioxidant and Germination

1. Introduction

Legumes are the edible seeds of leguminous plants. They are indispensable in human nutrition. Developing countries get substantial quantity of their daily food from legumes. They significantly contain relatively high proportion of protein comparable to other plant food stuff (Olanipekun *et al*., 2015) [21]. Many developing countries who have recorded increased occurrence of malnutrition which can be traced to low level of protein in diets has once again emphasized the prime place and importance of legumes as good and affordable source of legumes, most especially when consumed in a meal with cereal grains to which they act as extenders of protein. Recent studies showed that high intake of legumes protects the human body against the development of type 2 diabetes mellitus and also acts as protective factors against oxidative damage. (Savita *et al*, 2014) [25]. Pigeon pea is one of the most effective medicinal plants categorized to *Fabaceae* family. (Amalraaj and Ignacimuthus, 1998) [3]. In Africa, pigeon pea seeds are widely applied in treating hepatitis and measles while in China, they are used in stopping bleeding, relieve pain and as an expectorant (Abbiw, 1990) [1]. Research has shown that pigeon pea contain some polyphenols, such as flavonoids which plays an important role in preventing the development of such chronic diseases as cancer, heart disease and diabetes because of their actions (Prithviraj *et al*., 2009) [23]. Nutritionally, pigeon pea seeds have been reported to have high protein content and some amino acid like tryptophan, lysine and methionine. The protein content is comparable with those in cowpea and soyabean. It has high mineral quality and fibre content (EL-Tabey, 1992) [12]. Processing method such as germination is known to reduce anti-nutritional factors effectively thereby enhancing the nutritional quality and bioavailability of nutrients. (Urbano *et al*., 2005) [29]. Germination begins when the dry seeds absorbs moisture and it ends when the embryonic axis shoots out. Protective responses emerge via the production of phenolic the moment the seed breaks dormancy. Nicoli *et al.*, (1999) [20] reported that heat may change the phenolic content and antioxidant activity of cookies. In Nigeria biscuits are found to be the most popularly consumed bakery product. Some of the reasons for such wide popularity are their affordable cost, availability in different tastes, ready to eat form and longer shelf life (Gandhi *et al*., 2001) [13]. The aim of this study was to evaluate the effect of incorporation of different proportion of germinated pigeon as a composite flour in biscuit production and heat on the antioxidant and antioxidant activities and also sensory characteristic of antioxidant rich biscuits.

2. Materials and Methods

Dried white variety of pigeon peas (*Cajanus cajan*) were obtained from Ogbete Main Market in Enugu State, Nigeria. After due identification, they were rid of dirt and stored in plastic airtight containers at 4oC until needed.

2.1 Methods of Sample Preparation

Steeping and Germination Procedures

Five hundred (500 g) of the seeds were sterilized by soaking in 1% sodium hypochlorite for 20 min before steeping. The pigeon peas were methodically washed and steeped in water for 15 h. The steeped grains were spread on wet jute bags, covered with a cotton cloth and left to sprout at room temperature (28 + 2 ^{o}C) for 56 h. Germinated seeds were dried in a Gallenkamp oven (BS model OV - 160, Manchester, UK) at 50 ^{o}C for 24 h. The grain sprouts were separated from the grain (kernels) by rubbing off the germinated grain before milling and sieving through 100 µm mesh sieve. Ungerminated grain milled into flour served as the control.

2.2 Preparation of Biscuit from Germinated Pigeon Pea blended with Wheat flour and Ungerminated Pigeon Pea

The germinated pigeon pea and wheat flour were blended at various ratios to give five blends using sample A as control containing only wheat flour. The ratio were (wheat and pigeon pea flour) A (control) 100:0, B; 75:25, C; 50:50, D; 25:75, E; 0:100 respectively and F; ungerminated pigeon pea only. The blends were thoroughly mixed using a mixer (kenwood km 201, England). The biscuits were prepared with modifications according to the method described by Mousa (2014) [19]. 200 g of the various blends formulated were blended separately with the same quantity of other ingredients; 20 g of sugar, 10 ml of water, 200 g of baking fat, 0.50 g of sodium chloride, 2.50 g of sodium bicarbonate, 100 g of milk, 20 g of milk flavor, 20 ml of egg white and 10 ml of liquid vanilla flavor. Fat was creamed with sugar until fluffy, the other dry ingredients were added, and water was added until the preferred texture of the batter was achieved. The batter was kneaded on a rolling table to obtain the preferred thickness. The batter was then cut to different shapes using biscuit cutter. Thereafter, it was baked in the oven at 200 ^{o}C for 10 to 20 minutes, cooled and packaged.

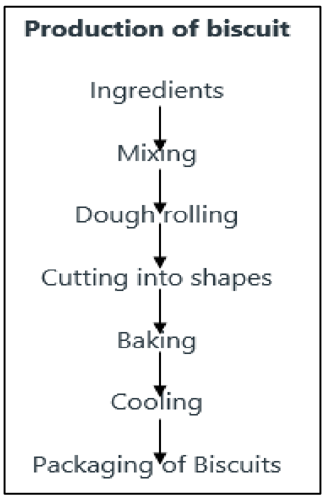


Fig 1: Flow chart for biscuit production

2.3 Sensory Evaluation of Biscuit

Sensory evaluation was also conducted on the biscuits. The samples were wheat and pigeon pea flour: A (control) 100:0, B; 75:25, C; 50:50, D; 25:75 and E; 0:100, F; ungerminated pigeon pea only. Fifteen (15) panelists were selected randomly from Institute of Management and Technology Enugu, Nigeria. The samples were coded and presented to the panelists. Water was provided for rinsing the mouth in between assessment. They were told to evaluate color, flavor, texture, crispness and overall acceptability. A 9 - point Hedonic scale was used for rating where 9 = Like extremely, 5 = neither like nor dislike, 1= Dislike extremely. (Ihekoronye and Ngoddy, 1985) [15].

3. Analytical Methods

The total phenol was determined using the method described by Makkar *et al*. (1993) [18], total flavonoid by Chang *et al*., (2002) [7], DPPH free radical scavenging activity by Blois (1958) [5], reducing power assay by Pin-Der *et al.* (2001) [22], and ferric reducing/antioxidant power by Benzie and Strain (1996) [4], Assay were carried out on the ungerminated and germinated pigeon pea flour and biscuits produced. All analyses were done in triplicates.

3.1 Statistical Analysis

A one-way analysis of variance (ANOVA) were carried out on the Anti-nutrients, chemical, functional properties, polyphenols and antioxidant activity data followed by Tukey's test (SPSS v. 20 for windows, SPSS Inc., Illinois, USA) to determine differences in the means.

4. Result and Discussion

Antioxidant and Antioxidant activities of germinated and ungerminated pigeon pea flour and different formulated biscuits

It was observed from table 1 that the polyphenol and antioxidant activities of germinated pigeon pea were higher than that of ungerminated pigeon pea. Polyphenol compound are a class of antioxidant agents which act as free radical terminators and scavenger (Shahidi and Naczk, 1995) [27] and many play a role in the prevention of certain diseases (Doss, 2011) [8]. Table I shows that antioxidant content and antioxidant activities in germinated pigeon pea were higher (p<0.05) than that of non-germinated pigeon pea. This increase in the amount of phenolic compound after germination agrees with the work done by Lopez-Amoros, (2006) [17] which indicated that germination modifies the quantity and quality of phenolic compounds in legumes.

Table 1: Antioxidant and Antioxidant activities of germinated and ungerminated pigeon pea flour.

Samples	Phenol (mg/100g dry weight)	Flavonoid (mg/100g dry weight)	DPPH (µg/ml)	Reducing power (µg/ml)	Frap (µmol/g)
Germinated pigeon pea	52.14^{a}±0.06	16.00 ^{a}±0.11	0.45 ^{a}± 1.21	69.11^{a}±0.72	66.00^{a}±2.01
Ungerminated pigeon pea	20.62^{b}±0.13	8.11^{b} ± 0.25	0.02^{b}± 0.01	49.00^{b}±0.34	47.81^{b}±1.03

Means of triplicate determinations + SD
Means within a column with the same superscript are not significantly different (p>0.05)

The phenolic compounds and antioxidant activity of the biscuit are presented in Table 2. Biscuit from wheat had the lowest phenol and flavonoid content of 10.13 + 0.68 mg /100g and 2.33 ± 0.27 mg /100g respectively, while those from germinated pigeon pea flour only had the highest phenol and flavonoid content of 31.49 ± 1.39 mg /100g and 5.49 ± 0.27 mg /100g respectively. This was followed by sample D (25:75 wheat/pigeon pea flour composite) 29.11 ± 0.02 mg /100g and 4.61 ±0.01 mg /100g while the values gotten from biscuit produced from ungerminated pigeon pea flour were significantly (p≤0.05) lower than that obtained from germinated pigeon pea F (100% ungerminated pigeon pea flour) 28.00 ± 0.00 mg /100g and 4.62 ± 0.41 mg /100g respectively. Phenol and flavonoid content of the composite biscuit increased with increasing level of pigeon pea flour in the blends. The phenol content of biscuit from samples C (50W:50P) and D (25W:75P) were not significantly (p≥0.05) different from each other while flavonoid content of biscuit from samples B (75W:25P), C (50W:50P) and D (25W:75P) were not significantly (p≥0.05) different from each other. It was observed that for all the antioxidant activity studied, biscuits from wheat alone had the lowest value while the biscuit from pigeon pea flour alone had the highest value followed by the biscuit from sample D (25W:75P) wheat/ pigeon pea flour, DPPH and reducing power content of the composite biscuits increased significantly ($p \leq 0.05$) with increasing levels of pigeon pea flour in the blends, while FRAP values were not significantly ($p \geq 0.05$) different for samples C (50W:50P) and D (25W:75P) wheat/ pigeon pea flour blends. The increase in phenolic compounds and antioxidant activity with increasing levels of germinated pigeon pea flour was anticipated because germination has been reported to increase the value of phenol, flavonoid and antioxidant activity in legumes Duenas *et al*. (2009) [9] and Savita *et al*. (2014) [25] had also reported high phenol, flavonoid and antioxidant activities in pigeon pea.

This increase of phenolic content in pigeon pea is also in agreement with Duenas *et al*. (2009) [9] who germinated lupin seeds (*Lupines angustifolius L.*) and documented an increase. However, relatively the phenolic content and antioxidant activity in the flour decreased when the batters were baked. Phenolic content decreased probably due to decomposition (Kikugawa *et al*., 1990) [16], volatilization (Hamama and Nawar, 1991) [14] and collaboration of the phenolics (Dykes and Rooney, 2006) [11] with other components of the dough. Antioxidant activity significantly reduced seemingly due to a decrease in phenolic content.

Table 2: Antioxidant and Antioxidant activities of biscuits prepared from germinated pigeon pea flour blended with wheat flour and ungerminated pigeon pea.

	Optimized biscuits	Phenol (mg/100g dry weight)	Flavonoid (mg/100g dry weight)	DPPH (µg/ml)	Reducing power (µg/ml)	FRAP (µmol/g)
	Wheat : Germinated pigeon pea					
A	100 : 0	$10.13^{d} \pm 0.68$	$2.33^{c} \pm 0.27$	$11.11^{e} \pm 2.12$	$0.10^{e} \pm 0.27$	$11.44^{d} \pm 0.02$
B	75 : 25	$20.41^{c} \pm 0.50$	$3.14^{b} \pm 0.05$	$18.42^{d} \pm 1.84$	$0.23^{d} \pm 0.19$	$29.05^{c} \pm 1.52$
C	50 : 50	$28.05^{b} \pm 0.47$	$4.00^{b} \pm 0.46$	$27.11^{c} \pm 0.00$	$0.31^{c} \pm 1.11$	$31.66^{b} \pm 0.03$
D	25 : 75	$29.11^{b} \pm 0.02$	$4.61^{b} \pm 0.01$	$30.22^{b} \pm 1.33$	$0.36^{c} \pm 0.02$	$31.91^{b} \pm 0.71$
E	0 : 100	$31.49^{a} \pm 1.39$	$5.49^{a} \pm 0.27$	$32.55^{a} \pm 0.61$	$0.41^{a} \pm 1.51$	$33.00^{a} \pm 0.88$
F	Ungerminated Pigeon pea	$28.00^{b} \pm 0.00$	$4.62^{b} \pm 0.41$	$27.21^{c} \pm 2.02$	$0.29^{d} \pm 0.81$	$30.00^{c} \pm 1.05$

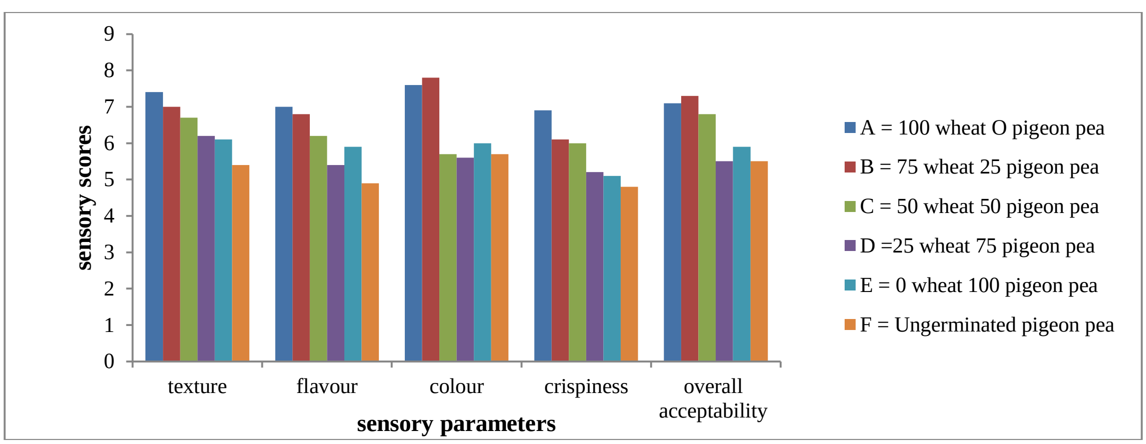
Means of triplicate determinations + SD

Means within a column with the same superscript are not significantly different ($p>0.05$)

However, biscuits containing wheat/ germinated pigeon pea in this study still contained considerable phenolic contents and exhibited antioxidant activities that are higher than commercially existing biscuits (Rasha *et al*., 2001) [24]. They were also higher than those found in biscuits made by Ajibola *et al*. (2015) [2]. Ajibola *et al*. (2015) [2] prepared biscuit from a composite blend of wheat, *Moringa oleifera* leaves and cocoa power and documented a phenol and flavonoid content of the biscuits in the range of 1.01 - 5.42 mg/g GAE and 0.22 - 1.39 mg/gQE) respectively. Even though baking reduced the levels of antioxidants in the flour, the biscuit produced still had antioxidant values higher than those produced from wheat.

4.1 Sensory Properties of the Biscuit Samples

Presented in bar diagram in Figure 2 are the sensory attributes of the biscuit produced with the flour obtained from germinated and ungerminated pigeon pea and wheat flour. There were no significant ($p \leq 0.05$) different in texture between samples A (control) and B (75W:25P), with the control ranking the highest followed by sample B (75W:25P). The flavor of the control (wheat based biscuit) was not significantly ($p \geq 0.05$) different from samples B (75W:25P) and C (50W:50P). Sample D (25W:75P) had the least rating by the panelist. The study showed that there were no significant ($p \geq 0.05$) difference in color between the control and sample B (75W:25P). The crispiness of the control was not significantly ($p \leq 0.05$) different from sample B (75W:25P) and C (50W:50P) while sample E (100P) had the least rating by the panelist. There were no significant ($p=0.05$) difference in terms of overall acceptability among the control (wheat biscuit) and samples B (75W:25P) and C (50W:50P).



The difference between the values with same letter are non-significant (p>0.05) to each other within every parameter.

Fig 2: Mean sensory score on parameters of biscuits prepared from germinated pigeon pea flour.

An essential feature of producing biscuits with better nutrient quality is the maintenance of the product's sensory characteristics because consumers' acceptability remains the key factor which determines the fruitful usage of a newly developed product. Germinating pigeon pea seeds improved the sensory characteristics of biscuit made from the flour over the ungerminated seeds. The texture scores between the 100W and 75W:25P did not show any significant (p>0.05) difference. The scores decreased to some extent with increase in pigeon pea flour addition. The texture scores were 'liked moderately'. This was because of cracks formed with the addition of gluten free pigeon pea. A Non-glutenous composite flour in biscuits preparation significantly reduces the textural strength of biscuits, such strength is observed to be dependent upon approximate levels of gluten development. This is because in disparity to bread, the gluten network in biscuits is only to some extent consistent without being too elastic (Scholar *et al*., 2003) [26] No significant (p>0.05) difference was observed in terms of taste between the 100W, 75W:25P and 50W:50P. This might also be due to the quantity of pigeon pea added which did not impart any additional flavour to the biscuits. Apart from 75W:25P, the control biscuit was significantly (p<0.05) different from other samples in colour. Biscuit from 100P had the least rating by the panelists. Broyart *et al*. (1998) [6] documented that the initial acceptance of baked products is much influenced by colour, which can also be an indicator of sufficient baking. Crispiness is noticed when food is chewed between molars, and is frequently expressed in terms of firmness and fracturability.

In this study, there were no significant (p>0.05) differences in crispiness among 100W, 75W:25P and 50W:50P. This observation was because not more than 50% of pigeon pea flour was used to substitute wheat in the biscuit preparation for 75W:25P and 50W:50P which did not affect the gluten network in the dough nor the development of an open internal structure upon baking Sudha *et al*. (2007) [28] had similar report in which oat bran was used to substitute wheat flour in biscuit formulation. They found that the acceptability of texture, taste, color and crispiness of the biscuits were not affected by up to 40% substitution with a very small increase in hardness. With respect to overall acceptability, biscuit prepared with up to 50% pigeon pea flour were similar with the control and can be baked with moderate acceptance.

5. Conclusion

The study showed that steeping and germination led to increase in some of the bioactive compounds like total phenol and total flavonoid with subsequent increase in antioxidant activity like DPPH, reducing power and FRAP. Biscuits produced had higher levels of antioxidants and antioxidant activity when compared with their respective controls. The products also had acceptable sensory quality. However, the phenolic content and antioxidant activity in the flour decreased when the batters were baked. As a result of the good functionality of the germinated pigeon pea seeds, the seeds would be very good in composite flours or even used alone depending on the product. Its use in food products will not only increase the nutritional intake of individuals, but improve problem of PEM in developing countries like Nigeria, reduce the risk of developing certain degenerative diseases such as cancer, diabetes, High blood pressure etc. Hence, the cultivation of pigeon pea and consumption of germinated pigeon pea seed should be encouraged while research efforts should continue for increased utility value of germinated pigeon pea.

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