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Composition of Polyphenols in Wheat Bread Supplemented with *Pleurotus ostreatus* Mushroom

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ABSTRACT

Pleurotus ostreatus mushrooms were blanched, milled into flour and incorporated into wheat flour at 5, 10 and 15%. These composite flours, together with composite breads developed from these formulations were evaluated for polyphenols, namely flavones and isoflavones. Wheat flour and wheat bread were used as control samples. Extraction of polyphenols was done using ethanol as a solvent and analyzed using gas chromatographmass spectrometry. It was not possible to make bread with 15% mushrooms. Quantities of different flavones and isoflavones evaluated increased significantly ($p<0.05$) with increasing supplementation of mushroom flour in wheat flour. Composite breads were also observed to have higher contents of flavones and iso flavones as compared to bread prepared from wheat flour only. Composite flour with 15% mushrooms had highest quantities of flavones and isoflavones while composite bread with 10% mushroom flour had significantly higher ($p<0.05$) levels of these compounds. Caffeic acid was the most abundant amongst all the flavones analyzed in wheat mushroom blends while genistein was the most abundant isoflavone. Flavones and isoflavones in human nutrition protect against oxidative stress and also provide medicinal properties such as antimicrobial, antiviral and anticancer benefits. *Pleurotus* mushrooms are a good source of these important compounds hence incorporating them in daily diet like bread would ensure constant supplementation for improved health and nutrition.

Key words: *Pleurotus ostreatus*, flavones, isoflavones, wheat mushroom composite flour

INTRODUCTION

Mushrooms are recognized widely for their culinary value as well as therapeutic benefits in different parts around the world. They are considered as functional foods that can provide health benefits beyond the nutrients they contain (Cheung, 2008). Edible mushrooms are valued for their nutritional and medicinal value with many species being used in traditional medicine to treat a broad range of diseases (De Roman *et al*., 2006). They are a good source of important bioactive compounds that have health promoting effects such as antioxidants, antimicrobial and anticancer properties (Patel *et al*., 2012).

In recent years, there has been a rise in consumption of mushroom due to their elevated polyphenol concentration, which correlates to elevated antioxidant activity (Alvarez-Parrilla *et al*., 2007). Polyphenols is a group of compounds whose structure contains an aromatic ring bearing one or more hydroxyl groups (Scalbert *et al*., 2005). They include simple phenols such as phenolic acids and derivatives, as well as complex structures such as flavones, flavonoids, anthocyanins, among

others (Alvarez-Parrilla *et al*., 2007). Polyphenolic compounds help to neutralize free radicals formed during cellular metabolism therefore preventing oxidative damage of cells in the body. High concentration of free radicals in the body has been associated with cancer, degenerative diseases, DNA damage and heart diseases (Tesoriere *et al*., 2004). Cowan (1999) observed that phenolic constituents in *Pleurotus ostreatus* mushroom exhibit antibacterial activity characterized by cell membrane lysis and inhibition of protein synthesis. Kosanic *et al*. (2012) established that acetonic and methanolic extracts of *Boletus aestivalis*, *Boletus edulis* and *Leccinum carpini* mushrooms exhibited frees radical scavenging activity hence high antioxidant potential. This study therefore set out to determine the quantities of flavones and isoflavones in wheat bread supplemented with *Pleurotus ostreatus* mushroom flour.

MATERIALS AND METHODS

Sample preparation: Commercial baker's grade wheat flour was purchased from Unga Limited (Nairobi, Kenya). Oyster mushrooms (*Pleurotus ostreatus*) were grown for 34 weeks under controlled conditions in the mushroom pilot plant at Kenya Industrial Research and Development Institute. Harvested mushrooms were chopped into small pieces and then blanched in boiling tap at 100°C for 3 min according to the method proposed by Vullioud *et al*. (2011). The blanched mushrooms were dried in a cabinet electric drier at 40°C for 6 h after which they were milled into flour using the Bauermeister milling machine (BauermeisterHamburg, Altona, Germany) with sieve size of 500 μm.

Blends formulation: Flour blends were prepared by mixing wheat flour to mushroom flour respectively at ratios of 100:0, 95:5, 90:10 and 85:15. These composite flours were then used to bake wheat mushroom composite breads.

Wheat and mushroom flours were blended in the ratio of 100:0, 95:5, 90:10 and 85:15. The other ingredients weighed on the flour weight basis were: water (60%), sugar (6.3%), salt (2.1%), fat (2.5%), bread improver (1.5%) and active dry yeast (3%). The ingredients were mixed using Kenwood mixer (Model 900, Watts KM264) for 6 min. The dough was covered with wet clean muslin cloth and rested for 15 min. It was then weighed, rounded and rested further for 10 min. The dough pieces were then placed in baking tins. They were then proofed for 40 min at 85% relative humidity and then baked at 200°C for 20 min.

Determination of flavones and isoflavones contents: Dry powdered sample was soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatman filter paper No.4 through 2 g sodium sulfate to remove the sediments and traces of water in the filtrate. The filtrate was then concentrated on rotary evaporator and finally by bubbling nitrogen gas into the solution. The extracts were injected directly for GC-MS analysis. Library-ms searches using Nist/Epa/Nih Mass Spectral Library (Nist 05) and Nist Mass Spectral Search Program Version 2.0d, Chemco and Adams data base were used for characterization purposes in the GCMS data system.

Experimental design and statistical analysis: The experimental design was a single factor completely randomized design experiment with three replicates.

The data obtained was analyzed statistically using SPSS software (IBM Inc, New York, USA).The results were subjected to one way analysis of variance (ANOVA) and differences in treatment means identified at $p<0.05$ by Duncan's multiple range test using PASW Statistics Edition 18 software.

RESULTS

Composition of flavones and isoflavones in composite flour: Caffeic acid was the most abundant flavone in all flours supplemented with mushroom. Composite flour at ratio 90:10 had the highest content of caffeic acid at 2.71±0.00 mg/100 g. The contents of xyloside flavone, ferulic acid, isovitexin and orientin increased significantly ($p<0.05$) with increasing mushroom content in the composite flours (Table 1).

Genistein was the most abundant isoflavones in all composite formulations (Table 2). Wheat flour (100:0) had the lowest content of genistein at 0.72±0.00 mg/100 g whereas composite flour at ratio 85:15 had the highest content (0.84±0.00 mg/100 g). The contents of other isoflavones increased with increasing mushroom content in composite flour.

Composition of flavones and isoflavones in wheat mushroom composite breads: Composite bread at ratio 85:15 did not develop. Caffeic acid was the most abundant amongst all the flavones analyzed (Table 3). Its content increased significantly ($p<0.05$) with increasing mushroom content in composite bread. Wheat bread (100:0) had the least content of caffeic acid (3.39±0.01 mg/100 g) whereas, composite bread prepared at ratio 90:10 had the highest amount (7.83±0.06 mg/100 g). Ferulic acid, acetylated dimethyl flavone, apigenin and procyanidin contents increased significantly ($p<0.05$) with increasing mushroom content in the composite breads. Isovitexin occurred in least amount in wheat bread (0.35±0.01 mg/100 g). Cxyloside, vicenin and luteolin increased with increasing mushroom content in the composite breads.

Genistein content was the most abundant amongst all the isoflavones analyzed (Table 4). Its content increased significantly ($p<0.05$) with increasing mushroom content in breads. Wheat bread had the least amount of genistein (1.13±0.00 mg/100 g) and composite bread prepared at ratio 95:5 had the highest (1.18±0.01 mg/100 g). Acetyldaidzn and daidzein increased with increasing mushroom content in composite bread while malonylglycitin content decreased significantly ($p>0.05$). Malonyldaidzin content in wheat bread was 0.58±0.00 mg/100 g. Its content decreased significantly ($p>0.05$) below 0.5 mg/100 g with increasing mushroom content in the composite breads.

DISCUSSION

Composite bread with 15% mushrooms did not develop as the dough for this ratio did not form a viscoelastic dough. Grube *et al*. (2001) reported that mushrooms are a good source of flavones and isoflavones. They demonstrated that flavones and isoflavones in wild edible mushrooms can inhibit aromatase activity and suppress breast cancer cell proliferation. Caffeic acid, a naturally occurring phenolic antioxidants in mushroom species has been reported to have a diversity of biological activities (Stojkovic *et al*., 2013). Genistein on the other hand has estrogenic activity and is used as a natural substitute for estrogen replacement therapy in postmenopausal women (Kim *et al*., 2006). Genistein has also been shown to possess antifungal activities. This isoflavone may also block uncontrolled cell growth associated with cancer by inhibiting activity of substances in the body that regulate cell division and cell growth (Weidenborner *et al*., 1990). A study by Chen *et al*. (2006) also concluded that oyster mushrooms may be an important dietary constituent for reducing the incidence of hormonedependent breast cancer in women. Regular consumption may be effective in preventing the initiation of breast tumors in women (Chen *et al*., 2006).

The present study found that isoflavones, acetyldaidzin and malonylglycitin also occurred in significant amounts in the wheatmushroom blends. These isoflavones are derivatives of daidzin and

Table 1: Flavones in wheat mushroom flours

Wheat mushroom flour	Caffeic acid	C-xyloside	Ferulic acid	Glycosylated dimethyl flavones	Isovitexin	Acetylated dimethyl flavone	Apigenin glucoside	Apigenin hexoside	Vicenin	Luteolin	Orientin	Procyanidin	Lucenin	Chlorogenic acid
100:0	1.40±0.01^{a}	0.04±0.00^{a}	0.39±0.00^{a}	0.29±0.00^{a}	0.24±0.00^{a}	0.27±0.00^{a}	0.03±0.00^{a}	0.01±0.00^{a}	0.03±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{a}	0.01±0.01^{a}	0.01±0.00^{a}	0.01±0.03^{a}
95:5	2.59±0.01^{b}	0.09±0.00^{b}	0.62±0.00^{b}	0.32±0.00^{a}	0.28±0.00^{b}	0.27±0.00^{a}	0.04±0.00^{a}	0.01±0.00^{a}	0.03±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{a}	0.02±0.00^{a}
90:10	2.62±0.02^{b}	1.33±0.01^{c}	0.75±0.00^{c}	0.32±0.00^{a}	0.28±0.00^{b}	0.27±0.00^{a}	0.05±0.00^{a}	0.01±0.00^{a}	0.04±0.01^{a}	0.03±0.00^{a}	0.05±0.00^{b}	0.02±0.00^{a}	0.01±0.00^{a}	0.02±0.00^{a}
85:15	2.71±0.00^{c}	1.37±0.00^{a}	0.93±0.00^{d}	0.32±0.00^{a}	0.31±0.01^{b}	0.27±0.00^{a}	0.03±0.00^{a}	0.12±0.00^{b}	0.04±0.01^{a}	0.30±0.00^{a}	0.08±0.00^{b}	0.04±0.02^{a}	0.02±0.00^{a}	0.03±0.00^{a}

Values are given on drymatter basis (mg /100 g), Values are means of three replicates. Means in the same column with different superscript are significantly different (p<0.05)

Table 2: Isoflavones in wheat mushroom flours

Wheat mushroom flour	Genistein	Malonylglycitin	Acetylglycitin	Malonyldaidzin	Acetyldaidzin	Acetylgenistin	Daidzein	Glycitein	Daidzin	Genistin	Glycitin
100:0	0.72±0.00^{a}	0.18±0.00^{a}	0.27±0.00^{a}	0.26±0.00^{a}	0.27±0.00^{a}	0.27±0.00^{a}	0.27±0.00^{a}	0.20±0.00^{a}	0.02±0.00^{a}	0.07±0.05^{a}	0.01±0.01^{a}
95:5	0.72±0.00^{a}	0.29±0.00^{b}	0.28±0.00^{b}	0.28±0.00^{b}	0.28±0.00^{b}	0.27±0.00^{a}	0.27±0.00^{b}	0.21±0.00^{b}	0.03±0.00^{b}	0.02±0.00^{a}	0.01±0.01^{a}
90:10	0.80±0.00^{b}	0.28±0.03^{c}	0.36±0.00^{c}	0.37±0.00^{c}	0.38±0.00^{c}	0.35±0.00^{b}	0.37±0.00^{c}	0.32±0.00^{c}	0.04±0.00^{c}	0.03±0.00^{a}	0.03±0.00^{b}
85:15	0.84±0.00^{c}	0.36±0.00^{d}	0.04±0.00^{d}	0.46±0.00^{d}	0.39±0.00^{d}	0.30±0.00^{c}	0.40±0.01^{d}	0.38±0.01^{d}	0.04±0.00^{c}	0.04±0.00^{a}	0.04±0.00^{c}

Values are given on drymatter basis (mg /100 g), Values are means of three replicates. Means in the same column with different superscript are significantly different (p<0.05)

Table 3: Flavones in wheat mushroom breads

Wheat mushroom bread	Caffeic acid	Ferulic acid	Acetylated dimethyl flavone	Apigenin hexoside	Procyanidin	Isovitexin	C-xyloside	Vicenin	Luteolin	Apigenin glucoside	Glycosylated dimethyl flavones	Orientin	Lucenin	Chlorogenic acid
100:0	3.39±0.01^{a}	0.76±0.00^{a}	0.47±0.00^{a}	0.39±0.00^{a}	0.08±0.00^{a}	0.35±0.01^{a}	0.08±0.00^{a}	0.06±0.00^{a}	0.06±0.00^{a}	0.29±0.01^{a}	0.47±0.01^{a}	0.04±0.01^{a}	0.04±0.00^{a}	0.28±0.00^{b}
95:5	6.36±0.04^{b}	0.95±0.01^{b}	0.94±0.01^{b}	0.71±0.01^{b}	0.52±0.00^{b}	0.55±0.01^{b}	0.49±0.00^{b}	0.48±0.01^{b}	0.47±0.00^{b}	0.49±0.01^{b}	0.46±0.01^{b}	0.22±0.02^{b}	0.31±0.00^{b}	0.18±0.00^{a}
90:10	7.83±0.06^{c}	0.97±0.01^{c}	0.79±0.01^{c}	0.71±0.01^{c}	0.59±0.01^{c}	0.55±0.01^{b}	0.64±0.00^{c}	0.62±0.00^{c}	0.62±0.01^{c}	0.49±0.01^{b}	0.47±0.01^{b}	0.39±0.01^{c}	0.46±0.04^{c}	0.30±0.01^{c}

Values are given on drymatter basis (mg/100 g), Values are means of three replicates. Means in the same column with different superscript are significantly different (p<0.05)

Table 4: Isoflavones in wheat mushroom breads

Wheat mushroom bread	Genistein	Acetyldaidzin	Malonylglycitin	Daidzein	Malonyldaidzin	Acetylglycitin	Acetylgenistin	Glycitin	Glycitein	Genistin	Daidzin
100:0	1.13±0.00^{a}	0.89±0.00^{a}	0.64±0.00^{b}	0.56±0.00^{a}	0.58±0.00^{c}	0.47±0.00^{a}	0.45±0.00^{c}	0.29±0.00^{a}	0.44±0.00^{c}	0.18±0.00^{c}	0.16±0.00^{b}
95:5	1.18±0.01^{c}	0.94±0.01^{b}	0.65±0.00^{b}	0.60±0.00^{c}	0.49±0.00^{b}	0.48±0.00^{b}	0.36±0.00^{b}	0.29±0.00^{b}	0.26±0.00^{b}	0.16±0.00^{b}	0.16±0.00^{a}
90:10	1.16±0.01^{b}	0.90±0.01^{a}	0.62±0.01^{a}	0.58±0.00^{b}	0.46±0.00^{a}	0.45±0.00^{a}	0.33±0.00^{a}	0.28±0.01^{b}	0.25±0.00^{a}	0.15±0.00^{a}	0.16±0.00^{a}

Values are given on drymatter basis (mg/100 g), Values are means of three replicates. Means in the same column with different superscript are significantly different (p<0.05)

glycitin. Daidzin is a natural organic isoflavone which is used in prevention of cardiovascular diseases and cancer preventive treatment by scavenging for free radicals (Popovic *et al*., 2013).

Study by Alves *et al*. (2013) to determine antimicrobial activity of phenolic compounds like caffeic and ferulic acid in *Pleurotus* mushroom species demonstrated antimicrobial activity against some antibioticresistant infections caused by some Gram negative and Gram positive bacteria. Ferulic acid exhibited an antimicrobial activity against *Neisseria gonorrhoeae*, a Gram negative bacterium that causes gonorrhoea.

Lin *et al*. (2002) assessed the antioxidant properties and functions of isovitexin and apigenin as scavengers for free radicals in the body. They concluded that these flavones have a potential to scavenge for free radicals that induce DNA strand breakage due to oxidative stress from heavy metal toxicity.

The contents of lucenin and chlorogenic acid flavones were in lowest amount in all the wheatmushroom blends. Chlorogenic, luteolin and procyanidin are not found in high amount in mushrooms (Sisler and Evans, 1958). Significant biological functions of chlorogenic acid include; improvement of retinal health due to antiinflammatory properties and inhibition of the release of glucose in the blood stream in management of diabetes type 2 (Farah *et al*., 2008). Luteolin on the other hand is noted for its anticancer, antiinflammatory and antitumor activities (Lin *et al*., 2008). It is found in abundant amount in *Agaricus* species of mushrooms (Mondal *et al*., 2013).

Besides the antioxidant properties of procyanidin, its extracts have been associated with growth promoting activity on hair epithelial cells (Kamimura and Takahashi, 2002).

Vicenin and orientin flavones also found in low amount in *Pleurotus* mushrooms but they are noted for their antioxidant as well as antiradiation properties (Patel *et al*., 2012).

CONCLUSION

This study established that adding *Pleurotus ostreatus* mushroom flour to wheat flour to develop bread increased the levels of flavanoids in the breads. Flavones and isoflavones are recognized for their antioxidant, antitumor, antiviral, antibacterial and antiarthritic functions hence their presence in mushrooms may provide significant health benefits.

Composite bread with 15% mushrooms did not form due to decreased dough strength despite blanching process to inactivate enzymatic activity in mushrooms. Further studies should be carried out to determine a feasible method to pretreat mushrooms hence improve dough strength. This will ensure a high polyphenolic profile of composite bread with over 15% mushroom flour.

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