

Article

# Phenolic compounds and antioxidant properties of roasted maize-peanut product (Zowey) and its potential to alleviate oxidative stress

Martin Adarkwah-Yiadom <sup>1,\*</sup>, Alexander Weremfo <sup>2</sup>, Daniel Awuah <sup>1</sup>, Franklin Brian Apea-Bah <sup>3</sup>

<sup>1</sup> Department of Drugs and Forensic, Ghana Standards Authority, Box 245, Accra, Ghana

<sup>2</sup> Department of Biochemistry, School of Biological Sciences, University of Cape Coast, Cape Coast, Ghana

<sup>3</sup> Department of Food and Human Nutritional Sciences, Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

\*Correspondence: Martin Adarkwah-Yiadom (martin.adarkwah-yiadom@gsa.gov.gh)

**Abstract: Background:** The study of phenolic compounds and their potential to contribute to health is a major interest in research. This work was to determine phenolic compound contents as well as antioxidant properties of roasted maize-peanut snack product with and without spices. **Methods:** HPLC was used to determine the phenolic composition of the maize flours, peanut flour and their composite snacks with and without spices. Total phenolic content (TPC), total flavonoid content (TFC), tannin content (TC) and radical scavenging activity (measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and hydrogen peroxide radical scavenging assays was also used. **Results:** TPC of the extract of roasted maize flour, roasted peanut flour and composite roasted maize-peanut flour ranged from 48.93 to 178.31 mg GAE/100 g, while the TFC was 3.18–25.94 mg CE/100 g and TC (0.22 – 0.73 mg CE/g). The dominant phenolic acid was protocatechuic acid ranged from 13.73 to 1643.54 µg/g. Among the flavonoids, quercetin and catechin were dominant. The extracts of the free soluble fraction exhibited 23.88 – 81.52 %, 49.59 – 85.17 % and 0.58 -5.13 µmol AAE/g of DPPH, hydrogen peroxide and ABTS radical scavenging abilities respectively. **Conclusion:** Maize-peanut product showed potential ability in contributing to alleviating radical induced oxidative stress.

**Keywords:** Phenolic Compounds, Cereals, Legume, Antioxidant Properties

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## 1. Introduction

Polyphenols are phenolic chemicals that are widely present in fruits, vegetables [1], cereals [2], legumes [3], and drinks [4]. Based on the amount of phenol rings and the structural components that bind these rings, polyphenols can be divided into four groups: phenolic acids, flavonoids, stilbenes, and lignans [5]. In plants, phenolic chemicals are not evenly distributed throughout tissues, cells, and subcellular structures. Plants inner layers have lower concentrations of phenolics than in their outer layers. Plant cell walls contain insoluble phenolics whereas cell vacuoles contain soluble phenolics [6]. Polyphenols are known to exhibit antioxidant properties [7].

Antioxidant compounds found in Cereals (Maize) [2] and legumes (Peanut) [8] have a beneficial effect in relation to diseases in which oxidative stress plays a role [7].

Oxidative stress is caused by an imbalance between the body's natural antioxidant defense systems' activity and the production of reactive oxygen [9]. This imbalance has the effect of rupturing cell membranes, damaging membrane proteins, and causing DNA

mutations, all of which can propagate the onset of numerous illnesses, including cancer, liver damage, and cardiovascular disease [10].

Peanut or “groundnut” (*Arachis hypogaea* L.) is the edible seeds of a legume. Peanut is rich in protein, oil and fibres and are widely used in the production of peanut butter, snack products, soup and desserts [10]. Peanut is a potential source of elimination of malnutrition in some populations in many Africa countries [10]. It is a potential source of phenolic compounds [8] with potent antioxidant activity [11]. Ma et al. [12] observed a significant increase in phenolics and antioxidant content when peanut was used in butter preparation.

For many individuals in sub-Saharan Africa, maize is a significant staple food. It is commonly eaten as a snack [13]. Maize is said to be the best traditional complementary food [14]. Maize is known for containing phenolic compounds, carotenoids, trace elements and vitamins [15]. The presence of these phenolic compounds has necessitated maize to be used widely in product formulation with improved functional activity. Various authors have used maize as a complimentary food in snack products, ready to eat foods [14, 16].

Composite cereal-legume foods are major food components in human nutrition and are beneficial due to the valuable nutritional and potential health benefits associated with their complementarity [17]. People living in sub-Saharan Africa and the Mediterranean still depend on complementary cereal -legume foods. Several countries have used cereal-legume composite food in the combat of malnutrition in children [18]. Cereal-legume foods are known at providing phenolics which have shown to have both antioxidant and antimutagenic activities. These phenolics has potential health benefits in humans [19]. Research on epidemiology suggest that, consuming diet high in phenolics, such as grain-based diets, may help prevent or reduce the incidence of chronic diseases because of their antioxidant properties [2, 20].

Although each grain and legume have an own nutritional composition, health benefits, and other functional qualities [17], Combining cereals and legumes can be a useful strategy to create foods with enhanced antioxidant and phenolic profiles, which are health-promoting.

The purpose of this study is to provide a scientific proof that compositing roasted white maize and peanut seed flour as an alternate peanut snack will provide a higher antioxidant health-promoting potential.

## 2. Materials and Method

### 2.1. Sample source and preparation

Two types of samples, white maize grain (*Zea mays* L) and peanut (*Arachis hypogaea* L) seeds were purchased from the local market in Accra, Ghana. One part of the maize grains and dehulled peanut seeds were cleaned and milled into a flour using a hummer mill (POLYMIX PX-mfc 90D, Switzerland) to pass through a 750 um screen.

The other part of the maize grains was dried, roasted and milled into a fine flour. It was then toasted on a low heat (70 °C) with constant stirring. The toasted flour was packed into a zip lock polyethylene bag and stored at -20 °C until required for analysis. The peanut seeds were roasted, dehulled and milled into powder. Part of the dehulled roasted peanut was ground together with spices (chilli, ginger), salt and sugar in a food processor machine (Panasonic MX-AC300 mixer grinder, Japan). The roasted peanut paste and the roasted maize flour in a ratio of 3:7 was mixed until a fine homogeneous paste was formed. It was then rolled into a ball, packed into a zip lock polyethylene bag and stored at -20 °C until required for analysis.

## 2.2. Extraction of free and bound phenolics

A modification of the method described by Apea-Bah *et al.* [16] was used to prepare free and insoluble bound phenolic extracts.

### Free phenolics extraction

20 ml 1% (v/v) Hydrochloric acid – methanol solvent was added to 2 g of each sample and extracted by magnetic stirring for 2 h. This was followed by centrifugation (JOUAN CR3i multifunction, France) at 1509 × g for 10 min. After separating the supernatant, the residue was rinsed three times with 10 ml of 1% (v/v) Hydrochloric acid–methanol solvent and centrifuged at 1509 × g for 10 min. The merged supernatants were stored at -20 °C in the dark until analyzed.

### Insoluble bound phenolics extraction (saponified extracts)

The residue left after the free phenolic extract was saponified for 4 hours at room temperature using 20 ml of 2 M NaOH. The saponified residue was adjusted to pH 2.0 ± 0.2 by concentrated Hydrochloric acid. The reaction mixture was centrifuged to get rid of the cloudy precipitate and the supernatant extracted four times using 10 ml of diethyl ether and ethyl acetate in a 1:1 ratio.

The organic phase was separated, dried with anhydrous sodium sulphate, filtered with Whatman No. 4 filter paper and evaporated to dryness under vacuum at 40 °C with a rotary evaporator the residue was re-dissolved in 20 ml 1% (v/v) HCl – methanol and stored at -20 °C in the dark until analysed.

## 2.3. Total Phenolic Compound (TPC)

The TPC of the extract prepared from roasted peanut-maize flour was determined using the Folin-Ciocalteu method described by Singleton *et al.* [21]. Extracts or gallic acid standard (0.5 mL) was dissolved in 1% (v/v) concentrated HCl in methanol and pipetted into a test tube and 0.2 ml of 10% v/v Folin-Ciocalteu reagent in water added. Thereafter, 0.5 mL of 700 mM Na<sub>2</sub>CO<sub>3</sub> and 8.8 mL of distilled water added. The reaction mixture was incubated in the dark for 60 min at room temperature. Gallic acid standard of concentration 10 to 90 mg/mL prepared in 1% (v/v) conc HCl in methanol were used for the calibration curve. The absorbance of the extract and gallic acid standards was measured at 765 nm using C-7200S UV/VIS spectrophotometer (PEAK Instruments, China). The experiment was conducted in triplicate and the results expressed as mg of gallic acid equivalents (GAE) per gram of dry sample (mg GAE/g).

## 2.4. Total flavonoid content

Total flavonoid content (TFC) of the extract was determined on a C-7200S UV/VIS spectrophotometer by the AlCl<sub>3</sub> method of Herald, Gadgil, and Tilley [22] with slight modification. The modification involved using reverse pipetting for mixing the reaction mixture before and after addition of NaOH, and absorbance was measured at 570 nm. All samples were measured against sample control to correct for colour interference. Concentration was expressed as mg catechin equivalents (CE) per g sample on dry weight basis.

## 2.5. Condensed tannin content

The condensed tannin content of the extracts was determined spectrophotometrically using the modified Vanillin-HCl method of Price *et al.* [23]. Briefly, 1 mL extract (or 1.5 mg/mL catechin standard) were transferred into test tubes containing 5 mL Vanillin- HCl reagent (prepared by mixing equal volumes of 8% HCl in methanol and 1% vanillin in methanol just before use) and vortexed. A colour blank determination was performed by transferring 1 mL extract or catechin to test tubes containing 5 mL 4% HCl in methanol and vortexed.

The extracts (or catechin standard) and colour blank were incubated at room temperature for exactly 20 min. The absorbance of the extracts, catechin standard and colour blank were read at 500 nm using a C-7200S UV/VIS spectrophotometer (PEAK Instruments, China). The experiment was conducted in triplicate and results expressed as mg catechin equivalents (CE) per g sample on dry weight basis.

### 2.6. DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The free radical scavenging potential of different extracts were determined according to the procedure of Bhatti *et al.* [24] with some modifications. An aliquot of 0.5 mL of sample extract was mixed with 9.50 mL of methanolic solution of 0.609 mM DPPH. The reaction mixture was incubated at 37°C for 30 min in the dark. The free radical scavenging potential of the extract was expressed as the disappearance of the initial purple colour. The absorbance of the reaction mixture was recorded at 515 nm using C-7200S UV/VIS spectrophotometer (PEAK Instruments, China) Ascorbic acid was used as the positive control. DPPH scavenging capacity was calculated by using the following formula:

$$\text{Percentage (\%)} \text{ DPPH scavenge} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

### 2.7. Hydrogen Peroxide Scavenging activity

The hydrogen peroxide scavenging ability of the extract was determined according to the method as described by Ruch *et al.* [25]. For this assay, 0.1 mL of the sample extract (0.1 mg/mL) was diluted in 9.4 mL of phosphate buffer saline (pH 7.4), followed by the addition of 0.5 mL of 4 mM H<sub>2</sub>O<sub>2</sub>, prepared in the phosphate buffer saline (pH 7.4). The reaction mixture was vortexed and after incubation for 30 min, the absorbance was measured at 230 nm using C-7200S UV/VIS spectrophotometer (PEAK Instruments, China). Ascorbic acid was used as the positive control. The H<sub>2</sub>O<sub>2</sub> scavenging ability of the extract was calculated as follows:

$$\% \text{ Hydrogen peroxide scavenge} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

### 2.8. High-Performance liquid chromatography

Reverse phase high-performance liquid chromatography (RP-HPLC) analysis was performed on the extracts of roasted maize, peanut and roasted maize-peanut flour according to Weremfo *et al.* [26] with some modifications. The HPLC system consisting of a binary pump, diode array detector, autosampler and column oven (Shimadzu, Kyoto Japan) was used. The samples were filtered through a 0.45 µm PTFE syringe filter and 10 µL of the filtered samples was injected into the HPLC system.

Sample separation was conducted on a Phenomenex Kinetex C<sub>18</sub> (100 × 4.6 mm, 2.6 µm) reverse phase column, and the analysis was carried out at a flow rate of 0.8 ml/min at 280 nm using a mobile phase consisting of A (0.1% Formic acid in water) and B (0.1% formic acid in acetonitrile) at 25°C. The gradient program used was as follows: 92% B, 0 min isocratic, 92%–91% 0–3 min; 91%–32% B, 3–30 min; 32%–91% B, 30–32 min; followed by 3 min re-equilibration of the column before the next run.

### 2.9. Statistical analysis

The data obtained were analysed by one-way analysis of variance (ANOVA). Differences between means were evaluated at the 95% significance level ( $P < 0.05$ ) using the Fisher's least significance difference (LSD) test. The statistical analyses were performed using IBM SPSS statistics version 20 (IBM SPSS, Tulsa, OK, USA).

### 3. Results

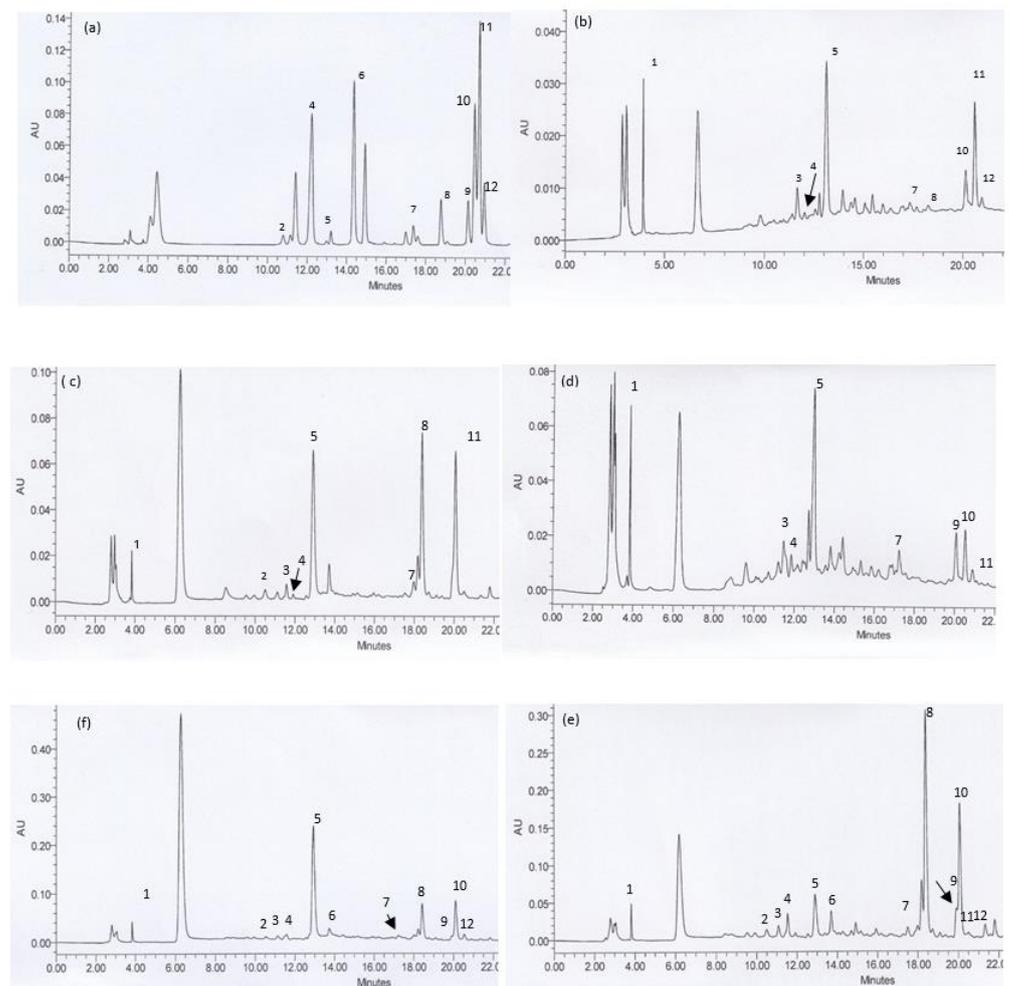
#### 3.1. Phenolic composition

Separation and identification of individual phenolic compounds in corn, peanut and maize-peanut snack extract were conducted by HPLC. Sample peaks were identified by matching against retention time of known phenolic standards under the same chromatography conditions (Figures 1a and 1b). The data generated from Figure 1a and Figure 1b is as represented in Table 1 and Table 2.

The results indicated that the contents of phenolic compounds of the acidified methanol extract of peanut flour and the maize-peanut product with and without spices were gradually increased upon roasting.

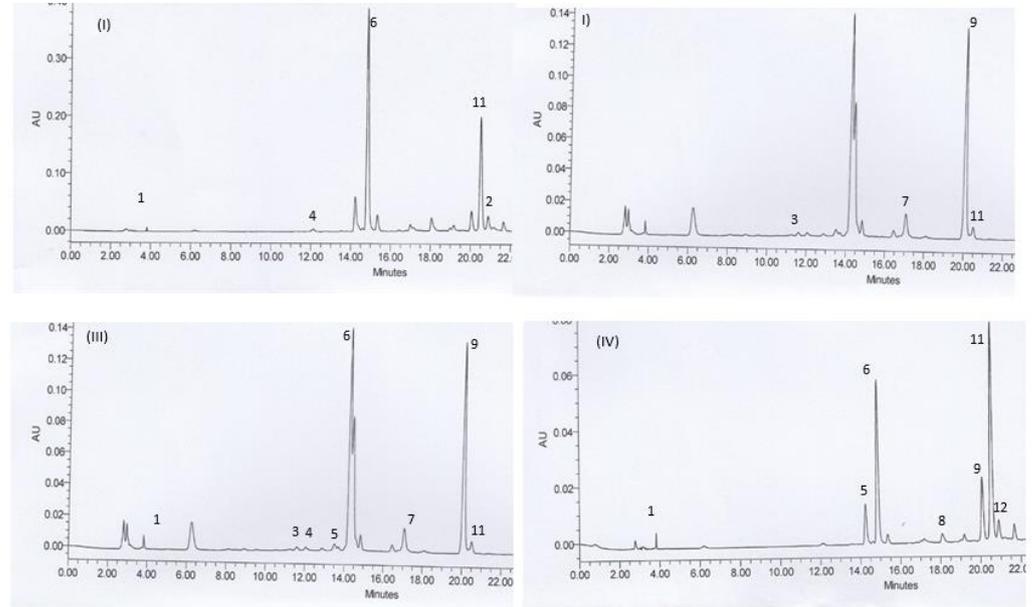
A large amount of phenolic acids namely *o*-coumaric acid, protocatechuic acid and caffeic acid and flavonoids (quercetin, catechin and rutin) were present in the acidified methanol extract of peanut upon roasting. Protocatechuic acid was the predominant phenolic acid in all extracts tested followed by caffeic acid and *o*-coumaric acid (Table 1) whilst with the flavonoids, quercetin followed by catechin and rutin (Table 2).

There was measurable amount of phenolic acids and flavonoids detected in the saponified residue of the roasted maize, roasted peanut and the combination product of maize-peanut with and without spices.



**Figure 1a.** Typical reverse phase HPLC chromatograph at 280 nm of phenolic standards (a), and acidified methanol extract of maize (b) peanut (c), roasted maize (d) roasted peanut (e) and roasted maize-peanut product (f)

Phenolic standards: 1, Gallic acid; 2, Catechin; 3, 4-hydroxybenzoic acid; 4, caffeic acid; 5, Syringic acid; 6, Rutin; 7, *o*-coumaric acid; 8, Protocatechuic acid; 9, Quercetin; 10, Apigenin; 11, Naringenin and 12, Kaempferol.



**Figure 1b.** HPLC chromatograms of saponified residue of roasted maize (I) peanut (II), roasted peanut (III) and roasted maize-peanut product (IV) extract.

Phenolic standards: 1, Gallic acid; 2, Catechin; 3, 4-hydroxybenzoic acid; 4, caffeic acid; 5, Syringic acid; 6, Rutin; 7, *o*-coumaric acid; 8, Protocatechuic acid; 9, Quercetin; 10, Apigenin; 11, Naringenin and 12, Kaempferol.

**Table 1.** Phenolic acid content ( $\mu\text{g/g}$  sample dry weight basis) of extracts from cereal flours, legume flours and their composites product

Sample	Maize flour	Roasted Maize Flour	Peanut flour	Roasted Peanut flour	Roasted Peanut flour (with spices)	Maize-peanut snack	Maize-peanut snack (with spices)
<i>Acidified methanol extract</i>							
Gallic acid	6.49 $\pm$ 0.01	8.39 $\pm$ 0.07	28.17 $\pm$ 0.03	69.56 $\pm$ 2.11	80.36 $\pm$ 1.57	61.92 $\pm$ 0.03	78.84 $\pm$ 0.05
Protocatechuic acid	13.73 $\pm$ 0.02	ND	497.67 $\pm$ 0.20	1568.02 $\pm$ 1.38	1643.54 $\pm$ 7.39	122.13 $\pm$ 0.93	126.24 $\pm$ 0.07
4-hydroxybenzoic acid	12.52 $\pm$ 0.01	11.83 $\pm$ 0.42	24.76 $\pm$ 0.03	175.27 $\pm$ 0.22	204.09 $\pm$ 0.45	87.81 $\pm$ 0.08	99.12 $\pm$ 0.14
Caffeic acid	7.39 $\pm$ 0.01	4.61 $\pm$ 0.04	12.89 $\pm$ 0.04	102.92 $\pm$ 0.01	115.37 $\pm$ 0.08	426.83 $\pm$ 0.49	433.09 $\pm$ 0.28
<i>p</i> -coumaric acid	30.41 $\pm$ 0.03	13.07 $\pm$ 1.13	28.49 $\pm$ 1.07	225.78 $\pm$ 0.15	258.37 $\pm$ 0.39	248.91 $\pm$ 0.30	303.56 $\pm$ 0.31
Syringic acid	21.73 $\pm$ 0.02	27.06 $\pm$ 0.26	40.53 $\pm$ 0.69	71.13 $\pm$ 0.04	74.07 $\pm$ 0.09	58.60 $\pm$ 0.39	61.63 $\pm$ 0.32
<i>Saponified residue</i>							
Gallic acid		7.76 $\pm$ 0.05		12.98 $\pm$ 0.01	10.84 $\pm$ 0.03	5.82 $\pm$ 0.08	3.97 $\pm$ 0.01
Protocatechuic acid		199.93 $\pm$ 0.10		ND		22.11 $\pm$ 0.03	23.27 $\pm$ 0.02
4-hydroxybenzoic acid		ND		6.62 $\pm$ 0.76	11.20 $\pm$ 0.07	ND	
Caffeic acid		2.49 $\pm$ 0.06		4.28 $\pm$ 0.02	4.61 $\pm$ 0.04	ND	
<i>p</i> -coumaric acid		ND		304.33 $\pm$ 0.62	104.62 $\pm$ 0.62	ND	13.02 $\pm$ 0.31
Syringic acid		23.70 $\pm$ 0.04		14.07 $\pm$ 0.04	14.68 $\pm$ 0.01	11.14 $\pm$ 0.01	ND

**Table 2. Flavonoid content of acidified methanol extract ( $\mu\text{g/g}$  sample dry weight basis) of cereal flours, legume flours and their composites product**

Sample	Maize flour	Roasted Maize Flour	Peanut flour	Roasted Peanut flour	Roasted Peanut flour (with spices)	Maize-peanut snack	Maize-peanut snack (with spices)
<i>Acidified methanol extract</i>							
Catechin	ND	ND	169.53 $\pm$ 0.02	462.65 $\pm$ 1.11	489.55 $\pm$ 0.72	232.95 $\pm$ 0.52	224.05 $\pm$ 0.30
Rutin	280.41 $\pm$ 0.28	138.50 $\pm$ 0.09	ND	111.75 $\pm$ 0.27	121.33 $\pm$ 0.29	74.54 $\pm$ 0.41	95.94 $\pm$ 0.04
Quercetin	ND	136.38 $\pm$ 0.20	757.61 $\pm$ 0.31	1865.43 $\pm$ 1.92	2123.92 $\pm$ 2.04	760.67 $\pm$ 0.02	774.19 $\pm$ 0.13
Kaempferol	5.05 $\pm$ 0.21	11.73 $\pm$ 0.10	ND	4.19 $\pm$ 0.03	6.03 $\pm$ 0.01	8.07 $\pm$ 0.01	9.54 $\pm$ 0.06
Naringenin	18.33 $\pm$ 1.37	21.10 $\pm$ 0.11	1.15 $\pm$ 0.08	12.09 $\pm$ 0.01	15.02 $\pm$ 0.02	15.64 $\pm$ 0.03	17.76 $\pm$ 0.05
Apigenin	13.25 $\pm$ 1.19	ND	ND	2.47 $\pm$ 0.11	2.71 $\pm$ 0.06	ND	ND
<i>Saponified residue</i>							
Catechin		ND		ND	ND	ND	ND
Rutin		135.11 $\pm$ 0.12		582.76 $\pm$ 1.99	79.82 $\pm$ 0.62	97.35 $\pm$ 0.15	60.83 $\pm$ 0.15
Quercetin		368.89 $\pm$ 0.91		1420.13 $\pm$ 2.03	943.19 $\pm$ 2.83	214.24 $\pm$ 0.16	98.18 $\pm$ 0.15
Kaempferol		92.55 $\pm$ 0.05		ND	ND	23.86 $\pm$ 0.02	ND
Naringenin		267.68 $\pm$ 0.49		10.12 $\pm$ 0.25	1.26 $\pm$ 0.03	103.31 $\pm$ 0.12	53.17 $\pm$ 0.11
Apigenin		ND		ND		ND	ND

### 3.2. Total phenolic content, total flavonoid content and Tannin content

Total phenolic content (TPC), tannin content (TC) and total flavonoid content (TFC) of the acidified methanol extract from unprocessed maize, dehulled peanut flour and roasted maize and peanut and their composite product with and without spices are shown in Table 3. TPC, TFC and TC of the acidified methanol extract of the maize, peanut and their composite product with and without spices ranged from 48.93 – 178.12 mg GAE/100g dry basis, 3.18 – 19.84 mg CE/100g dry basis and 0.22 – 0.73 mg CE/g dry basis. Whilst that of saponified extract ranges from 61.97 – 91.81 mg GAE/100g dry basis for TPC, TFC (0.83 -7.49 mg CE/g dry basis) and TC (0.33 -0.92 mg CE/g dry basis)

The TPC of the extract of unprocessed maize was significantly higher ( $P < 0.05$ ) that the extract of the unprocessed peanut. However, the TFC and TC of the extract of unprocessed maize was significantly lower ( $P < 0.05$ ) that the extract of the unprocessed peanut (Table 3). Generally, the acidified methanol extract of the roasted maize and peanut were significantly higher ( $P < 0.05$ ) than their respective unprocessed maize and peanut.

In comparing the soluble free phenolic fraction of the roasted samples for both TPC and TFC, there was a significant difference between the maize and the peanut and their composite product with and without spices. However, there was no significant difference in TPC between the peanut and the maize-peanut composite product (with or without spices). The TC recorded a significant difference between the soluble free phenolic fraction of the peanut (with or without spices) and the maize-peanut composite product (with or without spices).

Compositing the roasted maize flour and roasted peanut (with and without spices) flour increased the TPC, TFC and TC of the acidified methanol extract. To ascertain the effect of the cereal and legume composition on TPC, TFC, and TC, the observed means of

the cereal-legume composite product (with and without spices) were compared with the calculated expected mean. The expected mean was calculated as the sum of the product (corn and peanut) of the ratios of the individual component in the composite product. The effect was described as synergistic (Sy) when the observed value is higher than the expected value, antagonistic when the expected is higher than the observed and additive when there is no difference in value between the observed and the expected [42].

**Table 3. TPC, TFC, and TC of cereal flours, legume flours, and their composites product**

Sample	Maize flour	Roasted maize flour	Peanut flour	Roasted peanut flour	Roasted peanut flour (with spices)	Maize-peanut composite snack	Maize-peanut composite snack (with spices)
<b>Acidified methanol extract (Soluble free phenolics)</b>							
TPC	70.35 <sup>c</sup> ± 0.66	114.49 <sup>f</sup> ± 0.34	48.93 <sup>a</sup> ± 0.66	171.19 <sup>g</sup> ± 0.07	178.31 <sup>h</sup> ± 0.42	170.77 <sup>g</sup> ± 0.33	167.84 <sup>g</sup> ± 0.28
TFC	3.18 <sup>ab</sup> ± 0.33	10.58 <sup>e</sup> ± 0.17	15.56 <sup>f</sup> ± 0.21	19.36 <sup>gh</sup> ± 0.24	25.94 <sup>i</sup> ± 0.79	16.83 <sup>g</sup> ± 0.48	19.84 <sup>h</sup> ± 0.34
TC	ND	0.22 <sup>a</sup> ± 0.01	0.37 <sup>ab</sup> ± 0.04	0.71 <sup>c</sup> ± 0.02	0.73 <sup>cd</sup> ± 0.03	0.38 <sup>ab</sup> ± 0.02	0.36 <sup>ab</sup> ± 0.01
<b>Saponified residue (Insoluble bound phenolics)</b>							
TPC		91.81 <sup>e</sup> ± 0.31		71.40 <sup>c</sup> ± 0.07	76.12 <sup>d</sup> ± 0.07	63.82 <sup>b</sup> ± 0.19	61.97 <sup>b</sup> ± 0.27
TFC		4.20 <sup>bc</sup> ± 0.10		0.83 <sup>a</sup> ± 0.10	3.27 <sup>ab</sup> ± 0.34	7.49 <sup>d</sup> ± 0.31	6.27 <sup>cd</sup> ± 0.24
TC		0.47 <sup>b</sup> ± 0.07		0.35 <sup>ab</sup> ± 0.02	0.33 <sup>ab</sup> ± 0.11	0.88 <sup>cd</sup> ± 0.27	0.92 <sup>d</sup> ± 0.04
<b>TPC, TFC, TC and cereal legume interactive effect</b>							
TPC		206.3 ± 0.02		242.59 ± 0.01		O 234.59 ± 0.10 (Sy) E 217.19 ± 0.01	O 229.84 ± 0.02(Sy) E 220.71 ± 0.10
TFC		14.78 ± 0.05		20.19 ± 0.10		O 24.32 ± 0.12 (Sy) E 16.40 ± 0.03	O 26.11 ± 0.07(Sy) E 19.10 ± 0.03
TC		0.69 ± 0.04		1.06 ± 0.01		O 1.26 ± 0.18 (Sy) E 0.80 ± 0.02	O 1.26 ± 0.01 (Sy) E 0.80 ± 0.02

Key: TPC-Total phenolic content (mg GAE/100g sample, dry basis); TFC-Total Flavonoid content (mg CE/100g sample, dry basis) TC-Tannin content (mg CE/g sample, dry basis); GAE-Gallic acid equivalent; CE-catechin equivalent. O – Observed value; E – Expected value; Sy – synergistic effect

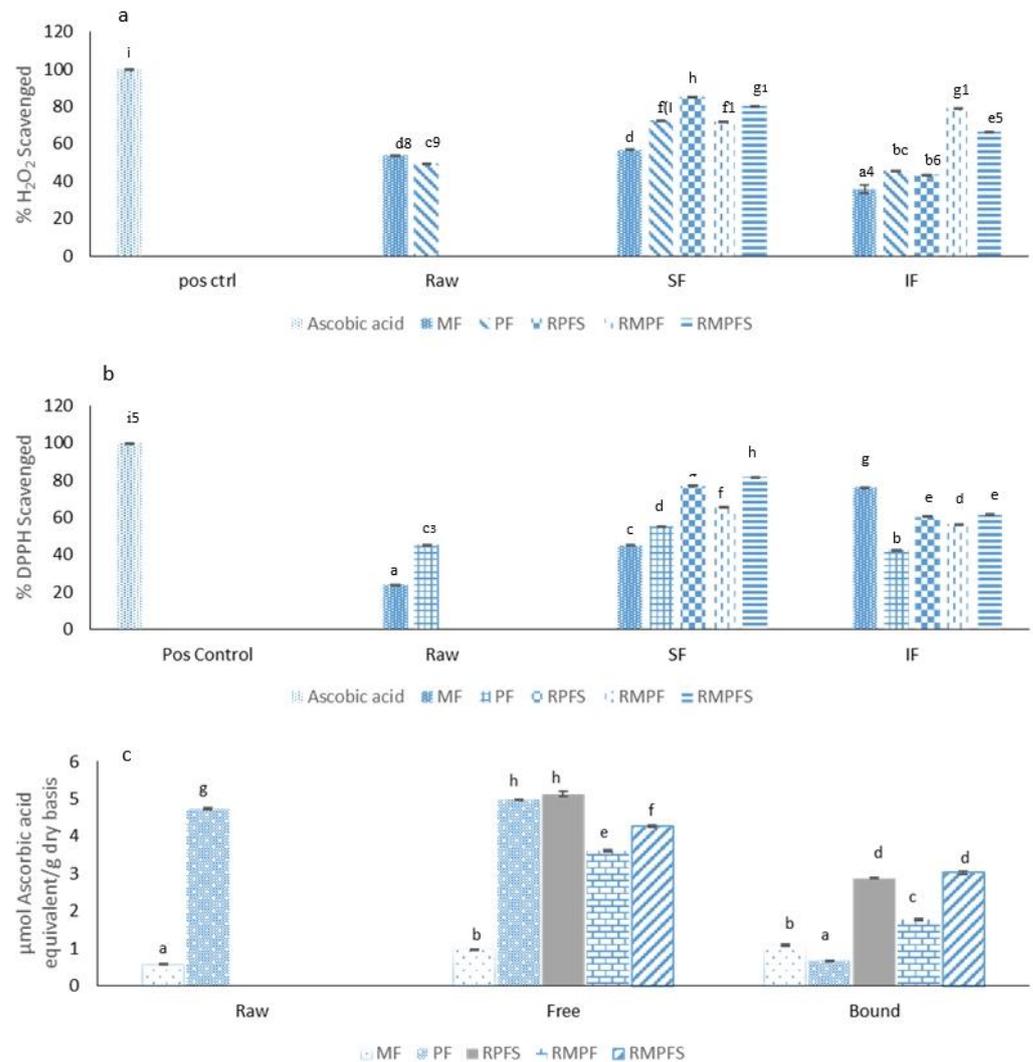
Values are means of triplicates ± standard deviation. Means in a column with different superscript letters are significantly different ( $P < 0.05$ ) from each other

### 3.3. Radical Scavenging Activity

Figure 2 shows the H<sub>2</sub>O<sub>2</sub>, DPPH and ABTS antioxidant scavenging activity of free (SF) and insoluble (IF) phenolic extracts of maize, peanut, and maize-peanut composite product with and without spices. The assays used in this study are based on their different action mechanisms and it demonstrates the antioxidant systems resilience and adaptability in quenching oxidants. Antioxidants are compounds or substances that in small quantities are able to inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions [43] in several ways including direct quenching of reactive oxygen species, inhibition of enzymes and chelating of metal ions (Fe<sup>3+</sup>, Cu<sup>+</sup>) [44].

Results from Figure 2 showed that the scavenging activities of the free phenolic extract of roasted peanut and maize-peanut product was significantly higher than free phenolic extract of maize. The DPPH scavenging activity for the maize-peanut product

for the free phenolics fraction was higher than the peanut and the maize by 15.6% and 30.9% respectively. The insoluble fraction of the maize-peanut product was significantly higher followed by peanut and then maize (Figure 2(a)) whilst in Figure 2(b and c), it did not follow the same pattern. Although there was no significant difference between the ABTS scavenging activity of the peanut flour and the peanut flour with spices in the soluble fraction (Figure 2c), the insoluble fraction showed a significant difference of about 76.4%.



**Figure 2.** H<sub>2</sub>O<sub>2</sub> (a), DPPH (b) and (c) ABTS radical scavenging activity by maize, peanut and their composite product. Values are mean ± standard deviation and bar with different letters are significantly different at  $p < 0.05$

MF- Maize flour, PF – Peanut flour, RPFS- Roasted peanut with spices, RMPF – Roasted maize-peanut product, RMPFS – Roasted maize-peanut product with spices

## 4. Discussion

### 4.1. Phenolic composition

Large amount of *o*-coumaric [27] and 4-hydroxy benzoic acids have been previously reported in peanut kernel by Win *et al.* [28]. The high amount in the roasted peanut with and without spices could be attributed to heat catalysed reactions of its bound form

releasing into the free form which is highly extractable in the acidified methanol solution. High temperature may cause cell wall rupturing leading to higher recovery of phenolic compounds in solvent [28].

Although roasting appeared to increase the extractability of most of the phenolic acids and flavonoids in the extract, the measurable amount in the saponified residue is an indicative that not all the phenolic compounds were extractable in the acidified methanol extract.

Various workers have reported on the interaction of phenolic compounds and food macromolecules [29, 30]. Adarkwah-yiadom and Duodu [31] suggests a possible interaction of proanthocyanidins, in sorghum with other food components such as protein and carbohydrates during high temperature cooking such as extrusion cooking process thus making these proanthocyanidins less extractable. Pessato *et al.* [32] also reported that whey proteins could interact with phenolics via noncovalent bonds.

The measurable amount in the saponified residue could be attributed to unextractable complex formation of macromolecules such as protein and carbohydrates with phenolic compounds in the acidified methanol sample.

#### **4.2. Total phenolic content, total flavonoid content and Tannin content**

The results support the findings of Awika *et al.* [33] and Zielinski and Kozłowska [20]. The authors stated that phenolic compounds are mainly concentrated in the pericarp and seed coat of the grain. Hag, Tinay and Yousif [34] also showed that dehulling decreased the TPC of pearl millets. The lower TPC value in the unprocessed peanut flour could be due to the fact that the peanut was dehulled before making it to flour. The higher TPC content of the roasted maize flour could be due to Maillard reaction that occurred during roasting. Millard reaction intermediate product, melanoidins, is known to contribute to increasing TPC in food samples [35].

Several authors have reported increase in TPC and TFC upon heating [36, 37]. Win *et al.* [28] stated that roasted peanut kernel showed higher TPC compared with the raw peanut kernel. Talcott *et al.* [27] also showed that, roasting peanut at 170 °C increased the phenol content from 0.913mg/g (raw kernel) to 0.949 mg/g (roasted kernel). The increase in TPC, TFC and TC as stated above may be explained as the liberation of bound phenolics from the cell walls, as well as possible breakdown of conjugated polyphenolic compounds into simple phenolics at high temperature and generally increased phenolic compounds extractability following roasting [38]. The occurrence of Millard reaction during roasting could contribute to the high TPC. Roasting results in phenol complexes such as pyrroles and furans [39] which is formed from the Millard reaction, contributing to the total phenolic compounds of the roasted samples [40].

The values of TPC TFC and TC in the free phenolic fraction of the roasted peanut flour was in agreement with that of Attree *et al.* [41] and was also higher than that of the roasted maize as reported by Chen *et al.* [15]. The increase in TPC, TFC and TC in the free soluble fraction of the roasted peanut with and without spices could be due to roasting which causes the release of phenolics attached to the cell walls of the food matrix [38] thereby increasing phenolics extractability. It has also been suggested that the observed increase in total phenolics may have caused by the release of bound phenolic compounds from cell walls during roasting. Roasting releases melanoidin and other components, a Maillard reaction product that have reducing properties [38].

From the results obtained in Table 3, no additive and antagonistic effect of the maize-peanut composite product was observed.

#### **4.3. Radical Scavenging Activity**

Ahn *et al.* [45] stated that phenolics demonstrate different antiradical property, depending on the method of analysis. This inconsistency in radical scavenging may be due to the reaction mechanism of the sample extract towards the free radical.

With DPPH assay, the antioxidant transfers an electron to the reactive free radical or oxidant in so doing reduces the potency of the reactive free radical or oxidant. Whilst in the H<sub>2</sub>O<sub>2</sub> assay, the antioxidant donated a hydrogen atom to bind with the reactive oxidant, thereby stabilizing or quenching it [46].

## 5. Conclusions

The results of this study showed that roasting had significantly affected the TPC, TFC, TC and the antioxidant activity of the maize-peanut composite snack with and without spices. Roasting disrupts plant tissue and helps in the release of more free and bound phenolic compounds in peanut. Compositing maize with peanut with and without spices increases the TPC, TFC, TC and antioxidant activities. The results provide the evidence of high level of health benefits due to the presence of phenolics and its ability to scavenge free radicals. This suggest that maize-peanut composite snack product has the potential to alleviate oxidative stress due to the presence of phenolics and its ability to scavenge free radicals. Therefore, maize-peanut composite snack could provide an inexpensive source of antioxidant that can be used in dietary supplement.

**Authors' contribution:** This work was carried out in collaboration between all the authors.

Author FBAP helped in the conceptualization of the research.

Author AW helped in the design of the study. Author DA helped in carrying out the experimental work. All authors oversaw the proof reading of the manuscript and agreed to be published.

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