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Effect of Roasting Regime on Phytochemical Properties of *Senna occidentalis* Seeds

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Abstract

Senna occidentalis seeds were roasted at varying temperatures of 190, 210 and 230 °C each for 10, 15 and 20 min. Phytochemicals of the roasted seeds were determined using standard methods. The phytochemicals analysed were tannins, saponins, flavonoids, alkaloids, glycosides, oxalate and phenolics. Phytochemicals are compounds hypothesized for much of the disease-protection provided by diets high in fruits, vegetables, legumes, cereals and plant-based beverages. This study has clearly shown that roasting time and temperature have significant effects on the seed parameters analyzed. There was an increase in tannin, alkaloid, saponin and phenolic contents and a decrease in the contents of flavonoids and oxalates.

Keywords: Coffee substitute; Time-temperature combination; Significant difference; Phytochemical properties

1 Introduction

The term 'substitute' refers to goods that are more or less interchangeable to a consumer (Encarta, 2009), such that one may be conveniently used in place of the other. "Coffee senna", botanically classified as both *Senna occidentalis* and *Cassia occidentalis*, seeds are chief substitutes of coffee and are thus sometimes roasted and made into a "coffee-like beverage" (Vashishtha, John, & Kumar, 2009; Abubakar & Sule, 2010; Sharma et al., 2013; Shittu et al., 2014; Teles, Fock, & Gomiak, 2015). Roasting is a primary preparation stage for coffee and coffee substitutes (Belitz, Grosch, & Schieberle, 2009). The effects of roasting include microbial inactivation, chemical damage, enzyme inactivation and physical changes (Lewis, 2006). Thus it alters sensory properties; improving palatability, extending the variety of tastes, aromas and textures in foods produced from similar raw materials.

Senna occidentalis seeds range in colour from greenish-brown to dark brown with a smooth surface that may have small bright coloured bands on the outer surface (Anonymous, 2012; Oshoke & Akinyemi, 2015). The seed was wrongly identified as *C. sieberiana* in earlier reports where physical and chemical properties of raw seed (Olapade, Ajayi, & Ajayi, 2014) and also changes in some physicochemical properties of the seed during roasting were studied (Olapade, Akinoso, & Oduwaye, 2012). No study has been done to date to evaluate the effect of roasting on phytochemical properties of the seed.

'Phytochemicals' are chemical substances, constitutive metabolites essential for survival, proper functioning, and protection against predators, amongst others (Molyneux, Lee, Gardner, Panter, & James, 2007). Although their inherent activities can have decidedly ad-

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verse effects on other organisms, especially animals, they confer good qualities beneficial to humans and are compounds hypothesized for much of the disease-protection provided by diets high in fruits, vegetables, legumes, cereals and plant-based beverages (Tyagi, Singh, Sharma, & Aggarwal, 2010; Teles et al., 2015). Phytochemicals analysed were tannin, saponin, flavonoid, alkaloid, glycoside, oxalate and phenolics.

Tannins affect the availability of amino acids and the utilization of protein. They inhibit the activities of digestive enzymes and are known for their ability to reduce digestibility of food proteins because they form insoluble complexes with proteins (Morrow, 1991; Griffiths & Moseley, 1980; Sharma & Sehgal, 1992; Ogunlade, Ilugbiyin, & Osasona, 2011). Saponins exhibit anti-fungal, anti-inflammatory, fungistatic, haemolytic, molluscidal and foaming activities and influence the body's immune system; lowering cholesterol levels and protecting against cancers (Shi et al., 2004). Of interest in beverages are the aesthetic appeal and a desirable mouth-feel. These are favoured by the presence of foam in or on the cup of the beverage. Saponins have foam causing properties and as such it is expected that they not only enhance health but stimulate the appeal to consume coffee substitutes.

Alkaloids and their derivatives have very important biological functions as analgesics and antispasmodics, and possess bactericidal activities (Hussain et al., 2011). Cyanogenic glycoside on hydrolysis yields toxic hydrocyanide acid (HCN). The cyanide ions inhibit several enzyme systems and depress growth through interference with certain essential amino acids and utilization of associated nutrients (Audu, Aremu, & Lajide, 2013). Oxalates are known to adversely affect mineral availability in foods. It was reported that a high intake of oxalates could result in gastro-intestinal irritation, blocking of the renal tubes, muscular weakness or paralysis (Innocentia, Ojotu, & Emmanuel, 2014). Phenols and phenolics compounds are greatly used in skin infections and other wound treatments, and also for healing and as antimicrobial agents (Hussain et al., 2011).

The aim of this research was to study the effect of roasting at various time-temperature combinations on the phytochemical properties of *Cassia occidentalis* as a coffee substitute.

2 Materials and Methods

2.1 Materials

The seeds used for this project were obtained from a settlement; Ajibode in Ibadan, Oyo state.

2.2 Sample preparation

The pods containing the seeds were opened and the seeds within removed. The seeds obtained were carefully sorted to make sure only good seeds were used. Seeds obtained were stored in polyethylene (PET) bottles before and after being roasted. Roasting was done using a gas oven at the Human Nutrition Department of the University and was carried out at 190, 210 and 230 ˚C each for 10, 15 and 20 minutes. Each experiment of 100 g seed was done in triplicate. The roasted seeds were cooled, coded, packed and later milled to powder. The raw seeds of the plant are shown in Plate 1. Samples were thoroughly mixed prior to sampling for analysis. The milled samples were stored in a low density polyethylene bag and polyethylene bottles in a cool and dry place to protect it from insect, dust, moisture gain and loss of flavour.

2.3 Phytochemical Analyses

All the determinations described below were run in triplicates.

Tannin content determination

Tannin content was determined as reported by Sharma and Sehgal (1992). 0.5 gram of sample was measured into a 50 ml beaker and 10 ml of water was added. The mixture was shaken, covered and left to stand for 1 hour. One ml of extract was transferred into a flask. Then, 5 ml distilled water and 2 drops 5% $FeCl_3$ aqueous solution were added and the solution was shaken to mix it properly. Thereafter, 4 drops of potassium ferrocyanide was added, and coloration developed. Preparations for a standard curve were made with 0.5 gram of tannic acid in 50 ml of

water. Aliquots of 0.0 ml, 0.1 ml, 0.2 ml, up to 1.0 ml were taken and each was made up to 10 ml with distilled water. The absorbance of sample and standard tannic acid solutions were read after colour development on a spectrophotometer at a wavelength of 620 nm and calculations were made.

Saponin content determination

The spectrophotometric method (Hussain et al., 2011) was used for saponin analysis. One gram sample was weighed into a beaker and 10 ml petroleum ether was added. The mixture was shaken to ensure uniform mixing. Thereafter, supernatant was decanted and then evaporated to dryness. 6 ml of 80 % ethanol was added to dried filtrate and swirled. From this, a 2 ml aliquot was transferred into test tube. 2 ml of 5 % $FeCl_3$ aqueous solution was added and the preparation was left to stand for 30 minutes meanwhile, colour developed. 0 to 10 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% $FeCl_3$ aqueous solution. The absorbance of the sample as well as standard saponin solutions were read after colour development on spectrophotometer at a wavelength of 550 nm.

Flavonoids content determination

Spectrophotometric method in Kathirvel and Sujatha (2012) was adopted and adapted as follows. 0.50 gram of sample was weighed. To this, 10 ml of ethanol was added and the mixture was left for 15 minutes. One ml of solution was transferred and 9 ml of distilled water added. Upon agitation, 1 ml was extracted. 6.1 ml distilled water and 0.3 ml of 5% $NaNO_3$ aqueous solution was added and left for 6 minutes. Thereafter, 0.6 ml 5% $AlCl_3$ aqueous solution was added in and it was again left for 5 minutes. Finally, 2 ml of 5% NaOH was added and sample absorbance read at 510 nm.

Alkaloids content determination

Determination of the alkaloid content was done by the alkaline precipitation method described by Harborne (1998) with modification. Five gram of the sample was dispersed in 20% acetic acid solution in ethanol to form a ratio of 2:8 (20%). The mixture was allowed to stand for 4 hours at 28 °C with lid and was later filtered. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop-wise addition of conc. aqueous NH_4OH until the alkaloid was precipitated. The precipitated alkaloid was received in a weighed filter paper, washed with 1% ammonia solution and dried in the oven at 80 °C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed (Hussain et al., 2011).

Glycoside content determination

To one gram of sample, 25 ml of 15 % lead acetate aqueous solution was added and the mixture was filtered. Two ml of chloroform was added to the filtrate and shaken vigorously. Clear fluid, i.e. lower layer, was collected and evaporated to dryness. Three ml of glacial acetic acid, 0.1 ml of 5 % $FeCl_3$ aqueous solution and 0.25 ml of conc. H_2SO_4 were added and the mixture thoroughly shaken. It was then allowed to stand in dark for 2 hours after which absorbance was read at 530 nm.

Oxalates content determination

A 0.5 gram portion of sample was weighed. 10 ml distilled water and 1 ml conc. H_2SO_4 were added. The solution was left to stand overnight. Thereafter, 39 ml distilled water was added. An aliquot of 5 ml was taken. This was titrated against 0.05 M standardized aqueous $KMnO_4$ solution till pink colour, which persists for 30 seconds, was obtained.

Phenolics content determination

The sample (0.5 g) was weighed into a conical flask and 10 ml ethanol was added. One ml of extract was taken and to it was added 9 ml of distilled water. A one ml aliquot was taken and 4.5 ml of Folin-Ciocalteu's reagent was added. Two ml of 7.5 % Na_2CO_3 aqueous solution was added and the mixture agitated. Solution absorbance was read at 610 nm.

2.4 Phenolics content determination

Results of each experiment were analysed to assess significant differences between raw and roasted samples' parameters at a significance level of $p>0.05$. All data was collated on Microsoft Excel sheet, analyzed using SPSS 20.0 and presented as mean ± standard deviation (Arunkumar & Muthuselvam, 2009).

3 Results and Discussion

3.1 Phytochemical content

The results of the phytochemical content of raw seed and seed roasted at 190 °C, 210 °C and 230 °C each at 10, 15 and 20 minutes respectively are presented in Table 1. Tannin content ranged from 0.13 % for roasting condition 190 °C /20 min to 0.25 % in 230 °C /20 min. Compared to raw sample, tannin content in samples undergoing roasting at 190 °C/15 min and 190 °C/20 min was reduced, while it was unchanged for 210 °C/10 min and 210 °C/15 min, but it increased for 190 °C/10 min, 210 °C/20 min and all roasted samples at 230 °C. With a roasting time of 15 min, the tannin content increased with increase in temperature. Tannin contents of both raw and roasted seeds of coffee senna were at least 90 % lower than the 2.27 and 2.73 % of raw and roasted seeds of coffee-like baobab (Innocentia et al., 2014). The tannin content of the water extract of the raw sample (0.16 %) was much lower than those of chloroform, methanol and petroleum ether extracts of raw *Cassia occidentalis* reported in Kathirvel and Sujatha (2012). With respect to other plants, the tannin content of samples undergoing roasting at 210 °C/20 min was comparable to that of *Sesbania pachycarpa* (Proll, Petzke, Ezeagu, & Metges, 1998) and watermelon seed kernel flour (El-Adawy & Taha, 2001); at 230 °C/15 min was comparable to that of fluted pumpkin leaf extract (Nworgu, Ogungbenro, & Solesi, 2007) and *Enterolobium cyclocarpium* (Proll et al., 1998); and at 230 °C/20 min was comparable to *Prosopis Africana* (Proll et al., 1998). All the roasted samples had lower tannin content than 0.32 % of protein isolate of *Jatropha curcas* (Makkar, Francis, & Becker, 2008); 0.38 % of *Lonchocarpus sericeus* (Proll et al., 1998); 0.448 % and 0.234 % of raw and processed mango seed flour (Arogba, 1997); 0.48 % of paprika seed and 0.69 % of *Pterocarpus osun* (Proll et al., 1998) but higher than 0.12 % and 0.127 % in *Adansonia digitate* and 100 % maize ogi flour respectively (Proll et al., 1998; Enujiugha, 2006).

The saponin content of raw and roasted samples ranged from 0.012 % to 0.019 % as revealed in Table 1. Raw samples had a saponin content of 0.013 %. The presence of saponin in coffee senna contradicts results of Veerachari and Bopaiah (2012), Mensah, Okoli, Turay, and Ogie-Odia (2009) and Olapade et al. (2014) who found no saponin in the seed. Of the roasted samples, only sample 190 °C/20 mins had a percentage of saponin content lower than that in the raw sample; though not significantly lower. According to Akinmutimi (2006) about 44.25 % reduction in saponin content occurred in jack beans cooked for 60 minutes; more than in those cooked for 20 and 40 minutes. A similar trend was observed with the roasted *Cassia occidentalis* seeds, where the saponin content was observed to reduce with an increase in duration of roasting from 10 to 15 minutes and then to 20 mins at 190 °C and 210 °C. The saponin contents observed in raw and roasted coffee senna seeds were lower than the values reported for raw (7.20 %) and fermented (6.71 %) *Cassia tora* (Adamu, Ushie, & Elisha, 2013) and for raw (3.26 %) and roasted (7.23 %) coffee-like baobab seed (Innocentia et al., 2014). The values were also lower than the ones stated for grain legumes (Khokhar & Apenten, 2003), raw S. obtusifolia (Ingweye, Kalio, Ubua, & Umoren, 2010) and all medicinal plants investigated by Edeoga, Okwu, and Mbaebie (2005) and by Aliyu, Musa, Oshanimi, Ibrahim, and Oyewale (2008). Flavonoids are beneficial phytochemicals. All the seeds; raw and roasted were found to contain flavonoids. All roasted samples except the ones roasted at 190 °C for 10 minutes had lower quantities of flavonoid when compared to raw seed. Roasting for more than 10 minutes at 190 °C and roasting at temperatures above 190 °C caused a significant reduction in flavonoids' content by as much as 87.1 %. Flavonoids' content reduced with in-

Table 1: Effect of roasting time on the phytochemical composition of seeds

Phytochemical (%)	Raw	190 °C			210 °C			230 °C		
		10 min.	15 min.	20 min.	10 min.	15 min.	20 min.	10 min.	15 min.	20 min.
Tannin	0.16±0.00 ^{d}	0.22±0.00 ^{b}	0.15±0.00 ^{d}	0.13±0.01 ^{e}	0.16±0.00 ^{d}	0.16±0.01 ^{d}	0.24±0.01 ^{a}	0.19±0.01 ^{c}	0.18±0.01 ^{c}	0.25±0.02 ^{a}
Saponin	0.013±0.001 ^{d,e}	0.019±0.001 ^{a}	0.014±0.000 ^{c,d,e}	0.012±0.000 ^{e}	0.017±0.001 ^{b}	0.017±0.001 ^{b}	0.016±0.002 ^{b,c}	0.016±0.001 ^{b,c}	0.015±0.001 ^{b,c,d}	0.014±0.003 ^{c,d,e}
Flavonoid	0.31±0.02 ^{b}	0.50±0.04 ^{a}	0.04±0.00 ^{e}	0.07±0.00 ^{c}	0.07±0.00 ^{c,d}	0.04±0.00 ^{e}	0.07±0.00 ^{c,d}	0.05±0.00 ^{d,e}	0.04±0.00 ^{e}	0.07±0.00 ^{c,d}
Alkaloid	2.00±0.00 ^{e}	2.00±0.00 ^{e}	5.50±0.71 ^{d}	4.50±0.71 ^{d,e}	9.00±0.00 ^{b,c}	10.00±1.41 ^{b}	6.50±0.71 ^{c,d}	9.00±1.41 ^{b,c}	13.00±1.41 ^{a}	6.00±2.83 ^{d}
Glycoside	0.014±0.000 ^{c}	0.028±0.001 ^{a}	0.031±0.001 ^{a}	0.010±0.001 ^{c}	0.022±0.002 ^{b}	0.022±0.001 ^{b}	0.032±0.002 ^{a}	0.023±0.003 ^{b}	0.031±0.001 ^{a}	0.023±0.002 ^{b}
Oxalate	0.21±0.01 ^{d}	0.20±0.01 ^{d}	0.17±0.01 ^{e}	0.16±0.00 ^{e}	0.22±0.02 ^{c,d}	0.22±0.01 ^{c,d}	0.28±0.01 ^{a}	0.24±0.01 ^{b,c}	0.22±0.00 ^{c,d}	0.25±0.03 ^{b}
Phenolics	0.27±0.02 ^{e}	0.24±0.04 ^{e}	0.76±0.01 ^{b}	0.38±0.01 ^{c}	0.20±0.01 ^{f}	0.88±0.00 ^{a}	0.75±0.02 ^{b}	0.24±0.01 ^{e}	0.90±0.01 ^{a}	0.35±0.01 ^{d}

Values of mean in rows; not followed by the same alphabet(s) are significantly different at $p<0.05$

creasing temperature in samples roasted for 10 minutes but remained constant despite variations in temperature in samples roasted for 15 minutes. The same consistency was observed for samples roasted for 20 minutes. Meanwhile, samples roasted for 20 minutes had slightly higher values than those obtained for seeds roasted at 190, 210 and 230 °C for 15 minutes. It was observed that flavonoids were best retained in seeds roasted at 190 °C and 10 minutes and then next in all seeds roasted for the duration of 20 minutes. Samples with the highest flavonoids content, i.e. 190 °C/10 mins, compared favourably with that of *Richardia brasilensis* (Edeoga et al., 2005) and *Anchomanes difformis* (Aliyu et al., 2008). While that of raw samples compared with *Cleome rutidosperma* (Edeoga et al., 2005) but was lower than that of *Cassia occidentalis* as reported in Kathirvel and Sujatha (2012). Alkaloids' content of seeds ranged from 2.00 ± 0.00 % in raw seeds and in roasted seeds at 190 °C for 10 minutes, to 13.00 ± 1.41 % in roasted seeds at 230 °C for 15 minutes. Alkaloids are phyto- constituents that intercalate with body deoxyribonucleic acid. No particular trend was observed with increasing duration of roasting, at each temperature, although most treatments for 10 minutes were significantly higher than in the raw sample except the treatment at 190 °C. The alkaloid content increased from 10 to 15 minutes of treatment and then reduced upon further exposure to roasting (i.e. till 20 minutes). This may be a result of decomposition of other components of the seed; thus increasing the percentage content of alkaloids rather than a conversion of other components into alkaloids. The highest alkaloid content was found in the sample roasted at 230 °C/15 minutes. Results obtained for alkaloids' content are higher than those studied by Edeoga et al. (2005) and in *Anchomanes difformis, Anisopus mannii* and *Pavetta crassipes* (Aliyu et al., 2008) but much lower than those in raw *Senna obtusifolia* (Ingweye et al., 2010). All roasted samples had an increased percentage of glycoside content except 190 °C/20 min. Raw samples presented 0.014 % glycosides and ranged between 0.010 and 0.032 % in roasted samples, which implies that roasting does not extensively destroy the cyanogenic glycosides. It is notable that the average percentage of content of glycosides in seeds is relatively low and is found to be lower than that found in raw seeds of *Senna obtusifolia* (Ingweye et al., 2010), leaves' extract of *Teleferia occidentalis* (Ekpenyong, Akpan, & Udoh, 2012) and Tanzanian locally available leaf and oil seed meals used as poultry feed ingredients (Mutayoba, Dierenfeld, Mercedes, Frances, & Knight, 2011). Results of samples' oxalate content presented in Table 1 clearly show that cooking, including roasting, has a minute effect on the oxalate's content of food. The oxalate content of the raw sample was 0.21 ± 0.01 % while that of the roasted samples ranged between 0.16 ± 0.00 % and 0. 28 ± 0.01 % for roasting conditions of 190 °C/20 min and 210 °C/20 minutes, respectively. Oxalate makes some mineral elements unavailable. Roasting at 190 °C for 10, 15 and 20 minutes resulted in a 4.76 %, 19.52 % and 23.80 % reduction in oxalate contents respectively (values of which were not significantly different from each other at $p < 0.05$). Meanwhile, the oxalate content in samples roasted at 210 °C and 230 oC were higher than that of raw samples. Akinmutimi (2006) recorded a 50 % reduction in oxalate content between raw and processed Jack bean seed while Innocentia et al. (2014) recorded a 40 - 88 % reduction between raw and roasted baobab. The percentage (%) of oxalate contents of raw and roasted coffee *senna* seed were found to be lower than that of raw and roasted seeds of coffee-like baobab and other tropical crops studied by Proll et al. (1998), but higher than that in 100 % maize-ogi flour (Enujiugha, 2006) and fluted pumpkin leaf extract (Nworgu et al., 2007). Phenolics have a strong antioxidant potential. The effect of roasting time-temperature combinations on sample phenolics ranged from 0.20 % to 0.90 %. Treatment for 10 minutes generally resulted in a sample with a phenolics content lower than that in the raw sample by as much as a 6.86 % reduction. Samples roasted for 15 and 20 minutes had higher phenolics content than raw samples. Seeds roasted for 15 min had a phenolics content higher than those roasted for 10 min and 20 min, increasing with increased roasting temperature applied as in Figure 1. The highest phenolics content was found in samples with roasting conditions 230 °C/ 15 min and the lowest in samples roasted at 210 °C for 10 mins. The phenolics content of raw and roasted *Senna*

occidentalis were higher than those in *E. heterophylla, R. bransilensis, S. dulcis, S. acuta, S. anthelmia, S.cyaenneusis* and *T. procumbeus* all of which are medicinal plants (Edeoga et al., 2005), but lower than that in protein isolates of seed cake of *Jathropha curcas* (Makkar et al., 2008) as well as *Anisopus mannii, Stachytarpheta angustifolia, Veronia blumeoides* (Aliyu et al., 2008). The phenolic content of the ethanol extract of raw coffee *senna* seeds in this study (0.27%) was comparable to that of the methanol extract of coffee *senna* seeds but lower than those of chloroform and petroleum ether extract as studied by Kathirvel and Sujatha (2012).

Figure 1: Seeds of *S. occidentalis*

4 Conclusions

This study has shown that the duration of exposure and the temperatures of roasting applied affect the seeds' phytochemical properties. While roasting did not totally eliminate the undesired phytochemicals (oxalate and tannin primarily), the resultant effect was that saponin and flavonoids contents reduced but phenolics, glycosides and alkaloids contents increased. The best phytochemical balance was observed in samples roasted at 210 °C for 15 min. Other favorable conditions for roasting of coffee senna seeds were 210 °C/10 min and 190 °C/20 min. The less favorable conditions were found to be the more severe roasting conditions of 230 °C/20 min and 210 °C/20 min.

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