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Phenolic composition and antioxidant properties of *koose*, a deep-fat fried cowpea cake

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ABSTRACT

Koose, a West African delicacy, is a side dish prepared by deep frying thick cowpea paste. The current research determined the effect of deep-fat frying of cowpea paste on its total phenolic content (TPC), phenolic composition and antioxidant properties. Four cowpea cultivars comprising two reddish-brown, a brownish-cream and cream phenotypes were used. Liquid chromatography-mass spectrometry was used to determine phenolic composition of the samples. TPC was determined using Folin-Ciocalteu method while radical scavenging capacities were by Trolox equivalent antioxidant capacity, oxygen radical absorbance capacity and nitric oxide scavenging assays. The phenolic acids identified included benzoic and cinnamic acid derivatives. The predominant flavonoid classes were flavan-3-ols and flavonols. Deep-fat frying of the cowpea pastes decreased their TPC, radical scavenging capacities and total quantified flavonoids. The *koose* inhibited radical-induced oxidative cellular and DNA damage. It is concluded that *koose* is a potential functional food that can contribute to alleviating radical-induced oxidative stress.

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain legume and forms an integral part of traditional cropping systems in the semi-arid regions of the tropics (Singh, Ajeigbe, Tarawali, Fernandez-Rivera, & Abubakar, 2003). It is believed to be indigenous to sub-Saharan Africa (Ehlers & Hall, 1997) and is an inexpensive source of plant protein and other nutrients in many developing countries (Giami, 2005; Giami, Akusu, & Emelike, 2001). It is a hardy crop adapted to dry conditions and makes a valuable contribution towards human food and livestock fodder (Singh et al., 2003). Cowpea may be prepared by boiling in water (Nderitu, Dykes, Awika, Minnaar, & Duodu, 2013) and eaten either alone or in combination with starch-based foods (Uwaegbute, Iroegbu, & Eke, 2000). It may also be used to fortify cereal-based weaning foods or processed into flour or paste and used as a food ingredient or starting material for a variety of local foods, such as *moin-moin* and *akara* which are common delicacies in West Africa (Giami et al., 2001).

Koose and *akara* are deep-fat fried cowpea cakes prepared in West Africa. While *koose* is prepared from whole seeds, *akara* is usually prepared from dehulled cowpea seeds. In both cases, the cowpea seeds are soaked in water, dehulled in the case of *akara* preparation, wet milled into a thick paste, and deep fried in vegetable oil into a golden brown colour. Cowpea seeds with lighter seed coat colour, such as Blackeye cultivar, are mostly used since darker seed coat-coloured cultivars produce darker products.

Cowpea is a good source of dietary phenolics mainly phenolic acids (Cai, Hettiarachchy, & Jalaluddin, 2003), flavonoids and anthocyanins (Ojwang, Dykes, & Awika, 2012), and proanthocyanidins (Ojwang, Yang, Dykes, & Awika, 2013). These compounds are reportedly responsible for the antioxidant and other health-promoting properties of cowpea. The effect of boiling (wet cooking) on the phenolic composition and antioxidant properties of cowpea has been reported (Hachibamba, Dykes, Awika, Minnaar, & Duodu, 2013; Nderitu et al., 2013). While some phenolic compounds are heat labile, others remain unaffected by wet cooking (Nderitu et al., 2013). According to Hachibamba et al. (2013), the total phenolic content and radical scavenging properties of some cowpea varieties decrease on boiling while these properties are unaffected in other varieties.

There is lack of information on the effect of other cooking methods such as deep-fat frying on the phenolic composition and antioxidant properties of cowpea. This information is important

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when discussing the health benefits of cowpea, since such cooking methods are applied to cowpea preparation for consumption, especially in developing countries. It also provides alternative preparation methods for cowpea and thus promotes its utilization. The objective of the study was therefore to determine the effect of deep-fat frying on the phenolic composition and antioxidant properties of different cowpea cultivars. This study will provide information on the potential of cowpea in the preparation of functional foods that could contribute to alleviating oxidative stress-related diseases when consumed.

2. Materials and methods

2.1. Materials

Two reddish-brown cowpea cultivars (Glenda, supplied by Agricol, Potchefstroom, South Africa, and Agrinawa, supplied by Premier Seed International, Pretoria, South Africa), a brownish-cream cowpea cultivar (Bechuana white, supplied by Agricol, Potchefstroom, South Africa) and a cream cowpea cultivar (Blackeye, procured from a local supermarket in Pretoria, South Africa), were used in this study. The Blackeye cultivar was used as reference sample for comparison, since it is the preferred cultivar used for making *koose* and *akara* in West Africa. Phenolic standards (gallic, *p*-hydroxybenzoic, protocatechuic, vanillic, syringic, caffeic, *p*-coumaric, and ferulic acids, (+)-catechin, (-)-epicatechin, kaempferol, kaempferol 3-*O*-*β*-d-glucoside, quercetin, myricetin, naringin, rutin hydrate and taxifolin), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), disodium fluorescein, dichlorofluorescein diacetate, Dulbecco's Modified Eagle's Medium (DMEM), and agarose powder were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Tris-acetate-EDTA (TAE) buffer pBR322 vector DNA was purchased from Promega (Madison, WI, USA). GelRed nucleic acid stain was procured from Biotium (Cambridge Bioscience Ltd., Cambridge, UK). Methanol, hydrochloric acid, potassium peroxodisulphate ($K_2S_2O_8$), Folin-Ciocalteu phenol reagent, sodium carbonate, sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium nitrite, sodium hydroxide, sodium nitroprusside, sulphanilamide, and N-(1-naphthyl)ethylenediamine dihydrochloride, aluminium chloride, were all purchased from Merck Chemical Co. (Johannesburg, Gauteng, South Africa).

2.2. Sample preparation

Clean, whole cowpea seeds were divided into three groups. The first group (dry seeds) was milled into flour using a hammer mill (IKA MF10 Laboratory Mill, Werke, Germany) to pass through a 500 μm mesh. The second group was washed two times with potable water and soaked in water for 5 h. The soaked seeds were blended in water into a thick paste (300 g soaked seeds in 200 ml water) using a laboratory blender. The thick paste was freeze dried and milled to pass through 500 μm mesh. The third group was similarly washed and soaked in water for 5 h and blended into a thick paste as the second group. Tablespoon scoops of the thick paste were deep fried in sunflower oil at 180 °C for 3 min. The fried cakes (*koose*) were broken into bits and defatted twice with hexane (200 g *koose* in 300 ml hexane) for 2 h. They were then air dried overnight (12 h, 25-30 °C) in a fume chamber. The defatted and dried samples were milled to pass through 500 μm mesh. Three samples were therefore obtained from each of the cowpea cultivars, and these are dry seed flours, soaked freeze dried seed flours and *koose* (deep-fried products) flours. A flow diagram for the sample preparation is shown in Supplementary Fig. 1. The flours were packed into zip-lock polyethylene bags and stored at −20 °C.

2.3. Extraction of phenolic compounds

Extraction was done according to the method of Kayitesi (2013) with slight modification. Flour of each sample (0.3 g) was extracted with 10 ml 1% (v/v) concentrated HCl in methanol for 2 h under magnetic stirring in a 100 ml capacity conical flask, covered with aluminium foil at ambient temperature (25-27 °C). The suspension was centrifuged at 3000*g*, 4 °C for 20 min and the supernatant collected into a clean centrifuge tube. The residue was extracted two more times, each time with 10 ml of the solvent for 20 min. It was then centrifuged and the supernatant collected as before. The pooled supernatants were aliquoted into 1.5 ml capacity Eppendorf tubes, and stored at −20 °C for analysis of total phenolic content and antioxidant properties. For phenolic composition determination, 1 g flour was extracted with 10 ml 1% (v/v) HCl in methanol under magnetic stirring and rinsed twice with 10 ml of the solvent as before. The pooled supernatant was concentrated under rotary evaporation (35 °C and 74.5 kPa) to 4 ml volume and centrifuged at 7500 g, for 5 min under ambient temperature (25–27 °C). The supernatant was removed and filtered through 0.2 μm Acrodisc PSF syringe filter (Pall Life Sciences, Ann Arbor MI, USA) for analyses.

2.4. Phenolic composition

The phenolic composition of the samples was determined according to the method of Apea-Bah, Minnaar, Bester, and Duodu (2014) using a Waters Acquity Ultra-Performance Liquid Chromatograph coupled to a Waters Synapt G2 Quadrupole-Time of Flight mass spectrometer (Milford, MA, USA). Identification was done by comparing phenolic compounds in extracts with external phenolic acid and flavonoid standards as well as comparison of mass and UV spectral data with phenolic compounds reported in literature. Quantification was done by comparing integrated peak areas of phenolic compounds in the extracts at 280 nm with that of the authentic standards. Leucine enkephalin (molecular weight 555 Da) was used as lock mass. Data were acquired using MassLynx v. 4.1 software (Waters, Milford, MA, USA).

2.5. Total phenolic content

Total phenolic content (TPC) of the samples was determined as described by Apea-Bah, Minnaar, Bester, and Duodu (2016) using a 96-well microplate reader (Multiskan FC, Thermo Fisher Scientific, Shanghai, China). (+)-Catechin was used as a standard and results were expressed as milligram catechin equivalents per gram flour (mg CE/g), dry weight basis.

2.6. Total flavonoid content

Total flavonoid content (TFC) of the samples was determined on a 96-well microplate by the $AlCl_3$ method of Herald, Gadgil, and Tilley (2012), as modified by Apea-Bah et al. (2016). The modification involved using reverse pipetting for mixing the reaction mixture before and after addition of NaOH, and absorbance was measured at 450 nm. Results were expressed as mg CE/g flour, dry weight basis.

2.7. Trolox equivalent antioxidant capacity

The method described by Apea-Bah et al. (2016) was used. Incubation of the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\bullet+}$) stock solution (7 mM ABTS and