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Total phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed Kenyan indigenous food ingredients

Catherine N. Kunyanga[a,1], Jasper K. Imungi[a,2], Michael W. Okoth[a,3], Hans K. Biesalski[b,4], Vellingiri Vadivel[b,*]

[a] Department of Food Science, Nutrition and Technology, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya
[b] Institute for Biological Chemistry and Nutrition, University of Hohenheim, 14 Emil-Wolff strasse, D-70593, Stuttgart, Germany

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ABSTRACT

Certain indigenous foods commonly consumed by Kenyan vulnerable groups (the malnourished; children under 5 years of age; pregnant and lactating women; malnourished adults in cases of vitamin or mineral deficiencies, TB, diabetes, cancer, AIDS; refugees; orphans the elderly and the disabled) are not yet evaluated for phenolic content and health relevant functionality. The present study was therefore designed to analyze the phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed food ingredients. The total phenolic contents of the cereals, legumes, oil seeds and vegetables were ranged from 0.41 to 3.00 g/100 g DM. Amaranth grain (*Amaranthus cruentus*) and drumstick leaves (*Moringa oleifera*) exhibited significantly higher phenolic content than the other samples. The methanolic extract of the investigated samples showed promising levels of DPPH radical scavenging activity (81–89%); ferric reducing/antioxidant power (FRAP, 44–744 mmolL^{-1} Fe[II]/g extract DM); α-amylase (10–45%) and α-glucosidase (13–80%) inhibition activities. The food ingredients with high phenolic content exhibited relatively higher antioxidant and antidiabetic activities. The results indicate that soaking + cooking is the mild processing method to preserve the phenolic compounds and their health relevant functionality in the presently investigated cereal, legume and oil grains, while cooking is suitable treatment for vegetables.

1. Introduction

In recent years, there has been increasing interest in consumption of plant-based foods including traditional staple foods which are widely perceived to possess health promoting benefits. These foods have a strong epidemiological link to reduce the risk of cardiovascular diseases, neuro-degenerative diseases and certain types of cancer (Anwar, Latif, Ashraf, & Gilani, 2007). Currently, there is a resurgence of consumption of indigenous vegetables to curb micronutrient deficiencies in Kenya. The consumption of such vegetables improves the nutrient quality of cereal-based diets through provision of affordable vitamins and minerals, especially provitamin A, vitamin C and iron (FAO/WHO, 2004). Therefore, indigenous cereals (finger millet and amaranth grain); legumes (pigeonpea and field bean); oil seeds (groundnut, sunflower and pumpkin seed); vegetables (pumpkin, butternut and sweetpotato) and leafy vegetables (drumstick, pumpkin and amaranth leaves) are playing a major role in attaining nutritional security of the low-income and vulnerable groups in Kenya (Neumann et al., 2003).

Plant foods are rich in macro- and micro-nutrients as well as bioactive compounds, and have been recognized as a major source of dietary antioxidants with potential therapeutic benefits (Prior & Cao, 2000). The non-nutritive health-promoting bioactive components present in foodstuffs have the potential to exert beneficial effects against certain chronic diseases such as diabetes, obesity, cardiovascular diseases and cancer (Art & Hollman, 2005). At present, the natural bioactive compounds like phenolics, carotenoids, phytates, isothiocyanates, phytosterols, phytoesterogens, organosulphur *etc* have been reported to exhibit many health benefits including excellent antioxidant property (Halvorsen et al., 2002). Among the various bioactive substances, phenolic

* Corresponding author. Tel.: +49 711 459 22948; fax: +49 711 459 23822.
E-mail addresses: nkirotekatie@yahoo.com (C.N. Kunyanga), imungijk@yahoo.com (J.K. Imungi), mwokoth@uonbi.ac.ke (M.W. Okoth), Biesal@uni-hohenheim.de (H.K. Biesalski), vadivelvellingiri@gmail.com (V. Vadivel).
[1] Tel.: +254 722873357.
[2] Tel.: +254 721468181.
[3] Tel.: +254 722870653.
[4] Tel.: +49 711 459 24112.

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compounds which are plant secondary metabolites and have been proven to exhibit many health protective effects, have received most attention (Vita, 2005). Phenolic compounds present in food ingredients such as cereals, legumes and vegetables are demonstrated to exhibit potential antioxidant (Prior & Cao, 2000), antimicrobial (Tapiero, Tew, Ba, & Mathe, 2002), anticancer (Fresco, Borges, Diniz, & Marques, 2006), anti-obesity (Hsu, Huang, & Yen, 2006), antidiabetic and anti-hypertensive (Randhir & Shetty, 2007) as well as anti-mutagenic properties (Islam, 2006). Epidemiological studies have also been suggested a positive role played by phenolics in the alleviation of oxidative stress and prevention of free-radical mediated diseases (Halvorsen et al., 2002).

For the last two decades, supplementary feeding programmes have been conducted by Non-Governmental Organizations (NGO's) like United Nations International Children's Emergency Fund (UNICEF) and United States Agency for International Development (USAID) to nourish the vulnerable groups in Kenya (Marchione, 2002). To formulate the supplementary foods, different food ingredients have been used on the basis of their nutritional profiles. However, due to the increasing incidence of many oxidative-stress related diseases, particularly Type II diabetes, it has been considered essential to include the bioactive compounds such as phenolics in the formulation of supplementary foods. Hence, the quest for natural food products with substantial antioxidant and antidiabetic characteristics has been the main focus of recent research efforts (Islam, 2006; Matsui et al., 2004). The outcome of such research on indigenous foods as in the present study could be vital to nutritionists, food manufacturers as well as consumers in formulating supplementary foods for vulnerable groups.

In Kenya, cereals and legumes are usually soaked + cooked or roasted, whereas the vegetables are cooked or blanched before use. During such thermal processing operations, the phenolic content and its functionality may be altered among different food ingredients and even within the same food and can result in either decrease (Randhir, Kwon, & Shetty, 2008) or increase of antioxidant activity of plant foods (Granito, Paolini, & Pérez, 2008). Hence, appropriate processing method should be established for each food ingredient in order to increase the health promoting properties of phenolic compounds in Kenyan indigenous food ingredients.

Even though few studies have reported the nutritional value of various indigenous food ingredients consumed by vulnerable groups in Kenya (Neumann et al., 2003), only limited information is available regarding their phenolic content and health relevant properties. Hence, the purpose of the present study was to determine the phenolic content, antioxidant and antidiabetic properties of methanolic extracts of certain selected raw and traditionally processed Kenyan indigenous foods. This was done to identify the elite food ingredient(s) with potential health benefits and also to select an optimal processing method(s) in order to utilize them in the formulation of supplementary foods for vulnerable groups living in Kenya.

2. Materials and methods

2.1. Chemicals

(+)-Catechin (Ref. No. 22-402-2), 2,2′-Diphenyl-1-picryl hydrazyl (DPPH, Ref. No. 217-591-8,), 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ, Ref. No. 3682-35-7), 4-Nitrophenyl-α-D-glucopyranoside (PGPP, Ref. No. 3767-28-0), Butylated Hydroxytoluene (BHT, Ref. No. 204-881-4), Polyvinylpolypyrolidone (PVPP, Ref. No. 25249-54-1), Starch (Ref. No. 232-679-6), α-Amylase (Ref. No. 9001-19-8) and α-Glucosidase (Ref. No. 9001-42-7) were obtained from Sigma Company, St Louis, MO, USA and all other chemicals were purchased from Merck, Darmstadt, Germany.

2.2. Sample collection

The samples were collected on the basis of food consumption pattern of vulnerable groups in Nairobi, Kenya. Samples included cereals such as finger millet (*Eleusine coracana* L. Gaertn. P-224) and amaranth grain (*Amaranthus cruentus* L.); legumes such as pigeonpea (*Cajanus cajan* (L.) Millsp. Kat/Mbaazi 3) and field bean (*Dolichos pupureum* L. Kat/DL-3); oil seeds such as groundnut (*Arachis hypogea* L.), pumpkin seed (*Cucurbita maxima* Duch. L.) and sunflower seed (*Helianthus annuus* L. PAN 7369). The vegetables selected were pumpkin (*C. maxima* L.), butternut (*Juglans cinerea* L.), sweetpotato (*Ipomoea batatas* [L.] Lamk. SPK 004) and leafy vegetables such as drumstick leaves (*Moringa oleifera* L.), amaranth leaves (*Amaranthus hybridus* L.) and pumpkin leaves (*C. maxima* Lam.). The food ingredients (1 kg each) were purchased from the local open-air market at Kangemi and Uchumi supermarket in Nairobi, Kenya.

2.3. Processing of cereals, legumes and oil seeds

The grains (cereals, legumes and oil seeds) were each randomly divided into three batches of 100 g each. The first batch constituted the control sample and was stored as such without any treatment. The second batch was washed with tap water and then soaked in 200 ml distilled water for 8 h in the dark at 25 ± 1 °C, then cooked at 90–95 °C for 120 min in fresh distilled water in the ratio of 1:4. The third batch was roasted in an open-pan iron container to a golden brown color for 30 min using traditional charcoal burner at 150 °C with continuous stirring to avoid burning of the seed coat. After cooking and roasting, the samples were cooled to room temperature.

2.4. Processing of vegetables

The fresh vegetables were randomly divided into three batches of 100 g each and the first batch was used as control. The second batch was cut into small pieces or cubes of 1 mm^3 each and immediately transferred to a vessel containing tap water in order to avoid oxidation. Then the samples were washed under running tap water and cooked in 200 ml of distilled water at 90–95 °C for 5 min. However, the sweetpotato, pumpkin and butternut were cooked for 15 min instead of 5 min to soften them. The vegetables in the third batch were cut into pieces (4 mm cubes or strips) and immediately transferred a vessel containing tap water to avoid oxidation and then blanched by immersing in boiling water for 5 min.

2.5. Preparation of methanolic extract

All the raw and differentially processed samples were dried in hot-air oven at 50 °C for 6 h and then powdered using a laboratory hammer mill (Type DFH48, No. 282521/UPM 6000, Glen Creston Ltd, London, England) and sieved through 100 micron sieve before analyses. The samples were defatted by adding petroleum ether in 1:10 ratio (w/v) and kept in an ultrasonic bath for 30 min. After centrifugation at 7558× *g* for 5 min, the supernatant was discarded and the residue was air-dried. One gram of defatted flour was sequentially extracted with 10 ml of HCl–methanol (1 ml/100 ml), methanol-water (80 ml/100 ml) and methanol-water (50 ml/100 ml) in an ultrasonic bath (Bandelin Sonorex, RK – 514 H, Berlin, Germany) for 30 min. After centrifugation at 7558× *g* for 5 min, all the supernatants were pooled and made up to a known volume (50 ml). The extract was purified by treating with 1 g of PVPP at 0 °C for 30 min and then the contents were passed through a Solid Phase Catridge (Strata-x-33 um polymeric sorbent, 8B-S100-FCH-S, Phenomenex, Aschaffenburg, Germany) and eluted with 10 ml of