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EFFECT OF BLANCHING CONDITIONS AND SOLAR DRYING ON SELECTED NUTRITIONAL, ANTI-NUTRITIONAL AND BIOACTIVE COMPONENTS OF FORMULATED AVOCADO SEED POWDER

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ABSTRACT

Avocado (*Persea americana* Mill) seeds are mainly considered as waste by-products of avocado fruit processing in many countries, including Tanzania despite their potential health and economic benefits. The present work investigated the effect of hot water blanching and solar drying treatments on nutritional, bioactive, and cyanogenic glycosides of avocado seed samples in an attempt to develop quality avocado seed powders (ASPs) by using solar drying technology. Results showed that total ash, crude protein, crude fiber, total phenolic, proanthocyanidins, and cyanogenic glycosides contents showed a significant reduction in avocado seed powders with respect to increased blanching temperature and time. On the other hand, crude fat and total carotenoids contents, showed a decline with respect to increased temperature only. Blanching treatment at 85 °C for 10 minutes retained a significant amount of total ash (86.9%), crude fat (100%), crude protein (78.8%), crude fiber (91.6%), total carotenoids (43.6%) and total phenol contents (47.0%) in developed avocado seed powder. Cyanogenic glycosides were highly reduced at 95 °C for 20 minutes (81.1%) than at 85 °C for 10 minutes (58.5%). However, the level of cyanogenic glycosides in all avocado seed powders are far below the lethal dose (36 mg/100 g) of hydrocyanic acid. The significant high losses of nutrients and phytochemicals was observed at a blanching treatment of 95 °C for 20 minutes. This could be due to prolonged leaching of soluble compounds to hot water and degradation of heat-sensitive bioactive compounds. The aforementioned results revealed that, different temperatures and times during hot water blanching significantly influence the quality of formulated avocado seed powder. Therefore, hot water blanching treatment at 85 °C for 10 minutes and solar drying technology could be employed to produce a nutrient-rich and palatable avocado seed powder that can find its use in normal food consumption and food processing industries, particularly in low income settings.

Key words: Avocado seed, avocado seed powder, solar drying, hot water blanching, utilization

INTRODUCTION

Avocado (*Persea americana* Mill) fruit, a native of Central America and Mexico is widely cultivated due to its nutritional and economical values. The processing of avocado fruits generates a large quantity of waste by-products (notably the seeds and peels), which cause serious environmental pollution [1]. Avocado seeds represent about 13% to 17% of the avocado fruit and are rich in various nutritional components, namely; polysaccharides, lipids, proteins, minerals, vitamins [2]. Additionally, the seeds contain significant amount of bioactive compounds such as; polyphenols (phenolic acids, flavonoids, and proanthocyanidins), vitamins (C and E), and carotenoids with several biological activities, including; antioxidant activity, anticancer activity, neuroprotective activity, anti-inflammatory activity, antimicrobial activity, and anti-diabetic activity [3,4].

On the other hand, seeds of avocados are known to contain anti-nutritional components such as alkaloids, saponins, tannins, and cyanogenic glycosides [5]. For instance, cyanogenic glycosides are water-soluble, natural plant toxicants that upon hydrolysis are converted to hydrogen cyanide, a potent respiratory inhibitor [6]. Signs of acute cyanide poisoning in humans include; headache, nausea, vomiting, abdominal cramps, metabolic acidosis, coma, respiratory depression, and death [7]. Moreover, avocado seeds contain polyphenol oxidase, an oxidative enzyme which is responsible for reddish brown color and astringent taste in crushed seeds. Nowadays, there is a global tendency towards utilization of avocado seed powder in food formulations due to its good nutritional and phytochemical profile [8]. In view of the increasing and emerging number of entrepreneurs who process and pack avocado seed powders in Tanzania, and other developing countries, little information exist on the anti-nutritional and functional properties leading to strong concern about the safety and quality of locally processed avocado seed powder [9,10]. Proper processing techniques that remove or reduce the levels of anti-nutrients while retaining the nutritional and bioactive compounds in avocado seed powder should be addressed to people, particularly in rural communities.

Several studies reported different processing techniques such as soaking, boiling, fermentation, microwave heating, or their combination to sufficiently remove or reduce the concentration of anti-nutritional compounds to acceptable safe levels, while retaining nutritional and bioactive compounds [11,12,13,14]. Hot water blanching process removes anti-nutrients through rupturing of the plant cell matrix, followed by leaching of soluble compounds into the blanching medium [15]. In addition, blanching influences both chemical and physical properties of plant material. The changes depend on the time duration, temperature, and the method of blanching [16,17]. However, drying technologies such as solar drying, hot air drying and freeze drying could further influence changes on blanched plant material. Currently, there is limited knowledge on the combination of hot water blanching and solar drying of avocado seeds. The present study is aimed at investigating the effect of hot water blanching conditions at various temperatures and times, and solar drying on nutritional, bioactive, and anti-nutritional compounds in avocado seeds. Understanding the optimal processing conditions and their effects is of paramount importance in order to efficiently add value to avocado seeds.

MATERIALS AND METHODS

Study Area and Design

Fresh avocado fruits were obtained in three districts of Kilimanjaro region: Hai (3° 09' 60" S; 37° 09' 60" E), Rombo (3° 00' 60" S; 37° 27' 59" E) and Moshi rural (3° 20' 5" S; 37° 20' 25" E). The region is located in Northern Tanzania, and characterized by a typical tropic climate, with a 'wet season' controlled by the inter-tropical convergence zone. The study area was purposely selected due to its prominence in growing local and exotic avocado varieties. Split plot design was used with block variety divided into three equal plots, and then samples were randomly collected in each plot.

Material Preparation and Processing

Exactly 320 avocado fruits of the Hass variety from a local cultivar (diameter 35-45 mm) were purchased from local markets of three districts. The local cultivar was selected for its dominance and being readily available in the Kilimanjaro region. The fruits were cleaned, the pulp was separated, and the resulting seeds were sliced into small pieces using a multifunctional kitchen shredder. The resulting slices were soaked in water for 5 minutes to reduce the effect of seed enzymes in color change.

The sliced avocado seeds were loosely tied in a muslin cloth and blanched in a thermostatically controlled double water bath at different temperatures and times. The processing conditions of blanching were chosen according to Chobpattana [18]. The established sample treatments were: T_1 = Non-blanching and solar dried; T_2 = Blanched at 85 °C for 10 minutes and solar dried; T_3 = Blanched at 85 °C for 20 minutes and solar dried; T_4 = Blanched at 95 °C for 10 minutes and solar dried; T_5 = Blanched at 95 °C for 20 minutes and solar dried. Samples were immediately cooled for 5 minutes in an ice water bath preceded by a draining process at ambient temperature (25 °C) for 5 minutes. The resulting samples were dried using a natural convection mixed solar drier at an average temperature of 55.56 ± 1.22 °C for 72 hours to a constant weight, followed by grinding into a powder using a grinder with grinding knives and passed through a 0.5 nm sieve to obtain fine powder.

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Subsequently, the dried samples were packed in labeled polyethylene zip-lock bags and stored in a refrigerator at 4 °C prior to analysis.

Analysis of Proximate Composition

The proximate composition was performed in avocado seed powders (ASPs). The moisture content, total ash, crude fat, crude protein, and crude fiber were determined by following the standard procedures 950.01, 942.05, 920.39, 945.01, and 985.29, respectively, according to The Association of Official Analytical Chemists (AOAC) [19].

Determination of Total Carotenoids Content

The total carotenoid content of ASPs was extracted and determined as described by Karnjanawipagul *et al.* [20] with minor modifications. Briefly, 0.5 g of ASPs was weighed and extracted with 50 mL of acetone (acetone refrigerated at -40 °C for 2 hours prior to analysis) using a homogenizer placed under the fume. About 40 mL of petroleum ether was put into a separating funnel and acetone was added. Distilled water was added slowly along the neck without shaking to avoid emulsion formation. After phase separation, the lower aqueous layer was discarded, and the upper layer was washed three times with distilled water (50 mL) to remove residual acetone. The upper phase was recovered, dried over anhydrous sodium sulphate and the absorbance was determined at 450 nm (UV/Vis spectrophotometer), and concentration of carotenoids in ASPs was calculated based on the standard curve of β-carotene and expressed as μg beta-carotene equivalents per g of dry weight (μg/g DW).

Determination of Total Phenolic Content (TPC)

The TPC were determined by the spectrophotometric method described by Arslan *et al*. [21] with some modifications. Exactly 10 mg of ASPs was extracted with 20 mL of aqueous methanol (50%) at 80 ºC for 1 hour, followed by filtration and volume made to 50 mL. Then, 1 mL of the prepared solution was put into a 50 mL volumetric flask and 20 mL of distilled water was added followed by 2.5 mL of Folin-ciocalteu reagent and 10 mL of sodium carbonate (17%). The mixture was homogenized and made to 50 mL with distilled water and incubated in a dark place at room temperature (27 ± 1 ºC) for 20 minutes before reading absorbance on UV/Vis spectrophotometer at 750 nm. The concentration of TPC in ASPs were calculated using the standard calibration curve of gallic acid at R^2 = 0.9951 and expressed as mg gallic acid equivalents per g of dry weight (mg GAE/g DW).

Determination of Total Proanthocyanidins Content

The total proanthocyanidin content in avocado seed powders (ASPs) was determined by a vanillin-sulfuric acid method as described by Noorul *et al.* [22]. Briefly, 1 mL of avocado seed extract (0.3 g of ASPs in 50 mL absolute methanol)

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were mixed with 2.5 mL of 1% (w/v) vanillin in absolute methanol followed by adding 2.5 mL of 25% (v/v) sulfuric acid in absolute methanol to undergo vanillin reaction with polyphenols in avocado seed extract. The blank solution was prepared in the same procedure without vanillin. The vanillin reaction was carried out at 26 °C in a thermostatic controlled water bath for 15 minutes. Then absorbance of resulting mixtures was read spectrophotometrically at 450 nm (UV/Vis spectrophotometer). The proanthocyanidins concentration in ASPs was extrapolated from a standard curve of (+)-catechin and expressed as mg catechin equivalents per 100g of dry weight (mg CE/100g DW).

Determination of Cyanogenic Glycosides Content

Cyanogenic glycosides content in ASPs was determined according to the method described by Egbuonu *et al*. [5] with some modifications. Briefly, 2 g of assay sample was mixed with 50 mL of 1% hydrochloric acid in methanol for 24 hours at ambient temperature (27 ± 1 ºC) and homogenized on an orbital shaker. Then, 1 mL of resulting solution was reacted with 5 mL of vanillin reagent solution (1:1 = 4% vanillin: 8% hydrochloric acid in methanol) for 20 minutes and read absorbance at 500 nm using a UV/Vis spectrophotometer. A catechin standard curve was used to calculate cyanogenic glycosides concentrations in ASPs.

Statistical Data Analysis

Sample preparations and measurements were performed in triplicate and data were presented as mean ± standard deviation (SD). The results were statistically analyzed by one-way ANOVA (Analysis of variance) with SPSS Statistics 21.0 (SPSS, Inc., Chicago, IL, USA). Duncan's Multiple Range Test (DMRT) was applied to evaluate the significant differences of the mean values of proximate compositions, total phenols, beta carotene, cyanogenic glycosides and proanthocyanidins at $p < 0.05$.

RESULTS AND DISCUSSION

Proximate composition of avocado seed powders

The proximate composition of ASPs is presented in Table 1. The moisture contents of ASPs ranging from 3.49% to 7.71%. The ASPs from non-blanched seeds had the lowest moisture content, while the ASPs from blanched seeds at 95 °C for 20 minutes presented the highest moisture content. The significant increase of moisture content as a result of blanching in hot water is due to water absorption. Studies by Kachhadiya *et al*. [23] and Szymanck *et al*. [24] observed a similar relationship when blanching sweet corn kernels in hot water. In this study, the moisture contents of ASPs from blanched samples at 85 °C for 10 minutes are considered microbiologically safe and stable during storage. This is in accordance to a study by Tontul and Topuz [25] who recommended a moisture content of about 5% for shelf stability of dried powders.

The total ash content of ASPs ranged from 1.55% to 2.22%. The highest value (2.22%) was recorded in ASP from the non-blanched seed sample. The observed loss of ash content in ASPs from blanched samples may be due to leaching of soluble inorganic salts during hot water blanching as similarly reported by Talabi *et al*. [11]. Further to this, Arslan *et al.* [21] reported that blanching in boiling water caused a significant reduction of water soluble minerals into the immersion media which was accelerated by the heating process. Moreover, longer blanching time appears to further influence the leaching of soluble minerals as both ASPs from blanched seeds for 20 minutes at both 85 °C and 95 °C showed significantly lower ash contents than their counterparts blanched for 10 minutes. However, avocado seeds contain a significant amount of various minerals such as phosphorus, calcium, sodium, potassium, zinc, iron, copper and cobalt, and thus ASPs could be incorporated in animal feed and human nutrition for alleviating micronutrient deficiencies [4].

The crude fat content in ASPs ranged from 1.27% to 1.36% as shown in Table 1. The ASPs from non-blanched seeds was 1.33%. As a result of blanching, the fat content of ASPs from blanched seeds at 85 °C for 10 and 20 minutes showed a slight increase, however, the values remain statistically similar to ASPs from non-blanched seeds. On the other hand, the fat content of ASPs statistically decreased to 1.29% and 1.27% at 95 °C for 10 and 20 minutes, respectively. Hot water blanching had little effect on the content of fat and fat soluble molecules. However, at higher temperature and longer blanching time, derivation of fat molecules might occur making them dissolve easily in water and eventually decrease the total fat content of food material as similarly observed by other researchers [27].

Protein is among the major macromolecules present in avocado seeds [5]. The mean protein content of ASPs from non-blanched seeds was 8.02%. As a result of blanching, it statistically decreased to 6.32% at 85 °C for 10 minutes, to 5.91% at 85 °C for 20 minutes, to 5.73% at 95 °C for 10 minutes, and to 5.36% at 95 °C for 20 minutes. Similar observations of protein reduction in hot water blanching were observed in sweet corn kernels [24] and moringa leaves [27].

The crude fiber content of formulated ASPs ranged from 3.03% (blanched seeds at 95 °C for 20 minutes) to 5.26% (non-blanched seeds). It was observed that fiber content significantly decreased as blanching temperature and time increases. However, no significant changes were observed after blanching seeds at 85 °C for both 10 and 20 minutes. The decrease of crude fiber contents might be due to the leaching of soluble fibers during hot water blanching and accelerated by elevated temperature and longer times. The quantity of fibers which are reported in all formulated ASPs are higher than 2.87%, as reported by Egbuonu *et al*. [5] for avocado seeds.

Bioactive components of avocado seed powders

Table 2 presents the content of carotenoids, total phenols, proanthocyanidins and cyanogenic glycosides in ASPs. Total carotenoids content in ASPs from non-blanched seeds was 3.49 µg/g Dry Weight (DW). As a result of blanching, it dropped significantly ($p < 0.05$) to the lowest value of 0.37 µg/g DW in ASP from blanched seeds at 95 °C for 20 minutes. Further, ASPs from blanched seeds at 85 °C have significantly higher ($p < 0.05$) carotenoids contents than ASPs from blanched seeds at 95 °C. At their respective blanching temperature, carotenoids contents in ASPs slightly decreased ($p > 0.05$) when blanching time increased to 20 minutes. A study by Ishiwu *et al*. [28] reported a sharp decrease (61.4%) in beta-carotene content of tomato pulp as boiling time increased to 30 minutes. The significant decrease of the total carotenoids in blanched ASPs was due to leaching of some carotenoid molecules in the blanching water. Sicari *et al.* [17] found a significant amount of total carotenoids in blanching water, particularly higher than in blanched samples. It is noteworthy that high temperature and light can degrade carotenoids molecules due to their highly unsaturated structure leading to isomerization and oxidation [29]. Therefore, in addition to hot water blanching, solar drying could also contribute to the observed losses of total carotenoid contents in ASPs.

The total phenolic content (TPC) of ASPs ranged from 0.35 to 1.25 mg GAE/g DW, with the lowest value coming from blanched seeds at 95 °C for 20 minutes. The ASPs from non-blanched seeds have significantly higher ($p < 0.05$) TPC with the value of 1.25 mg GAE/g DW than all blanched samples. The TPC significantly decreased ($p < 0.05$) when both blanching temperature and time increased. Similar observations have been reported by several studies [17,30]. Therefore, blanching in hot water and longer time significantly reduce TPC due to thermal degradation and leaching of polyphenolic compounds in water. Additionally, softening of cell walls during hot water blanching increases the efficiency of extraction of polyphenols in water.

Proanthocyanidins (basically condensed tannins) are oligomers or polymers of flavan-3-ols (such as epicatechin, catechin, gallocatechin, epigallocatechin, afzelechin, and epiafzelechin) which are connected by C–C linkages (B-type) or by C–O–C linkages (A-type) [3]. Although proanthocyanidins are sometimes classified as anti-nutrients, their biological benefits as antioxidant, anticancer, anti-obesity, immunomodulatory, cardio-neuro protective are profound Rauf *et al*., [3]. In the present study, the total proanthocyanidins content of ASPs significantly decreased

($p < 0.05$) from 0.027 mg CE/100g DW (non-blanched seeds) to 0.003 mg CE/100g DW (blanched seeds at 95 °C for 20 minutes). The ASPs from blanched seeds at 85 °C for 10 minutes retained more proanthocyanidins than other blanched samples, while ASPs from blanched seeds at 85 °C for 20 minutes and at 95 °C for 10 minutes showed significantly similar ($p > 0.05$) proanthocyanidins content. The observed decline could be due to leaching of low molecular weight proanthocyanidins molecules into the blanching hot water and the extent of losses is positively influenced by blanching temperature and time.

With respect to cyanogenic glycosides, the results revealed that the application of blanching treatments (temperature and time dependent) had a significant ($p < 0.05$) effect on the reduction of cyanogenic glycosides of ASPs. The percentage reduction of 58.49%, 71.70%, 77.36%, and 81.13% in the content of cyanogenic glycosides was observed during T2 (85 °C for 10 minutes), T3 (85 °C for 20 minutes), T4 (95 °C for 10 minutes), and T5 (95 °C for 20 minutes) blanching treatments of avocado seed samples, respectively. The observed losses could be attributed to leaching of free forms of cyanogenic glycosides in blanching medium and acute heat stress which destroy the liberated hydrogen cyanide by volatilization. Several researchers observed similar reduction of cyanogenic glycosides during hydrothermal treatments [14]. Egbuonu *et al*. [5] reported a higher amount of cyanogenic glycosides (1.02 mg/100 g) in non-blanched and sun-dried avocado seeds, in comparison to our current findings. However, the level of cyanogenic glycosides in all ASPs seems to be negligible when compared with the lethal level (36 mg/100 g) of hydrocyanic acid [7]. Thus, it is safe to be included in industrial food formulations, or as part of the daily diet.

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

In this study, the different contributions of hot water blanching temperatures and times on the formulation of ASPs were investigated. Increasing temperature and time during hot water blanching caused substantial variations in proximate, bioactive and anti-nutrient contents of ASPs. Blanching treatment at 85 °C for 10 minutes followed by solar drying proved to be most effective in retaining a significant amount of total ash (86.9%), crude fat (100%), crude protein (78.8%), crude fiber (91.6%), total carotenoids (43.6%) and total phenol contents (47.0%) in developed avocado seed powder. Cyanogenic glycosides were highly reduced at 95 °C for 20 minutes (81.1%) than at 85 °C for 10 minutes (58.5%). This study might provide the basis for hot water blanching and solar drying of avocado seeds to effectively exploit their potentials and opening up possibilities for its use in food industries. However, further studies about the effects of hot water blanching and solar drying techniques on specific food molecules such as fatty acids, phenolic compounds, amino acids, and sugars are required.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Table 1: Proximate composition of raw avocado seed and processed seed powder

Treatments	Proximate components				
	Moisture (%)	Total Ash (%)	Crude Fat (%)	Crude Protein (%)	Crude Fiber (%)
T_1	3.49±0.07[d]	2.22±0.08[a]	1.33±0.03[a]	8.02±0.13[a]	5.26±0.08[a]
T_2	5.45±0.29[c]	1.93±0.01[b]	1.36±0.02[a]	6.32±0.33[b]	4.82±0.07[b]
T_3	6.20±0.20[b]	1.78±0.01[c]	1.35±0.01[a]	5.91±0.03[c]	4.78±0.27[b]
T_4	6.41±0.20[b]	1.63±0.01[d]	1.29±0.01[b]	5.73±0.05[c]	4.37±0.06[c]
T_5	7.71±0.08[a]	1.55±0.02[e]	1.27±0.02[b]	5.36±0.06[d]	3.03±0.24[d]

Means with the same superscript in the same column are not significantly different ($p > 0.05$). T_1 = Non-blanching and solar dried; T_2 = Blanched at 85 °C for 10 minutes and solar dried; T_3 = Blanched at 85 °C for 20 minutes and solar dried; T_4 = Blanched at 95 °C for 10 minutes and solar dried; T_5 = Blanched at 95 °C for 20 minutes and solar dried

Table 2: Bioactive components of avocado seed powder segregated by treatment

Treatments	Bioactive compounds			
	Carotenoids (µg/g)	Total Phenols (mg GAE/g)	Proanthocyanidins (mg CE/100g)	Cyanogenic glycosides (mg/100g)
T_1	3.49±0.39[a]	1.25±0.04[a]	0.027±0.001[a]	0.53±0.120[a]
T_2	1.52±0.03[b]	0.72±0.04[b]	0.017±0.002[b]	0.22±0.130[b]
T_3	1.46±0.40[b]	0.62±0.04[c]	0.01±0.007[c]	0.15±0.001[c]
T_4	0.40±0.03[c]	0.45±0.03[d]	0.01±0.003[c]	0.12±0.001[d]
T_5	0.37±0.03[c]	0.35±0.02[e]	0.003±0.002[d]	0.10±0.001[e]

Means with the same superscript in the same column are not significantly different ($p > 0.05$). T_1 = Non-blanching but solar dried; T_2 = Blanched at 85 °C for 10 minutes and solar dried; T_3 = Blanched at 85 °C for 20 minutes and solar dried; T_4 = Blanched at 95 °C for 10 minutes and solar dried; T_5 = Blanched at 95 °C for 20 minutes and solar dried. All values are per Dry Weight (DW)

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