Assessment of bioactive compounds, physicochemical properties and microbial attributes of hot air-dried mango seed kernel powder: An approach for quality and safety evaluation of hot air-dried mango seed kernel powder

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Abstract

The presence of carbon and nitrogenous compounds in mango processing by-products makes them excellent substrates for the biosynthesis of many microbial metabolites using fermentation processes. Pre-treatment of the substrate with retention of crucial growth supporting compounds is vital for designing and optimizing fermentation media for enhanced production of desired metabolites. The present study investigated the effect of hot air drying (HAD) (50, 60, 70, and 80°C) on the bioactive compounds, physio-chemical and minerals profile, fermentable sugar, and microbial safety of mango (Cv-chausa) seed kernel powder. Results indicated that different drying temperatures non-significantly ($P < 0.05$) affected the carbohydrates, starch (except at 60 and 80°C), nitrogen and protein content. The pH (except at 70°C), total phenolics, and antioxidant activity decreased with an increase in drying temperatures. Inductively coupled plasma-optical emission spectrometry (ICP-OES) analysis revealed the increase in concentrations of majority minerals with incremental drying temperature. The microbial load of powdered seed kernel after 30 days of room temperature storage was within safe limits, as samples were devoid of food pathogens. Briefly, the study suggests HAD (at 70–80°C) to convert mango kernels into stable powdered form for prolonged storage. The powdered kernels can be utilized in diversified food industry and as a feedstock (with safe storability, preserved bioactive, mainly carbon and nitrogen compounds) for biosynthesis of valuable metabolites via microbial fermentation route.

Introduction

Food processing industries generate a vast quantity of by-products/waste that results in severe environmental problems and greenhouse gas emissions (Al Khawli et al. 2019, Karic et al. 2022, Munekata et al. 2022). Food processing waste disposal is a costly affair and increases overall production costs. Food waste contains ample carbohydrate polymers (cellulose, hemicelluloses, starch, pectin, and sugars like glucose, sucrose, and fructose), protein, oil, minerals, and fat (Pateiro et al. 2020, Lai et al. 2022). Consequently, they have immense biotechnological potentialities, can be valorized into many value-added products, and can be utilized in a broad array of microbial and enzymatic processes. However, their utilization is limited, possibly due to the scanty understanding of their nutritional and economic importance (Kannah 2020).

Mango (*Mangifera indica* L.) is a widely cultivated fruit crop in the tropical and subtropical regions of the globe and is annually growing at 2.7% (Nadeem et al. 2016). In India, it is commercially cultivated with more than 1,500 varieties. The “chausa” cultivar is extremely sweet and mainly grown for table and processing purposes in north Indian states like Uttar Pradesh, Bihar, Punjab, Himachal Pradesh, and West Bengal (Directorate of Marketing and Inspection 2013). Consumers highly appreciate mango owing to the presence of health-beneficial substances, such as dietary fiber, mineral elements, phenolic and antioxidants, vitamin C, and carotenoids (Lamilla et al. 2021). To improve shelf-life, mangoes are processed into various storable products such as concentrated juice, puree, jam, and chutney (Lamilla et al. 2021). Mango processing industries generate 25–40% by-products, mainly in mango peel and seed, which could be utilized in the circular economy concept (Gómez-Caturla et al. 2022). Peel constitutes 15–20% of the whole fruit weight (Serna-Cock et al. 2015), while seed constitutes from 20–60% of the entire fruit mass. Mango seed kernel (MSK) represents approximately from 45–75% of the total seed’s weight. Globally, mango seed is among the foremost agro-industrial wastes, with the approximate generation of 123,000 metric tons of seeds annually (Reddy et al. 2016). The extraction of bioactive compounds and the development of value-added products from mango processing wastes have greatly interested among researchers (Nagel et al. 2014). For instance, carbon and nitrogenous compounds in these byproducts make them excellent substrates for the biosynthesis of many microbial metabolites using fermentation processes. Therefore, enzymatic and microbial technology could facilitate the recycling of mango and other fruits processing waste into numerous industrially important metabolites/compounds. Many valuable compounds such as organic acids, bioethanol, enzymes, biofertilizers, single-cell protein, and biogas can be produced through microbial fermentation by utilizing food waste as a feedstock (Kannah et al. 2020).

To guarantee the continued supply of MSK to various industries, it should be a storable commodity with preserved quality and storability. The main problem in the revalorization of MSK is its limited shelf-life and susceptibility to enzymatic and microbiological degradation due to high moisture. Therefore, MSK has to be processed into a self-stable by-product by quick-drying; hence, it is essential to develop a stabilization process to preserve the keeping qualities and ensure its highest potential reuse. The shelf-life attributes of MSK can be enhanced using the drying approach. The drying of MSK must be rapid and costlier with the preservation of temperature-sensitive high-value compounds (Vásquez-Caicedo et al. 2007; Pott et al. 2003). Further, drying inactivates metabolic enzymes accountable for degrading bioactive compounds and reducing microbial infection. However, drying conditions (such as temperature and time) affects the functioning and stability of bioactive compounds owing to their enzymatic, chemical, and thermal decomposition. Therefore, drying conditions significantly determine the quality of the final product, chiefly in terms of its bioactive and physiological compositions (Dorta et al. 2012).

Convective hot air drying (HAD) is a suitable approach for preserving the storability of high moisture foods. HAD is simple in operation and cost-effective (Ma et al. 2021). However, as reported by several researchers, HAD may induce many changes in the chemometric profile by affecting the bioactive compounds, antioxidant activity, and functional properties of processing by-products (Ancos et al. 2018; Dorta et al. 2012; Sanchez-Camargo et al. 2019; Sogi et al. 2013). Before using MSK as a feedstock (owing to carbon and nitrogenous compound) in the microbial fermentation process, the impact of HAD parameters on biochemical, especially carbohydrates and nitrogen characteristics, nutrient profile, reducing sugar, and other physicochemical changes need to be assessed. These ingredients are pivotal in designing, developing, and optimizing the fermentation process for the surplus production of valuable metabolites. Intensive work has revealed the promising potential of food waste for its bioconversion into various microbial-based bio-products. However, there is no information about the stability of these compounds as affected by different HAD temperatures before their utilization in the microbial fermentation process. Furthermore, most of the previous drying studies on mango processing by-products were performed using a limited range of HAD temperatures (Dorta et al. 2012; Sogi et al. 2013). However, how drying conditions influence the different nutritional and bioactive compounds of MSK was not clearly elucidated. To the best of our knowledge, there is no complete study on how different HAD temperatures affect the bioactive

compounds, physicochemical and functional attributes, minerals profile, microbial safety, and fermentable sugar from the MSK (Cv-chausa and any other varieties). The drying process stabilizes the product and preserves several bioactive constituents. The inappropriate drying pretreatment may often induce physicochemical reactions, resulting in the loss of minerals, bioactive compounds, and textural properties. Therefore, optimizing the drying of MSK would ensure the final dried product has desired quality.

With this background, the present study was conducted to assess the impact of different HAD temperatures (50, 60, 70, and 80 ^{o}C) on (a) the bioactive compounds, physiochemical characteristics, functional attributes, and nutrients profile, (b) the recovery of reducing sugar from dried MSK (using optimized temperature) using acidic pretreatment, and (b) microbial safety of dried MSK powder. These findings will provide the theoretical basis for possible uses of MSK powder as starting substrate (with the stability of carbon and nitrogenous compounds) in the microbial fermentation process, along with their potential use as ingredients in developing functional foods.

Materials And Methods

Raw material and sample preparation

Fruits of mango (cultivar-*Chausa*) were procured from the Agricultural Produce Market Committee (APMC) market, Abohar, Punjab. The selected fruits were semi-ripe, physiologically mature, uniform in shape and size, defect-free and devoid of fungal and insect infestation. Mangoes were immediately brought to the Horticultural Crop Processing laboratory and kept in the cold room (Temp: 10± 2 °C, relative humidity: 60-65%) before further processing. The analytical grade chemicals and reagents (Merck, India) of uppermost commercially available pure grade were used for all the analysis. All the analysis and measurements were performed in triplicates. Physiologically ripe mango fruits were washed 2-3 times with running potable water to remove debris or attached surface particles, followed by air drying at room temperature (25±2 ^{o}C, RH: 60-70%). The peel and stones of mangoes were removed using sterilized stainless-steel knives. Mango pulp was removed and collected in a separate utensil. Subsequently, stones were sundried and the seed kernels were extracted manually using a stainless-steel knife. Before keeping for HAD, seed kernels of equal thickness were uniformly cut sliced into small pieces (1-2 cm) to achieve uniform drying.

Drying experiment

The initial moisture content of seed kernel samples was determined by drying them at 102±0.1 °C to constant weights and bone-dry mass of the sample was determined by hot air oven at 105-110 °C for 8-10 h. The MSK samples of known (≈500 g) weight were spread uniformly in the tray (810 ×400 ×30 mm) and subjected to HAD using thermostatically controlled hot air dryer (Model No-MSW-216, Macro Scientific Works Private Limited, New Delhi, India). The drying experiment was conducted at four different temperatures (50 °C, 60 °C, 70 °C, and 80 °C) with constant fan air flow rate of 1,000 rpm (≈ 1.5 m/s air flow) in triplicate. The drying of MSK was carried out until the equilibrium was reached (AOAC 2005). After drying, the dried seed kernel was powdered using laboratory grinder (1600n Disc code no 640080) and sieved. The powdered MSK samples (average particle size was ≤ 500 µ) were packed in air tight containers and stored at 4 °C. The steps involved in the processing, HAD, preparation of fine powder and characterization of dried mango seed kernel are shown in Figure 1.

Effect of different drying temperature on the carbohydrates and nitrogen characteristics of MSK powder

Total carbohydrates

Total carbohydrates content was estimated as per the method given by Hedge and Hofreiter (1962). 0.1 g MSK powder was hydrolyzed by keeping in the water bath (Model-MSW 274) at temperature 80 ^{o}C for 3 h with 5 mL of 2.5 N HCL (5 mL) and was neutralized with Na_2CO_3 after cooling it to room temperature. Sample volume was made to 100 mL, centrifuged (4,000 rpm for 10 min), and then 0.1 mL aliquot was mixed with 4 mL of anthrone reagent in a test tube. Finally, sample tubes were boiled for 5 min, cooled rapidly, and the intensity of green color was measured at 630 nm using UV-Vis spectrophotometer (UV 2550, Shimadzu Corporation, Kyoto, Japan). Total carbohydrates content was calculated from the graph of the glucose standard curve and calculated values were expressed in percentage (%).

Starch content

Starch content was estimated by the anthrone method (Sadasivam & Manickam, 1992). Exactly 0.1 g MSK powder sample was homogenized in hot 80% ethanol and centrifuged. Then, the pellet was mixed and extracted with 5 mL of water and 6.5 mL of 52% perchloric acid at 4 °C for 20 minutes. Subsequently, the sample was centrifuged (4,000 rpm for 15 min), and 1 mL (0.1 mL of aliquot + 0.9 mL of water) was mixed with 4 mL of anthrone reagent. Finally, sample tubes were boiled for 5 min, cooled rapidly, and the intensity of green color was measured at 630 nm using UV-Vis spectrophotometer. Starch content was calculated from the graph of the glucose standard curve and values were expressed as a percentage (%).

Nitrogen and Protein content

Nitrogen content in MSK powder samples was estimated as per the standard Kjehdahl method (AOAC 2000) using nitrogen estimation system (Model: KEL PLUS Classic DX VATS (E), Pelican Equipments, Chennai, India). After determination, the factor 6.25 was used to obtain the protein content in MP powder samples.

Effect of HAD on physicochemical and functional characteristics of MP powder

Surface acidity (pH)

Surface acidity was measured by determining the pH of the sample. 1 g of dried MSK powder was mixed in 50 mL of double-distilled water and kept in an incubator shaker (150 rpm, 30 °C and 24 h). Subsequently, the mixture was filtered, and its final pH was measured using a pH meter (model- Eutech instruments pH tutor bench meter-2440677) (Pathak et al. 2016).

Ash content

A sample of 1.0 g was placed in a muffle furnace at 580-600 °C for four-six and total ash content was recorded. The residual ash was weighed and was expressed as percentage of mass of ash with respect to mass of the original sample.

Ascorbic acid

Ascorbic acid in the samples was measured as per the method described Nath et al. (2022). The concentration of L-AA was calculated using a standard curve. Measurement was done at 520 nm using UV-Vis spectrophotometer (UV 2550, SHIMADZU CORPORATION, Kyoto, Japan).

Water absorption capacity

The water absorption capacity of MSK powder was estimated by mixing 1.0 g of MSK powder with ten volumes of distilled water. Then, the sample mixture was vortexed properly and allowed to stand at room temperature for 30 min. Later, the samples were centrifuged at 4,000 rpm for 15 min. The sediment was weighed after draining off the supernatant. Water absorption capacity was expressed as the weight of absorbed water (g) per gram of MSK powder sample (Cheng and Bhat 2016).

Oil absorption capacity

1 g MSK powder sample was thoroughly mixed with soybean oil (10 mL) in a pre-weighed centrifuge tube. Then, the sample mixture was vortexed properly and incubated at room temperature for 30 min. Later, the samples were centrifuged (4,000 rpm for 15 min) to separate and remove oil from the sample. Finally, oil absorption capacity was calculated as the amount of oil absorbed (g) per gram of MSK powder (Sosulski et al. 1976).

Total phenolics

Total phenolic content in the MSK powder sample was determined using the Folin-Ciocalteu method (Singleton et al. 1999). 2 g MSK powder was extracted twice with 30 mL of 80% ethanol by stirring for 30 min in the dark. Then, the obtained homogenate was centrifuged (4,000 rpm for 20 min), and the supernatant was used as sample extract to estimate total phenolics and antioxidant activity. 0.2 mL of sample extract was mixed with 1 ml of 1:10 diluted Folin-Ciocalteu reagent and allowed to stand for 5 min. Then, the sample mixture was neutralized using sodium carbonate (0.8 mL) followed by incubation (25 ºC for 2 h). Finally, the absorbance was measured at a 725 nm UV-Vis spectrophotometer. The experiment was conducted in triplicate, and results were expressed as gallic acid equivalent (mg GAE/g sample).

Antioxidant activity

The antioxidant activity of the MSK powder samples was determined from the radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Scherer & Godoy, 2009). 0.1 mL of MP powder sample (ethanolic extract) was added to a 3.9 mL ethanolic solution of DPPH (0.2 mM). The samples mixture was incubated for 30 min at room temperature in a dark place. Finally, the absorbance was read at 517 nm, and the results were expressed as percent inhibition of the DPPH radical calculated according to the following equation:

$$\text{DPPH inhibition (\%)} = \frac{\text{ABS(DPPH)} - \text{ABS(Sample)}}{\text{ABS(DPPH)}} * 100$$

Where ABS_{DPPH} is the absorbance of the DPPH solution without extracts and ABS_{Sample} is the absorbance of the sample solution.

Mineral analysis using inductively coupled plasma-optical emission spectrometry (ICP-OES)

Mineral analysis of dried MSK using ICP-OES (Thermo Fisher Scientific, UK) was performed at citrus estate laboratory, State Horticulture Department, Abohar (Punjab), India. Samples (0.2 g) were acid digested using a commercial high-pressure laboratory microwave oven (Multiwave Microwave 3000, Anton Paar, Austria) operating at 2450 Hz frequency with 900 watts (W) of energy output. Mono-elemental and multi-elemental stock solution (100 mg/L) of the high-purity grade used in the analysis was purchased from Merck (Darmstadt, Germany). The instrument ICP-OES was furnished with a solid-state detector, mist chambers (Stumar-master), and nebulizer (V-groove). The operating conditions of the ICP-OES included radio frequency incident power: 1.15 kW; plasma argon flow rate: 15 L/min; auxiliary argon flow rate: 0.5 L/min and nebulizer argon flow rate: 0.6 L/min. Based on the earlier interference, the emission lines showing low interference with high analytical signal and background ratios were chosen for mineral analysis. An internal standard was used for all ICP-OES measurements in order to quantify the elemental composition of the samples. The mineral concentrations in the samples were expressed in mg/kg (Dukare et al. 2020b).

Color analysis

Color attributes of MSK powder samples were determined using a spectrophotometer (Model No. Y54580, Shenzhen Three NH Technology Co., Ltd., China) in terms of CIE (*Commission Internationale de L'Eclairage*) L* (lightness and darkness), a* (redness and greenness), and b*(yellowness and blueness). The instrument was calibrated with a white and a black tile before actual measurements.

Recovery of reducing sugar from powdered peel: optimizing dilute acid as a pre-treatment for maximum reducing sugar recovery from stable peel powder

Based on the overall assessment of carbohydrates, nitrogen, physicochemical qualities and nutritional profile of different hot dried MSK, stable MSK powder (dried at 70 ^{o}C) showing enhanced and promising retention of these attributes were further selected for obtaining maximum fermentable sugar. The effect of different concentrations (1, 2, 3, 4 and 5%) of acid treatment such as hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) on the recovery of reducing sugar from MSK powder was estimated. Reducing sugar content in MSK powder was determined by dinitrosalicylic acid (DNS) method (Miller 1959). In test tubes, 3 mL of MSK powder extract was thoroughly mixed with the 3 mL of DNS reagent and kept in boiling water bath (model-MSW274) for 5 min for color development. Then, 1 mL of 40% Rochelle salt was added in still warm test tubes, followed by cooling under running tap water. The absorbance of the solution was measured at 575 nm using spectrophotometer. Starch content was calculated from the graph of the glucose standard curve and expressed as µg/mL of extract.

Microbiological analysis

The population size of cultivable microorganisms from the dried MSK powder was estimated using standard growth media and incubation conditions. Roughly 10 g of MSK powder was aseptically suspended in 90 mL of sterilized saline diluents (SD; 0.85%, w/v NaCl), in 250 mL conical flasks and shaken vigorously (at 150 rpm for 30 min) to facilitate the release of microbes into SD. Thereafter, sample suspension was subjected to serial dilutions and an aliquot of 0.1 mL from respective diluted tubes was plated in triplicate on the respective solidified agar media.

The quantity of total viable plate count (TVC) was enumerated (CFU/g) via the plate counting method on plate count agar (Standard Methods Agar) media comprising tryptone (5 g/L); yeast extract (2.5 g/L); dextrose (1 g/L); agar (15 g/L) with final adjusted pH: 7.2 ± 0.2 at 25 °C. The media was supplemented with cycloheximide (1 µg/mL). The fungal count (CFU/g) was determined by plating suspension on the potato dextrose agar media comprising potato infusion (20 g/L), dextrose (20 g/L), and agar (20 g/L), supplemented with antibiotic **chloramphenicol** (25 mg/L). Plates were incubated at 37 °C for 24 hrs and 25 °C for 48 h for TVC and fungi, respectively. The quantity of *Salmonella* species and *Escherichia coli* was enumerated (CFU/g) via the plate counting method on the standard recommended media. Colony counts were done after incubating plates at standard incubation conditions recommended for each different organism group.

Statistical analysis

All the quality parameters were measured in triplicate and means were reported. Duncan's multiple range test (DMRT) and ANOVA was performed to test the statistical differences in these properties as affected by different processing conditions. SPSS software (version 16.0) was used to conduct the tests. The significance was accepted at 5% levels of significance (P<0.05).

Results And Discussion

Drying Time and Residual Moisture Content

The initial moisture content of MSK varied between 36 and 38% (wb). The MSK were dried to final moisture content ranged from 6.29±0.17 to 9.00±0.35% (wb). The time required for drying of MSK varied significantly with an increase in hot air-drying temperature. For instance, the time taken for drying of seed kernels to a desired final moisture content at 70 °C and 80 °C drying temperature were 472 and 390 min, respectively. The lowest drying temperatures (50 °C and 60 °C) took more time to dry MSK to reach equilibrium moisture content (data not shown).

The result indicated that an increase in drying temperatures noticeably reduced the time required for drying of seed kernel till moisture reaches to the equilibrium. The increased drying temperatures rapidly decreased moisture from produce, which further accelerates the moisture migration out of the food (Mphahlele et al. 2019). Similar findings have been obtained concerning drying of various agricultural by-products such as olive cake (Vega-Gálvez et al. 2010), mango seed kernels (Ekorong et al. 2015), pomegranate peel (Mphahlele et al. 2019), and prickly pear seed (Motri et al. 2013) using different temperatures. Further, the average drying rate (data not shown) was more significant at the beginning of the drying process, conceivably due to evaporation of moisture from the seed surface, which subsequently declined with falling moisture content for all the drying temperatures.

Carbohydrates characteristics

The effect of different drying temperatures on the total carbohydrates and starch content of MSK was evaluated and results are represented in Table 1. The increasing drying temperatures has no significant effect (P<0.05) on carbohydrates content of MSK. The carbohydrate content varied between 71.32±3.30 and 79.20±2.04%. The starch content in MSK increased significantly (P<0.05) after subjecting to drying with increasing temperatures. Drying at higher temperatures (70 and 80°C) had significant positive effect on starch content of MSK as compared to lower drying temperatures (50 and 60 °C). The highest starch content (72.98±5.55%) was observed in MSK samples dried at 80 °C, while the lowest value (44.41±6.82%) was recorded in MSK samples dried at 50 °C.

The experimental results indicated that the seed kernel of mango (Cv-chausa) is a good source of carbohydrates and starch. The obtained values of total carbohydrates in powdered MSK after drying at different hot air temperatures were comparable with previous reported findings. For instance, carbohydrates content in the air-drying (soaking for 30 min, followed by boiling for 15 min in water, then finally drying at 65 ^{o}C for 16 h) and pretreated flour of dried seed kernel of mango was found to be 72.07% (Das et al. 2019). Similarly, carbohydrate content of 73% was reported in mango seed kernels, which were subject to blanching and drying (at 85 ^{o}C for 24 h) (Uzombah et al. 2019). The composition of dietary fibers in the mango processing by-products depends on both the cultivar and the fruit ripening stage (Ajila et al. 2008). In the present study, the obtained values of starch content (ranged from 44 to 72%) in seed kernel of mango after was reported similar to the reported by previous researchers (Patiño-Rodríguez et al. 2020; Ferreira et al. 2019; Tesfaye et al. 2018). It is evident that starch content in seed kernel manly depends on the genotype and growing climatic conditions. The categorization of MSK starch is chiefly based on the size of its granules, shape, and proportion of amylase and amylopectin (Mwaurah et al. 2020). Digestibility studies on starch have revealed the presence of more of resistant starch than readily and slowly digestible starch (Sandhu and Lim 2008). Resistant starch can be absorbed in the small intestines, hence; it gets fermented by the microbiota in the large intestines (Patiño-Rodriguez et al. 2020). The extraction starch can be used as stabilizers, thickeners, production of alcohol, and in the cosmetic, paper, and textile industries. Concerning reducing sugar, to the best of knowledge, there is no report on how reducing sugar from MSK get affected by different drying temperatures. However, the extraction of reducing sugar from the extract of seed kernel has been reported (Bangar et al. 2021). The amount of reducing sugar recovered decides the efficiency of pretreatment method and will aid in making microbial fermentative metabolite production more economical (Premjet et al. 2018).

Nitrogen and protein characteristics

Nitrogen content (%), hence the protein content of dried MSK samples varied non-significantly across the drying temperature conditions. Nitrogen content in the studied MSK samples ranged from 0.97±0.15% (at 60 °C) to 1.01±0.19% (at 80 °C). The highest protein content (6.30±1.18%) was found in the MSK dried at 80 °C (Table 1). The analyzed powdered samples of MSK showed a reasonable amount of nitrogen and protein content. However, different drying temperatures had no significant (P<0.05) impact on nitrogen and protein (%) in MSK flour. In the case of the effect of drying on protein, the more or less similar value of protein has been reported in MSK dried using different hot air temperatures. For instance, MSK showed the protein values of 8.3% (dried at 65 °C for 16 h), 7.76% (drying at 50 °C), and 6.20% (drying at 85 °C for 24 h) (Das et al. 2019; Ashoush and Gadallah 2011; Uzombah et al. 2019). Previous studies indicated that MSK flour contains reasonable quantities of proteins (from 6 to 7.76%) (Nzikou et al. 2010; Olajumoke 2013).

Protein quality and essential amino acid index of MSK are high, showing the standard quality of the proteins. The bioavailability of MSK protein can be positively compared with the standard protein obtained from eggs (Abdalla et al. 2007). Protein content in mango by-products such as peel and MSK can be correlated with pectin modification during the fruit maturity stages. Besides use as ingredients in functional food development, MSK, being a good source of nitrogen and protein, can be used as feedstock increased production of microbial polyhydroxybutyrate polymer (McAdam et al. 2020).

Effect of different drying temperature on different physicochemical characteristics

Physicochemical characteristics of MSK powder prepared after drying 50-80 °C temperatures are given in Table 2. Significant change in ash content of MSK was not observed after drying at 50-70 °C, however, the ash content was significantly lower for MSK dried at higher temperature. The pH of MSK powder solution changes due to the leaching of MSK compounds to the solution. Specific trend was not observed in surface pH of MSK dried at increasing temperature, however the values were significantly (P<0.05) different at each treatment level. Highest pH value of 5.44 was observed for MSK dried at 70 °C. Ascorbic acid content (mg/100 g sample) was lowest in samples dried at lower temperature an increased significantly (P<0.05) after drying at 60 °C and remained unaffected by increasing drying temperature till 80 °C. Similar trend was also observed in water holding capacity of the MSK powder which remained unaffected by increasing drying temperature after 60 °C. Significant (P<0.05) reduction was observed in OHC of MSK powder as hot air temperature increased from 50 °C to 60 °C. The OHC values increased significantly (P<0.05) with drying temperature increasing from 70 °C to 80 °C.

Surface pH provides information about the change in pH of water when a known amount of MSK is added to it. The changes in pH occur due to the leaching of seed kernel compounds to the solution. In foods, ash content denotes the amount of mineral content as an inorganic portion (Kaur and Srivastav 2018) and as an incombustible solid material. The level of ash (0.70-1.5%) obtained in the present study is comparable to the previous research findings (Okpala and Gibson-Umeh 2013; Ashoush and Gadallah 2011). In the present study, ascorbic acid (2.45-3.27 mg/100 g) values in seed kernels dried using different temperatures are comparatively less than those previously reported (Sogi et al. 2013; Mwaurah et al. 2020). Sogi et al. (2013) studied the effect of drying on the ascorbic acid content of MSK. They reported a significant difference in the ascorbic acid content of MSK subjected to hot air (at 60 °C), vacuum (at 60 °C), infra-red, and lyophilized drying. The drying methods also affect the ascorbic content, and higher drying temperatures reduce the ascorbic acid content (Somsub et al. 2008). At higher drying temperatures, ascorbic acid is rapidly oxidized to dehydroascorbic acid, converted to 2, 3-diketogulonic acid, and finally, polymerized to other nutritionally inactive compounds (Nath et al. 2022). WHC and OHC are the important functional properties of mango kernel. WHC is mainly relies on the amount and types of the hydrophilic constituents, to some extent on the pH and nature of the protein (Owuarnanam et al. 2013). Additionally, several other factors such as porosity charge dependency and pectin structure can influence the WHC of food (Shivamathi et al. 2022). In a similar way, OHC of food is due to its hydrophilic and overall charge density constituents (Bayar et al. 2018). Similar to our findings, the water retention (1.22 g/g) and oil retention capacity (0.94 g oil/g) was reported in the thermal pretreated (Soaking for 30 min followed by boiling for 15 min and drying at 65 °C for 16 h) flour of MSK (Das et al. 2019).

Total phenolics and antioxidant activity

The effect of different HAD temperatures on phenolics and antioxidant content of powdered seed kernel is presented in Figure 2. The total phenolic content of MSK decreased gradually from 8.33±0.23 mg GAE/g to 4.98±0.03 mg GAE/g with increasing drying air temperature from 50 to 80°C. The reduction was significant (P<0.05) at all the drying temperature levels except at highest drying temperature of 80 °C. The drying temperature had significant (P<0.05) negative impact on antioxidant activity of the MSK powder. The antioxidant activity measured in terms of DPPH % scavenging assay significantly (P<0.05) reduced from 80.69±2.76% (at 50 °C) to 61.15±0.76% (at 80 °C).

The phenolics and antioxidant metabolites are the groups of bioactive compounds that perform specific biological actions besides being used as functional food ingredients (Granato et al. 2020). Natural antioxidants act against oxidative stress, reactive oxygen species, and free radicals produced by the body during diverse metabolic processes (Ma et al. 2011, Pateiro et al. 2021). MSK is a good source of phenolic compounds and antioxidants (Castro-Vargas et al. 2019). In our findings, both phenolics and antioxidant activity of MSK were reduced with an increase in HAD temperatures. Similar results on the negative effect of drying on phenolics and antioxidants action of MSK have been reported. For example, hot air oven drying reduced the total polyphenolic content in MSK from 1.20 mg/g (at 40 °C) to 0.20 mg/g at 80 °C (Ekorong et al. 2015). A similar trend was also noticed in the case of total antioxidant activity (Ekorong et al. 2015). In mango by-products, phenolic compounds such as xanthones and flavonoids are susceptible to degradation at higher temperatures (Ancos et al. 2018). At higher temperatures, phenolic compounds are reduced in MSK, probably due to their degradation caused by chemical and enzymatic action and thermal decomposition. Additionally, the possible explanation for the reduction in phenolics content at higher temperatures is a gradual inactivation of polyphenol oxidase (Dibanda et al. 2020). The antioxidant activity is correlated positively with their bioactive compounds, namely with phenolic compounds (Dorta et al. 2012). Thus, MSK subjected to higher drying temperatures has reduced antioxidant activity and phenolic content (Dibanda et al. 2020).

Mineral elemental profile affected by different drying temperatures

The results on the effect of different drying temperatures on the major and micro minerals of MSK powder are tabulated in Table 3. The observed concentrations of major nutrients studied were much higher than micronutrients. Though, the specific trend in terms of concentrations of minerals was not visible. The result indicated that concentrations (mg/kg of the sample) of most analyzed nutrients increased with increasing temperature. For instance, MSK samples dried at 50 °C showed the highest concentrations of K (3,438.5±65.50 mg/kg sample) and Ca (726.50±11.30 mg/kg sample). The highest concentrations of P (1,810.50±34.50 mg/kg sample), Mg (1,395.00±15.00 mg/kg sample), and S (888.75±14.15 mg/kg sample) was observed in samples dried at 70 °C. While the maximum concentrations of all micronutrients such as Fe (80.62±2.58 mg/kg sample), Mn (11.23±0.04 mg/kg sample), and Zn (8.01±0.18 mg/kg sample) was observed in MSK dried at 80 °C. The observed concentrations of nutrients in MP in decreasing order were as follows: K>P>Mg>S>Ca>Fe>Mn>Zn.

The ICP-OES investigation revealed the presence of macro (K, P, Ca, Mg, and S) and microelements (Fe, Mn, and Zn) in all MSK subjected to drying at different HAD temperatures. These nutrients have been detected in the seed kernel of various cultivars of mangoes (Mwaurah et al. 2020). MSK is an acceptable source of minerals and could be used to develop functional foods to alleviate micronutrient deficiency. These nutrients have a pivotal role in human body metabolism. Additionally, MSK with varied concentrations of mineral nutrients may be exploited to design and standardize the fermentation media for higher production of specific microbial metabolites such as polyhydroxy butyrate. The current outcome of the study supports the findings related to the presence of several macro (K, P, Mg, S, and Ca) and micro (Fe, Zn, Mn, etc.) minerals in the MSK (Lasano et al. 2019). However, the reported values for some analyzed mineral nutrients were lower than the findings of the present investigation. The differences in these results could be attributed to the distribution of vascular tissue, sink characteristics, and metabolic rate of the plants (Lasano et al. 2019). The increase in mineral concentration in the by-products of the dried fruit following drying treatment has been reported (Rafiq et al. 2019; Mohammed et al. 2020). This could be due to the excessive desiccation and the substantial increase in dry matter of the dried produce (Mohammed et al. 2020). In addition, Suna et al. (2014) reported that the dry matter and mineral nutrients in dried produce correlated positively.

Effect of different drying temperature on color profile of MP powder

As seen from the colour profile of MSK powder (Table 4) the L* values of mango stone kernels showed non-significant (P<0.05) marginal increase with increasing drying temperatures. Browning index was significantly higher for kernels dried at 80 °C as compared to the lower drying temperatures. Whiteness index of MSK powder bears great importance as it may directly affect the color of starch being extracted as end product. Whiteness index of powder was significantly higher for MSK dried at lower temperature of 50 and 60 °C and decreased significantly with increase in drying temperatures till 70 °C. WI values showed significant (P<0.05) decrease with increasing drying temperature beyond 70 °C. Browning index of powder was significantly (P<0.05) higher for MSK dried at 80 °C as compared to lower drying temperatures owing to higher L* values and lower a* and b* values. The obtained color values are more or less similar to that of reported by Das et al. (2019). Slight deviation in the results may be due to the varietal difference, determination process and error.

Optimization of dilute acid treatment for recovery of reducing sugar from dried stable powdered peel

The suitability of using dilute acids for optimum extraction of reducing sugar from MSK (dried at 70 °C) is expressed in Figure 3. Use of increasing concentrations (from 1% to 3%) of HCl has negative correlation with extraction of reducing sugars from MSK wherein, the values decreased from 398.80±12.49µg/mL to 348.17±13.75µg/mL. However, as the HCl concentration increased to 4 and 5%, the observed values for extraction were significantly (P<0.05) higher (416.22±34.71 µg/mL and 415.78±21.81µg/mL, respectively). The ability of HCl at 4 and 5% concentration to assist higher extraction of reducing sugars from MSK did not vary significantly (P<0.05), and 4% HCl solution was found optimum for the task. Varying concentrations

of dilute H_2SO_4 has non-significant (P<0.05) effect on extraction of reducing sugars from dried MSK. Different levels of dilute H_2SO_4 were able to extract reducing sugars in the range from 364.41±93.53 µg/mL to 374.72±50.50 µg/mL.

The powdered MSK samples pretreated with varying concentrations of dilute acids improved recovery of reducing/fermentable sugars. To the best of our knowledge, there are no scientific studies dealing with the recovery of reducing sugar from hot air dried MSK powder. However, few researchers have attempted to analyze reducing sugar obtained from fruit processing waste using either acid or alkaline pretreatment. Reddy et al. (2011) also tried to recover the fermentable sugar from mango peels. In the present study, dilute acid hydrolysis pretreatment was used to break down the hemicellulosic and lignocellulosic components of MSK. Dilute acid as a pretreatment can enhance simple sugar release from lignocelluloses biomass. Dilute acid is a promising technique to convert hemicelluloses into monomeric sugars by modifying the chemical structure of lignocelluloses biomass. This pre-treatment yields better extraction of fermentable sugars from fruit processing by-products and other agro biomass (Fernandes et al. 2021). The preliminary treatment of biomass achieves pre-fermentation bioconversion, enabling the availability of sufficient substrate for the activity of fermentative microorganisms. Furthermore, more cellulose in the biomass is made more accessible to the microbial/enzymatic action (Chaudhary et al. 2021), which eliminates the need for cellulase/hemicellulase enzymes mixtures required for the breakdown of cellulose and hemicelluloses. The recovered fermentable/reducing sugar efficiently increases microbial metabolism to produce the surplus amount of desired metabolites of industrial importance. Bacteria and yeast efficiently metabolize fermentable monosaccharides (such as glucose and fructose) as a carbon source. Reducing sugar is a good carbon source in fermentation to synthesize several industrial metabolites from microbes (Fabricio et al. 2022).

Microbial analysis/safety of MSK powder

After 30 days of ambient storage, only a bacterial population (total plate count, CFU/g) was observed among the microbes under consideration (Table 5). A bacterial population, within the safe limit, was observed only in samples dried using 50 and 60 °C. On the contrary, population count of other microbes such as fungi (CFU×10^3/g), *Salmonella* spp. (CFU×10^2/g), and *E. coli* (CFU×10^2/g) was nil for all MSK samples dried at different temperatures.

Mango processing wastes are generally susceptible to microbial attack, probably due to high moisture content and bioactive compounds, limiting their reuse in the food industry (Ajila et al. 2007; Sogi et al. 2013). The drying process can overcome this limitation as pretreatment. Drying limits, the activities of microbes and enzymes accountable for degrading MSK and improves safe storage and transportation. The presence of antimicrobial activities in the MSK has been reported. In the current study, MSK samples dried at higher temperatures (60-80 °C) were devoid of any significant microbial presence. Their absence could be linked to the higher drying temperature, lack of moisture, or substrate's antimicrobial nature. The potent antimicrobial activity in South African MSK against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans* has been demonstrated (Ahmed et al. 2005). The antimicrobial and antibacterial property of MSK and plant-based compounds is probably due to polyphenolic, flavonoids, tannins, terpenes, and coumarin compounds (Mutua et al. 2016). The capability of different phenolic compounds in inhibiting rot-causing fungi in multiple foodstuffs has been documented (Dukare et al. 2020a). Further, convective HAD can significantly limit the activity of mesophilic bacteria and bacterial pathogens growing on processed products of fruits and vegetables (Alp and Bulantekin 2021). In food processed using higher drying temperatures, microbial growth is restricted probably due to the cell wall damage and protein denaturation (Alp and Bulantekin 2021).

Conclusions

The collection, storage, transportation, and pretreatment of biomass-based feedstock constitute major expenses for a biorefinery. As a result, standardizing these unit operations should prove beneficial. The present study demonstrated the effectiveness of convective HAD for quicker drying of MSK (Cv-chausa) with retention of most bioactive and physiochemical constituents, minerals, fermentable sugar, and enhanced microbial safety. Even though values were slightly different, carbohydrates (total sugar, starch, and reducing sugar) and nitrogenous attributes (nitrogen and protein) were non-significant after drying at different temperatures. The majority of the physicochemical qualities (pH, ascorbic acid, total phenolics, and antioxidant) were adversely affected by increase in drying temperatures, revealing their heat labile nature. On the contrary, concentrations of majority minerals enhanced with increment in drying temperature, possibly due to excessive desiccation and sizeable dry matter increase. The dry matter and mineral contents in dried produce were correlated positively. Regarding microbial safety, microbial count in all samples was within the safe limit after 30 days of ambient storage. Based on the comprehensive analysis, we suggest (1) possible use of convective hot air (at 70–80°C) for drying and conversion of MSK into stable powdered form for prolonged storage (2) powdered seed kernel (with safe storability, preserved bioactive, mainly carbon and nitrogen compounds) as a potential feedstock for round the year use in the microbial fermentation process, and (3) optimized acid-based pretreatment for recovery of reducing/fermentable sugar from the powdered seed kernel (dried at 70°C). Briefly, preserving key food ingredients that are vital for microbial growth and augmenting the bioavailability of simple sugars of the feedstock at a fixed pretreatment will lessen overall capital and operating costs of fermentation process targeted for the biosynthesis of industrially important metabolites. This study can be considered a potential reference for future investigations to using dried (at 70–80°C) peel as a substrate for microbial growth during their biotransformation and value addition of agro-horticultural based processing waste/by-products.

Declarations

Author Contributions:

A.D.: Conceptualization, methodology, investigation, validation, formal analysis, writing-original draft preparation; M.K.S.: methodology, investigation, validation, formal analysis, contribution in writing; B.B.: methodology, investigation, validation, formal analysis and contribution in writing in relevant

section; S.D.: data analysis, writing-review and editing, final draft supervision and monitoring. M.K.: review and editing, final draft supervision and monitoring; J.M.L.: review and editing, final draft supervision and monitoring. All authors read and approved the final manuscript.

Declarations

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Conflicts of Interest:

In this research, authors are declaring no conflict of interest.

Availability of data and material:

All the data used in the manuscript are available in the tables and figures.

Code availability:

Not applicable.

Ethics approval:

Not applicable.

Consent to participate:

All authors has given their full consent to participate.

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Tables

Table 1

Effect of different hot air-drying temperatures on the carbohydrates and nitrogen characteristics of powdered MSK

Drying temp.	Carbohydrates and nitrogen characteristics			
	Total carbohydrates (%)	Starch (%)	Nitrogen (%)	Protein (%)
50 °C	71.32±3.30[a]	44.41±6.82[a]	0.99±0.23[a]	6.19±1.42[a]
60 °C	71.60±3.06[a]	53.51±2.27[a]	0.97±0.15[a]	6.06±0.94[a]
70 °C	79.20±2.04[a]	69.82±3.38[b]	0.98±0.06[a]	6.13±0.35[a]
80 °C	72.43±10.20[a]	72.98±5.55[b]	1.01±0.19[a]	6.30±1.18[a]

*Values are the mean of triplicate determinations ± standard deviation. Different letters denote statistically significant differences (P<0.05, DMRT test)

Table 2

Effect of different drying temperatures on different physicochemical and functional characteristics of MSK powder

Drying temp.	Physicochemical qualities				Functional qualities	
	Residual moisture (%)	Ash (%)	Surface pH	Ascorbic acid (mg/100gm sample)	Water holding capacity (%)	Oil holding capacity (%)
50 °C	8.66±0.17[a]	1.37±0.02[a]	5.33±0.00[c]	2.45±0.82[b]	1.33±0.01[a]	0.86±0.02[a]
60 °C	9.00±0.35[a]	1.22±0.22[a]	5.29±0.01[a]	3.27±0.00[a]	1.20±0.02[b]	0.62±0.00[b]
70 °C	6.29±0.17[c]	1.50±0.05[a]	5.44±0.00[d]	3.27±0.00[a]	1.20±0.00[b]	0.57±0.02[b]
80 °C	7.42±0.13[b]	0.70±0.35[b]	5.31±0.00[b]	3.27±0.00[a]	1.19±0.01[b]	0.77±0.09[a]

*Values are the mean of triplicate determinations ± standard deviation. Different letters denote statistically significant differences (P<0.05, DMRT test)

Table 3

Effect of different drying temperatures on mineral content of MSK powder

Drying temp.	Major nutrients (mg/kg)					Minor nutrients(mg/kg)		
	P	K	Ca	Mg	S	Fe	Mn	Zn
50 °C	1752±31.00^{a}	3438.5±65.50^{a}	726.50±11.30^{a}	1350.00±27.00^{ab}	825.35±8.95^{b}	68.44±0.54^{b}	10.84±0.28^{a}	6.43±0.11^{a}
60 °C	1662.50±77.50^{b}	3256±162.00^{b}	726.15±31.45^{a}	1296.00±56.00^{b}	826.65±32.65^{b}	69.72±9.39^{b}	10.79±0.43^{a}	7.58±1.88^{a}
70 °C	1810.50±34.50^{a}	3372.5±36.50^{ab}	708.35±48.15^{a}	1395.00±15.00^{a}	888.75±14.15^{a}	62.26±2.05^{b}	11.15±0.20^{a}	6.60±0.19^{a}
80 °C	1747.50±03.50^{ab}	3423±3.00^{ab}	681.65±7.65^{a}	1367.00±5.00^{a}	870.65±4.45^{a}	80.62±2.58^{a}	11.23±0.04^{a}	8.01±0.18^{a}

*Values are the mean of triplicate determinations ± standard deviation. Different letters denote statistically significant differences (P<0.05, DMRT test)

Table 4

Effect of different drying temperatures on color attributes of MSK powder

Drying temp	Color profile of MSK powder				
	*L** value	*a** value	*b** value	*Whiteness index (WI)*	*Browning Index (BI)*
50 °C	68.94±7.41^{a}	4.61±0.34 ^{a}	33.73±3.12 ^{a}	44.49±5.54^{a}	275.37±11.49^{b}
60 °C	73.16±0.60^{a}	5.86±0.64 ^{a}	26.29±1.29 ^{a}	27.25±2.73^{b}	337.33±20.80^{b}
70 °C	70.47±0.51^{a}	3.23±1.42^{c}	10.49±3.72^{b}	13.80±3.15^{c}	343.93±91.41^{b}
80 °C	73.12± 1.07^{a}	4.50±2.29^{b}	7.64±6.34^{b}	5.80±4.94^{c}	555.20±54.76 ^{a}

*Values are the mean of triplicate determinations, *L**-Lightness or darkness, *a**-redness or greenness, *b**-yellowness or blueness. *Values are the mean of triplicate determinations ± standard deviation. Different letters denote statistically significant differences (P<0.05, DMRT test)

Table 5

Microbial load of mango seed kernel powder prepraed by drying at different temperatures

Drying temp.	Total plate count (CFU×10^4/g)	Fungal count (CFU×10^3/g)	*Salmonella* count (CFU×10^2/g)	*E. coli* (CFU×10^2/g)
50 °C	0.33±0.34^{a}	nd	nd	nd
60 °C	0.33±0.33^{a}	nd	nd	nd
70 °C	nd*	nd	nd	nd
80 °C	nd	nd	nd	nd

*nd- not detected

Figures

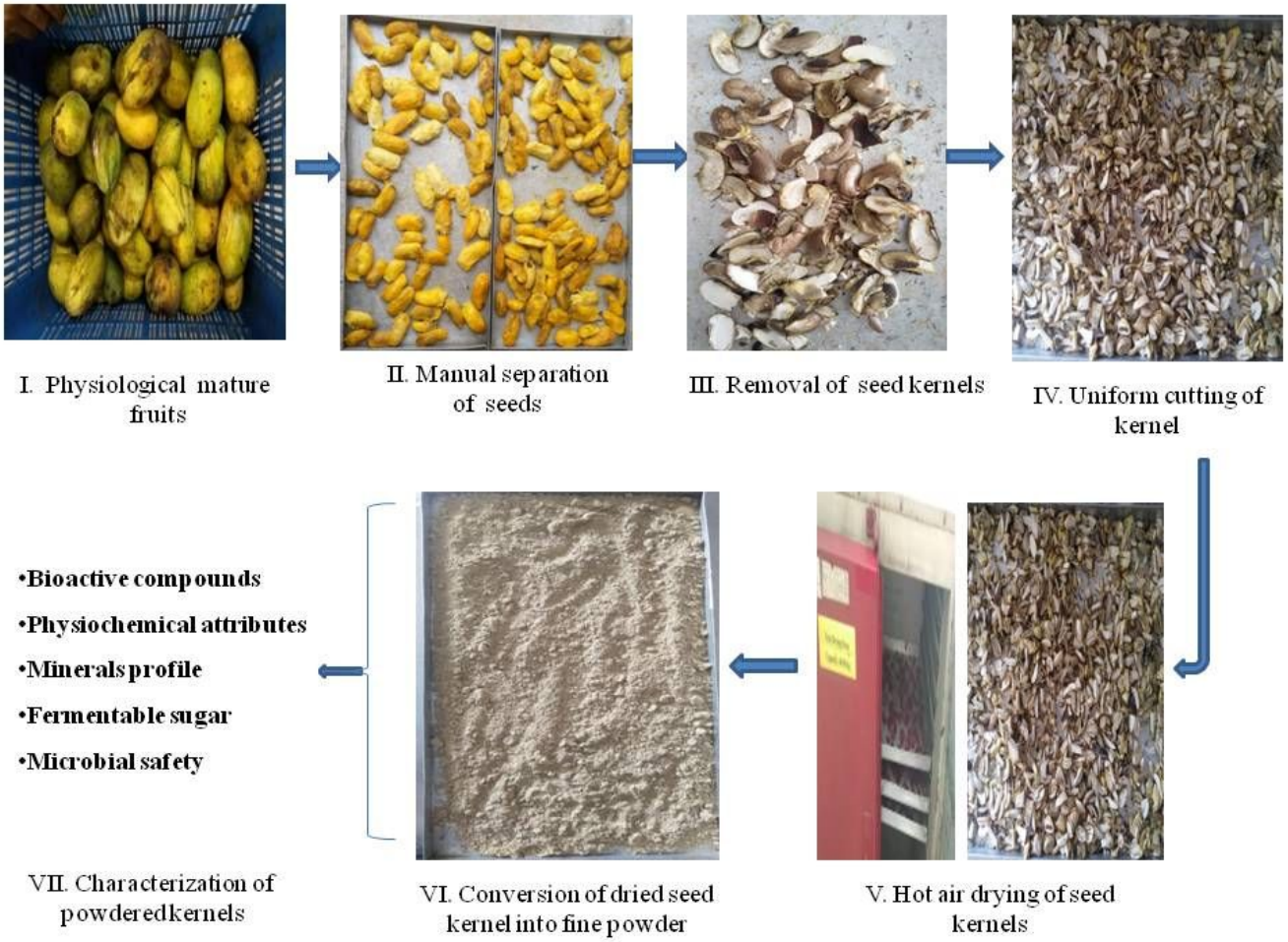

Figure 1

Pictorial charts showing the steps involved in the raw material processing, hot air drying, and characterization of dried mango seed kernel

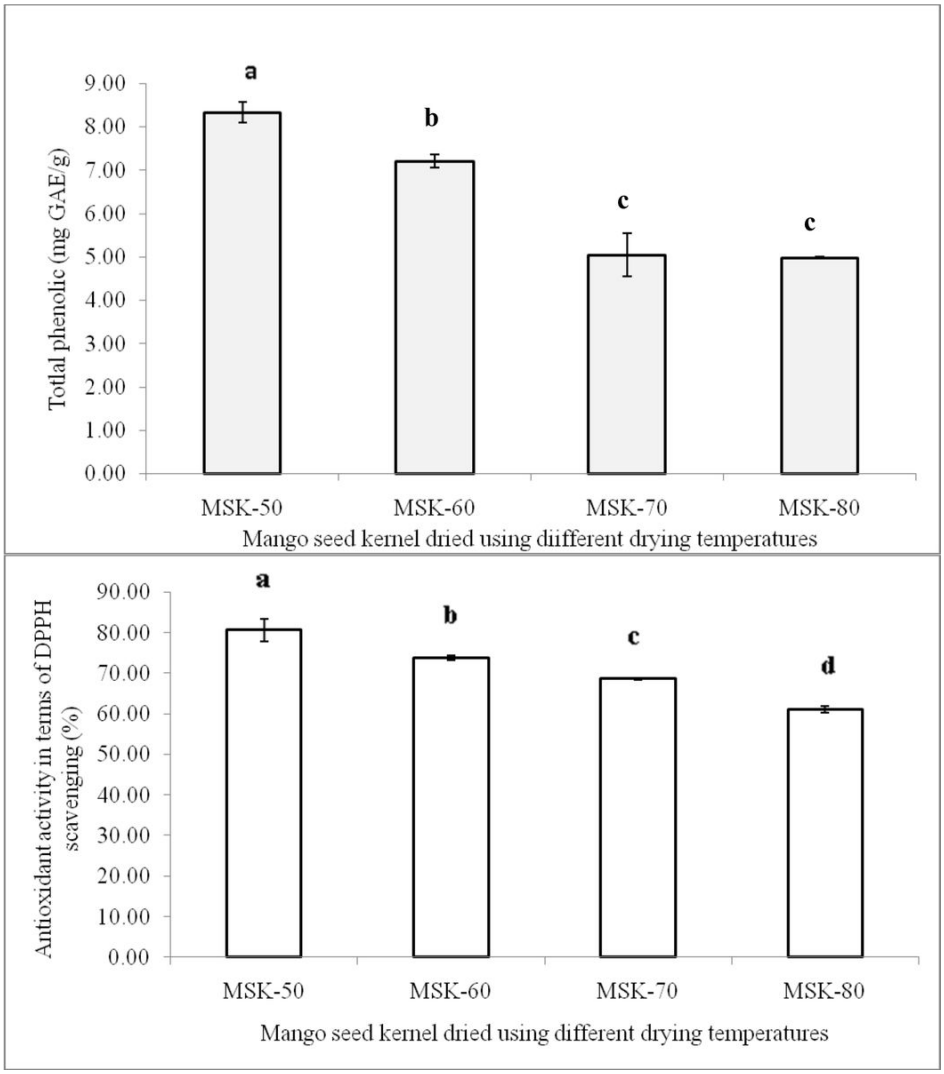

Figure 2

Total phenolics and antioxidant activity in dried mango seed kernel as affected by different hot air-drying temperatures (50, 60, 70, and 80 ^{o}C)

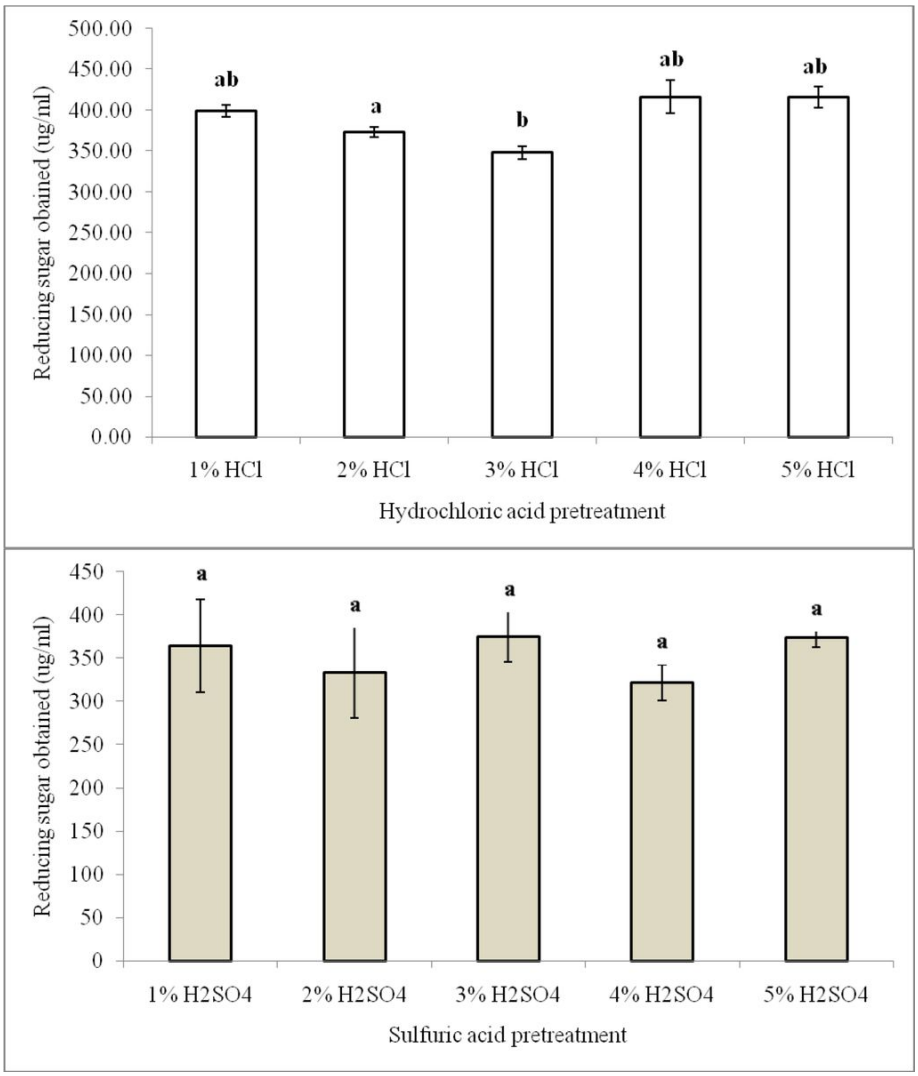

Figure 3

Effect of different concentrations of hydrochloric and sulfuric acid on the recovery of reducing sugar from powdered seed kernels (dried at 70 ^{o}C)