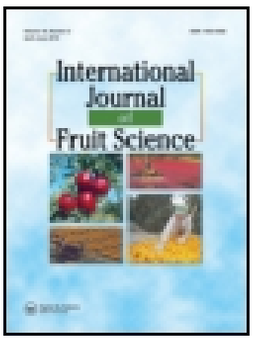

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Antioxidant Potential, Physico-chemical, and Sensory Attributes of Cookies Supplemented with Mosambi Peel Extract

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ABSTRACT

The core objective of the present investigation was to isolate and quantify antioxidants from mosambi (sweet orange) peel. In the proposed research, different solvents were used for the extraction of antioxidants from mosambi peel. Among these solvents, ethanol resulted in better antioxidant yield compared to aqueous extract. Ethanolic extract of mosambi peel showed higher total phenolic contents (19.3 ± 0.3 mg/g) as compared to aqueous extracts (18.2 ± 0.04 mg/g). Considering the safety health concern, aqueous extract of mosambi peel was incorporated in cookies at different concentrations of 1%, 2%, and 3%, and further subjected to physico-chemical and sensory attributes. Proximate composition (moisture, crude protein, crude fat, crude fiber, ash, and niitrogen-free extract (NFE) contents) of fortified cookies varied non-significantly. However, sensory evaluation rated aqueous extract of mosambi peel (2%) with better hedonic response. Mosambi peel antioxidants have a great potential to be used in various functional foods and the ability to improve storage stability.

KEYWORDS

Mosambi peel; antioxidant activity; 2,2-diphenyl-1-picrylhydrazyl (DDPH); sensory attributes; fortified cookies

Introduction

Natural antioxidants are gaining popularity because of their health promoting perspectives and limited side effects (Suleria et al., 2015). For this reason, consumers are focusing more toward natural products as compared to synthetic (Siró et al., 2008; Sultan et al., 2011). In the recent era, various research investigations have been carried out to explore numerous plants and their by-products for the isolation and purification of antioxidant bioactives like phenolic acids, anthocyanins, flavonoids, carotenoids, coumarins, tannins, and lignins (Manach et al., 2004; Rates, 2001). In developing countries like Pakistan, huge amounts of food processing wastes are generated

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annually. These processing wastes can be a substantial source of functional ingredients if managed properly, e.g., the citrus industry produces lot of waste mainly in the form of peel, which accounts for almost 50% of the total fruit weight (Raskin et al., 2002).

Citrus, belonging to family *Rutaceae*, has different variations like sweet orange, bitter orange, persium lime, lemon, and grapefruit. Among them, mosambi (sweet orange) is rich in naturally occurring flavonoids present in the peel portion. The mosambi peel is comprised of an appreciable quantity of flavonoids, i.e., polymethoxylated flavones and glycosylated flavanones. Plant flavonoids, including flavanones, flavanone glycosides, and polymethoxylated flavones, are generally located in the leaves, seeds, and flowery portion. Plant flavonoids possess health-promoting characteristics because of their prominent antioxidant activity (Gundgaard et al., 2003; Hashimoto et al., 2002). These flavonoids of mosambi peel act as antioxidants and are capable of preventing or inhibiting oxidation processes in food systems (Taguri et al., 2004). Lipid peroxidation in food products not only deteriorates their quality by oxidative spoilage but also generates free radicals and reactive oxygen species (ROS), which are dangerous for human health (Decker et al., 2005).

Antioxidants extracted from mosambi peel have a great potential to be utilized in various food products. Accordingly, many promising techniques have been introduced for their extraction, such as solvent, enzyme aided, and supercritical extraction processes. In the current research project, a concerted effort has been made to extract and quantify antioxidant fractions from mosambi peels using a solvent extraction technique (ethanol and water). The antioxidant potential of extracted fractions was estimated through total polyphenols, antioxidant activity, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Moreover, cookies were developed using different concentrations of mosambi peel extract and their physico-chemical and sensory attributes assessed.

Materials and methods

The current investigation was carried out at the National Institute of Food Science & Technology, University of Agriculture, Faisalabad, Pakistan. Mosambi peel was washed and then dried using an oven-dried method at 60 °C. Afterwards, the dried sample was ground to make a fine powder and sieved through a 30-mesh sieve. Finally, prepared peel powder was examined for moisture, ash, crude fat, crude protein, and crude fiber using methods of the AACC (2000).

Preparation of antioxidant extracts

The prepared powder was used to extract antioxidants using a solvent extraction method. Powder and solvents (ethanol and water) at a

concentration of 1:5 were maintained and placed on an orbital shaker followed by centrifugation at 7000 rpm for 15 min at 4 °C. The supernatant was filtered through Whatman filter paper while the solvent was separated by using a rotary evaporator (Eyela, Tokyo, Japan). The resultant antioxidant extracts were stored at 4 °C for further analysis (Imran et al., 2015).

Total phenolic content (TPC)

Total phenolics were determined by Folin-Ciocalteu method (Sun et al., 2007). Accordingly, 50 µL of extract was added to a test tube containing 250 µL of Folin-Ciocalteu's reagent, 750 µL of 20% sodium carbonate solution, and volume was made up to 5 mL with distilled water. After 2 h, absorbance was measured at 760 nm using a UV/visible light spectrophotometer (CECIL CE 7200, Cecil Instruments Limited, Cambridge, UK) against control. Total polyphenols were recorded and values were computed in gallic acid equivalent (mg gallic acid/100g).

Antioxidant activity

Antioxidant activity based on coupled oxidation of ß-carotene and linoleic acid was calculated by using the method of Taga et al. (1984). In this procedure, 2 mg of ß-carotene were added in 20 mL of chloroform and then a 3-mL aliquot of the solution was placed in a 50-mL beaker. Further, 40 mg of linoleic acid and 400 mg of Tween 20 were added, whereas chloroform was removed by purging with nitrogen. This mixture was diluted with 100 mL of distilled water and was mixed properly by a vortex mixer to prepare emulsion. Then, 3 mL of β-carotene emulsion as well as 0.12 mL of phenolic extracts were placed in test tubes and thoroughly mixed. Afterward, test tubes were incubated at 50 °C in a water bath for 30 min. Absorbance of each sample was measured at 470 nm on a UV/visible light spectrophotometer. The degradation rate of the extracts was also calculated according to the first-order kinetic reaction using the following expression:

$$In(a/b) \times 1/t = \text{sample degradation rate},$$

where In = natural log; a = initial absorbance (470 nm) at time zero; b = absorbance (470 nm) after 40 min; and t = time (min).

The antioxidant activity was expressed as percentage inhibition (%) relative to the control by the following equation:

$$\text{AA}(\%) = \frac{\text{Degradation rate of control} - \text{Degradation rate of sample}}{\text{Degradation rate of control}} \times 100.$$

DPPH scavenging activity

The free radical scavenging activity of solvent extracts of musambi peel was determined by the method of Conforti et al. (2006). For this purpose, a sample solution was prepared by dissolving 0.025 mL of sample extract in 10 mL of respective solvent. Afterwards, 3 mL of freshly prepared DPPH solution in respective solvent (6 × 10 – 5 M) was mixed with 77 µL sample extract. Each sample was kept in a dark place for about 15 min at room temperature and the decrease in absorbance was measured at 517 nm on a UV/visible light spectrophotometer. Similarly, a blank sample absorbance having the same amount of solvent and DPPH solution except extract was estimated at the same wavelength. The free radical-scavenging activity of each extract can be presented as a percentage reduction in DPPH:

$$\text{Reduction of absorbance}\,(\%) = [(AB - AA)/AB] \times 100,$$

where AB = absorbance of blank sample at t = 0 min and AA = absorbance of tested extract solution at t = 15 min.

Preparation and analysis of cookies

In the current study, aqueous extracts of mosambi peel powder were used to formulate functional cookies at different concentrations, such as 1%, 2%, and 3%. For preparing the cookies, creaming of shortening, sugar, and fresh eggs were added and mixed thoroughly. Subsequently, flour and baking powder were added and stirred to prepare the homogeneous dough. The cookie dough was cut with a mold and shifted to baking trays. Afterwards, the cookies were baked at 170 °C in the baking oven for 20 min.

The prepared cookies were investigated for physical attributes, such as thickness, width, and spread factor, following the protocols of AACC (2000). The proximate composition, including moisture, crude protein, crude fat, crude fiber, ash, and nitrogen-free extract (NFE) contents, were also determined (AACC, 2000). Finally, a sensory evaluation of the cookies was carried out by a panel of experts considering different traits like taste, color, flavor, texture, crispness, and overall acceptability (Meilgaard et al., 2007).

Statistical analysis

The collected data was subjected to statistical analysis using a statistical package, i.e., Cohort V-6.1 (Co-Stat-2003, Insightful Corporation, Seattle, WA, USA). Furthermore, an analysis of variance technique was applied to determine the level of significance as described by Steel et al. (1997).

Results and discussions

Proximate analyses and antioxidant activity with different solvent

A proximate composition like moisture, ash, crude fat, crude protein, crude fiber, and NFE was found in mosambi peel (11.2 ± 6.5, 5.7 ± 0.2, 6.7 ± 0.1, 9.5 ± 0.02, 15.2 ± 0.5, and 62.9 ± 17.3%, respectively) (Table 1). Moisture, protein, and fiber are in accordance with the earlier findings of Oluremi et al. (2007). It was also observed that ethanolic extracts exhibited a better yield as compared to aqueous extracts, i.e., 11.0 ± 0.04 and 10.8 ± 0.5%, correspondingly. Wang et al. (1996) reported ethanol as an effective solvent for extraction of antioxidants. Similarly, Brigita et al. (2008) observed twice the anthocyanins and polyphenols contents in ethanolic extracts compared to aqueous extract.

Moreover, it was also noticed that ethanolic extract of mosambi peel showed higher total phenolic content (19.3 ± 0.3 mg/g) as compared to aqueous extracts (18.2 ± 0.04 mg/g) (Table 2). The findings of recent research are coherent to the investigations of Alasalvar et al. (2005). Furthermore, ethanolic extract of mosambi peels also exhibited higher free radical scavenging activity (DPPH) followed by aqueous extract 44.5 ± 0.1 and 41.4 ± 0.1%, respectively. Likewise, ethanolic extract showed better antioxidant activity (65.6 ± 0.04%) as compared to aqueous extract (60.1 ± 0.05%). It was concluded that both ethanolic and aqueous extracts exhibited considerable antioxidant and free radical scavenging activity (Table 2). Antioxidant activity is efficiently judged by DPPH, antioxidant activity, and β-carotene/linoleic acid emulsion methods (Brand-Williams et al., 1995; Wang et al., 1996). Antioxidant activity of ethanolic extract of mosambi peel was higher because of greater solubility of bioactive molecules in ethanol as compared to other

Table 1. Proximate composition of mosambi peel.

Characteristics	Amount (%)
Moisture	11.2 ± 6.5
Crude fiber	15.2 ± 0.5
Ash	5.7 ± 0.2
Crude protein	9.5 ± 0.02
Fat	6.7 ± 0.1
NFE[z]	62.9 ± 17.3

[z]NFE = Nitrogen free extract.

Table 2. Antioxidant potential of mosambi peel extracts.

Parameters	Ethanol	Aqueous
Yield of extracts	11 ± 0.04	10.8 ± 0.6
Total polyphenols contents (%)	19.3 ± 0.3	18.2 ± 0.04
DPPH[z] (%)	44.5 ± 0.1	41.4 ± 0.1
Antioxidant activity (%)	65.6 ± 0.04	60.1 ± 0.05

DPPH = 1,1-Diphenyl-2-picrylhydrazyl.

solvents (Anagnostopoulou et al., 2006; Sun et al., 2005; Zhang and Hamauzu, 2004). The results suggested that the solvent extraction methods could be used for extraction of antioxidants from different food processing waste at an industrial scale.

Physico-chemical and sensory attribute of fortified cookies

Aqueous extract of mosambi peel was selected for product development because of health concerns. Aqueous extract is safer than other solvents, such as ethanol or methanol. For this reason, aqueous extract of mosambi peel was added into cookies using different concentrations, i.e., 1%, 2%, and 3%, namely T_1, T_2, and T_3. It was found that supplementation of cookies with aqueous extracts imparts no substantial difference on physical and chemical attributes. The physical parameters (Table 3) including thickness, width, and spread factor were 51.4 ± 0.4 to 51.5 ± 0.6 cm, 241.0 ± 0.5 to 245.0 ± 0.2 cm, and 47.8 ± 0.2 to 49.0 ± 0.9 cm, respectively. Moreover, proximate composition like moisture, crude protein, crude fat, crude fiber, ash, and NFE contents of cookies varied non-significantly from 3.09 ± 0.1 to 3.1 ± 0.07, 6.27 ± 0.1 to 6.31 ± 0.2, 21.9 ± 1.8 to 22.3 ± 0.7, 1.08 ± 0.1 to 1.2 ± 0.2, 0.4 ± 0.06 to 0.6 ± 0.1, and 66.2 ± 0.04 to 66.4 ± 1.8%, respectively, in all treatments (Table 4).

Finally, sensory evaluation of cookies prepared from aqueous extracts (Table 5) showed better hedonic response compared to the control. Color improved from 6.8 ± 0.3 to 7.7 ± 0.5 by the addition of 2% aqueous extract, while further addition of extract reduced color scores. Flavor, texture, taste, and overall acceptability were maximum in T_2. Flavor score was minimum in T_1 (6.5 ± 0.4) while maximum in T_2 (7.2 ± 0.4) and texture ranged from 6.5 ± 0.4 to 7.0 ± 0.5. Regarding the taste of the cookies, T_2 (7.7 ± 0.4) showed the best followed

Table 3. Effect of treatments on physical parameters (cm) of cookies.

Treatments[z]	Thickness	Width	Spread factor
T_0	51.5 ± 0.6	241.0 ± 0.5	47.9 ± 0.5
T_1	52.0 ± 0.5	243.2 ± 0.6	47.8 ± 0.2
T_2	51.4 ± 0.4	243.0 ± 0.3	48.4 ± 0.6
T_3	50.7 ± 0.4	245.0 ± 0.2	49.0 ± 0.9

[z] T_0 = control, T_1 = 1% water extract of mosambi peel, T_2 = 2% water extract of mosambi peel, and T_3 = 3% water extract of mosambi peel.

Table 4. Effect of mosambi peel extracts on various characteristics of cookies (%).

Treatments	Moisture	Protein	Fat	Fiber	Ash	NFE
T_0	3.1 ± 0.1	6.30 ± 0.03	21.9 ± 1.8	1.1 ± 0.2	0.6 ± 0.1	66.2 ± 0.04
T_1	3.1 ± 0.08	6.29 ± 0.2	22.1 ± 1.5	1.1 ± 0.2	0.5 ± 0.06	66.4 ± 1.8
T_2	3.09 ± 0.1	6.27 ± 0.1	22.2 ± 1.1	1.08 ± 0.1	0.5 ± 0.06	66.2 ± 1.0
T_3	3.1 ± 0.07	6.31 ± 0.2	22.3 ± 0.7	1.2 ± 0.2	0.4 ± 0.06	66.3 ± 1.2

Table 5. Effect of mosambi peel extracts on sensory parameters of cookies.

Treatments	Color	Flavor	Texture	Taste	Overall acceptability
T_0	6.8 ± 0.3	6.9 ± 0.1	6.5 ± 0.4	7.1 ± 0.3	6.8 ± 0.2
T_1	6.4 ± 0.3	6.5 ± 0.4	6.6 ± 0.4	6.8 ± 0.2	6.5 ± 0.2
T_2	7.7 ± 0.5	7.2 ± 0.4	7.0 ± 0.5	7.7 ± 0.4	7.5 ± 0.4
T_3	7.2 ± 0.2	7.1 ± 0.5	6.9 ± 0.4	7.1 ± 0.2	6.9 ± 0.3

by T_4 (7.1 ± 0.2) and T_0 (7.1 ± 0.3) while least in T_1 (6.8 ± 0.2). Moreover, T_2 (7.5 ± 0.4) also recorded the maximum score for overall acceptability.

Overall, results indicated that physical and chemical properties of cookies remained unaffected as a function of aqueous extract addition. Findings of the current study are in accordance with the explorations of Camire et al. (2005), Gouveia et al. (2007), and Paradiso et al. (2008); they determined the improved oxidative stability of the products with addition of antioxidants supplements. Finally, it is proposed that mosambi peel could be utilized on a commercial basis for the extraction of natural antioxidants to develop various functional foods.

Conclusions

Food processing wastes are a substantial source of functional foods if managed properly, e.g., the citrus industry produces the bulk of processing waste mainly in the form of peel. Extraction of antioxidants from mosambi peel using a solvent extraction method (ethanolic and aqueous extract) is one of the prime objectives of the current research. Moreover, the resultant aqueous extract of mosambi peel was used in cookies and it is concluded that it has multifarious objectives, i.e., antioxidant in food and improving the health status. Finally, the current research also suggests to target different food processing wastes that have a tendency to produce value-added products in other food and pharma industries.

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