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Extraction of phenolic compounds from (*Mangifera indica* L.) and kinnow (*Citrus reticulate* L.) peels for the development of functional fruit bars

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Abstract

An investigation was carried out to extract polyphenols from mango and kinnow mandarin peels for the development of functional fruit bars and their storage stability. Solvent ethanol at 50%, 80% and 100% concentration was employed for polyphenols extraction from mango and kinnow peels through ultrasound assisted extraction technique. Higher polyphenols were extracted from mango peels with comparatively more antioxidant activity than kinnow peels. Fruit bars fortified with different levels of mango and kinnow peel polyphenolic extracts subjected to ambient and refrigerated storage conditions revealed a gradual decline in total polyphenols, antioxidant activity and sensory attributes score during storage period. Microbiological parameters though increased during storage but remained well within the permissible limits. Ambient temperature storage fruit bars were discarded after three months whereas at refrigeration temperature conditions remained sensory acceptable for five months. Overall, 2% mango peel extract fortified fruit bars scored highest by the sensory panelists while 3% kinnow peel extract fortified fruit bars scored lowest. It was concluded that mango and kinnow peel extract could be utilized as ingredient for the preparation of functionl foods.

Keywords: phenolic compounds; peel extracts; antioxidant activity; functional foods; fruit bars.

Practical Application: Polyphenols are the natural antioxidants in plants having a significant role in human health by prevention of certain degenerative diseases related to oxidative stress. Mango and kinnow peels are the major agro-industrial wastes in fruit juice processing which are not further utilized despite being an abundant source of phenolic compounds. Mango and kinnow peels polyphenolic extract fortified fruit bars and other functional foods may be prepared and utilized as a preventive and alternate therapeutic measure against certain oxidative stress related diseases.

1 Introduction

A positive correlation between fruits and vegetables intake with a decrease in the rate of certain oxidative stress related disorders such as cardiovascular problem, atherosclerosis, aging, hypertension, certain cancers, rheumatoid arthritis etc. has been reported by different epidemiological studies owing to the antioxidant activity of polyphenols in fruits and vegetables (Huang et al., 2011; Sesso et al., 2012). During industrial processing of fruits, huge quantities of agro-industrial wastes i.e. peels, seeds, stones and other residues are generated which contain valuable bioactive phenolic compounds that may be converted into value-added by-products. Fruit peels have comparatively higher concentration of phenolic compounds than in fruit pulp and thus have more antioxidant activity (Goulas & Manganaris, 2012).

Mango (*Mangifera indica* L.) and kinnow mandarin (*Citrus reticulate* L.) peels are the major by-product during fruit processing which constitutes approximately 15-20% and 35-40% of the fruit weight respectively. Beneficial effects of citrus peel against certain degenerative diseases such as anti-inflammatory, anti-carcinocegenic agent as well as reducing the risk of coronary heart disease has been reported (Tripoli et al., 2007). Ultrasound assisted extraction is nowadays widely employed technique for the extraction of polyphenols from plant sources due to its comparatively higher efficiency and better yield than conventional extraction methods (Rosello-Soto et al., 2015). During a study on orange peel polyphenols Khan et al. (2010) compared conventional solvent extraction process with ultrasound-assisted extraction and observed that significantly higher extraction yield and polyphenols flavanone concentration was observed at ultrasound frequency 25 kHz and 15 minutes treatment time as compared to conventional extraction at 40 °C for 60 minutes. Fruit bars are the snacks with highly nutritional and sensory attributes owing to elevated concentration of carbohydrates, proteins, vitamins and minerals. They may be prepared from different fruits along with other ingredients such as cereals, legumes, nuts, chocolate coatings etc. Since fruit bars are prepared from fruit pulp as major ingredient, they have high nutrients, flavour, better consumer appeal and utilized as a good nutritional supplement. Functional foods are natural or processed foods similar to conventional foods in appearance but besides providing basic nutrition, they also furnish additional physiological benefits in the management,

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prevention and treatment of certain chronic diorders. Functional foods play an important role in modern life because people are now more health conscious and seek for foods that not only provide necessary nutrients but also improve physical and mental well-being by preventing certain diseases (Orrego et al., 2014). Functional fruit bars may be prepared by incorporating bioactive compounds such as polyphenols as ingredient to the fruit bars thus utilizing them as functional food. Mango peel powder (MPP) and mango kernel powder (MKP) as rich sources of phenolic compounds at different fortification levels in biscuits were assessed by Ashoush & Gadallah (2011). It was concluded that mango peel powder and mango kernel powder could be utilized as functional food ingredients due to inherent phenolic compounds. Keeping in view the above-mentioned facts, a research study was designed to extract polyphnols from mango and kinnow peels, determination of antioxidant activity, development of functional fruit bars and assessment of quality attributes during storage.

2 Materials and methods

2.1 Plant material

Mango (*Mangifera indica* L.) of Chaunsa variety and Kinnow mandarin (*Citrus reticulate* L.) were procured from fruit market Islamabad and taken to Food Science Research Institute (FSRI) research laboratory, National Agricultural Research Center (NARC). Fruits were thoroughly washed under tap water to remove dirt, dust, micro flora and surface pesticide residue. Peeling of fruits was carried out by stainless steel knife, oven-dried at 50 °C for 48 hours in hot air oven. Dried peels were grinded to fine powder through Tecator cyclotec 1093 sample mill, Sweden with sieve size 0.5 mm. Mango and kinnow peel powder were packed in air-tight polyethylene zip bags and stored at refrigeration temperature.

2.2 Extraction of polyphenols

Ultrasound-assisted extraction technique was employed for polyphenols extraction of polyphenols from mango and kinnow peel powders according to procedure depicted by Bimakr et al. (2013) with minor modifications. Peel powders samples were extracted with solvent ethanol at 50%, 80% and 100% concentration levels, sample to sovent ratio 1:20, at extraction temperature and time 45 °C and 60 minutes into 125 mL (diameter: 57 mm/height 105 mm) reagent bottles placed at 35 kHz frequency in a sonicator (Transsonic 700 Elma) set at 35 kHz frequency with 100% amplitude level. Peel extracts were filtered, centrifuged, solvent evaporated by vacuum evaporator (BUCHI Rotavapor) and microfiltered through 0.45 µm cellulose membrane filter (Merck Millipore), collected in amber glass bottles and stored at refrigerated temperature.

The total polyphenol content of mango and kinnow mandarin peel extracts was measured by the Folin-Ciocalteau method as described by Singleton et al. (1999) and the absorbance was measured at 765 nm with UV-VIS Spectrophotometer (Agilent 8453, USA). The antioxidant activity of mango and kinnow mandarin peel extracts was carried out by DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay according to the method of Brand-Williams et al. (1995) with slight modifications and the absorbance was measured at 517 nm with UV-VIS Spectrophotometer (Agilent 8453, USA). The scavenging activity was calculated based on the DPPH radical percentage scavenged.

2.3 Development of fruit bars

Mangoes(Chaunsa variety), skimmed milk powder, roasted corn flour, sugar, gum arabic were procured from local market and taken to FSRI, NARC. Mangoes were thoroughly washed and pulp was taken by passing through mango pulper. Mango pulp was pasteurized at 85 °C for 10 min in a pan placed in the water bath. Pasteurized pulp was kept at freezing temperature till further processing. Sugar and roasted corn were grinded to fine powder by passing through Tecator cyclotec sample mill, packed in air-tight polyethylene zip bags and stored at refrigeration temperature. Fruit bars were prepared according to the method described by (Nadeem et al., 2012). Sugar, roasted corn flour, skimmed milk powder were added to mango pulp. Mango and kinnow peel polyphenol extract at different ratios 1%, 2%, 3% (T_1, T_2,T_3 for mango and T_4, T_5, T_6 for kinnow peel extracts alongwith control bars T_0) were fortified and mixed with mango pulp in the blender. Then the mixture was transferred to sheeting and cutting table where sheeting was carried out with sheeting roller and cut into mango bars of 7 cm length, 2.5 cm width and I cm height. Each bar of approximately 25 g was packed in aluminum foil and stored at refrigerated (6 ± 1 °C) and ambient temperature (25 ± 3 °C). Fruit bars were subjected to physicochemical, microbiological and sensory evaluation at 30 days intervals till storage period of 150 days.

Total polyphenols content and antioxidant activity of fruit bars

Total polyphenol content and antioxidant activity of fruit bars of fruit bars during storage were determined similarly as already described for mango and kinnow peel extracts.

Microbiological analysis of fruit bars

Total plate count, yeast and mould of fruit bars were determined by the standard methods of American Association of Cereal Chemists (2000). Plate count agar and potato dextrose agar were used for total plate count, yeast and mould determination. For total plate count estimation, 30 to 300 colonies on petri plates were counted and multiplied by the dilution factor whereas for yeast and mould less than 50 colonies were counted and multiplied by the dilution factor. Results were expressed as cfu/g.

Sensory evaluation of fruit bars

Fruit bars were sensory evaluated by a panel of twelve judges, males and females of diverse age groups at storage intervals of 0, 30, 60, 90, 120 and 150 days according to 9-point Hedonic scale as described by Land & Shepherd (1988).

2.4 Statistical analysis

Data was statistically analyzed by applying analysis of variance (ANOVA) technique to determine significance level (Steel et al., 1997). Least square design test was used to calculate

least significant difference among means. Minitab software was used for conducting statistical analysis of data.

3 Results and discussion

Solvent ethanol at 50%, 80% and 100% concentration level was employed for polyphenols extraction from mango as well as kinnow peels. Highest polyphenols were extracted in mango peels (67.58 ± 0.21 mg GAE/g of extract) at 80% concentration level whereas kinnow peel 100% ethanolic extract exhibited lowest phenolic compounds (24.39 ± 0.28 mg GAE/g of extract) as evident in Figure 1A. LSD-test indicated that ethanol concentration levels had a significant effect on extraction of polyphenols and were significantly different from each other for both mango and kinnow peel extarcts at all concentration levels. During polyphenol extraction from lemon peel, Haya et al. (2019) observed that aqueous organic solvent concentration led to higher extraction yield and total polyphenol content as compared to absolute methanol or ethanol . Among twenty different fruit peel extract samples analyzed for polyphenol content, Suleria et al. (2020) reported that mango peel extracts had significantly higher phenolic compounds than other fruit peels samples studied.

DPPH radical scavenging activity of mango and kinnow mandarin peel ethanolic extracts at different concentration levels (Figure 1B) reveals higher antioxidant activity of all sample extracts. However, maximum radical scavenging activity (83.19 ± 0.96%) was exhibited by mango peel samples at 80% concentration level followed by mango peel 50% ethanolic extract (70.63 ± 1.07%) whereas kinnow peel 50% ethanolic extracts had the minimum scavenging activity (56.52 ± 0.92%). Aqueous ethanol peel extracts showed higher inhibitory activity against DPPH radical than corresponding absolute ethanol peel extracts which may be attributed to higher polyphenol content in these peel extracts. However, DPPH radical scavenging activity of mango and kinnow peel extracts was lower than standard ascorbic acid (95.83 ± 0.75%). During a study on natural antioxidants from citrus mandarin peels, Karsheva et al. (2013) observed that 50% ethanolic extracts had highest DPPH radical scavenging activity than 20% and 70% ethanolic extracts.

Solvent ethanol 80% concentration was further employed for polyphenols extraction from mango and kinnow peels to prepare polyphenolic extracts for fortification in mango bars.

3.1 Development and storage studies of fruit bars

Total polyphenol and antioxidant activity of fruit bars content of fruit bars

Total polyphenol content of fruit bars fortified with different levels of mango and kinnow peel extracts varied significantly among treatments. Maximum total polyphenols was analyzed in fruit bars T_3 (405.91 ± 11.79 mg GAE/fruit bar) whereas control fruit bars T_0 had the minimum polyphenol content (78.05 ± 4.94 mg GAE/fruit bar) at 0 day (Figure 2A). During storage period total polyphenols of fruit bars gradually decreased but the rate of decline in fruit bars held at refrigerated storage was significantly less than ambient temperature storage. Total polyphenols of different treatment fruit bars ranged from 65.32 ± 5.16 mg GAE/fruit bar (T_0) to 390.47 ± 8.24 mg GAE/fruit

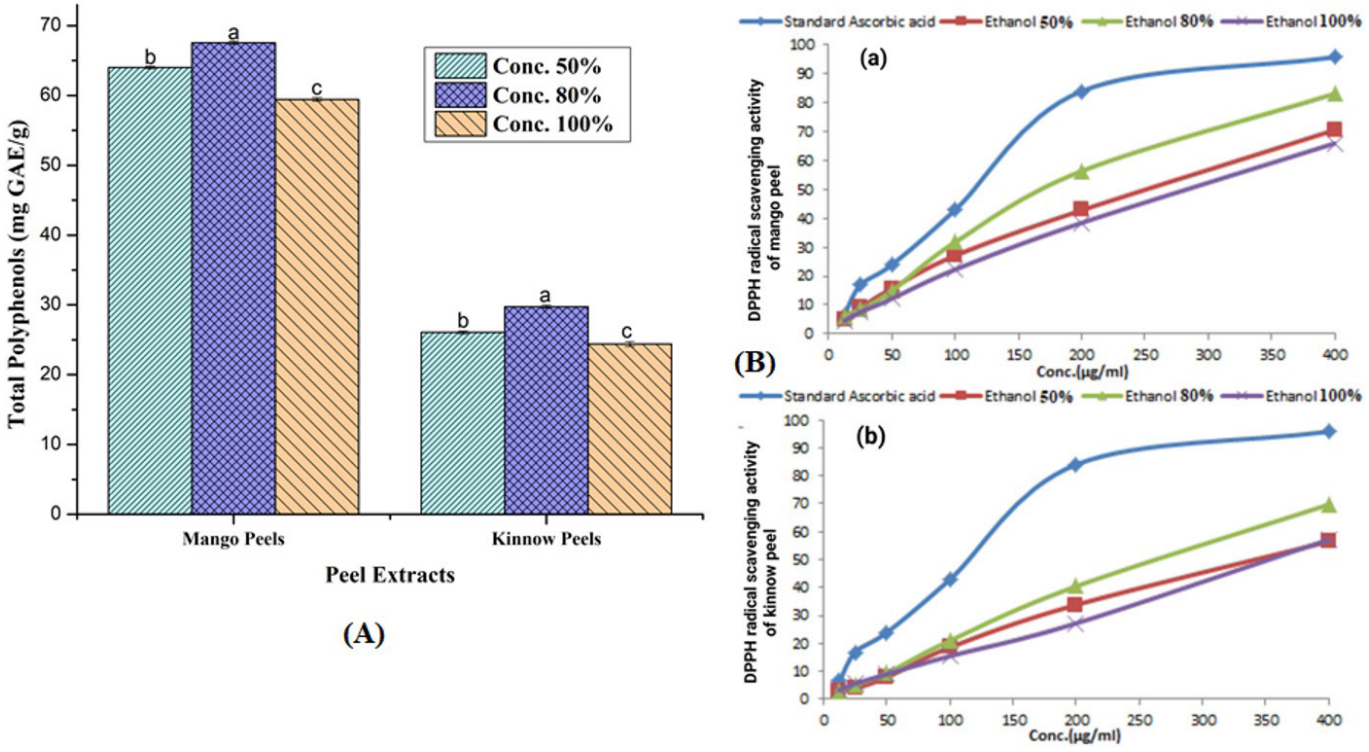

Figure 1. Effect of ethanol concentration levels on (A) total polyphenols and (B) DPPH scavenging activity of mango (a) and kinnow (b) peel extracts.

bar (T_3) after 90 days ambient temperature storage. In case of refrigerated temperature storage, total polyphenols of fruit bars ranged from 70.64 ± 4.12 mg GAE/fruit bar (T_0) to 398.42 ± 5.23 mg GAE/fruit bar (T_3) and 68.12 ± 3.65 mg GAE/fruit bar (T_0) to 394.16 ± 7.13 mg GAE/fruit bar (T_3) after 90 and 150 days refrigerated storage period respectively. LSD-test showed that storage temperature had significant effect on total polyphenols of fruit bars and treatment T_3 fruit bars were significantly different from other treatment bars at both storage temperature. The decrease in total polyphenols content of fruit bars might be attributed to oxidation of the phenolic compounds during storage (Liu et al., 2014).

DPPH radical scavenging activity of fruit bars fortified with different levels of mango and kinnow peel extracts varied significantly among treatments (Figure 2B). Highest scavenging activity or percent inhibition was recorded in fruit bars T_3 (73.37 ± 1.32%) whereas control fruit bars T_0 had the lowest radical scavenging activity or percent inhibition (23.15 ± 1.32%) at 0 day. During storage period radical scavenging activity/antioxidant activity of fruit bars held at ambient and refrigerated storage conditions gradually decreased.

As regards ambient temperature storage, the decreasing trend of percent inhibition was more in control fruit bars T_0 (10.84%) and least in T_5 (3.92%). In case of refrigerated storage fruit bars, rate of decline was comparatively less than ambient temperature storage. LSD-test revealed that storage temperature had significant effect on radical scavenging activity of fruit bars and control fruit bars(T_0) scavenging activity were significantly

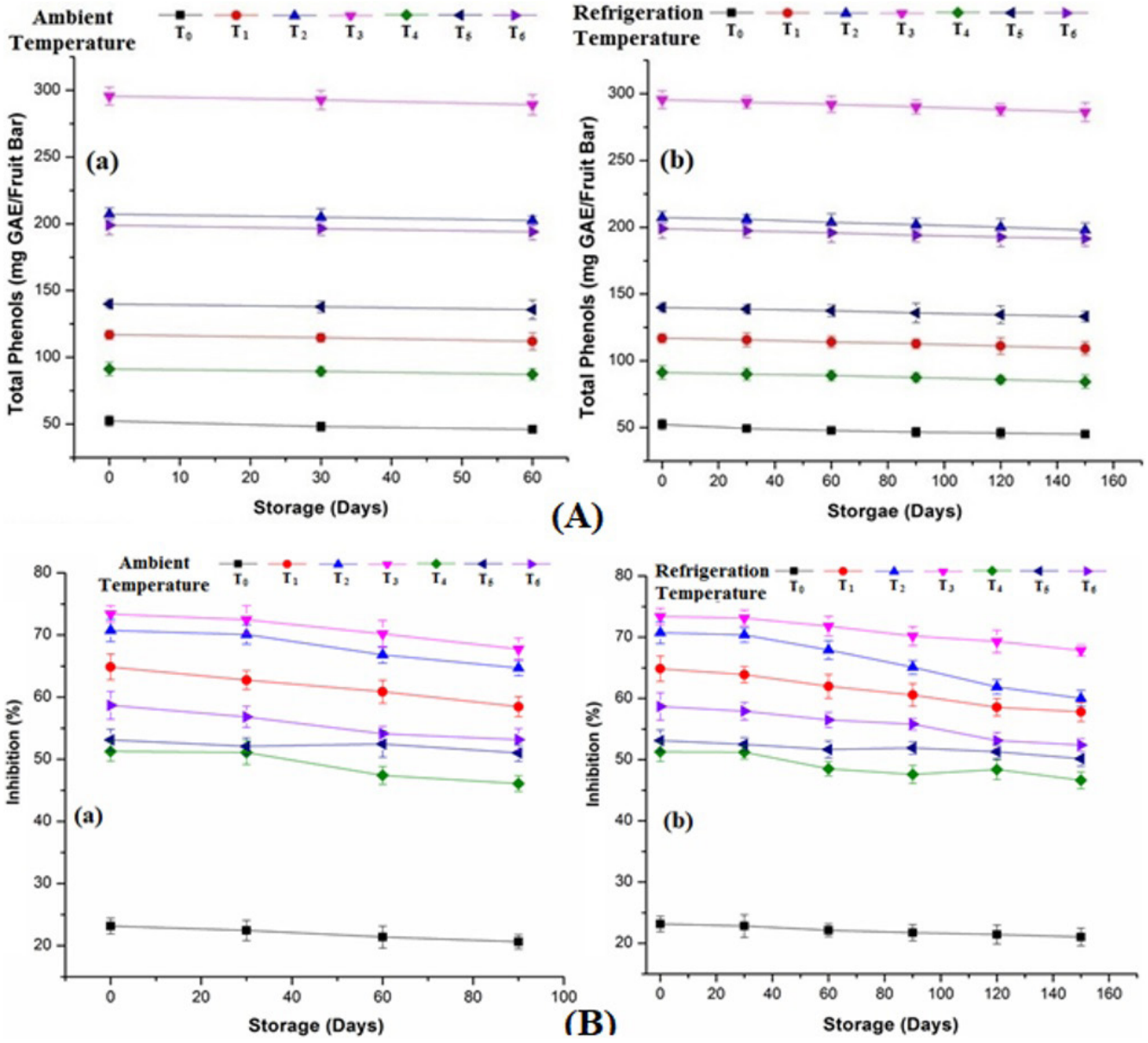

Figure 2. Effect of storage period on (A) total polyphenols and (B) DPPH radical scavenging activity at ambient (a) and refrigeration (b) temperature of fruit bars.

different from other treatment bars at both storage temperature. The antioxidant activity of fruit bars fortified with elevated concentration of polyphenolic extracts was comparatively more than control fruit bars and other low polyphenolic extract fortified fruit bars which indicated that the antioxidant activity of fruit bars was ascribed to the total polyphenolic content of fruit bars. The decrease in radical scavenging activity of fruit bars might be attributed to oxidation of the phenolic compounds during storage. The oxidation reactions were comparatively higher at ambient temperature than refrigerated temperature storage of fruit bars. Saci et al. (2015) reported a significant decrease in antioxidant activity of carrot and mango beverages during storage at 25 and 35 °C for 90 days.

Microbiological parameters of fruit bars

Fruit bars were analyzed for total plate count (TPC), mould and yeast count at 30 days intervals during storage at ambient and refrigerated conditions. Maximum TPC was determined in fruit bars T_4 (3.30 x 10^2 ± 0.20 cfu/g) whereas fruit bars T_2 had the minimum TPC (2.10 x 10^2 ± 0.10 cfu/g) at 0 day (Figure 3A). Highest mould count was observed in fruit bars treatment T_4 (2.50 x 10^2 ± 0.10 cfu/g) whereas fruit bars T_2 had the lowest mould content (1.40 x 10^2 ± 0.12 cfu/g) at 0 day (Figure 3B). As regards yeast count, fruit bars T_4 (0.60 x 10^2 ± 0.05 cfu/g) and treatment T_2 (0.40 x 10^2 ± 0.03 cfu/g) exhibited highest and lowest yeast count respectively (Figure 4C). LSD-

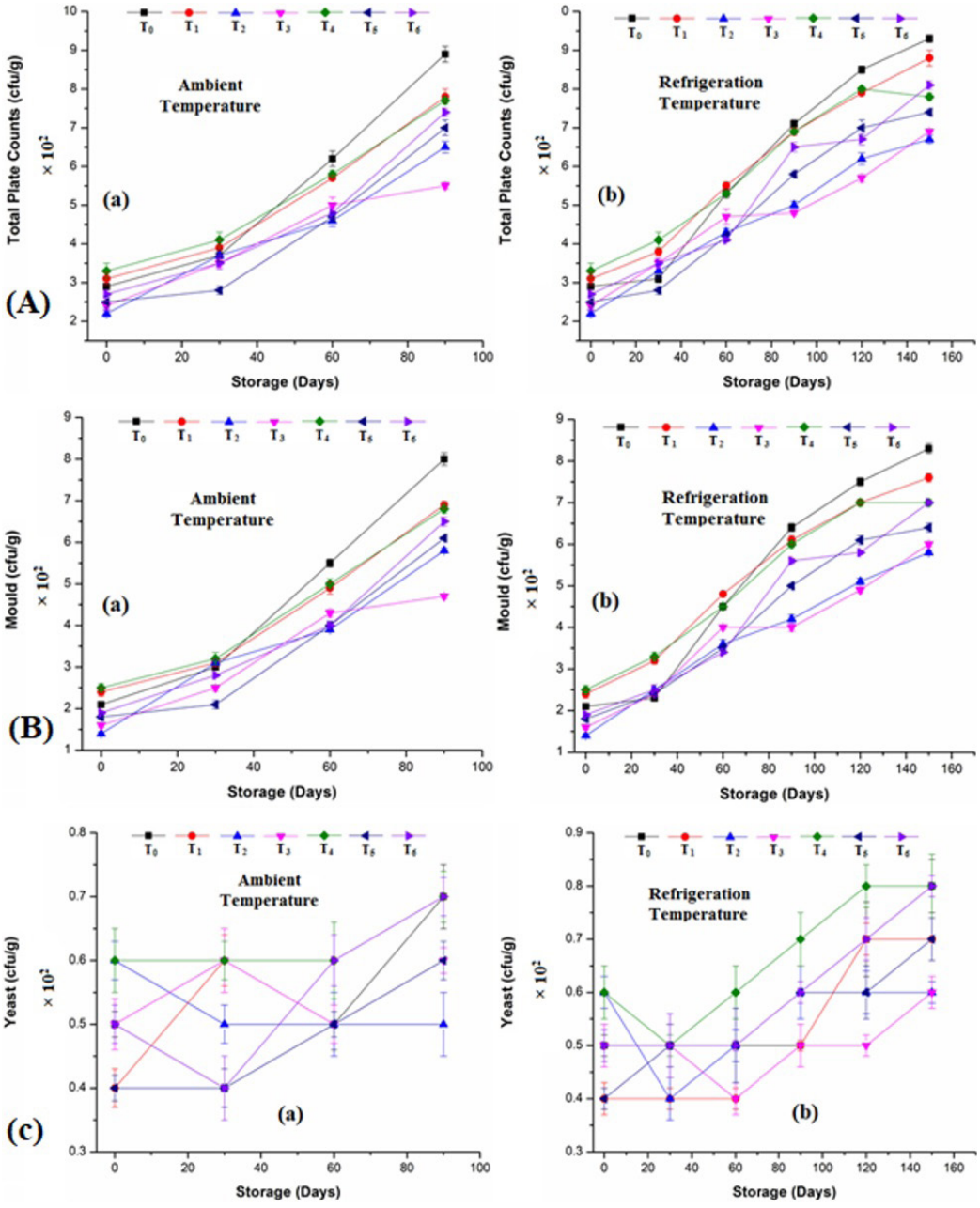

Figure 3. Effect of storage period on (A) total plate count (B) mold count and (C) yeast count at ambient (a) and refrigeration (b) temperature of fruit bars.

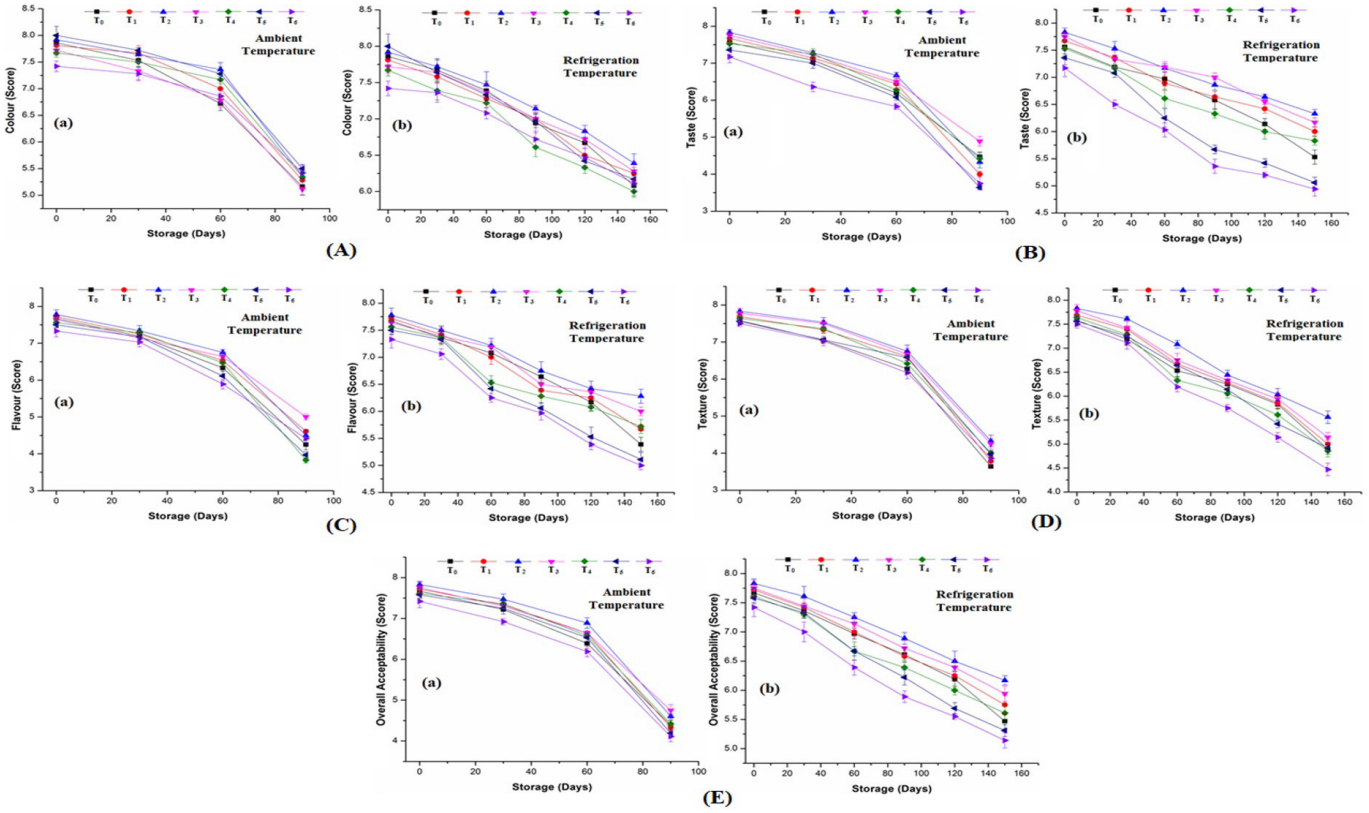

Figure 4. Effect of storage period and temperature on (A) color (B) taste (C) flavour (D) Texture and (E) ovrall acceptability score of fruit bars T0: Control bar, T1: 1% Mango peel extract bar, T2: 2% Mango peel extract bar, T3: 3% Mango peel extract bar, T4: 1% Kinnow peel extract bar, T5: 2% Kinnow peel extract bar, T6: 3% Kinnow peel extract bar.

test indicated that microbial growth of control fruit bars(T_0) was significantly different from other treatment bars at both storage temperature. During storage period microbial growth of fruit bars held at ambient and refrigerated storage conditions slightly increased but remained within permissible limits of U.S. Food and Drug Administration (2001) which stated that the aerobic plate count/total plate count, moulds and yeast in snack foods should be less than 1 x 10^4, 1 x 10^3 and 1 x 10^2 cfu/g respectively. Therefore, different treatment fruit bars at ambient and refrigerated storage conditions might be adjudged safe for consumption. The relatively less microbial activity during storage period in different treatment fruit bars might be attributed to low water activity of fruit bars which impeded the microbial especially bacterial growth during storage. Silva et al. (2013) developed cassava flour-based fruit bars and observed that fruit bars remained microbiologically safe for consumption after 210 days storage at 27 to 30 °C.

Similar gradual increase in total plate count, mould and yeast count of different apple-date fruit bars during storage was reported by Akhtar et al. (2014).

Sensory characteristics of fruit bars

Colour/appearance score of fruit bars revealed that treatment T_5 got maximum colour/appearance score (8.00± 0.17) while treatment T_6 had the minimum colour/appearance score (7.42 ± 0.10) at 0 day (Figure 4A). During storage period, a declining trend in colour score of fruit bars held at ambient and refrigerated storage conditions was observed. As regards ambient temperature storage, the decreasing trend of colour score was more in control fruit bars T_0 (34.35%) and least in T_6 (26.95%). In case of refrigerated storage fruit bars, rate of decline in colour score was comparatively less than ambient temperature storage. Colour score of fruit bars held at refrigerated temperature ranged from 6.61 ± 0.13 (T_4) to 7.14 ± 0.05 (T_2) and 6.00 ± 0.10 (T_4) to 6.39 ± 0.13 (T_2) after 90 and 150 days refrigerated storage period respectively. LSD-test showed that colour score of treatment T_2 fruit bars was significantly different from other treatment bars at both storage temperature. Decrease in colour score of fruit bars might be due to oxidation of pigments especially anthocyanins during storage (Karki, 2011; Kaur et al., 2013). The oxidation reactions were comparatively higher at ambient temperature than refrigerated temperature storage of fruit bars. Similar decreasing trend in colour/appearance score were reported in guava leather (Chavan & Shaik, 2015).

Taste score of fruit bars fortified with different levels of mango and kinnow peel extracts varied significantly among treatments. Fruit bars treatment T_2 awarded highest taste score (7.83 ± 0.08) whereas treatment T_6 got the lowest taste score (7.17 ± 0.08) by the sensory panelists at 0 day (Figure 4B). During storage period, a decreasing trend in taste score of fruit bars held at ambient and refrigerated storage conditions was observed. The

taste of fruits bars held at ambient temperature was disliked by sensory panelists after 90 days ambient storage period and fruit bars were therefore rejected. In case of refrigerated temperature storage, taste score of fruit bars ranged from 5.36 ± 0.13 (T_6) to 7.00 ±0.08 (T_3) and 4.94 ± 0.13 (T_6) to 6.33 ± 0.10 (T_2) after 90 and 150 days refrigerated storage period respectively. Decline in taste score of fruit bars during storage might be attributed to change in acidity, pH and brix/acid ratio (Malundo et al., 1997).

Flavour score of fruit bars fortified with different levels of mango and kinnow peel extracts varied significantly among treatments. Fruit bars treatment T_2 awarded highest flavour score (7.78 ± 0.13) whereas treatment T_6 got the lowest flavour score (7.33 ± 0.16) by the sensory panelists at 0 day (Figure 4C). During storage period, a declining trend in flavour score of fruit bars held at ambient and refrigerated storage conditions was observed. In case of ambient temperature storage, the declining trend of flavour score was maximum in fruit bars T_4 (49.34%) and minimum in T_3 (34.55%). As regards refrigerated storage fruit bars, rate of decrease in flavour score was comparatively less than ambient temperature storage. The flavour of fruits bars held at ambient temperature was disliked by sensory panelists after 90 days ambient storage period and fruit bars were therefore rejected. As regards refrigerated temperature storage, flavour score of fruit bars ranged from 5.97 ± 0.13 (T_6) to 6.75 ± 0.17 (T_2) and 5.00 ± 0.08 (T_6) to 6.28 ± 0.13 (T_2) after 90 and 150 days refrigerated storage period respectively. All treatment fruit bars held at refrigeration temperature neither liked nor disliked after 150 days storage period except fruit bars fortified with 2% mango peel extract (T_2) which remained acceptable for flavour score after 150 days refrigerated storage period. Decrease in flavour score of fruit bars during storage might be attributed to change in brix/acid ratio, pH and degradation of volatile flavouring compounds (Jitareerat et al., 2007). Higher flavour scores of refrigerated storage fruit bars might be attributed to comparatively better retention of volatile compounds and slower chemical reactions than ambient temperature stored fruit bars. Texture refers to those characteristics of a food product that can be evaluated visually or by touch. Texture score of fruit bars fortified with different levels of mango and kinnow peel extracts varied significantly among treatments. Fruit bars treatment T_2 were appreciated most and got highest texture score (7.83 ± 0.08) whereas treatment T_6 got the lowest texture score (7.50 ± 0.10) by the sensory panelists at 0 day (Figure 4D). During storage period, a decreasing trend in texture score of fruit bars held at ambient and refrigerated storage conditions was observed. As regard ambient temperature storage, the decreasing trend of texture score was highest in control fruit bars T_0 (51.98%) and least in T_2 (44.70%). In case of refrigerated storage fruit bars, rate of decline in texture score was comparatively less than ambient temperature storage. Texture score of different treatment fruit bars ranged from 3.64 ± 0.05 (T_0) to 4.33 ± 0.16 (T_2) after 90 days ambient temperature storage. The texture of fruits bars held at ambient temperature was disliked by sensory panelists after 90 days ambient storage period and fruit bars were therefore rejected. As regards refrigerated temperature storage, texture score of fruit bars ranged from 5.75 ± 0.08 (T_6) to 6.44 ± 0.10 (T_2) and 4.47 ± 0.13 (T_6) to 5.56 ± 0.16 (T_2) after 90 and 150 days refrigerated storage period respectively. Control fruit bars (T_0) and kinnow peel extract fortified fruit bars (T_4, T_5 and T_6) held at refrigeration temperature were rejected based on texture characteristics after 150 days storage period. Decrease in texture score of fruit bars during storage might be attributed to hardening of fruit bars resulting from moisture loss during storage period (Aggarwal & Kaur, 2015).

Overall acceptability score of fruit bars fortified with different levels of mango and kinnow peel extracts varied significantly among treatments. Fruit bars treatment T_2 got highest score (7.83 ± 0.13) whereas treatment T_6 had the lowest overall acceptability score (7.42 ± 0.16) by the sensory panelists at 0 day (Figure 4E). During storage period, a declining trend in overall acceptability score of fruit bars held at ambient and refrigerated storage conditions was observed (Figure 4E). As regard ambient temperature storage, the declining trend was highest in fruit bars T_6 (44.61%) and least in T_3 (38.71%). In case of refrigerated storage fruit bars, rate of decrease in overall acceptability score was comparatively less than ambient temperature storage. The overall acceptability of fruit bars held at ambient temperature was disliked by sensory panelists after 90 days ambient storage period and fruit bars were therefore rejected. All treatment fruit bars held at refrigeration temperature neither liked nor disliked after 150 days storage period except fruit bars fortified with 2% mango peel extract (T_2) which remained acceptable after 150 days refrigerated storage period. Kinnow peel fortified fruit bars especially 2% (T_5) and 3% kinnow peel fortified fruit bars (T_6) were disliked by the panelists due to their comparatively bitter taste. However, mango peel fortified fruit bars especially 2% fortified bars (T_2) were highly appreciated by sensory panelists and were significantly different from other treatment bars as indicated by LSD-test. Similar decreasing trends in overall acceptability score during storage were reported in guava nectar (Bal et al., 2014) and mango bar (Parab et al., 2014).

4 Conclusion

Higher polyphenols were extracted from mango peels with comparatively more antioxidant activity than kinnow peels. Fruit bars fortified with different levels of mango and kinnow peel polyphenolic remained sensory acceptable for five months at refrigerated storage conditions. Overall, 2% mango peel extract fortified fruit bars scored highest and were appreciated most by sensory panelists while 3% kinnow peel extract fortified fruit bars scored lowest and were disliked most. It was concluded that mango and kinnow peel extract being a potential source of polyphenols could be utilized as ingredient for the preparation of functionl foods such as fortified fruit bars.

Conflict of interest

The authors declare that they have no competing interests.

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