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Antioxidant activities and physical properties of stirred yoghurt fortified with pomegranate peel extracts

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Abstract Pomegranate peels (outside, inside and whole) were oven (40 °C) and solar dried. Aqueous and methanolic extracts were prepared from the dried peels, and their antioxidant activities [radical scavenging activity (RSA %), ABTS radical scavenging, total phenolic content (TPC) and total flavonoids content (TFC)] were determined. The aqueous extract of the dried whole peel showed the highest antioxidant activities as compared to other pomegranate peel extracts (PPEs). Stirred yoghurt was prepared from reconstituted skim milk powder fortified with 5%, 10%, 15%, 20%, 25%, 30% and 35% of the PPE, before and after inoculation with the traditional yoghurt starter. Addition of PPE before inoculation with the starter resulted in stirred yoghurt of higher antioxidant activities than that with PPE added after inoculation with the starter. Also, increasing the percentage of the added PPE increased significantly the antioxidant activities of stirred yoghurt up to 25% and further increase in the percentage of added PPE led no significant effect. Addition of PPE had no significant effects on the sensory attributes (appearance, body & texture and flavor) as compared to the control sample. Increasing the percentage of the added PPE resulted in decrease in the viscosity of the stirred yoghurt, but samples containing 20% and 25% PPE led almost the same viscosity.

Introduction

Yogurt is among the most common dairy products consumed around the world (Saint-Eve et al., 2006). It is mainly obtained by fermenting fresh milk or reconstituted milk with lactic acid bacteria, and favored by the customer because of its effects of improving the intestinal environment and enhancing the body

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immunity (Michael et al., 2010). There are increasing interests in applying fruit processing wastes as functional food ingredients since they are rich source of dietary fiber, and most of the beneficial bioactive compounds are remained in those by-products (Balasundram et al., 2006). Additionally, waste products (e.g. fruit peels) from processing of agricultural commodities could offer practical and economic sources of active antioxidants which could replace the synthetic ones (Moure et al., 2001; Balasundram et al., 2006; Reddy et al., 2007). The pomegranate plant (*Punica granatum* L., *Punicaceae* family) is a shrub and its fruit is a rich source of bioactive phytochemicals such as tannins and other phenolics. It is a native plant to the Mediterranean region and has been used extensively in folk medicine of some countries in Asia and other parts of the world. Interestingly, it was stated that pomegranate peels have been used since antiquity in the Middle East as colorant for textiles because of their high tannin and phenolic contents (Li et al., 2006).

Stomachic, inflammation, fever, bronchitis, diarrhea, dysentery, vaginitis, urinary tract infection, and, among others, malaria have been treated using various parts of pomegranate including fruit peels. Several Studies have reported that the phenolic content of pomegranate peels was 10 times higher than that found in the pulp (Li et al., 2006). The phenolic constituents, ellagic tannins and ellagic acid, are among the potent antioxidants in peels (Murthy et al., 2002; Negi and Jayaprakasha, 2003; Reddy et al., 2007; Iqbal et al., 2008; Madrigal-Carballo et al., 2009).

The objective of the current study was to use pomegranate peel extract in the manufacture of stirred yoghurt. Antioxidant and organoleptic properties were determined. Sensory acceptability of stirred yoghurt was assessed.

Materials and methods

Materials

Skim milk powder: Low heat powder imported from USA. It had 34% protein, 51% lactose, 1.2% fat, 8.2% ash, 4% moisture. Fully ripe pomegranate fruits (*P. granatum* L.) were obtained from the local market. 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), Gallic acid: "monohydrate reagent grade", rutin, ammonium persulphate and ABTS from Sigma–Aldrich Inc. (St. Louis, MO, USA). Folin–Ciocalteus phenol reagent was purchased from Fluka Chemical Co. Methanol was purchased from Sd Fine-Chem. Limited.

Methods

Preparation of pomegranate peel powder

Pomegranate fruits were washed by distilled water peeled carefully separate into two parts; the outside and the white inside part of the peel. Samples of outside and inside and whole peel were dried by the oven and solar drying methods.

1. Oven drying

The peels were air dried in a ventilated oven at 40 °C for 48 h and ground to a fine powder.

2. Solar drying

The pomegranate peel was placed on shelves of a solar drier made of aluminum mesh. The temperature inside of the dryer was about 50 °C for 2 h to get dried pomegranate peel, and then grouned to fine powder.

Preparation of pomegranate peels extracts (PPE)

According to Shiban et al. (2012), pomegranate peel powder (5 g) was separately blended for 2 min with 300 ml of 80% methanol or with distilled water. Each mixture was then left, in the dark; at room temperatures for 1 h prior to filtration (Whatman No. 1) and centrifuged at 3500 rpm for 10 min. Extracts were kept at −20 °C prior to analysis. The antioxidant activities of aqueous PPE are (RSA 97.214%, ABTS 89.561%, TPC 16.343 mg Gallic acid/g and TFC 6.863 mg RE/g) and acidity was 4.83% and pH 3.5.

Manufacture of stirred yoghurt fortified with PPE

Reconstituted skim milk (12% T.S) was heated to 90 °C for 5 min and cooled to (40 °C). The aqueous PPE of whole peel was added at the ratios of 5%, 10%, 15%, 20%, 25%, 30% and 35%, then 3% traditional starter culture was added and the mixtures were incubated at 45 °C until the gel structure was formed. The gel was stirred and stored at refrigerator (6 ± 2 °C) (Experimental I). The same experiment was done the same as obvious except that whereas PPE was added after inoculation of the mixtures (Experimental II).

Antioxidant activity of pomegranate peel extracts

Radical scavenging activity (RSA %) assay. Free radical scavenging activity (RSA) of the samples was measured using the method of Brand-Williams et al. (1995). An aliquot 100 µl of the sample solution was mixed with 2.9 ml of 60 µM DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol solution are vortexed. The reaction mixture was left in the dark for 30 min, after which the absorbance was measured at 517 nm. Methanol was used as blank. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

$$\text{RSA}\,\% = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

ABTS radical scavenging assay. The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay was done according to the method of Re et al. (1999). Five milliliters of ABTS solution (7 mM) was mixed with 88 µl of ammonium persulphate solution (140 mM) and then incubated in the dark at 25 °C for 12–16 h, and then diluted with phosphate buffer (pH7.2) until the absorbance at 734 nm was 0.70 ± 0.02. Aliquot (100 µl) of each sample was mixed with 3 ml of the prepared ABTS working solution and the change in absorbance was observed at 734 nm. The ABTS radical scavenging capacity of the sample was calculated by the following formula:

$$\text{ABTS radical scavenging activity} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$