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Infusion of fructooligosaccharide in Indian gooseberry (*Emblica officinalis*) fruit using osmotic treatment and its effect on the antioxidant activity of the fruit

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ABSTRACT

Osmotic dehydration is the most reliable method for the partial removal of water from fruits and vegetables because it is less energy intensive and retains the natural colour, texture and flavour of the food materials. The present work deals with the infusion of fructooligosaccharide (FOS) into Indian gooseberry (amla) fruit. The infusion of FOS was found to increase over a range of temperatures from 30 to 50 °C. The water loss and solid gain during the infusion process were higher at higher temperatures. Furthermore, the antioxidant activity and phenolic as well as flavonoid contents of the FOS osmotic dehydrated samples were also found to be significantly higher as compared to fresh fruits in both *in vitro* and cell line assays. The present study indicated that the osmotic treatment can be used as a method for the infusion of FOS in solid food matrix such as Indian gooseberry.

1. Introduction

Indian gooseberry or amla (*Emblica officinalis*) is a euphorbiaceous plant, which is widely distributed in subtropical and tropical areas of India, China and Indonesia. Amla fruit is mainly used as raw, cooked, pickled, as well as in many traditional medicinal systems, such as Chinese herbal medicine, Tibetan medicine and Ayurvedic medicine (Khan, 2009). Amla fruit is reported to enhance the immunity of the body and it is effective against cancer, diabetes, liver disorders, heart trouble, ulcer, anemia and other diseases. The fruit also has antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive, gastroprotective and antimicrobial properties (Khan, 2009). Furthermore, it was found to be effective in the prevention of hypercholesterolemia (by degradation and elimination of cholesterol) as well as atherosclerosis (Anila and Vijayalakshmi, 2002; Kim et al., 2005). Amla is a good source of phenolic compounds having high anti-inflammatory and antioxidant activity (Arunachalam et al., 2011).

Unfortunately, the fruit has a very short shelf life (5–6 days) and is not available throughout the year. Therefore, attempts have been made at extension of their shelf life by processing (Bijendra et al., 2014). Osmotic dehydration was demonstrated as a method of extending the shelf life of the fruits (Moazzam, 2012), besides preserving the important nutrients of the fruit (Matusek et al., 2008; Castagnini et al., 2015). During osmotic dehydration, the moisture content of fruits is partially reduced by immersing the fruits in hypertonic solution for specific amounts of time. Unlike other methods of dehydration, osmotic dehydration preserves the cellular constitution by preventing thermal stress (Pheeraya et al., 2012). Previously, Pratibha et al. (2010) and Adsare et al. (2016) subjected amla fruits to osmotic dehydration in sucrose solutions. But, sucrose is not suitable for diabetic patients. Hence, considering the needs of diabetic patients, alternative osmotic solutes need to be explored. Fructooligosaccharides (FOS) is one such compound that can be used as an osmotic agent. It is a non-digestible oligosaccharides and can be used as an alternative sweeteners, dietary fibres or prebiotic (Matusek et al., 2008). A prebiotic is defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacterial species in the colon, thus improving host health or colon microflora composition (Gibson and Wang,

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1994; Gibson and Roberfroid, 1995; Collins and Gibson, 1999; Boeckner et al., 2001). FOS reduces the glucose levels in the blood and helps in the prevention of colon cancer (Torres et al., 2014).

FOS present in breast milk contributes to the increase of Bifidobacteria in the intestines of breastfed infants, thus enhancing the resistance to infections (Rotimi and Duerden, 1981; Gnoth et al., 2000; Dai et al., 2000). It resists hydrolysis and digestion in the upper gastrointestinal tract, but are hydrolysed and fermented in the large bowel by colon bacteria, such as Bifidobacteria (Kleessen et al., 1997). It also helps in increasing the concentration of beneficial bacteria, such as *Lactobacilli* and *Bifidobacteria*, which help in modulating the activity of the immune system. It also decreases the concentration of pathogenic organisms, such as *Clostridia* and protein-degrading bacteriodes (which produce tumour-promoters from metabolites of proteins that escape digestion in the upper gut) (Rastall et al., 2005; Sanders, 2006). It also increases calcium and magnesium absorption by increasing the production of short-chain fatty acids and fermentation of end products, which lower the pH of faecal content. Lower faecal pH increases mineral solubility leading to improved mineral absorption by forming complexes with the minerals (Remesy et al., 1993; Wang and Gibson, 1993; Trinidad et al., 1993, 1997; Bouhnik et al., 1997; Campbell et al., 1997).

The objectives of the present study were to study effect of time and temperature of osmotic treatment so as to enhance the infusion of FOS into amla fruit as well as to study the effect of infusion on phytochemical profile or biological activity of amla fruit.

2. Materials and methods

2.1. Materials

Amla fruit (*E. officinalis*) was purchased from a local market in Mysore. Fructooligosaccharides (FOS) used in the study was produced by the transfructosylation of sucrose using FTase enzyme obtained by submerged fermentation using *Aspergillus oryzae* MTCC 5154 (Sangeetha et al., 2003). Sodium nitroprusside (SNP), methanol, ammonium molybdate, sulfuric acid, sodium phosphate, ethylene diamine tetra acetic acid (EDTA) trichloroacetic acid, ascorbic acid, Folin- Ciocalteu's reagent, isopropanol, aluminium chloride and sodium acetate were purchased from M/s. Sisco Research Laboratories, Mumbai. Griess' reagent, 2-thiobarbituric acid, Dulbecco's Modified Eagle Media (DMEM), Penicillin- Streptomycin antibiotic, Oil red O stain was purchased from M/s. Sigma-Aldrich, India. Fetal bovine serum, gallic acid, sodium bicarbonate, potassium bromide and sodium chloride were purchased from M/s. Himedia Laboratories, India and ethanol was purchased from M/s. Merck, India. RAW 264.7 cell lines were purchased from the National Centre for Cell Sciences (NCCS), Pune, India.

2.2. Sample preparation and osmotic dehydration

The fruits were subjected to open steam at atmospheric pressure for 5 min to loosen the fibres. After steaming, the fruits were cut into pieces of uniform sizes and completely immersed in FOS solution of 70°Brix in a fruit to a FOS ratio of 1:5 W/V. The samples were stirred at 100 rpm at three different temperatures 30, 40, or 50 °C using magnetic stirrer. The samples were withdrawn at regular time intervals (0, 2, 4, 6, 10, 12 and 24 h). After thoroughly washing with distilled water, the samples were weighed and dried in an oven at 70 °C till uniform weight was attained.

2.3. FOS content estimation

The fruit pieces infused with FOS at different time intervals after drying in hot air oven at 50 °C were suspended in a known amount of distilled water (fruit to water ratio 1:10) and agitated for 2 h at 200 rpm. The samples were centrifuged at 8000 rpm for 20 min to obtain a clear supernatant. The supernatant was filtered through a 0.45 μm cellulose nitrate filter (M/s. Millipore India Pvt Ltd., India) and appropriately diluted with triple distilled water and analysed by HPLC (Sangeetha et al., 2002). FOS content was expressed as kg of FOS/kg Initial dry solids.

2.3.1. Water loss and solid gain calculations

The weight loss during osmotic dehydration can be expressed by the following relation,

$$\text{Weight loss} = \text{Water loss} + \text{Solid gain} \quad (1)$$

The moisture, solid and FOS content (kg/kg of initial dry solids) at any time can be calculated as follows,

$$\text{Moisture content (kg/kg) at any time on dry basis} = (\text{Initial water present} - \text{Water loss})/\text{Initial dry solids} \quad (2)$$

$$\text{Solid content (kg/kg) at any time on dry basis} = (\text{Initial dry solids} + \text{Solid gain})/\text{Initial dry solids} \quad (3)$$

2.3.2. Determination of moisture, solid or FOS diffusion coefficients

Fick's unsteady state diffusion equation can be written as (Crank, 1975):

$$\frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial x^2} \quad (4)$$

where 'C' is the concentration, 'D_e' is the diffusion coefficient, 'x' is the diffusion path and 't' is the time.

The solution of Equation (4) for diffusion from an infinite flat plate (thickness 2a) being subjected to osmotic dehydration from both the major faces with assumptions (a) uniform initial moisture distribution (b) negligible external resistance to mass transfer (c) no shrinkage during osmotic dehydration; and the following initial and boundary conditions

$$C = C_o \text{ at } t = 0;\ -l < x < +l \quad (5)$$

$$C = C_1 \text{ at } t > 0;\ x = l \quad (6)$$

It results in the well-known following Equation (7) for the transfer of moisture, solid or FOS (Crank, 1975; Rastogi et al., 2002):

$$X_i = \frac{(x_{ti} - x_{\infty i})}{(x_{oi} - x_{\infty i})} = \sum_{n=1}^{\infty} C_n \exp\left[-D_{ei} t q_n^2 \left(\frac{1}{a^2}\right)\right] \quad (7)$$

Defining the Fourier number for moisture, solid or FOS (F_{oi}) as $D_{ei}t/a^2$ and substituting its value into Equation (7) results in the following equation:

$$X_i = \frac{(x_{ti} - x_{\infty i})}{(x_{oi} - x_{\infty i})} = \sum_{n=1}^{\infty} C_n \exp\left[-F_{oi} q_n^2\right] \quad (8)$$

where X_i is the dimensionless moisture, solid or FOS ratio; the subscripts o, ∞ and t represent the relevant concentrations initially, at equilibrium, and at any given time; the subscript 'i' takes values 'm', 's' and 'c' for moisture, solid and FOS content, respectively; D_{ei} is the effective diffusion coefficient; and C_n is equal to $2\alpha(1+\alpha)/(1+\alpha+\alpha^2 q_n^2)$ where, q_n's are the non-zero positive roots of the