



JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal



Published by
Faculty of
Biotechnology and
Food Sciences

Arya et al. 2013 : 2 (6) 2440-2443

USE OF JAMBHUL POWDER IN THE DEVELOPMENT OF BIOACTIVE COMPONENTS ENRICHED MILK KULFI

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ARTICLE INFO

Received 7. 3. 2013
Revised 23. 4. 2013
Accepted 29. 4. 2013
Published 1. 6. 2013

Regular article



ABSTRACT

Jambhul (*Syzygium cumini L*) is an indigenous minor fruit of India which is especially available in summer season and is very good source for medicinal purpose. In the present investigation jambhul was preserved by using infrared drying technology and jambhul powder was utilized to develop Kulfi which is popular Indian frozen dessert. The infrared jambhul powder showed TPC (27.61 mg/g), TF (28.75 mg/g), Anthocyanins (2.59 mg/g), ABTS (27.19 μ M/g), and DPPH (375.32 μ M/g). Kulfi prepared by using 3% of jambhul powder which was optimized on the basis of 9- Point Hedonic Scale. Milk kulfi showed enhancement in TPC (78.68%), TF (100%) anthocyanin (100%), ABTS capacity (66.20%), DPPH (91.22%) by incorporation of 3% of jambhul powder. Freezing showed significant decrease ($p < 0.05$) in nutraceutical component (phenolic, flavonoid, anthocyanin and antioxidant capacity by ABTS and DPPH). Anthocyanin and flavonoids were additional to milk kulfi. Thus, Jambhul powder could be used as functional ingredient in the development of Kulfi.

Keywords: *Kulfi*, jambhul powder, total phenolic, flavonoid, anthocyanin, ABTS, DPPH

INTRODUCTION

Natural bioactive compounds include a broad diversity of structures and functionalities that provide an excellent pool of molecules for the production of nutraceuticals, functional foods, and food additives. Bioactive components are useful in maintenance of good health and helps in disease prevention (Gil-Chávez et al. 2013). These compounds efficiently interact with proteins, DNA, and other biological molecules to produce a desired outcome, which could be exploited for designing natural products-derived therapeutic agents (Ajikumar et al. 2008).

Jambhul (*Syzygium cumini L*) is an indigenous minor fruit of India. It is especially available in summer season. Jambhul fruit is universally accepted to be very good source for medicinal purpose especially for curing diabetes because its effect on pancreas. Jambhul fruit and seeds are sweet, acrid and sour (Bopp et al. 2009). Jambhul has a potent prophylactic anti-septic effect that is associated to a recruitment of activated neutrophils to the infectious site and to a diminished systemic inflammatory response (Maciel et al. 2008). Chaudhary and Mukhopadhyay (2012) reported that jambhul has potential source for nutraceutical.

Infrared radiation can be used as a deliberate heating source and is also gaining popularity as a safe drying method giving a better quality product (Wang and Sheng 2006). The drying time can be reduced by infra red energy, which is rapidly absorbed by the water molecules in the product, resulting in rapid evaporation of the water and thus a higher drying rate. Moreover, infra red application has been reported to improve product qualities, such as aroma and to result in faster and better rehydration compared to hot-air drying alone.

Phenolic compounds are crucial in the sensory and nutritional quality of fruits, vegetables and other plants and also known as secondary plant metabolites (Tomas-Barberan et al. 2000). Polyphenols play crucial role in preventing obesity, coronary heart disease, colon cancer, gastrointestinal disorders and can also reduce the risk of diabetes (Jitaru et al. 2005; Luthria and Pastor-Corrales 2006; Altiok et al. 2008; Ross et al. 2009). Polyphenols are also known for their ability to prevent fatty acids from oxidative decay, and provide a defense against the oxidative stress of oxidising agents and free radicals (Slusarczyk et al. 2009).

Kulfi is popular Indian frozen dessert prepared from concentrated sweetened milk or cow or buffalo milk with or without added nuts and flavors. It is mostly known for refreshingly cool and delightfully sweet characteristics and daily consumable. Addition of fruit with milk will help to increase the nutritive value of food and which also help to maintain good health and prevent from degenerative disease. Various dairy products were prepared by using fruit pulp

such as milk beverage containing fruit pulp (Kumar et al. 2001), Mango burfi (Kadam et al. 2009), Fruit Shrikhand (Vagdalkar et al. 2002) etc. Dairy food consumption is associated with overall diet quality and nutrient adequacy (Ballew et al. 2000; Weinberg et al. 2004).

The consumer is shifting towards 'light' foods with low calories and adopting 'functional foods' that is going to improve their health and well-being too. In fact, blending functional ingredients into dairy based foods helps increased sale of dairy foods (Berry, 2002). Hence, there is a need to merge non-dairy ingredients with dairy based ingredients and products to attain the previous mentioned objectives with attendant savings in cost, enhanced appearance, taste, texture and even functionality.

Hence in the present investigation jambhul was preserved by infrared drying. The effect of infra red drying on phenolic content, flavonoid content, anthocyanin content and total antioxidant capacity in jambhul powder was studied. This powder was utilized as ingredient in the preparation of milk kulfi and was optimized on the basis of sensory evaluation. Further effect of freezing on bioactive components of kulfi was also studied.

MATERIAL AND METHODS

Materials

Fresh jambhul fruits, corn flour, sugar, milk were purchased from local market of Mumbai. Folin-Ciocalteu reagent, sodium carbonate anhydrous, sodium hydroxide was purchased from FINAR Chemicals, Mumbai, India. Vanilline, Pet ether (60- 80^o C) was purchased from HiMedia, Mumbai, India. HCl, gallic acid, sodium acetate, potassium chloride, potassium per sulphate, were purchased from SD Fine Chemicals, Mumbai, India. 2, 2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Torolox, catechin were obtained from Sigma-Aldrich. Methanol (HPLC grade) from Merck and Ethanol from sd fine chem. All other chemicals and reagents used in the present study were of analytical grade.

Methods

Infrared drying of jambhul

Infrared drying was performed with laboratory scale infrared dryer (Gel Engineering India Pvt. Ltd). Drying temperature and time were controlled by using control knob. The pulp from thawed jambhul was separated manually and homogenized using laboratory blender. This homogenized jambhul pulp was

subjected to infra red drying at 80°C until the constant weight of the sample is obtained. The dried pulp was ground using analytical mill. Prepared powders were kept in airtight plastic jars and utilized for the preparation of *kulfi*.

Preparation of Kulfi from jambhul

The *kulfi* was prepared from 250 ml milk. Milk was then reduced to half by continuously stirring it. Powdered sugar (25 g), corn powder (2.5 g) and sodium alginate (0.39 g) were initially dissolved in hot milk and then added to milk (to avoid the lumps formation in *kulfi*) and stirring was done continuously to make milk thick and then this was transferred to mold. These molds were then allowed to cool. They were then kept for freezing at (-20°C) for 12 hrs. The jambhul *kulfi* was prepared by adding (3 to 9% of infrared dried jambhul powders) at the end of the cooling with even distribution of powders. The product was optimized on the basis of sensory evaluation and further it was analyzed for proximate and biochemical analysis.

Sensory analysis

Sensory analysis was carried out according to the method of Lawless and Heymann (2010). Sensory evaluation was carried out using 9-point hedonic rating scale in laboratory at ambient conditions. Ten semi-trained panelists were selected. They were healthy postgraduate students (M.Tech and Ph.D Research Scholars) of food technology between age group of 23 to 30 years without any medical disorder. Sensory panelists were asked to rate and give score for different parameters as appearance, colour, texture, mouth feel, flavour, taste and overall acceptability.

Extraction of phenolics

The homogenized jambhul pulp, jambhul powder, milk *kulfi* and jambhul *kulfi* (1 g) was extracted for 3 hour with 10 ml of methanol solvent on an orbital shaker set at 180 rpm (30 ± 1°C). The sample suspension was centrifuged at 10,000 g for 15min at 10°C, the supernatant was collected filtrate was stored at -20°C. Analysis was completed within two days.

Determination of total phenolic content

The total phenolic content was measured by Folin - ciocalteu method explained by Singh et al (2002). For phenolics gallic acid was used as a standard. Methanol extracts of phenolics (0.2 ml) from jambhul and milk *kulfi* was added with 1 ml of Folin - ciocalteu reagent and was diluted with distilled water in a ratio of 1:10 and 0.8 ml sodium bicarbonate (7.5%) was added to this mixture. This mixture was allowed to stand for 30 min at room temperature in dark and absorbance was measured at 765 nm. The standard curve was linear between 0 and 100 µg/ ml gallic acid. Results were represented as mg of GAE/g Wet basis.

Determination of total flavonoid content

The total flavonoid content was measured by Vanillin-HCL method as explained by Rebecca et al. (2010). Methanol extracts of phenolics (0.5 ml) jambhul and milk *kulfi* was dispensed into test tube and 2.5 ml of vanillin reagent (8% HCl in methanol/ 4% vanillin in methanol, 1:1, v/v) was added to the sample and incubated in water bath for 20 min at 30°C. The absorbance was taken at 500nm. The standard curve was linear between 0 and 250 µg/ ml catechin. The flavonoids were represented as mg of CE/g Wet basis.

Determination of total monomeric anthocyanin pigment content

The samples were analyzed for its total monomeric anthocyanin pigment content by pH differential method (Lee et al. 2005). Test portion was diluted with pH 1.0 and pH 4.5 buffer at 520 and 700 nm until absorption within linear range of spectrophotometer which were measured within 20-50 min of preparation. Result of anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents, were calculated and expressed as follows:

$$\text{Anthocyanin pigment (cyanidin - 3 - glucoside equivalent, } \frac{\text{mg}}{\text{L}}) = \frac{A \times MW \times DF \times 10^3}{\epsilon \times l}$$

where A = (A_{520nm} - A_{700nm})pH 1.0 - (A_{520nm} - A_{700nm})pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor established in D; l = path length in cm; ε= 26 900 molar extinction coefficient, in L · mol⁻¹ · cm⁻¹, for cyd-3-glu; and 10³ = factor for conversion from g to mg.

ABTS Assay free radical-scavenging Assay

Antioxidant activity was measured using Hitachi Spectrophotometer using the improved ABTS method (Re et al. 1999). The ABTS reagent was prepared freshly and used within 2days. The reagent was made by mixing 7mM ABTS + 2.45 mM potassium persulfate and incubated for 16 hours at 37°C. The ABTS

cations diluted with ethanol to set O.D at 0.7 (±0.02) at 734 nm(1:30, v/v). 3.9 ml (absorbance of 0.700±0.02) was added to the 0.1 ml of tasted sample and mixed thoroughly and absorbance was measured at 734 nm immediately after 6 min. The standard curve was linear between 0 and 20 µM Trolox. Results are expressed in µM Trolox equivalents (TE)/g Wet basis.

DPPH free radical-scavenging assay

The ability to scavenge DPPH free radicals was determined based on the method (Sahreen et al. 2010) with little modification in the mixture of test sample concentration and DPPH concentration. 0.1mM of DPPH prepared in ethanol was diluted to set the absorbance below 1.2(±0.02) at 517 nm and added to the 1ml of test sample in test tube and shake it vigorously and kept for 15 min incubation in dark room and absorbance was measured at 517 nm. The standard curve was linear between 0 and 30 µM Trolox. Results are expressed in µM Trolox equivalents (TE)/g Wet basis.

Chemical composition

Jambhul and milk *kulfi* were subjected to proximate analysis such as moisture, fat, protein, ash by AOAC Method, (AOAC 1995).

Statistical analysis

Statistical analysis was performed by using an MS Excel (2007) t-test. All data were expressed as mean from triplicate samples. Differences were considered statistically significant at p<0.05 level.

RESULTS AND DISCUSSION

Effect of infrared drying on phenolic, flavanoid, monomeric anthocyanin and antioxidant capacity

Jambhul was dehydrated within 2 hrs by using infrared drying. Effect of infra red drying on phenolic content, flavonoid content, monomeric anthocyanin content and antioxidant capacity by ABTS and DPPH of jambhul powder was shown in Table1. Infra red dried jambhul powder showed total phenolic content (27.61 mg/g), total flavonoid content (28.75 mg/g), total monomeric anthocyanin content (2.59 mg/g), and antioxidant capacity shown by ABTS (27.19 µM/g) and DPPH (375.32 µM/g). This freshly dried powdered sample was used in the preparation of milk *kulfi*. Madrau et al (2009) observed degradation of phenolic acid content (neochlorogenic acid and chlorogenic acid) and flavonoid content (catechin, epicatechin, quercetin 3 O-glucoside, and rutin) in apricots after drying at 55°C and 75°C as compared with fresh apricots. We observed similar trend in our sample. Polyphenoloxidase (PPO) enzymatic activity responsible for degradation of phenolics in apricots (Raynal et al. 1989; Radi et al. 1997) During the dehydration process PPO activity remains high for longer periods when the drying temperature is around 55–60°C, whereas shorter exposure period are needed to inactivate the enzyme at temperatures of 75–80°C (Raynal et al. 1989; Arslan et al. 1998). In our case, although PPO activity was not studied, it is likely that degradation kinetic was the same as in the works cited. Madrau et al. (2009) reported that degradation of flavonoids is sensitive to temperature and temperature dependant. The degradation of anthocyanin, total phenolic content and antioxidant capacity found in raspberries as compared to fresh after drying was observed by various methods such as freeze drying, hot-air drying, microwave-vacuum (MIVAC) drying and Hot-air and microwave-vacuum combination drying (Mejia-Meza et al. 2010)

Table1 Effect of infra red drying on bioactive components (TPC,TF, ANTHOCYANINS, ABTS, DPPH) in jambhul pulp and jambhul powder

Bioactive Composition	Jambhul pulp	Jambhul powder (IR dried)
TPC (mg/g)	87.37 ±0.53	27.61 ±1.38
TF (mg/g)	266.85±0.93	28.75 ± 1.31
Anthocyanins (mg/g)	27.4 ±0.09	2.59 ±0.09
ABTS (µM/g)	141.20±0.47	27.19 ± 0.59
DPPH (µM/g)	396.09±0.77	375.32 ±3.71

N.B: TPC= Total Phenolic Content; TF = Total Flavanoids; TMAC- anthocyanin content, , DPPH- (2,2-diphenyl-1-picrylhydrazyl), ABTS -(2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)
Mean± SD of three determinations

Effect of jambhul powder on the sensory parameters of Kulfi

Sensory evaluation of jambhul *kulfi* is presented in Table 2. *Kulfi* prepared by incorporating 3% jambhul solids received sensory score 6.57 which is near to the control *kulfi* (6.59). Incorporation of 3% of jambhul powder in milk *kulfi* showed highest sensory overall acceptability score in taste and flavor as compared with the control milk *kulfi*. As the concentration of jambhul powder increased; the astringent taste was also increased hence panelists disliked or slightly liked the product. The body and texture score was influenced by the incorporation of jambhul powder. It ranged from 6.32 to 6.06.

Table 2 Effect of jambhul powder on the sensory parameters of Kulfi

Sample	Taste	Body and Texture	Color and appearance	Flavor	Overall acceptability
Control Milk Kulfi	6.4±0.28	6.62±0.31	7.18±0.23	6.1±0.19	6.59±0.29
3% Jambhul powderKulfi	6.7±0.26	6.32±0.28	6.82±0.29	6.4±0.25	6.57±0.26
6% Jambhul powder Kulfi	6.03±0.19	6.24±0.18	6.56±0.17	6.1±0.16	6.22±0.18
9% Jambhul powder Kulfi	5.53±0.16	6.06±0.18	6.38±0.19	5.3±0.16	5.82±0.16

Score Card of 9- Point Hedonic Scale

A 9-extremely like, 8-like very much, 7- like moderately, 6 -like slightly, 5- Neither like nor dislike,4-dislike slightly, 3- dislike moderately, 2- dislike very much, 1- extremely dislike
 Mean± SD of three determinations

Chemical composition of jambhul kulfi

Chemical composition of jambhul and milk *kulfi* is presented in Table 3. Total solid content of milk *kulfi* 23.67 but after incorporation of jambhul powder, it increased upto 35.94. Incorporation of jambhul powder in milk *kulfi* shows increased in fat, ash, and acidity. There is no significant difference in protein content of milk and jambhul kulfi. **Bhadakawad et al (2009)** reported that golden kulfi prepared from 40:60 blends of buffalo milk and safflower milk had lower fat, protein, total solid and ash content compared to control golden kulfi. **Ayar and Elgün (2003)** found there was no significant difference (p< 0.01) in Fat, protein and acidity by addition of strawberry (0.2%) and banana (0.2%) with yoghurt.

Phenolic content, flavonoid, anthocynin and antioxidant capacity was determined for jambhul and milk *kulfi* before freezing to study the effect of freezing on above active components. These components were analyzed after

freezing. As seen from the Table 4 there was value addition in milk kulfi such as TPC (78.68%), TF (100%) anthocynin (100%), ABTS capacity (66.20%), DPPH (91.22%) by incorporation of 3% of jambhul powder, whereas total flavonoid and anthocynin were additional to the milk *kulfi*. Also anthocynin provide color to the milk *kulfi*.

Table 3 Chemical composition of Kulfi

Composition (% basis)	Milk Kulfi	Jambhul Kulfi
TS	23.67 ±0.09a	35.94±1.11b
Fat	3.97±0.14a	4.95±0.15b
Protein	12.03±0.04a	11.89±0.3a
Ash	1.73±0.03a	2.38±0.09b
Carbohydrates (by difference)	82.27±3.27a	80.78±2.45b
% TA	0.18±0.01a	0.36±0.01b

Mean± SD of three determinations

Effects of freezing on bioactive nutrients of Kulfi

After 12 hrs of freezing of jambhul and milk *kulfi* at (-20°C); percentage losses in milk *kulfi* 18.75 in TPC, 17.24 in ABTS, 41.17 in DPPH where as % losses in jambhul *kulfi* 4.68 in TPC,17.86 in TF,32.6 in anthocynin, 15.45 in ABTS capacity, 3.71 in DPPH capacity. Total phenolic content and antioxidant capacity shown by ABTS significantly decreased (p < 0.05) in milk *kulfi* and jambhul *kulfi*. Milk *kulfi* showed significant decrease in antioxidant capacity shown by DPPH but in jambhul *kulfi* there were no significant differences after freezing. ABTS and DPPH assay is based on the antioxidant ability to react with ABTS and DPPH radical cation generated in the assay system. These methods are widely used to evaluate antioxidant activity in foods and biological systems. Total flavonoid content and anthocynin content in jambhul *kulfi* were significantly (p < 0.05) decreased after freezing shown in Table 4. Many research studies have reported that enzymes are responsible for degradation of phenolic, flavonoid, anthocynin (**Shahidi and Nacz 2004; Wrolstad 2004; Madrau et al. 2009; Odriozola-Serrano et al. 2009**). In our experiment pasteurized milk and infra red dried jambhul powder were used for the preparation of jambhul *kulfi* hence it is considered as activity of enzyme is zero, losses which occurred in bioactive components of milk *kulfi* and jambhul *kulfi* could be due freezing process at -20°C for 12 hours.

Table 4 Effects of freezing on bioactive components (TPC, TF, ANTHOCYANINS, ABTS, DPPH) on milk kulfi and jambhul kulfi

Bioactive components (wet basis)	Milk Kulfi		Jambhul Kulfi		Value addition (%)
	Before Freezing	After Freezing	Before Freezing	After Freezing	
TPC (mg/g)	0.16±0.01 ^a	0.13±0.01 ^b	0.64±0.03 ^c	0.61±0.03 ^d	78.68
TF(mg/g)	-	-	17.02±0.68 ^a	13.98±0.55 ^b	100
Anthocyanins (mg/g)	-	-	0.46±0.01 ^a	0.31±0.01 ^b	100
ABTS (µM/g)	0.29±0.01 ^a	0.24±0.01 ^b	0.84±0.02 ^c	0.71±0.01 ^d	66.20
DPPH (µM/g)	0.85±0.01 ^a	0.50±0.02 ^b	5.92±0.18 ^c	5.70±0.29 ^c	91.22

*TPC= Total Phenolic Content; TF = Total Flavonoids; DPPH- (2,2-diphenyl-1-picrylhydrazyl), ABTS -(2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt),
 Mean± SD of three determinations

CONCLUSION

Incorporation of 3% of jambhul powder showed value addition in the milk kulfi in terms of enhanced phenolic and antioxidant capacity. Flavonoid and

anthocynin content in milk kulfi in the form of jambhul powder as functional ingredient. It was observed that degradation of phenolic, flavonoid, anthocynin, and antioxidant capacity by ABTS and DPPH after drying and freezing. Nowadays consumers are very conscious about health promising products hence

addition of fruit pulps or fruit powders with dairy based products will help to fight the degenerative diseases and also enhance the diet.

Acknowledgments: The authors gratefully acknowledge University Grant Commission (UGC) for the financial support in carrying out this study.

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