Composition and functionality of whole jamun based functional confection

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Abstract Whole jamun based functional confection (WJFC) was developed from an optimized blend (through response surface methodology) containing 26.585 % paste of jamun pulp with adhering skin, 2 % jamun seed powder, hydrocolloid mixture (2.289 % agar, 1.890 % pectin and 27.236 % polydextrose), antimicrobials (0.022 % benzoic acid and 0.085 % sorbic acid), and 40 % added water. The confection also contained 0.08 % sucralose, 0.06 % citric acid and 100 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ /g pectin. The confection was found to be rich in minerals like Ca, Mg, K, Na and P, with prebiotic activity and low glycemic index (48.1). Additionally, WJFC had reduced calorie (1.48 kcal/g) and high dietary fiber content (15.49 ± 0.058 g/100 g (db)). The antioxidant potential measured as DPPH radical scavenging activity and FRAP with different extraction solvents was found to range between 0.26 ± 0.01 and 0.98 ± 0.04 mg BHA/g and 2.57 ± 0.97 and 18.17 ± 1.30 μM Fe^{2+} /g, respectively, with highest yield obtained for 50 % aq. ethanolic extract. Moreover,

the antioxidant potential was observed to be dose dependent with IC_{50} values as 9.89 and 2.75 mg (db) against DPPH and superoxide anion radicals, respectively. WJFC was found to suppress α -amylase activity and retard glucose dialysis depicting the antidiabetic effect.

Keywords *Syzygium cumini* · Confection · Antioxidant · Antidiabetic · Amylase inhibition · Glycemic index · Prebiotic effect · Glucose dialysis retardation

Introduction

The fusion of physiological benefits like health promoting and disease preventing with the basic function of supplying nutrients conceptualized the development of functional foods. In addition to health benefits tested by appropriate methodology, functional food should be sensorially acceptable.

Jamun (*Syzygium cumini* L.), a seasonal and perishable fruit, indigenous to India, grows mainly in tropical and subtropical parts of the world. It is highly underutilized as no organized farming is practiced. The fruit is rich in minerals and phytochemicals, sweet and sour in taste with little astringency attributed to its high tannin content, and shows high antioxidant potential heading towards its therapeutic effectuates (Schwag and Das 2014). Pharmacological investigations have proven its health benefits, to name a few, antioxidant (Benherlal and Arumughan 2007), antidiabetic (Helmstädter 2008), hypolipidemic (Ravi et al. 2005), hepatoprotective (Das and Sarma 2009), and radioprotective (Jagetia et al. 2005). Among these studies, antidiabetic effect is widely documented. Helmstädter (2008) and Rekha et al. (2008) observed considerable reduction in blood glucose level of induced diabetic rat on administration of feed containing jamun seed and pulp. A few attempts on post-harvest processing of

Highlights

1. The laboratory developed whole jamun fruit based functional confection (WJFC) was characterized for its functional properties.
2. The confection is found to be nutritious with high mineral content.
3. The product is superior to the existing jamun based/containing products in terms of TPC and antioxidant potential.
4. WJFC has prebiotic effect and is a low glycemic food with high carbohydrate content.
5. WJFC has α -amylase inhibition activity and retards the glucose diffusion, and therefore, possesses potential antidiabetic action.

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this fruit have been undertaken for making different foods, namely squash, jam, juice (Shahnawaz and Sheikh 2011), wine (Chowdhury and Ray 2007) and fruit powder (Sonawane et al. 2013). However, report on utilization of jamun fruit or its fraction for development of functional foods is unavailable.

Confection is a group of sweet and carbohydrate rich products which primarily includes sugar (sucrose) along with optional addition of chocolate, nuts, fruits, vegetables or gums, possessing texture from hard to soft delicacies. Its popularity and demand have opened a huge business possibility worldwide at a tune of US\$ 200 billion in 2014 with an annual growth rate of 2 % during last five years. To reduce the calorie intake by the obese and diabetic people as well as to satisfy their appetite for sweet, several sugar-free (low sugar) sweetmeats are appearing in the market. Among the documented information on fruit based confections, the combination of low/no added sugar with health claim is not available. The existing literature documented the confection as either no added sugar without any health claim (Savant 2012) or functional confection with added sugar (Bali 2007). A potential solution of functional confection can be served by integrating whole jamun (skin, pulp and seed) for its health benefits into a confection without added sugar; however, it is a challenge to maintain its functional properties intact. Reports on nutritional composition and functional property of fruit based confections, in general, and jamun in particular is scarce. In this study, the nutritional and functional properties of the laboratory developed whole jamun based functional confection were characterized.

Materials and methods

Materials

Ingredients used for WJFC

Jamun was procured in the peak growing season (June–July 2013) from local markets of Kharagpur, West Bengal, India. Pulp with adhering skin and seeds were separated manually. The pulp portion was stored at -18°C while the seed was dried at 60°C for 24 h in an incubator and powdered in hammer mill to pass through 120 μm sieve. The jamun seed powder (JSP) was stored under refrigeration until use. At the time of confection preparation, the frozen pulp was thawed and made into paste (JP) in mixer. All food grade additives like agar (E-406), low methoxyl pectin (Food grade, E-440), polydextrose (E-1200), calcium chloride (E-509) and citric acid (E-330) were purchased from companies viz., Calpro Foods Pvt. Ltd. India,

CPKelco Pvt. Ltd. India, Brenntag Ingredients (India) Pvt. Ltd. India, Hi-Media Laboratories Pvt. Ltd. India and Jason Chemical Works, Kolkata, India.

Reagents

All the reagents used in the chemical analyses were of analytical grade and were procured from Merck, India and HiMedia, India, while the enzymes and microbial cultures used for analysis were purchased from Megazymes, India and MTCC Chandigarh, India, respectively. Unless otherwise mentioned, water used as solvent for dissolution/dilution was Millipore water (mw).

Methods

Confection preparation

Three stages of numerical optimization through response surface methodology (RSM) using Design Expert ver. 7.0.0' statistical software (Stat-Ease Int. Co., Minneapolis, USA) with D-optimal mixture design at first two stages and rotatable central composite design (RCCD) at third stage were applied sequentially to obtain whole jamun based functional confection (WJFC). The first step involved optimization of hydrocolloid mixture using agar (ranging within 1 to 3 g), pectin (1 to 3 g) and polydextrose (24 to 28 g) as variables to obtain 30 g of hydrocolloid mixture. For each experiment, gel was prepared by dissolving 30 g hydrocolloid mixture in 70 ml aquaguard water (aw) (Aquaguard cooler cum purifier, HECCP, Uttarakhand, India) by heating, followed by molding of the hot solution in cells of 8 ml capacity each. Each cell was preloaded with 200 μl CaCl_2 solution (100 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ /g pectin dissolved in 2 ml distilled water (dw)). Just after pouring the hot solution into the cell, the solution was manually stirred twice with a spatula. The mixture was allowed to set at room temperature for 15 min. The set gels were carefully removed from the molds, wrapped in commercially available transparent cling film (Rana et al. 2015) as primary packaging material, followed by keeping the wrapped confections in LDPE zip lock pouch as secondary packaging material. The pouches were stored under refrigeration (10°C), and the confections analyzed for hardness, springiness and cohesiveness as responses. Optimization was performed to achieve optimized hydrocolloid mixture (OHM) yielding maximum of these responses. Secondly, OHM (25 to 35 g) dissolved in 40 ml aw along with JP (20 to 30 g) and JSP (1 to 5 g) were optimized to arrive 60 g of optimum confection formulation (OCF) with a target of maximising sensory quality (appearance, flavor, taste, mouthfeel and overall acceptability using 9 point hedonic

scale and 25 panelists), total phenolics content and texture characteristics (hardness, springiness and cohesiveness) as the responses. Preparation and storing of the confections were same as above. For the first two stages of optimization, responses of the end products were measured within five days of preparation. Lastly, the optimization of antimicrobial agents (benzoic acid: 0 to 1500 ppm and sorbic acid: 0 to 2000 ppm) to be added in OCF was carried out considering the time of storage at which the product was beyond safety limit, i.e., yeast and mold count (YMC) ≥ 100 cfu/g (Food Safety and Standard Authority of India, FSSAI 2011) as response. For preparation of OCF containing benzoic acid and sorbic acid, the antimicrobials were added at the stage of dissolution of hydrocolloids; rest of the procedure was exactly same as discussed, however, the ziplock pouches were stored at ambient condition (temperature fluctuating between 32.2 °C maximum and 24.4 °C minimum and relative humidity between 80 % maximum and 76.4 % minimum). The optimized formulation, thus arrived was used for preparation of WJFC. For this, 2.289 g agar, 1.890 g pectin, 27.236 g polydextrose, 0.06 g citric acid, 0.08 g sucralose, 0.022 g benzoic acid and 0.085 g sorbic acid were dissolved in 40 ml aw. To the dissolved mixture, 26.585 g JP and 2.00 g JSP were added and heated for 1 min. The mixture was set in molds as described above to form WJFC. About 8–10 numbers of set confections, each one individually wrapped in cling film, was put in ziplock LDPE pouches (265 mm $\times$ 175 mm) and stored under refrigeration (10 °C). Details of the methodology has been described elsewhere (Schwag 2016). The samples were analyzed within 10 days of preparation.

Proximate composition

Moisture content, crude protein, crude fat, crude fiber and ash content of WJFC were measured using standard methodologies (AOAC 2012). The carbohydrate content was determined by difference.

Reducing sugar content

Reducing sugars was extracted from 1 g dried WJFC using ethanol by refluxing. The ethanol was evaporated, residue dissolved in water, and the solution estimated by 3,5-dinitrosalicylic acid (DNS) assay (Sadasivam and Manickam 2008). The results were expressed as mg glucose/100 g WJFC (db).

Dietary fiber

The dried and defatted WJFC was analyzed for total dietary fiber comprising of the soluble and insoluble categories by

enzymatic-gravimetric method (AOAC 2012) using K-TDFR megaenzyme dietary fiber kit.

Elemental composition

Wet ashing of WJFC was performed using H₂SO₄, HNO₃ and HClO₄ mixture (Ranganna 2007). The elements, namely K, Na, Ca, Mg, Fe, Zn, Cu and Mn were then determined using atomic absorption spectroscopy (Perkin Elmer, AAnalyst 700, USA), and phosphorus was estimated colorimetrically (Elico SL 210 UV-Vis spectrophotometer, Kolkata, India) using AOAC (2012) protocol.

Vitamin C

Vitamin C was estimated by following direct colorimetric method using 2,6-dichlorophenol-indophenol dye (Ranganna 2007). For this, 5 g WJFC was extracted by maceration with 20 ml of 2 % metaphosphoric acid (HPO₃), followed by centrifugation at 3500 g for 15 min. The extract was assayed to determine vitamin C and results were expressed as mg ascorbic acid (AA)/100 g WJFC (db).

Calorific value

The calorific value was determined using bomb calorimeter and factorial modified Atwater model. For bomb calorimeter, 1 g sample was loaded in a closed ignition vessel/bomb at a pressure of 30 bar. The heat released was absorbed by the surrounding water which was directly displayed on the display board of the machine (IKA®c200 bomb calorimeter). In factorial modified Atwater model, the quantity of fat (F), protein (P), available carbohydrate (AC, determined by difference, excluding dietary fiber) and unavailable carbohydrate (UC, total dietary fiber) were multiplied by 9.0, 4.04, 4.04 and 2.0 kcal, respectively (equation (eq.) 1), to obtain the calorific value (Zou et al. 2007).

$$CV(\text{kcal}/100\text{g}) = (9.0)F + (4.04)P + (4.04)AC + (2.0)UC \quad (1)$$

Functionality

Anthocyanin content (ANT) ANT was estimated using pH differential method (Lee et al. 2005). One gram of WJFC was extracted with 25 ml of acidified methanol (0.1 ml of conc. HCl added in 100 ml of absolute (99.99 %) methanol) by shaking in incubator shaker at 27 °C for 3 h, followed by centrifugation at 5800 g for 15 min. The extract was diluted 5 times separately by 0.4 M acetate buffer (pH = 4.5) as well as 0.025 M potassium chloride-HCl buffer (pH = 1), and allowed

to stand for 15 min in dark. Absorbance of the mixtures was measured at 520 and 700 nm, and ANT was evaluated as mg malvidin-3-glucoside (M3G)/100 g using Eqs. 2 and 3.

$$A = (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH}=1} - (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH}=4.5} \quad (2)$$

$$\text{Anthocyanins} = \frac{A \times MW \times DF \times 1000}{\epsilon} \quad (3)$$

where A is absorbance calculated using Eq. 2, MW is molecular weight of M3G, i.e., 493.2 g/mol, DF is dilution factor, ϵ is molar absorbance for M3G, i.e., 28,000.

TPC and antioxidant activity Extraction of phenolics and antioxidants is highly dependent on condition of extraction like temperature, pH, nature of solvent, solid solvent ratio, etc., the most crucial one being the nature of solvent. Therefore, WJFC was extracted with different solvents for assessment of TPC and antioxidant activity.

WJFC (5 g) was extracted (3 h at 27 °C) with 20 ml of water, absolute ethanol, 50 % (v/v) aqueous (aq.) ethanol, absolute methanol, 50 % (v/v) aq. methanol, absolute acetone and 50 % (v/v) aq. acetone in shaking incubator (Atala et al. 2009). Following centrifugation (5800 g for 15 min) the supernatants were stored at −20 °C for further estimations. The extract(s) was used for the assessment of total phenolics content (TPC) using a modified Folin-Ciocalteu assay (Singleton et al. 1999). Antioxidant activity of the extract(s) was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging (Kim et al. 2004), ferric reducing antioxidant property (FRAP) (Benzie and Strain 1999), and photochemiluminescent (PCL) assay (for 50 % aq. ethanolic extract only) using antioxidant analyser (Photochem® instrument, Analytik Jena, Leipzig, Germany) following manufacturer's protocol (Chemical kit

number: 360:003.24). For PCL assay, data acquisition and analysis were done through inbuilt software that generated an area of the plot of signal (V) vs. time (s) to calculate the % inhibition using Eq. 4:

$$\% \text{Inhibition} = \frac{\int \text{AUC}_{\text{Blank}} - \int \text{AUC}_{\text{Sample}}}{\int \text{AUC}_{\text{Blank}}} \times 100 \quad (4)$$

where $\text{AUC}_{\text{Blank}}$ is the integral of the area under the curve for blank and $\text{AUC}_{\text{Sample}}$ is for sample. The antioxidant potential was expressed as nm Trolox/g WJFC (db).

IC_{50} represents the amount of material required for 50 % inhibition of a radical (Upadhyay and Mishra 2014). The value was determined using 50 % aq. ethanolic extract against DPPH radical and superoxide anion radical using DPPH and PCL assays.

Prebiotic activity Prebiotic activity was estimated according to Moongngarm et al. (2011) using MTCC 10307 *Lactobacillus acidophilus* (LAB) and enteric bacterial culture, MTCC 443 *Escherichia coli* (*E. coli*). Following revival using MRS broth and tryptone soy (TS) broth for LAB and *E. coli*, the cultures were employed for prebiotic assay. For this, LAB was separately incubated in MRS broth containing 1 % glucose ($\text{LAB}_{\text{control}}$) and 1 % freeze dried WJFC ($\text{LAB}_{\text{sample}}$), while in case of *E. coli* only MRS broth was replaced by M9 broth. Colonies were enumerated at 0 and 24 h (after incubation at 37 °C) using spread plate technique in MRS agar plate for LAB and TS agar plate for *E. coli*, and prebiotic activity score (PAS) was calculated using Eq. 5.

$$\text{PAS} = \frac{\text{LAB} \log\left(\frac{\text{cfu}}{\text{g}}\right)_{\text{Sample at 24 h}} - \text{LAB} \log\left(\frac{\text{cfu}}{\text{g}}\right)_{\text{Sample at 0 h}}}{\text{LAB} \log\left(\frac{\text{cfu}}{\text{g}}\right)_{\text{Control at 24 h}} - \text{LAB} \log\left(\frac{\text{cfu}}{\text{g}}\right)_{\text{Control at 0 h}}} - \frac{\text{E.coli} \log\left(\frac{\text{cfu}}{\text{g}}\right)_{\text{Sample at 24 h}} - \text{E.coli} \log\left(\frac{\text{cfu}}{\text{g}}\right)_{\text{Sample at 0 h}}}{\text{E.coli} \log\left(\frac{\text{cfu}}{\text{g}}\right)_{\text{Control at 24 h}} - \text{E.coli} \log\left(\frac{\text{cfu}}{\text{g}}\right)_{\text{Control at 0 h}}}$$

Glycemic index Glycemic Index (GI) was estimated using in vivo as well as in vitro methodologies.

For in vivo GI test, 4 non-diabetic male BALB/c mice (a strain of an albino), each of 30 g body weight (DFRL Animal house, Mysore) were used. After overnight fasting, the mice were force fed with 125 mg of freeze dried WJFC powder (~100 mg available carbohydrates). Onwards this, blood samples were taken from the tip of the tail after 15, 30, 45, 60 and 90 min, to measure the plasma glucose. For fasting plasma glucose levels, i.e., 0 min, the blood sample was collected before feeding. Similar methodology was followed using the

same group of mice, but feeding 100 mg glucose per mouse as reference carbohydrate. The two tests were performed on two different occasions with an interval of about 7 days.

A graph was plotted for the blood glucose level at different intervals of time (Björck et al. 2000). Then, GI was calculated as the percentage ratio of incremental AUC over the baseline for the test sample to that of reference (glucose at 100 mg/animal).

In vitro analysis was done following the method of Goñi et al. (1997). It involved starch hydrolysis of freeze dried WJFC (50 mg) with pepsin, α -amylase and amyloglucosidase

and the glucose release was timely monitored. The same procedure was followed for reference food, that is, commercially available white bread of a popular brand, WB (freshly collected from a retail outlet). Hydrolysis Index (HI) for WJFC was determined as a ratio of area under the curve (AUC) for WJFC to that of reference food, expressed as percentage. GI and glycemic load (GL) of WJFC was calculated using empirical Eqs. 6 and 7.

$$GI = 39.71 + (0.549 \times HI) \quad (6)$$

$$GL \text{ per } 100g \text{ WJFC} = \frac{GI \times \text{Carbohydrates (excluding dietary fiber) in } 100g \text{ of WJFC}}{100} \quad (7)$$

In vitro antidiabetic assay α -Amylase is a major enzyme responsible for release of glucose from food. In diabetic people, the inhibition in activity of the enzyme is desirable to control the blood glucose level. Blood glucose level is also controlled by the extent of glucose diffusion from digestive system. Antidiabetic effect of WJFC was assessed in terms of α -amylase activity (Kotowaroo et al. 2006) and glucose dialysis (Chau et al. 2003). For the former, to an aliquot of 50 % aq ethanolic extract of WJFC 1 % starch solution and α -Amylase (1Unit/ml) was added. The mixture was incubated at 25 °C for 15 min and the reaction was stopped by adding 1 N acetic acid. The remaining starch concentration was estimated by adding iodine reagent and noting the absorbance at 580 nm in spectrophotometer. Percent inhibition was calculated using Eq. 8.

$$\% \text{Inhibition} = \frac{\text{starch unhydrolysed}}{\text{total starch}} \times 100 \quad (8)$$

For glucose dialysis retardation index (GDRI) (Eq. 9), freeze dried WJFC was mixed with 20 mM glucose solution (in 0.9 % NaCl solution, pH = 7.0). The mixture was taken in a dialysis sac (molecular weight cut off =2000) and dialyzed against 0.9 % NaCl solution (pH = 7.0) at 37 °C in orbital shaking condition. The dialyzate was timely collected, and glucose content was determined using DNS assay. Similar setup was run for control (without sample) and the standard drug, acarbose (10 mg).

$$GDRI = 100 - \left(\frac{\text{Glucose concentration in dialyzate for sample}}{\text{Glucose concentration in dialyzate for control}} \times 100 \right) \quad (9)$$

Statistical analyses

Except *in vivo* GI investigated with the help of DFRL where replication was four, all the objective experiments were

replicated thrice (sample replicate), unless otherwise mentioned. The mean and standard deviation (SD) was evaluated. The effect of parametric variation on the mean was investigated by one way analysis of variance (ANOVA, F-test). Calculations were done by Microsoft excel 2007.

Results and discussion

Composition

The composition of WJFC including moisture, protein, fat, crude fiber, ash and carbohydrates along with elemental composition, reducing sugar, dietary fiber, and vitamin C content is presented in Table 1. The analysis exhibits that carbohydrate is the major component of WJFC. This is due to the fact that formulation of WJFC comprises 31.32 % of carbohydrate based hydrocolloids and 28.51 % of jamun part that also contains carbohydrate as major component viz. 14–19 % on wb for pulp and 72 % db for seed (Sehwag and Das 2014). Since no protein rich and fatty material were added in the

Table 1 Chemical composition of WJFC

Parameter	WJFC [^]
Proximate composition (%db)	
Protein	1.079 ± 0.008
Fat	0.000 ± 0.000
Ash	0.486 ± 0.006
Crude fiber	0.337 ± 0.003
Carbohydrates (excluding crude fiber)	98.097
Carbohydrates (including crude fiber)	98.343
Moisture Content (%wb)	54.817 ± 0.259
Total dietary fiber [#]	15.714 ± 0.014
Soluble dietary fiber [#]	9.859 ± 0.004
Insoluble dietary fiber [#]	5.855 ± 0.009
Reducing sugar	16.981 ± 0.147
Elements (mg/100 g, db)	
Potassium	81.595 ± 0.470
Sodium	21.200 ± 0.622
Calcium	100.097 ± 0.365
Iron	5.019 ± 0.533
Zinc	2.214 ± 0.035
Magnesium	30.184 ± 0.342
Manganese	1.612 ± 0.172
Copper	3.054 ± 0.672
Phosphorous	15.514 ± 0.083
Vitamin C (mg/100 g, db)	28.359 ± 0.667
Anthocyanin (mg M3G/100 g, db)	126.287 ± 0.969

[^] Whole jamun based functional confection; [#] for marked parameters, mean ± SD with *n* = 2, for other parameters, mean ± SD with *n* = 3

formulation, WJFC contains negligible amount of these components. Moisture content (54.817 %) of WJFC is somewhat higher than that of reported values for confections prepared with added sugar for instance: soft fruit candies enriched by grape skin powder contains 40 % moisture (Cappa et al. 2015), starch and corn syrup based strawberry confection contains 24 % (Fisher et al. 2014) and apple marmalade candy 44.78 % (Muizniece-Brasava et al. 2011).

The reducing sugar content of WJFC is estimated to be 16.981 ± 0.147 g glucose equivalent/100 g WJFC (db). Nasrin et al. (2007) reported the reducing sugar content of shelf stable anola, carambola, papaya, pineapple and watermelon rind candies in the range of 55.56–58.71 %. Dar et al. (2011) reported the content as 27.47 % for candied cherry fruit. Thus, in comparison to the data for fruit based confection containing added sugar, WJFC has lower content of reducing sugar, probably because of avoiding addition of sugar during its formulation. In case of confection containing added sugar, the sucrose can undergo hydrolysis and followed by inversion producing reducing sugars i.e., glucose and fructose, during the undertaken processing.

From Table 1, total dietary fiber content of WJFC is found to be 15.714 g/100 g dry matter (i.e., 7.07 g/100 g wb) which comprises of 62.74 % soluble and 37.26 % insoluble fraction. According to the European claims-Regulation (EC) No. 1924/2006, the fruit candies containing more than 6 g fiber/100 g product can be labeled as “high in fiber”. Therefore, in the present case, WJFC can be claimed and labeled as high fiber confection.

It is worth mentioning that on an average an adult excretes 20–30 g of mineral salts out of body. These mineral salts comprise of chlorides, sulphates and phosphates of K, Na, Mg and Ca. Thus, these elements are most important from physiological point of view, and should be incorporated in diet in adequate amount on daily basis. Also, K and Na are electrolyte of human body to regulate blood pressure. Mg also helps in maintaining blood pressure, whereas Ca along with P is responsible for cellular metabolism and bone strengthening. A functional food must be rich in major minerals required for proper functioning of the metabolism. WJFC is found to be rich in calcium, magnesium, potassium, sodium, phosphorous and iron and follows the order as $\text{Ca} > \text{K} > \text{Mg} > \text{Na} > \text{P} > \text{Fe}$ (Table 1). The high Ca content is explicable by the formulation, i.e., addition of CaCl_2 as firming agent during formulation of the confection. Also, WJFC has ferric reducing antioxidant power (discussed below), i.e., the antioxidants present in WJFC are capable of reducing ferric to ferrous, and thus increasing the bioavailability of the iron present therein.

Vitamin C is a natural antioxidant and its presence in body serves many biochemical functions. Vitamin C content in WJFC is estimated to be 28.359 ± 0.667 mg ascorbic acid/100 g dry matter (Table 1). Vitamin C content of fruit based confection has been reported to depend on the type and quality

of the fruit used, formulation and mode of processing. For instance, Sawate et al. (2005) reported the value of papaya candy prepared by immersion in cold sugar syrup as 22.1–25.02 mg/100 g, Wijaya et al. (2012) reported 18–36 mg ascorbic acid/100 g of soft candy prepared from various ratios of yoghurt with red fleshed guava extract, while Pietrzyk et al. (2010) have reported quite high amount in candied black currant (268.4 mg/100 g) and black chokeberry (78.69 mg/100 g). However, Pietrzyk et al. (2010) purposefully enriched the candies with Vitamin C during the processing. Thus, WJFC is comparable to other reported fruit based confection in respect of vitamin C content, though its preparation involves a heating step.

Calorific value

Calorific value (CV) calculated using factorial modified Atwater model and Bomb calorimeter are found to be 3.67 and 4.00 ± 0.01 kcal/g of dry matter of WJFC, respectively; on wet basis the value amounts to 1.48 and 1.80 kcal/g WJFC, in that order. The difference in value is due to the fact that factorial method is empirical calculation of the energy derived from digestible part of the food product whereas bomb calorimeter estimates the energy generated by complete combustion of the product. As discussed above, the confection has high dietary fiber content which has combustible properties but are not fully digested and assimilated by the body to extract calories. In comparison to market available branded fruit based jelly confection with added sugar (3.18 kcal/g wb), WJFC (1.48 kcal/g wb) have 53 % lower calories on wet basis. According to FDA and FSSAI labeling and nutrition guidelines, the WJFC lies under the category of reduced calorie product.

Functionality

Anthocyanin content

Anthocyanin content of WJFC is estimated to be 126.287 ± 0.969 mg M3G/100 g db (i.e., 56.67 mg M3G/100 g wb). The pigment is not only responsible for the color of WJFC but also possesses health benefits like prevention of cardiovascular diseases and obesity (He and Giusti 2010). Since ancient times, humans are consuming anthocyanin rich fruits with no adverse impact on health. However, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that anthocyanin-containing extracts had a very low toxicity (He and Giusti 2010). In this concern, Clifford (2000) reported the acceptable daily intake of ANT for humans as 2.5 mg/kg body weight/day. An adult with 50 kg weight cannot exceed 125 mg M3G intake in a day. On this basis, even 100 g of WJFC could be considered safe to be consumed by consumers weighing more than 50 kg.

TPC and antioxidant activity

Phenolic compounds are bioactive components present in food, and are responsible for the functional and health beneficial properties. The TPC and antioxidant activity of WJFC evaluated using different extraction solvent are presented in Table 2. The phenolic content of WJFC is found to range between 1.62 ± 0.01 and 3.72 ± 0.03 mg GAE/g dry matter. Few researchers have utilized jamun to develop products like wine (Chowdhury and Ray 2007) and milk kulfi (Sonawane et al. 2013) reporting TPC (GAE) values as 1.7 mg/100 ml wb and 1.74 mg/g db. In comparison to these values on dry matter basis, WJFC have higher TPC content. Thus, the product developed in the present study is superior to the existing jamun based products in terms of TPC.

The dependency of TPC and its antioxidant activity on nature of solvent is affirmative for WJFC (F-test, $p < 0.05$). However, there is no significant difference in TPC and antioxidant values for absolute ethanol, methanol and acetone extracts (LSD, $p < 0.05$). The values of TPC and antioxidant activity follow the order as: 50 % aq. ethanol > 50 % aq. acetone > 50 % aq. methanol > absolute ethanol $\equiv$ absolute methanol $\equiv$ absolute acetone > water.

Overall, the antioxidant potential of WJFC ranges between 0.26 ± 0.01 and 0.98 ± 0.04 mg BHA/g (db) by DPPH assay and 2.57 ± 0.97 and 18.17 ± 1.30 μ M Fe^{2+} /g (db) by FRAP assay. The antioxidant potential against superoxide anion radical as evaluated using PCL is 0.42 ± 0.02 μ M Trolox/g dry matter.

From Table 2, it is observed that the value of TPC increased with increase in aqueousity of any absolute solvents. This effect was also reported by Chavan et al. (2001) for different peas, where more yield was recovered in 70 % aqueous acetone than absolute acetone. In present case, 50 % aq. ethanolic extract yielded maximum TPC, and thus used to calculate IC_{50} values.

Table 2 Effect of solvent on TPC and antioxidant activity of WJFC[^]

Solvent	TPC [#] (mg GAE/g)	DPPH [#] (mg BHA/g)	FRAP [#] (μ M Fe^{2+} /g)
Aqueous	1.62 ± 0.01	0.26 ± 0.01	2.57 ± 0.97
50 % aq. Ehanol	3.72 ± 0.03	0.98 ± 0.04	18.17 ± 1.30
Absolute Ethanol	$1.98 \pm 0.01^*$	$0.66 \pm 0.00^*$	$5.69 \pm 0.35^*$
50 % aq. Methanol	2.93 ± 0.02	0.79 ± 0.01	7.47 ± 0.88
Absolute Methanol	$1.83 \pm 0.01^*$	$0.64 \pm 0.02^*$	$6.91 \pm 1.04^*$
50 % aq. acetone	3.42 ± 0.01	0.84 ± 0.01	12.49 ± 1.74
Absolute acetone	$1.98 \pm 0.02^*$	$0.67 \pm 0.01^*$	$6.82 \pm 0.51^*$
LSD _{0.05}	0.15	0.03	1.86

[^] Whole jamun based functional confection; [#] mean $\pm$ SD with $n = 3$;

* difference between mean is non-significant ($p > 0.05$)

The value of %inhibition and % radical scavenging activity (RSA) for different mass of Trolox, BHA and WJFC is presented in Table 3. It is found that all these substances exhibit dose dependent antioxidant activity (F-Test, $p < 0.05$) and the activity increases significantly with weight (LSD, $p < 0.05$). The corresponding empirical relations with R^2 values are shown in the table. The values of IC_{50} derived from these equations are also included in the table. Both the trends of the effect of mass of WJFC on superoxide anion radical and DPPH[•] are linear, however, the slopes are different with IC_{50} values as 2.75 and 9.89 mg dry matter, respectively. From Table 3, it is noticed that WJFC has less antioxidant potential than trolox, and BHA, as lower the IC_{50} value more is the antioxidant potential. Surinut et al. (2003) reported the range of IC_{50} values of some fruits like grape skins, mulberries, mango, carambola, guava, and lichee as 1.10 to 9.60 mg using 0.5 mM DPPH[•] and marked the range as high antioxidant potential. Since the unit for expressing IC_{50} is not yet standardized (Maria et al. 2010), different scientists have adopted different modes. In order to provide comparable antioxidant podium, the literature reported IC_{50} values of Surinut et al. (2003) were converted to μ g DPPH[•] scavenged/mg of sample, i.e., 10.26–89.54 μ g DPPH[•] scavenged/mg. Thus, WJFC with antioxidant potential as 15.17 μ g DPPH[•] scavenged/mg lies within the range of high antioxidant foods.

Prebiotic score

Prebiotic activity is a property of a substance to support the growth of healthy bacteria such as *Bifidobacteria* and *Lactobacilli* in the gut and successively maintain health of human gut by increase resistance to invading pathogens. Prebiotic activity score of WJFC using Eq. 5 is 2.16 ± 0.05 . Moongngarm et al. (2011) reported PAS for inulin, fructooligosaccharide, garlic, onion and shallots as 2.22, 1.45, 2.15, 1.94 and 2.09, respectively. In comparison to this data, WJFC has considerable prebiotic activity, which could be attributed to the presence of polydextrose in the formulation. Slavin (2013) reported increase in growth of *Lactobacilli* and *Bifidobacteria*, the probiotic microorganisms by polydextrose and further expressed the prebiotic effect in dose-dependent manner. Since 2nd part in Eq. 5 containing count of *E. coli* is to be subtracted, the antibacterial action of jamun pulp and seed against *E. coli* (Sehwag and Das 2014) probably has contributed synergistically in increasing the PAS value.

Glycemic index

GI is a scale to calibrate the food by virtue of its potential to raise the blood glucose level. The GI scale varies from 1 to 100. Based on this, foods are categorized into three groups, that is, high GI foods ($\text{GI} \geq 70$, rapid rise in blood glucose levels), intermediate GI foods ($56 \leq \text{GI} \leq 69$, medium rise in

Table 3 Effect of antioxidants concentration on % inhibition and % RSA of WJFC

PCL Assay				DPPH Assay			
Trolox		WJFC [^]		BHA		WJFC [^]	
W* (μg)	%Inhibition [†]	W* (mg)	%Inhibition [†]	W* (μg)	%RSA [†]	W* (mg)	%RSA [†]
0.5	23.4 ± 0.0	2.02	49.0 ± 0.1	2	19.03 ± 0.43	0.00	0.00
1	40.3 ± 0.1	4.04	65.4 ± 0.2	4	44.71 ± 2.70	5.05	36.12 ± 2.43
2	52.6 ± 0.2	5.05	77.0 ± 0.1	6	64.41 ± 0.30	10.11	61.86 ± 1.49
3	72.0 ± 0.0	10.11	84.2 ± 0.1	8	74.16 ± 1.65	15.17	77.23 ± 0.37
5	92.0 ± 0.1	n.d.	-	10	83.80 ± 0.25	20.22	93.89 ± 0.18
LSD _{0.05}	2.1		2.1		1.93		1.36
Equation							
$W(\mu\text{g}) = \frac{0.0017\% \text{Inhibition}}{1-0.008\% \text{Inhibition}} R^2 = 0.99$		$W(\text{mg}) = \frac{0.022\% \text{Inhibition}}{1-0.009\% \text{Inhibition}} R^2 = 0.98$		$W(\mu\text{g}) = \frac{\% \text{RSA}}{9.214} R^2 = 0.99$		$W(\text{mg}) = \frac{\% \text{RSA}}{5.057} R^2 = 0.95$	
IC ₅₀ (db) 1.488 μg		2.75 mg		5.424 μg		9.89 mg	

[^] Whole jamun based functional confection; n.d. not determined; * weight on dry basis; [†] mean ± SD with *n* = 3

blood glucose level) and low GI foods (GI ≤55, slow rise in blood glucose level) (Kaur and Das 2015).

The *in vivo* study shows that WJFC exhibits slow increase in the blood sugar level of mice in comparison to reference carbohydrate, i.e., glucose. The blood glucose level monitored with time for reference food and WJFC is depicted in Fig. 1a where AUC of WJFC is less than that of reference food. GI of WJFC expressed as percentage of AUC of the test sample to that of the reference (glucose) is found to be 49.17.

Concerned to *in vitro* study, it is clearly noticed that WJFC is slowly hydrolyzed into glucose in comparison to white bread (Fig. 1b) leading to GI of 48.55 with 40.12 GL per 100 g of WJFC (db). The value is similar to the one obtained from *in vivo* method. According to GI based classification, WJFC is low glycemic food. It is worth to mention the GI values of some fruit based confectionery products like fruit bars (50–61), fruit leathers (90–99), fruity bitz- vitamin and mineral enriched dried fruit snack (35–42), and jelly beans (76–80) (Foster-Powdell et al. 2002).

In both the assays, WJFC is digested slowly into glucose in comparison to reference foods. The reason could be attributed to

high dietary fiber of WJFC, as the fibers lead to the formation of a polymeric matrix that makes the enzymatic attack more difficult (Gourgue et al. 1994; Chung et al. 2006). Also, the glycemic effect depends on the texture and particle size of food, type of starch, food processing and presence of ingredients, such as sugar, fat, protein, dietary fiber and anti-nutrient (Reyes-Pérez et al. 2013). Besides the influence of compositional factors, WJFC possesses α-amylase inhibitory action (as discussed later) leading to slow digestion vis-à-vis slow release of glucose into blood.

GI has relevance with chronic diseases like cancer, obesity and diabetes. The relevance is based on fiber hypothesis, suggesting that fiber consumption reduces the rate of nutrient influx from the gastrointestinal track (Jenkins et al. 2002). Augustin et al. (2001) and Franceschi et al. (2001) reported that the risk of breast and colorectal cancer decreases with incorporation of a low GI diet. A low glycemic food helps in body weight regulation. McMillan-Price and Brand-Miller (2006) suggested that a diet with low GI/GL rather than a diet with low fat content is more successful in reducing body weight. The latter could lead to post prandial hyperglycaemia whereas the former is associated with reduced energy release

Fig. 1 Time dependent variation in (a) blood glucose level of rats fed with whole jamun based functional confection (WJFC) and glucose, and (b) starch hydrolysed from white bread and WJFC

from carbohydrates. It has also been reviewed by Jenkins et al. (2002) and McMillan-Price and Brand-Miller (2006) that the reduction in portion of energy obtainable from carbohydrates in the diet improves the rate of fat loss. Whole jamun based functional confection is found to be low glycemic food with high carbohydrate content. As per the above discussion, it may be apprehended that WJFC might be able to combat obesity along with controlling diabetes.

In vitro antidiabetic activity

The α -amylase activity was found to be inhibited in the presence of WJFC where %inhibition was varying with change in weight of WJFC as 18.42 ± 0.10 for 20.13 mg, 27.04 ± 0.08 for 40.27 mg, 40.03 ± 0.06 for 60.40 mg, 48.22 ± 0.20 for 80.53 mg and 55.05 ± 0.15 for 100.66 mg. Since the methodology followed for estimation of α -amylase inhibition activity by different researchers is different, for example, Kunyanga et al. (2012) and Ali et al. (2013) expressed the activity in terms of purified extracts, it is impossible to compare the efficacy on an absolute scale. However, some of their results are quoted in the present discussion for easy reference: thus, the %inhibition with 100 μ l extract was 28.33, 10.67, 19.33, 11.33, 17.33 and 27.33 for pumpkin, butternut, sweetpotato, drumstick leaves, pumpkin leaves and amaranth leaves.

The α -amylase inhibition activity of WJFC is found to be significantly dose dependent (F-test, $p < 0.05$) and increases linearly with increase in WJFC amount. The trend was regressed and the equation thus obtained is $\text{WJFC (mg, db)} = \% \text{Inhibition} / 0.596$. The acceptability of the equation is supported by the R^2 (0.96). Thus, the amount of WJFC capable of suppressing 50 % of enzymatic activity, i.e., IC_{50} is found to be 83.89 mg (db). Such type of direct relation between α -amylase inhibition and test sample has also been reported by Reddy et al. (2010) and Ali et al. (2013) for extracts of *Asystasia gangetica* Linn. and *Phaleria macrocarpa* fruits.

Chethan et al. (2008) reported that certain plant phenolics have the ability to partially inhibit the amylase activity by non-competitive inhibition. Same results were also demonstrated by Karthic et al. (2008) using jamun seed polyphenolic extract against α -amylase. In non-competitive inhibition, the phenolics bind the α -amylase at sites other than active site and concomitantly change its structural conformation, so that starch will bind to the active site but the enzyme cannot produce glucose. This is due to unstable transitional stage of α -amylase-phenolics-starch complex. Therefore, it can be stated that the polyphenolics present in WJFC might be responsible for the α -amylase inhibition potential.

The glucose dialysis study reveals the tendency of WJFC to inhibit the diffusion of glucose through semi-permeable membrane. The pattern of increasing glucose concentration in dialysate with advent in time for control, acarbose and WJFC is presented in Fig. 2. It is clear from the figure that mean

Fig. 2 Effect of whole jamun based functional confection (WJFC) on glucose diffusion through semi permeable membrane

glucose concentration is increasing with time in all the three cases, however the cases are significantly different from each other (F-test, $p < 0.05$). It is also observed that at any particular time the glucose concentration in dialysate follows the order of: control > WJFC > acarbose. In the study, WJFC displayed considerable inhibition of glucose diffusion w.r.t. acarbose.

López et al. (1996) stated that GDRI is an in vitro index to calculate the effect of substance on the delay of glucose dialysis in the gastrointestinal track. Higher the GDRI, lower the glucose diffusion, hence higher is the antidiabetic potential. At regular interval of time i.e., after 30, 60, 120, 180 and 240 min, GDRI evaluated (Eq. 9) were found to be 13.97, 9.71, 11.84, 12.13 and 20.89 for WJFC and 26.54, 41.78, 24.33, 19.47 and 30.12 for acarbose, respectively. It is observed that GDRI of WJFC is less than acarbose.

Many studies have depicted that dietary fiber may increase GDRI (Chau et al. 2003; Nuñez-López et al. 2013). López et al. (1996) reported that insoluble dietary fiber is more effective in this regard than that of soluble ones. The mechanism postulated by researchers includes: 1) fiber absorbs glucose and hence reduces the diffusion (Chau et al. 2003) and 2) fiber creates physical obstacles by entrapping glucose molecules into complex network, concomitantly showing high GDRI (Ahmed and Urooj 2010). WJFC has high dietary fiber with almost 37.26 % insoluble fraction (Table 1) which might be responsible for decrease in glucose diffusion through semipermeable membrane.

Conclusion

WJFC developed through sequential optimization of ingredients had high dietary fiber and reduced calories. It possessed functionality as being prebiotic, low glycemic, and antioxidant with antidiabetic potential. WJFC retarded α -amylase activity and the glucose diffusion, and helped in maintaining low blood glucose level.

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