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DEVELOPMENT AND PHYSICOCHEMICAL ANALYSIS OF WOOD APPLE JELLY INCORPORATED WITH IMMOBILIZED *L. ACIDOPHILUS*

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Abstract
Nowadays, people prefer food products with health benefits. Fruit based products with additional benefit of probiotics also gaining popularity worldwide. The objective of this study was to develop, and analysis of physicochemical parameters and sensory attributes of wood apple jelly incorporated with probiotic organisms. Control jelly (WAJ) was prepared from wood apple pulp and sugar. Experimental jelly (PWAJ) was prepared same as control jelly incorporated with immobilized probiotic organisms. Total phenol, flavonoid and antioxidant activity was measured from different extracts wood apple pulp. WAJ and PWAJ were analysed for pH, titratable acidity, colour, texture, total phenol, flavonoid, total antioxidant capacity and sensory attributes using standard methods. The result revealed that methanolic extract of wood apple pulp showed significantly higher total phenol, flavonoid, and antioxidant activity. Comparing the result of WAJ and PWAJ, no significant difference was found in pH, titratable acidity, colour, texture, total phenol, flavonoid, DPPH radical scavenging activity, ferric reducing antioxidant power. The sensory attributes of both the jellies were well accepted. During storage of one month, no significant change was observed in any of the sensory attribute except flavour. The probiotic count in PWAJ at zero day was 7.18 log cfu which was not changed significantly up to 15 days. The present study concludes that wood apple is a potential source of antioxidants. Addition of probiotic strain in wood apple jelly was found to be acceptable and it can be served as functional food.

Keywords: Wood apple, jelly, probiotics, antioxidant activity.

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1. INTRODUCTION

Functional foods provide health benefits beyond the macro and micronutrient they have (Hasler, 2002). These health benefits are mainly attributed to the nutracetuicals like bioactive components dietary fibre, bioactive peptides, prebiotics and probiotics. These compounds are beneficial for boost up immunity and in prevention of life style disease such as obesity, diabetes, hypertension, cancer, osteoporosis, stoke and cardiovascular disease (Gabay et al., 2010). Fruits and vegetables are reported to possess antioxidants, dietary fibres including different vitamin and minerals (Singh et al., 2016)

The wood apple (*Limmoniaaccidicema*) is one of the common names of an edible fruit from several trees, mainly those belonging to genus *Limmoniaaccidicema*. The fruit is also known as elephant-apple, monkey fruit or crud fruit. It is commonly known as “kaitha” in India and “kotha” in Gujarati.It has woody and extremely hard outer shell (rind) which very difficult to creak and open. Inner pulp is brown, mealy, aromatic, resinous, and sweetish with many small seeds with it (Pandey, Satpathy et al., 2014). The fruit pulp is used in preparations like, jellyes and jams, Syrups, drinks. (Senthikumar and Venkatesau, 2013).

The wood apple pulp is a rich source of Beta carotene, significant amount of vitamins-B such as riboflavin and thiamine good amount of protein, dietary fibre, ascorbic acid, magnesium, calcium, and other minerals It has various phytochemicals like alkaloids, saponisis, flavonoids, total phenols. (Kumar and Deen, 2017; Pandey, Satpathy et al., 2014).

Wood apple to possess beneficial effects against, dysentery, diarrhoea, piles, scurvy. It is reported as tonic for liver and heart and have protective effects against skin cancer (Ahmed et al., 2008)

Among the conventional food preserves, jellies have been widely used allowing the off-season consumption of fruits. Jelly is a gelatinous, semisolid product prepared with mixture of fruit juice and sugar. Jelly is forms when pectin, sugar, acid and water are in suitable concentration. (Codex Standard 296, 2010)

Pectin is the most important substance in jelly to provide proper structure and strength to jelly. Pectin is formed from a parent compound protopectin, during the ripening of fruit. Wood apple pulp is rich in pectin (3-5%) and is an excellent material for preparation of jelly. (Kumar and Deen, 2017).

Nowadays, many researches carried out on fruits based products with additional benefits of probioitcs as it provides dual benefits of antioxidants and probiotics such as probiotic yogurt with annona pulp (Senadeera et al., 2018) and Antioxidant rich fruit supplemented probiotic yogurt (Kumar et al.,2016), probiotic juice (Shah et al., 2016).

(Pandey, Naik et al., 2015). Probiotics especially lactic acid bacteria are helpful for maintain gut health (Raid and G, 2008) treatment of intestinal disorders, inflammatory diseases, allergies (Parvez et al, 2006). Antitoxin and diarrhoea reduction effects by dietary supplements of probiotics are proved to improve gut health and nutrient digestibility (Balkrishnan and Floch, 2012).

Lactobacillus acidophilus is a specious of gram positive bacteria. It is a homofermentative, microarophilic species, fermenting sugar into lactic acid, and grows readily at rather low pH value (below pH 5.0). It possesses many health benefits such as reduced overgrowth of pathogens in human digestive tract, relives irritable bowel syndrome and gut dysbiosis, treat respiratory infections like bronchitis and increases immune response.(Gomes et al., 1999) Extreme processing conditions can damage these cells. Several methods have been proposed to improve the viability of probiotics, and cell immobilization appears to be the most promising among these methods (Cai et al., 2014; Sathyabama and Vijyabharti, 2014). Microencapsulation is a promising technology that may be useful for the oral delivery of live probiotic bacteria (Malmo et al., 2013). The goal of microencapsulation of probiotics is to protect microorganisms from adverse conditions, enabling the arrival in the intestine at the concentration required to exert its beneficial effect (Kailasapathy at al., 2006)

2. MATERIALS AND METHODS

2.1 Procurement of raw material:

The raw materials namely wood apple and sugar for jelly preparation were procured from the local market of Anand. All the materials were directly brought to the Food Biotechnology laboratory of Post Graduate Department of Home Science, Sardar Patel University, Vallabh Vidyanagar, Gujarat.

2.2 Extraction of sample for antioxidant activity:

2 gm of wood apple pulp was extracted using 25 ml of solvent. Three solvents were used namely acidified methanol: water solvent (80:20) with pH 2.0, acetone (as such) and distilled water. The sample was extracted for 30 minutes in shaker (Remi Ltd.) at room temperature followed by centrifugation (Remi Ltd.) for 10 minutes at 6000 rpm. The supernatant was collected and the residues were extracted again with the respective solvent. The process was repeated three times and final volume of extracts was made to 100 ml. The extracts were stored in -20°C till further use. The same extracts were used for further analysis of total phenol, flavonoids and antioxidant activity.

2.2.1 Total phenol estimation using Folin Ciocalteu method:

Total phenol estimation was done by folin ciocalteu reagent method (Singleton et al, 1999). Appropriate aliquot of extracts was treated with folin ciocalteu reagent (1:1) and 7.5% sodium

carbonate. After incubation of 30 minutes at 37°C, the blue color developed in tubes was read at 750 nm in spectrophotometer (Systronics Ltd.) against distilled water as blank. For standard, Gallic acid (5µg to 20µg) was used and a result is expressed in mgGAE/100gm.

2.2.2 Determination of total Flavonoid:

The total flavonoid content was determined using a colorimetric assay (Chang et al., 2002). Appropriate aliquot of sample was taken and treated with distilled water and incubated for 5 minutes at 37°C. Then 5% sodium nitrite and 10% aluminum chloride were added and incubated for 6 minutes at 37°C. After incubation 1 N sodium hydroxide was added. The developed pink color was read at 520 nm against. Rutin (50 µg to 200 µg was used as a standard and the results is expressed as Rutin equivalents (RE) mg/100g.

2.2.3 DPPH (2, 2-Diphenyl-1-picrylhy-drazyl) radical scavenging activity (DPPH RSA):

The DPPH RSA measures of total antioxidant activity (Benzie and Strain, 1999). Appropriate aliquot of sample mixed with DPPH reagent and incubated for 20 minutes at 37°C. Absorbance was read at 517 nm against methanol as blank. As a standard, trolox (5 µg to 20µg) and the result is expressed as Trolox equivalents (TE) in mg/100gm.

2.2.4 Ferric reducing antioxidant power (FRAP) assay:

FRAP assay was done using spectrophotometric method (Brand Williams et al., 1995). The appropriate aliquot of sample was mixed with FRAP reagent and tubes were incubated for 10 minutes at 37°C. The developed blue color complex was read at 593nm against distilled water as blank. Trolox (0.5 µg to 2.0µg) was used as standard, and the result is expressed as Trolox equivalent (TE) in mg/100gm.

2.3 Immobilization of probiotic microorganisms:

Lactobacillus acidophilus was used as probiotics. The stock culture of *L. acidophilus* was activated in 100 ml MRS Broth and incubated at 37°C for 24 hrs. Culture was harvested after growth by centrifugation for 15 min at 7000 rpm, washed twice in phosphate buffer saline and then suspended in same buffer. Optical density was adjusted to 1.00 at 600 nm with PBS (phosphate buffer saline) to obtain the desired cell concentration. It is mixed with sterile solution of 2% sodium alginate solution (1:1). The uniform size beads of this mixture were introduced into 4% sterile calcium chloride solution at room temperature and allow it to stand for 30 min for complete gelation. Then the beads were washed with distilled water. They were transferred to sterile solution of 0.4% calcium chloride and stored in refrigerator (4°C) until further use.

2.4 Preparation of control and probiotic jelly

Control jelly (WAJ) was prepared from wood apple pulp. 100 gm of wood apple pulp was boiled in 150 ml of water for 10 minutes and the mixture was filtered. The filtered pulp was mixed with 75% of sugar and mixture was heated with continuous stirring. The end point of cooking was judged by achieving 65 brix using refractometer. The mixture was brought to warm temperature and poured into sterilized glass container and allowed to set. The probiotic jelly (PWAJ) was prepared by the same method. Probiotic beads were added before pouring into to sterilized glass container. The developed WAJ and PWAJ were stored in a cool place and used for the evaluation for analysis of physicochemical parameters, sensory attributes and microbial analysis.

2.5 Analysis of control (WAJ) and probiotic jelly (PWAJ):

2.5.1 Titratable acidity and pH:

Titratable acidity of the jelly samples was analyzed by acid base titration. The result was expressed as % citric acid. The pH values of the control and probiotic jelly was determined using digital pH meter (Systronics Ltd.).

2.5.2 Antioxidant activity:
The samples were extracted as mentioned in 2.2. The extracted samples were analyzed by total phenol, flavonoid, DPPH radical scavenging activity and ferric reducing antioxidant activity.

2.5.3 Color and texture analysis:
Color measurement was done by hunter L, a, b color scale (Hunterlab Ltd.). The instrument was standardized, and the sample was transferred into glass cup and read on the scale of L, a and b.
Texture analysis was done by texture analyzer (Brookfield Ltd.). For the evaluation of texture TA3/100 probe was used. The compression test type was used with 10 mm target; 10 gm trigger load and 1mm/s test speed. The hardness and stringiness were measured.

2.5.4 Microbial analysis:
For microbial analysis 5 gm of jelly was taken aseptically and diluted to 45 ml of sodium citrate and then subjected to serial dilution with distilled water. Nutrient agar was used for total plate count, potato dextrose agar was used for yeast and mold count for both the jelly samples. Probiotic count was estimated from PWAJ using MRS agar. The microbial analysis of jellies was carried out at regular intervals during storage.
2.5.5 Evaluation of sensory attributes:
For sensory evaluation of the jelly samples, composite score card was used. Color, flavor, texture, taste and overall acceptability were evaluated by semi trained panel members. The evaluation was done by the same panel members at regular intervals during storage.
2.5.6 Stastical analysis:
All results were presented as mean and standard error of mean. t-test, ANOVA and regression analysis was carried out. All the analysis was done in SPSS (Version 20.0).

3. RESULTS AND DISCUSSION

3.1 Antioxidant activity of wood apple:
The result of total phenol, flavonoid, DPPH radical scavenging activity and frap of different extracts of wood apple are shown in table 1. Methanol extract of wood apple showed the highest total phenol content (335.12 mg GAE/100gm), followed by acetone extract (98.93 mg GAE/100gm) and aqueous extract (76.25 mg GAE/100gm). A significant difference was noted in total phenol content pertaining to type of solvent used for extraction. The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids. (Chang et al., 2002). In the present study, the potential of solvent for extracting of flavonoid content of wood apple extract was observed in pattern of methanol (mgRE/100g)> acetone (mgRE/100g)> distilled water (mgRE/100g).
Darshini et al. (2013) reported that the highest percentage of phenol and flavonoid contents were observed in methanol and the lowest content was found in chloroform extract of wood apple. In another study methanolic extract showed the best capacity to extract the highest antioxidant compounds from dessert bananas as well as the TPC and TFC was also highest in methanolic extract (Ramlan et al.; 2017).
The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Moreover, solvent polarity will play a key role in increasing phenolic solubility (Naczk & Shahidi, 2006).
The percentage of inhibition of DPPH within the assay time will reflect the antioxidant capacity of the extract assessed. (Gil, Tomas-Barberan, Hess-Pierce, Holeroft, & Kader, 2000). A significantly higher DPPH-RSA was found in methanolic extract (56.83 mg TE/100gm) as compared to other solvent extracts.
The antioxidant capacity of fruits extracts is determined by the ability of the antioxidants in these extracts to reduce ferric iron to ferrous in FRAP reagent, which consists of 2, 4, 6-tris (1-pyridyl)-5-triazine (TPTZ) prepared in sodium acetate buffer, pH 3.6. The reduction of ferric

iron in FRAP reagent will result in the formation of a blue product (ferrous – TPTZ complex) whose absorbance can be read at 593 nm. (Alothman et al., 2009)

The highest FRAP was observed for the methanolic extract (684.94 mg TE/100gm) of wood apple followed by acetone extract (134.3 mg TE/100gm) and aqueous extract (58.35mg TE/100 gm). Similar observation reported by Felhi (2017). Methanolic extracts of various parts of plant like leaf, seeds and peels showed the highest DPPH RSA and ferric reducing antioxidant power.

The extraction of phenolic compounds and its antioxidant activity affected by many factors namely type of solvent used, polarity of solvent (Alothman et al., 2009), pH of solvent, plant material to solvent ratio, time and temperature of the extraction (Robards, 2003; Dorta, 2013), the degree of polymerization of phenols and their interaction (Djeridane et al., 2006). For polar antioxidants, acetone: water mixture is proved to be good solvent (Lu & Foo, 1999; Luximon-Ramma, Bahorun, & Crozier, 2003; Sun et al., 2002). While organic solvents such as methanol, ethanol are mainly used for extraction of phenolics (Antolovich, Prenzler, Robards, & Ryan, 2000; Luthria & Mukhopadhyay, 2006).

Regression analysis between total phenol and antioxidant capacity as well as flavonoid and antioxidant capacity is presented in figurer 1 to figure 4. A positive and significant ($P<0.01$) relation was observed between total phenols and DPPH radical scavenging activity ($R^2=0.978$, $F=442.28$, $P=0.000$) and flavonoid and DPPH radical scavenging activity ($R^2=0.967$, $F=292.85$, $P=0.000$). Similarly total phenol ($R^2=0.992$, $F=127.68$, $P=0.000$) and flavonoid ($R^2=0.940$, $F=155.60$, $P=0.000$) also showed a significant ($p<0.01$) and positive relation with ferric reducing antioxidant power. This reveals that antioxidant capacity of wood apple is attributed to total phenol and flavonoid content of wood apple.

3.2 Physicochemical parameters of control (WAJ) and experimental jelly (PWAJ):

3.2.1 pH and Titratable acidity:

The results of pH and titratable acidity of control (WAJ) and experimental jelly (PWAJ) are expressed in table 2. No significant change ($P>0.05$) was observed in pH WAJ during storage of one month. A similar trend was also observed for PWAJ. While comparing pH of WAJ and PWAJ at regular interval of storage, no significance difference ($P>0.05$) was noticed. Titratable acidity of WAJ ranged from 2.36% to 2.5% However, no significant changes was observed during storage. Similarly no significant change in titratable acidity was observed in experimental jelly during storage. Moreover, titratable acidity of WAJ and PWAJ was not significant varied at regular period of storage.

Table 1. Total phenol, flavonoid and antioxidant activity of different extracts of wood apple

Extracts	Flavonoid (mg RE/100gm)	Total phenol (mg GAE/100gm)	DPPH radical scavenging activity (%inhibition)	Ferric reducing antioxidant power (mg TE/100gm)
Methanol	60.72^{c} ±0.83	335.12^{c} ±19.12	56.83^{c} ±0.16	684.94^{c} ±4.59
Acetone	39.07^{b} ±0.88	98.93^{b} ±2.92	33.49^{b} ±1.00	134.3^{b} ±4.77
Distilled water	27.23^{a} ±0.45	76.25^{a} ±3.91	27.64^{a} ±0.581	58.35^{a} ±4.78
F-value	2044.11^{**}	614.76^{**}	2084.48^{**}	2099.99^{**}

Values are mean±SD of three observations, NS indicates no significant difference, mean value with different superscripts within the column differ significant difference ($p\leq0.05$)

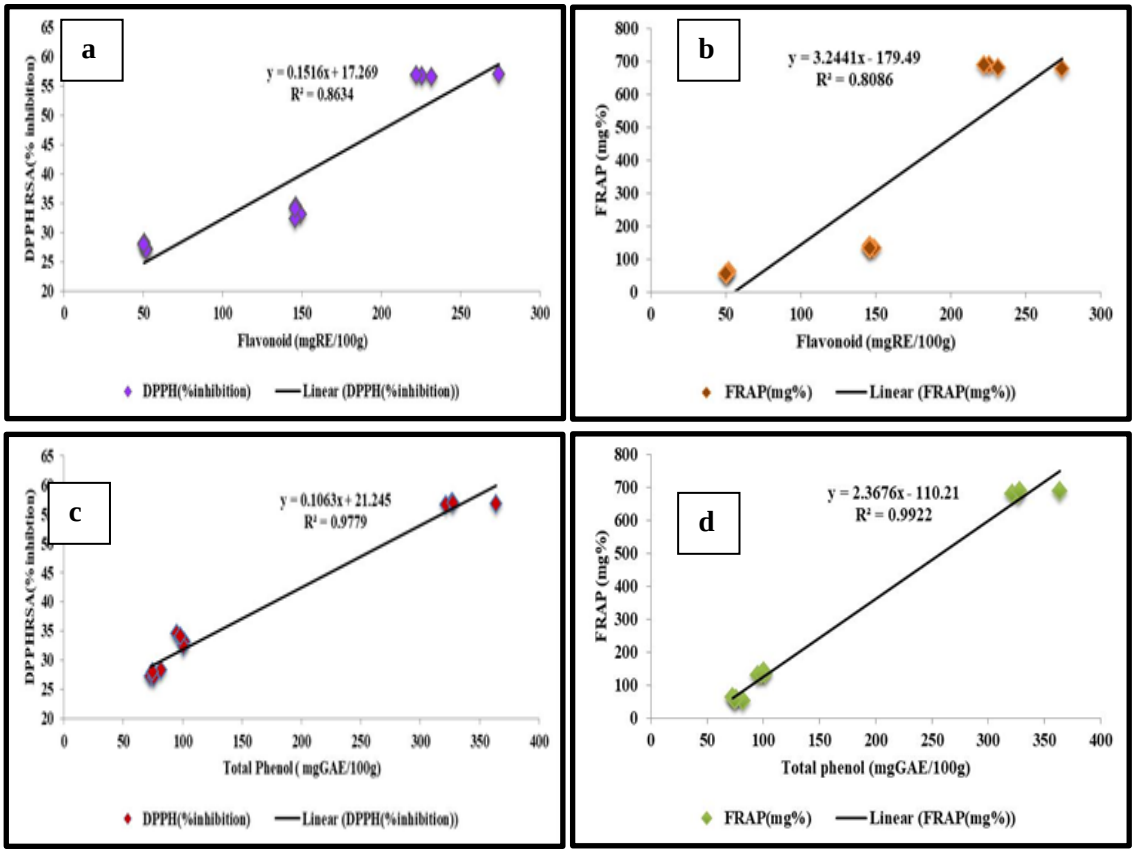

Figure 1. Correlation between(a) flavonoid and DPPH radical scavenging activity (b) total phenol and DPPH radical scavenging activity (c) flavonoid and ferric reducing antioxidant power (d) total phenol and ferric reducing antioxidant power of wood apple extracts

As free probiotic bacteria are likely to have consumed carbohydrates and produced organic acids, the pH diminishes during storage. The microencapsulation of the probiotic bacteria was corroborated to provide stable protections for products over time (Kailasapathy 2006; Saarela et al. 2006). Irrespective of bacterial strain, samples containing protected probiotics with sodium alginate have a more stable environment during storage (Ding and Shah 2008).

Table 2. pH and Acidity of WAJ and PWAJ

	Sample	0 Day	15 Day	30 Day	F-Value
pH	**WAJ**	3.70^{b} ±0.15	3.30^{a} ±0.26	3.70^{ab} ±0.2	4.30^{NS}
	PWAJ	3.60^{a} ±0.20	3.70^{a} ±0.26	3.30^{a} ±0.15	20.20^{NS}
	t-value	0.671^{NS}	1.852^{NS}	2.295^{NS}	
Acidity	**WAJ**	2.36^{a} ±0.40	2.42^{a} ±0.10	2.5^{a} ±0.1	0.217^{NS}
	PWAJ	2.64^{a} ±0.12	2.5^{a} ±0.17	2.51^{a} ±0.05	0.801^{NS}
	t-value	1.136^{NS}	1.140^{NS}	0.249^{NS}	

Values are mean±SD of three observations, NS indicates no significant difference, mean value with different superscripts within the column differ significant difference ($p \leq 0.05$), WAJ= Wood apple jelly, PWAJ= Probiotic wood apple jelly

3.2.2 Antioxidant capacity of WAJ and PWAJ:

The result of total phenol, flavonoid, and Antioxidant capacity of WAJ and PWAJ is presented in Table 3. Total phenol content of WAJ was significantly (p>0.01) higher than PWAJ. While PWAJ had significantly higher flavonoid content as compared to WAJ. Similar findings of Ferric reducing antioxidant power (FRAP) were observed. Pertaining DPPH radical scavenging activity had no significant difference was observed between the jelly samples.

The result of total phenol, flavonoid, and Antioxidant capacity of WAJ and PWAJ is presented in Table 3. Total phenol content of WAJ (225 mg GAE/100gm) was significantly (P<0.01) higher than PWAJ (196 mg GAE/100gm). While the flavonoid content was significantly (P<0.01) higher in PWAJ (18.40 mg RE/100gm) as compared to WAJ (15.34 mg RE/100gm). Similar findings were observed for ferric reducing antioxidant power (FRAP). Pertaining DPPH radical scavenging activity, no significant (P>0.05) difference was observed between the jelly samples.

3.2.3 Colour and texture analysis:

Table 4 presents L, a, b value of control and experimental jelly. 'L' value indicates perceptual lightness of sample. 'a' value indicates axis was relative to green-red opponent colours and 'b' value indicates axis was represents the blue-yellow opponent's colours. Pertaining to the colour scale L, a, and b, no significant (P>0.05) difference was observed between the jelly samples. It reveals that addition of probiotic beads has no significant (P>0.05) changed the colour of the jelly. WAJ has significantly (P<0.05) higher hardness (3130 gm) as compared to PWAJ (2777.50 gm). Similarly, stringiness of WAJ (13.22mm) was significantly higher (6.82mm) than PWAJ.

3.2.4 Sensory evaluation of WAJ and PWAJ:

Table 5 presents the result of sensory evaluation of WAJ and PWAJ during storage. There is no significant difference (P>0.05) was noted for any of the sensory attributes while comparing WAJ and PWAJ. During storage, the colour of WAJ and PWAJ not significantly changed. Similar findings were noted for texture, taste and overall acceptability of jelly samples. For flavour, the mean score was significantly (P<0.05) reduced for both the jelly samples during the storage period. The findings revealed that jelly incorporated with probiotic beads was well accepted and had good stability of sensory attributes during storage. Talebzadeh et al. (2014) also reported the overall the jelly samples incorporated with microcapsule were well accepted pertaining to flavour, odour, colour, mouth texture and overall acceptance while jelly samples incorporated with free bacterial was not well accepted by panel members.

Table 3. Total phenol, flavonoid and antioxidant activity of WAJ and PWAJ

Sample	Total phenol (mg GAE/100gm)	Flavonoid (mg RE/100gm)	DPPH radical scavenging activity (%inhibition)	Ferric reducing antioxidant power (mg TE/100gm)
WAJ	225.0 ±6.66	15.34^{c} ±0.12	66.88^{b} ±0.19	85.51^{b} ±0.16
PWAJ	196.60^{c} ±4.17	18.40^{c} ±0.07	65.60^{c} ±0.57	90.39^{c} ±0.13
t-value	6.266**	35.826**	2.696^{NS}	39.845**

Values are mean±SD of three observations, NS indicates no significant difference, mean value with different superscripts within the column differ significant difference (p≤0.05), WAJ= Wood apple jelly, PWAJ= Probiotic wood apple jelly

Table 4. Colour score and Texture of WAJ and PWAJ

	Colour score			Texture	
Sample	**L**	**a**	**b**	**Hardness (gm)**	**Stringiness**
WAJ	9.35 ±0.47	3.32 ±0.02	0.44 ±0.02	3130.0 ±150.0	13.22 ±0.38
PWAJ	9.33 ±0.18	3.33 ±0.03	0.36 ±0.02	2777.50 ±117.50	6.82 ±0.030
t-value	0.084^{NS}	0.795^{NS}	0.066^{NS}	3.204*	29.081**

Values are mean ± SD of three observations, NS indicates no significant difference, mean value with different superscripts within the column differ significant difference ($p \leq 0.05$), WAJ= Wood apple jelly, PWAJ= Probiotic wood apple jelly

Table 5. Mean score for colour of WAJ and PWAJ

	Sample	0 Day	15 Day	30 Day	F-Value
Colour	**WAJ**	8.33^{a} ±0.51	8.00^{a} ±0.5	7.66^{a} ±0.51	2.14^{NS}
	PWAJ	8.16^{a} ±0.40	8.5^{b} ±0.54	7.83^{a} ±0.40	3.15^{NS}
	t-value	0.620^{NS}	1.464^{NS}	0.620^{NS}	
Flavour	**WAJ**	8.00^{a} ±0.00	7.5^{ab} ±0.5	7.33^{a} ±0.51	3.82*
	PWAJ	8.00^{b} ±0.00	7.33^{a} ±0.51	7.83^{b} ±0.40	5.00*
	t-value	0.000^{NS}	0.542^{NS}	1.861^{NS}	
Texture	**WAJ**	7.50^{a} ±0.84	7.33^{a} ±0.82	7.17^{a} ±0.075	0.259^{NS}
	PWAJ	7.50^{a} ±0.84	7.17^{a} ±0.98	7.33^{a} ±0.82	0.214^{NS}
	t-value	0.000^{NS}	0.319^{NS}	0.368^{NS}	
Taste	**WAJ**	7.00^{a} ±0	7.5^{a} ±0.5	7.16^{a} ±0.40	2.50^{NS}
	PWAJ	7.66^{a} ±0.51	7.66^{a} ±0.51	7.16^{a} ±0.40	2.143^{NS}
	t-value	3.162^{NS}	0.562^{NS}	0.00^{NS}	
Overall Acceptability	**WAJ**	7.50^{a} ±0.00	7.30^{a} ±0.4	7.40^{a} ±0.00	0.081^{NS}
	PWAJ	7.50^{a} ±0.83	7.50^{a} ±0.83	7.50^{a} ±0.83	0.000^{NS}
	t-value	0.00^{NS}	0.346^{NS}	0.00^{NS}	

Values are mean±SD of ten observations, NS indicates no significant difference, mean value with different superscripts within the column differ significant difference ($p \leq 0.05$), WAJ= Wood apple jelly, PWAJ= Probiotic wood apple jelly

3.3 Microbial analysis

It was observed that mean log cfu of probiotic wood apple jelly was 7.18 at zero day which was significantly ($p<0.05$) reduced to 6.29 on 15^{th} day. The count was further declined to 5.51 on 30^{th} day of storage. This indicates experimental jelly in the present study possess probiotic properties up to 15^{th} days as any food product that contain 10^{6} cfu/gm is considered as probiotic food product. Talebzadeh et al. (2017) also observed reduction in viable count while extending storage period. Microencapsulation technique helps in increasing the stability of cells (Lee et al. 2004; Anal and Singh 2007) and

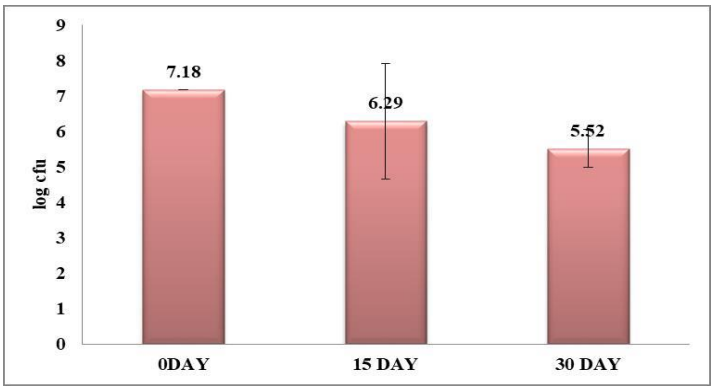

Figure 2. Mean value of log cfu probiotic of PWAJ

to sustain viability in harsh conditions such as high acidity and temperature (Chandramouli et al. 2004; Picot and Lacroix 2004; Saarela et al. 2006). There was no growth of other bacteria, yeast and mould was observed in both the jelly samples throughout the storage study period.

4. CONCLUSIONS

In conclusion, methanolic extract of wood apple contained significantly higher content of total phenol, flavonoid, DPPH radical scavenging activity and ferric reducing antioxidant power. Wood apple jelly with and without incorporation of immobilized probiotic organisms were well accepted pertaining to sensory attributes. Incorporation of probiotics not affected pH, acidity, colour, texture, phenols, flavonoids and antioxidant activity of wood apple jelly. Hence, incorporation of immobilized cells of probiotics is suggested for value addition in fruit jellies.

Conflict of Interest:
There is no conflict of interest with reference to research and authorship of this paper.

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