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DEVELOPMENT OF JAMUN SYNBIOTIC SMOOTHIE

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Abstract: In the present study smoothie was prepared using different inclusion levels of jamun and yoghurt. The inclusion level of jamun: yoghurt ratio at 20:80 (JS_3) per cent was found optimum by sensory analysis. Jamun juice exerted maximum prebiotic effect on *L.plantarum*. *L.plantarum* was examined for its probiotic properties, microencapsulated in jamun juice and incorporated into smoothie to make it synbiotic.Shelf life studies (7days) of the synbiotic smoothie included analysis of physicochemical, functional, microbial and sensory characteristics. The range of total phenolic content (TPC), antioxidant activity and ascorbic acid was from 19.23 to 19.80µg/mg; 56.06 to 56.07mgFe^{2+}/100ml and 0.80to 0.74mg/100ml during 1 to 7 days of storage respectively. Coliform, yeast and mould count were not detected during storage at refrigerated temperature in the synbiotic smoothie.
Keywords: Synbiotic Smoothie, functional smoothie, jamun, probiotic, microencapsulation, Fruit based smoothie.

Introduction

In the past two decades, there has been a tremendous demand for functional foods among the consumer due to their potential for providing health benefits. Smoothies are fruit based dairy ideal food format to deliver health-promoting polyphenols (PP's) and dietary fibres (DF's) and antioxidants to consumers and act as functional food.Coloured fruits are excellent sources of phytochemicals. Jamun (*Syzygium cuminii*) fruit a seasonal perishable berry, grows mainly in tropical and sub-tropical parts of the world. It is rich in minerals, phytochemicals and shows high antioxidant potential heading towards its therapeutic effects.

Fermented dairy products like yoghurt etc. are ideal food matrix associated with probiotics and beneficial bacteria and in increasing their viability. According to FAO/WHO (2001), Probiotics are defined as "live microorganisms which when administered in adequate amounts confer health benefits on the host". Encapsulation tends to increase the stability and maintain the culture viability during the storage of the product. The combination of alginate

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with prebiotics offers an enhanced protection for probiotics in food systems due to the symbiotic relationship.

In as much as milk and its products being considered a complete food, it lacks functional qualities like polyphenols, antioxidant and dietary fibres that are beneficial to health. Hence to facilitate the consumers' access to efficacious functional dairy foods the present study focussed on the preparation of synbiotic smoothie using and jamun juice and evaluating the functional property of the product.

Materials and methods

Skim milk, skim milk powder, yoghurt culture (NCDC- 144) and sugar was used for the preparation of yoghurt. Jamun was obtained for the preparation of smoothie. Probiotic culture *Lactobacillus plantarum* (NCDC- 21), sodium alginate and calcium chloride were obtained for preparation of microencapsulated probiotic beads with fruit juice.

Preparation of Yoghurt

Yoghurt was prepared as per the method adopted by De (1980).

Optimizing the inclusion level of jamun juice into yoghurt for preparation of jamun smoothie by sensory evaluation (modified method of Kale, 2007)

Jamun smoothie was prepared using different inclusion levels of jamun juice into yoghurt as depicted below.

Proportion of Composite Mixes (per cent)				
Ingredients (g)	**Jamun smoothie (Jamun juice: Yoghurt)**			
	JS_1	**JS_2**	**JS_3**	**JS_4**
Jamun juice	40	30	20	10
Yoghurt	60	70	80	90

Assessment of probiotic characteristics for microencapsulation

L.plantarum was phenotypically identified as gram positive rods by using Grams stain under microscope. Tolerance of *L.plantarum* to bile salts (0.2, 0.4 and 0.6 per cent w/v) was carried out as per the method adopted by Prasad *et al.* (1999). The tolerance of *L.plantarum* to acidity was evaluated at pH 3 with HCl (5N) at 0, 90 and 180 mins (Zinedine and Faid, 2007).

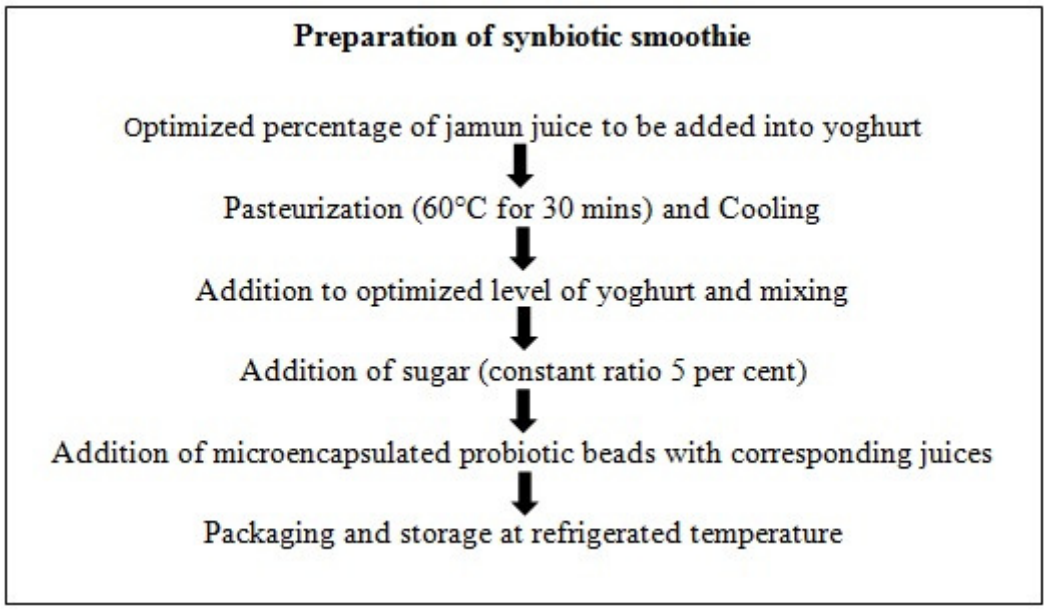
Assessment of prebiotic effect of jamun juice on *Lactobacillus plantarum* for microencapsulation (Ida *et al.*, 2005)

Varying concentrations of skim milk to jamun juice (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 9:1 and 0:10) were used to study their respective prebiotic effects on *L.plantarum* by adopting pour plate method after 48 hours of post incubation.

Microencapsulation of *Lactobacillus plantarum* with jamun juice was prepared through extrusion method (Krasaekoopt *et al.,* 2003)

Preparation of synbiotic smoothie

Preparation of synbiotic smoothie with optimized ingredients is illustrated below (Kale *et al.,* 2007).



Estimation of nutrient analysis of control and synbiotic smoothie

Proximate analysis of the smoothie product was done by AOAC (2000) method for determination of moisture, protein, fat, ash and NFE.

Assessment of shelf life of control and synbiotic smoothie at refrigeration temperature

The jamun smoothie enriched with 5 per cent of microencapsulated probiotic beads were assessed for their shelf life and storage stability based on physicochemical (pH, acidity and viscosity), functional (TPC, Antioxidant activity and ascorbic acid), microbial (total viable count, coliform, yeast and mould) and sensory characteristics.

Results and Discussion

Optimizing the inclusion level of jamun juice into yoghurt for preparation of jamun smoothie by sensory evaluation

Table 1 and Figure 1 shows the respective mean ± SE sensory values for control (yoghurt) and smoothie prepared by optimizing the inclusion level of jamun into yoghurt through sensory evaluation using 9-point hedonic scale. From the table it was concluded that the ratio of 20 per cent inclusion of pomegranate juice to 80 per cent yoghurt was considered the ideal composition for the preparation of synbiotic smoothie (JS_3). The present results were in agreement with Lakshmi *et al*. (2015) revealed that 9 per cent inclusion of jamun pulp scored maximum followed by 7 per cent. The inclusion level of increasing per cent of jamun juice showed significant difference in colour and was liked by the panelists. This is due to the bright purple colour of the fruit which was also reported by Chaudhary and Mukhopadhyay (2012) that consumers preferred jamun fruit as a natural colourant.

Figure 1: Optimizing the inclusion level of jamun juice into yoghurt for preparation of jamun smoothie by sensory evaluation

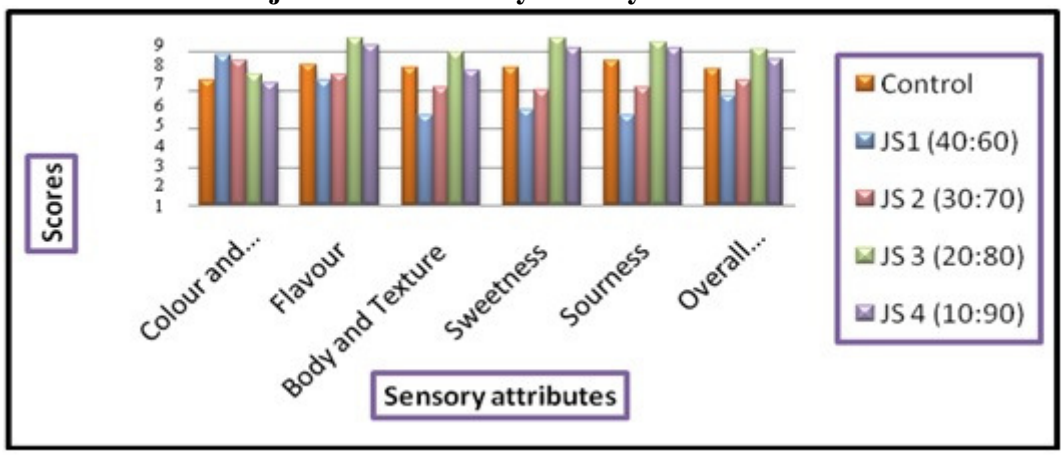


Table 1: Optimizing the inclusion level of jamun juice into yoghurt for preparation of jamun smoothie by sensory evaluation (Mean± SE)[@]

Inclusion level of Jamun:Yoghurt (%)	Sensory attributes (9 point hedonic scale)					
	Colour and Appearance	Flavour	Body and Texture	Sweetness	Sourness	Overall acceptability
Control	6.50^{a}±0.223	7.33^{a}±0.333	7.16^{b}±0.307	7.16^{b}±0.166	7.50^{a}±0.341	7.10^{b}±0.177
JS_1 (40:60)	7.83^{b}±0.307	6.50^{a}±0.619	4.66^{a}±0.666	5.00^{a}±0.516	4.66^{a}±1.115	5.73^{a}±0.111
JS_2 (30:70)	7.50^{b}±0.223	6.83^{a}±0.477	6.16^{a}±0.600	6.00^{a}±0.365	6.16^{a}±0.477	6.53^{b}±0.190
JS_3 (20:80)	6.83^{a}±0.307	8.66^{b}±0.210	8.00^{b}±0.516	8.66^{c}±0.210	8.50^{b}±0.341	8.13^{c}±0.122
JS_4 (10:90)	6.30^{a}±0.210	8.30^{b}±0.210	7.00^{b}±0.365	8.10^{b}±0.166	8.10^{b}±0.166	7.54^{b}±0.136
F value	**2.88***	**5.43****	**6.12****	**22.92****	**6.94****	**34.16****

Characteristics of *Lactobacillus plantarum* for probiotic properties

Tolerance of *Lactobacillus plantarum* to bile salts and acidity at pH 3

Table 2 and 3 shows *L.plantarum*'s tolerance to maximum bile concentration with a viability of 6.58±0.326 (log_{10}cfu/ml). This was further affirmed by Prasad *et al*. (1999), who reported that one of the criteria for lactic acid bacteria to be called probiotic is its tolerance to bile salt. Statistical analysis revealed highly significant difference (P≤ 0.01) in the viable counts of culture after 90 (log6.03cfu/ml) and 180 (log3.42cfu/ml) minutes of incubation at pH 3 when compared to 0 hour incubation. The findings were also in consonance to the observations of McLauchlan *et al.* (1998) that acid tolerance is an important quality for a probiotic.

Table 2 Tolerance of *Lactobacillus plantarum*[#] to bile salts

Name of the culture	Control	Percentage of bile salt			F value
		0.2%	0.4%	0.6%	
Lactobacillus plantarum	10.58^{c}±0.327	8.37^{b}±0.219	7.25^{a}±0.251	6.58^{a}±0.326	**78.63****

Table 3 Tolerance of *Lactobacillus plantarum*[#] to acidity at pH 3

Name of the culture	Incubation period in minutes			F value
	0	90	180	
Lactobacillus plantarum	10.58^{c}±0.327	6.03^{b}±0.357	3.42^{a}±0.108	**89.66****

Assessment of prebiotic effect of jamun juice *on Lactobacillus plantarum* for microencapsulation

Ratio of inclusion level of skim milk and jamun juice to assess prebiotic effect on *Lactobacillus plantarum*

Table 4.There was a significant difference (P≤ 0.05) in the viable count between 4 parts of jamun juice and increasing ratio of addition of jamun juice to skim milk. The increase in viability of *L.plantarum* in jamun juice was justified with the findings of Shahnawaz *et al*. (2009) who reported immense nutritive value and rich carbohydrate content of jamun juice.

Microencapsulated *Lactobacillus plantarum* in jamun juice through extrusion method

Plate 1 shows the microencapsulated probiotic beads of JS_3 prepared by extrusion method. This was in agreement with the findings of Gnaanappriya *et al*. (2013) who reported viability of encapsulated *L. plantarum* using fruit juices.

Plate 1: Microencapsulated *Lactobacillus plantarum* in jamun juice through extrusion method

Table 4: Ratio of inclusion level of skim milk and jamun juice to assess prebiotic effect on *Lactobacillus plantarum*[#] (Mean± SE)[@]

Composition Ratio of Skim milk:Jamun juice	Count (log_{10}cfu/ml)
10:0	10.58^{a}±0.327
9:1	10.61^{a}±0.198
8:2	10.63^{a}±0.183
7:3	10.91^{a}±0.244
6:4	10.96^{a}±0.210
5:5	11.03^{b}±0.286
4:6	11.04^{b}±0.247
3:7	11.30^{b}±0.134
2:8	11.40^{b}±0.125
1:9	11.43^{b}±0.139
0:10	11.43^{b}±0.139
F value	**2.55***

Estimation of nutrient composition of control and synbiotic smoothie

Proximate analysis of control and jamun (JS_3) synbiotic smoothie

Table 5 shows the nutrient composition of control and synbiotic smoothie. This was in agreement to Walsh *et al*. (2014) who also reported lower fat content in the preparation of carbonated drinkable yoghurt due to the use of skim milk. Increase in NFE content was due to the increase in amount of pomegranate juice was in agreement to the findings of Driessen (1988). Chaudhary and Peter's (2015) observation for: moisture 76.47 per cent, protein 3.40

per cent, fat 2.32 per cent, ash 0.67 per cent in fruit smoothies at wet basis was is in agreement with the values in the present study.

Table 5: Proximate analysis of control and jamun (JS_3) synbiotic smoothie

Treatments	Proximate composition on dry matter basis (%)				
	Moisture	Protein	Fat	Ash	Nitrogen Free Extract
Control	76.53±0.003	20.65±0.003	0.30±0.003	3.02±0.003	76.03±0.010
JS_3	77.44±0.002	16.66±0.004	0.34±0.002	3.11±0.002	79.89±0.031

Effect of storage on pH, acidity, viscosity and total viable count of control and jamun synbiotic smoothie at refrigeration temperature

Table 6 shows the mean ± SE pH, acidity and viscosity values of control and synbiotic jamun based smoothie at 0^{th}, 3^{rd}, 5^{th} and 7^{th} days of storage. This was in agreement with the findings of Lakshmi *et al.* (2015) who also reported that the pH of set type yoghurt containing jamun remained higher than stirred yoghurt during storage. The acidity values in the present study were in accordance to the range of 0.528 and 0.984 per cent/100g for banana and apple based smoothies as observed by Stan and Popa (2013). The viscosity of JS_3 was significantly higher than other treatments due to the high total solid content, as opined by Ezkauriatza *et al*. (2008). Coliform, yeast and mould count was not detected and total viable count was decreased during storage which was in accordance to Kneifel *et al.* (1993) who reported that the loss of cell viability was due to the decreased pH caused by the accumulation of small organic acids in yoghurt.

Table 6: Effect of storage on pH, acidity (%), viscosity (cp) and TVC of control and JS_3

Days	Days							
	Control				JS_3			
	pH	Acidity (%)	Viscosity (cp)	TVC	pH	Acidity (%)	Viscosity (cp)	TVC
0	4.34^{bB}±0.015	0.79^{aA}±0.003	16.65^{aB}±0.050	9.51^{c}±0.387	4.29^{bA}±0.009	0.80^{aB}±0.003	16.40^{aB}±0.002	10.25^{b}±0.225
3	4.33^{bB}±0.015	0.80^{aA}±0.003	16.88^{bB}±0.016	9.03^{cA}±0.273	4.28^{bA}±0.011	0.81^{aB}±0.003	16.80^{bA}±0.036	11.16^{cB}±0.035

5	4.30^{aB}±0.015	0.80^{aA}±0.010	16.98^{bB}±0.016	7.38^{bA}±0.290	4.26^{bA}±0.008	0.83^{bB}±0.004	16.90^{bA}±0.036	9.78^{bB}±0.219
7	4.27^{aB}±0.021	0.83^{bA}±0.004	16.90^{bA}±0.036	5.47^{aA}±0.209	4.17^{aA}±0.028	0.84^{bB}±0.001	16.98^{bA}±0.040	7.87^{aB}±0.073

Effect of inclusion of pasteurized/unpasteurized jamun juice into yoghurt in the preparation of JS_3 on the total phenolic content (µg/mg), antioxidant (mg Fe^{2+}/100ml) and ascorbic acid (mg/100ml) during storage at refrigeration temperature

Table 7 shows the mean ± SE TPC, AA and ascorbic acid values of pasteurized and unpasteurized jamun juice for preparation of synbiotic smoothie at 0^{th}, 3^{rd}, 5^{th} and 7^{th} days of storage. The increase in TPC during storage was in agreement to Balaswamy *et al*. (2013) who reported that it might be due to the release of bound phenols from the cell walls and disassociation of dimers to monomers during storage. The prolonged storage of pomegranate yoghurt causes the transformation of phenolic compounds which are unstable and undergo numerous enzymatic and chemical reactions during storage (Cheynier, 2005). The declination of vitamin C is in agreement with the discussion of Coultate (2002). This may be reasoned due to the fact that vitamin C may retard PP degradation and be used to regenerate PPs. Therefore, the vitamin C initially present in the smoothie might be used up more quickly.

Table 7: Effect of inclusion of pasteurized and unpasteurized jamun juice into yoghurt in the preparation of JS_3 on TPC, AA and ascorbic acid during storage at refrigeration temperature

Storage period in days	**Treatments**					
	JS_3 with pasteurized juice			**JS_3 with unpasteurized juice**		
	TPC	**AA**	**Ascorbic acid**	**TPC**	**AA**	**Ascorbic acid**
0	19.23^{a}±0.013	56.06^{a}±0.002	0.80^{bA}±0.003	19.41^{a}±0.014	56.06^{a}±0.003	1.00^{cB}±0.002
3	19.35^{a}±0.009	56.06^{a}±0.002	0.80^{bA}±0.003	19.48^{a}±0.008	56.07^{a}±0.002	0.95^{bB}±0.002
5	19.55^{a}±0.005	56.07^{b}±0.003	0.77^{aA}±0.003	19.70^{a}±0.006	56.07^{b}±0.002	0.85^{aB}±0.002
7	19.80^{b}±0.000	56.07^{b}±0.003	0.74^{aA}±0.003	19.93^{b}±0.000	56.08^{b}±0.002	0.79^{aB}±0.003

Sensory evaluation of control and jamun synbiotic smoothie during storage at refrigeration temperature

Table 8 shows the mean ± SE sensory evaluation values for control and JS_3 at 0^{th}, 3^{rd}, 5^{th} and 7^{th} days of storage. There was no significant difference in colour characteristics within control and JS_3 during all storage days and a significant difference ($P \leq 0.05$) was also noticed between control and JS_3 on all days of storage.A significant difference ($P \leq 0.05$) between control and JS_3 was noticed in flavour during storage andin body and texture from 3^{rd} day of storage. A high significant difference ($P \leq 0.01$) was also noticed in sweetness, sourness and overall acceptability between control and JS_3 from 3^{rd} day storage. The result was in agreement with the findings of Lakshmi *et al.* (2015) that smoothies blended with jamun pulp were found acceptable on sensory analysis.

Table 8: Sensory evaluation of control and jamun synbiotic smoothie during storage at refrigeration temperature

Sensory attributes	Treatments	Storage period in days			
		0	3	5	7
Colour and appearance	C	$6.50^{A}\pm0.223$	$6.33^{A}\pm0.333$	$6.00^{A}\pm0.000$	$5.83^{A}\pm0.166$
	JS_3	$6.83^{A}\pm0.307$	$6.83^{A}\pm0.307$	$6.66^{A}\pm0.210$	$6.50^{A}\pm0.223$
Flavour	C	$7.33^{bA}\pm0.333$	$7.00^{bA}\pm0.258$	$6.33^{aA}\pm0.210$	$5.66^{aA}\pm0.210$
	JS_3	$8.66^{bB}\pm0.210$	$7.33^{bB}\pm0.333$	$7.00^{aB}\pm0.365$	$6.33^{aB}\pm0.333$
Body and texture	C	$7.16^{b}\pm0.307$	$6.83^{bA}\pm0.307$	$6.33^{aA}\pm0.333$	$5.33^{aA}\pm0.421$
	JS_3	$8.00^{b}\pm0.516$	$7.83^{bA}\pm0.401$	$7.00^{aA}\pm0.447$	$6.16^{aA}\pm0.477$
Sweetness	C	$7.16^{bA}\pm0.166$	$7.00^{bA}\pm0.365$	$6.00^{aA}\pm0.447$	$5.66^{aA}\pm0.421$
	JS_3	$8.66^{bB}\pm0.210$	$8.33^{bB}\pm0.210$	$7.00^{aB}\pm0.258$	$6.33^{aB}\pm0.210$
Sourness	C	$7.50^{bA}\pm0.341$	$6.50^{bA}\pm0.223$	$6.00^{aA}\pm0+.365$	$5.16^{aA}\pm0.307$
	JS_3	$8.50^{bB}\pm0.341$	$7.30^{bB}\pm0.223$	$7.00^{aB}\pm0.258$	$6.16^{aB}\pm0.166$
Overall acceptability	C	$7.10^{bA}\pm0.177$	$6.73^{bA}\pm0.176$	$6.13^{aA}\pm0.204$	$5.53^{aA}\pm0.122$
	JS_3	$8.13^{bB}\pm0.122$	$7.56^{bB}\pm0.130$	$6.93^{aB}\pm0.143$	$6.30^{aB}\pm0.112$

Conclusion

The development of new functional food from locally available raw materials confers the advantage of value addition. The product prepared may be considered as functional due to the polyphenolic content, antioxidant activity and the inclusion of *L.plantarum* beads that may

exert health benefits. However there is a need to develop technologies that would optimize production processes on an industrialized scale.

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