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Development and evaluation of probiotic jam from watermelon rind with microencapsulated *Lactobacillus rhamnosus* and *Lactobacillus casei*

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ABSTRACT

Watermelons are generally consumed fresh, but their rind is disposed of as waste. In the present study, jam variants of watermelon (cv. Sugar Baby) were prepared with rind and pulp. Jam variants were inoculated with chitosan-coated calcium alginate beads with prebiotic inulin microencapsulating probiotic *Lactobacillus rhamnosus* (ATCC 7469) and *Lactobacillus casei* (ATCC 393). Micro-encapsulated probiotics survived better in simulated intestinal juices than in gastric juices. During ninety days of storage, jam variants' physicochemical, sensory and microbial properties were assessed. Micro-encapsulated probiotics had a non-significant effect on the physico-chemical characteristics of watermelon jam variants during storage. In the sensory analysis of watermelon jam variants, jam having 50% endocarp and 50% rind was most accepted. A probiotic count of 10^7 colony forming unit per gram was observed in the rind jam variant after ninety days of storage, and no coliform count was observed. Present work implies that a novel synbiotic (probiotic with prebiotic) jam could be prepared using watermelon rind.

Key words: Watermelon, rind probiotics, microencapsulation, jam, shelf-life.

INTRODUCTION

Watermelon (*Citrullus lanatus* cv. Sugar Baby) is a member of *Cucurbitaceae* family. Watermelon generate waste through their rind and seeds. Present work has mainly focused on the utilization of waste from watermelon to prepare jam, however, watermelon waste, especially the rind, can be repurposed in various ways, including bioethanol production, folk medicine, bacterial cellulose production and development of functional foods. In recent years, research has focused on utilizing watermelon rind in developing different food products. Efforts were made to prepare watermelon jam by incorporating all its parts. Rind could produce quality jam with enormous amounts of pectin and solid matter. Jam can be used as a way to carry probiotics like *Lactobacillus rhamnosus* (ATCC 7469) and *L. casei* (ATCC 393) in microencapsulated form. Factors such as jam processing temperature, product acidity, and gastrointestinal conditions may influence the viability of probiotics (Garg *et al.*, 4). Encapsulation is usually employed to enhance the viability of the bacteria. The relationship between probiotics and prebiotics is called synbiotics (Kaur and Katyal, 5). Microcapsules can be used to add probiotic culture coated in prebiotic polymer to develop synbiotic food products. In the area of fruit and vegetable research, recent work has mainly focused on the development of synbiotic drinks (Donthidi *et al.* 2; Kaur and Katyal, 5). Research on synbiotic watermelon jam is very scarce. With research and insights into the production of synbiotic watermelon jam, watermelon rind will continue to be helpful and have various health benefits. Therefore, the present study emphasized using watermelon rind biowaste to develop probiotic jam with prebiotic coating in microencapsulation.

MATERIALS AND METHODS

Pure cultures of probiotic *L. rhamnosus* (ATCC 7469) and *L. casei* (ATCC 393) were procured from American Type Culture Collection(ATCC), HiMedia Limited, India. The cultures were sub-cultured every 30 days using De Man Rogosa Sharpe (MRS) agar at 37°C. Watermelon (*Citrullus lanatus* cv. *Sugarbaby*) fruits were obtained from the local market. Analytical and laboratory-grade chemicals were procured from HiMedia Limited, India.

After twenty-four hours of incubation, cell suspension of *L. rhamnosus* and *L. casei* were harvested by centrifuging at 4000 rpm, 10 min at 4°C. The probiotic cell suspension [10% w/v having 10^9 colony forming unit per gram (CFU/g) of *L. rhamnosus* and *L. casei,* separately] and calcium alginate (3% w/v) was used in microencapsulation by an extrusion method and collected in calcium chloride solution (2% w/v). For prebiotic coating, inulin (3% w/v) and maize starch, (2% w/v) (Sigma-Aldrich S4126) were used in

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which microcapsules were coated with chitosan (0.1% w/v) (Donthidi *et al.*, 2; Krishna and Rao, 6) (Fig. 1).

Microcapsule bead morphology and diameter were observed using an optical microscope with 100x magnification. Encapsulation yield (%) and release of microencapsulated cultures were determined by spread plating on MRS agar plates, incubated for 48 h at 37ºC. Survival of microencapsulated *L. rhamnosus* (MLR) and *L. casei* (MLC) was observed on MRS agar by pour plating after incubation of 30, 60, 90, and 120 min at 37°C in the simulated gastric and intestinal juices (Zanjani *et al.*,13). Jam variants with different ratios of watermelon flesh (endocarp): rind (mesocarp + exocarp) were prepared (Table 1).

Jam variants *viz.*, JR (Jam Red: Jam made from 100% red/endocarp part of watermelon); JRW (Jam Red White: Jam 50% red/ endocarp part & 50 % white/ mesocarp part); JRG (Jam Red Green: Jam 50% red/endocarp part & 50% green/rind part); JG (Jam Green: Jam 100% green/rind part); were formulated with sugar, citric acid and pectin (FAO, 3) with some modifications (Fig. 2).

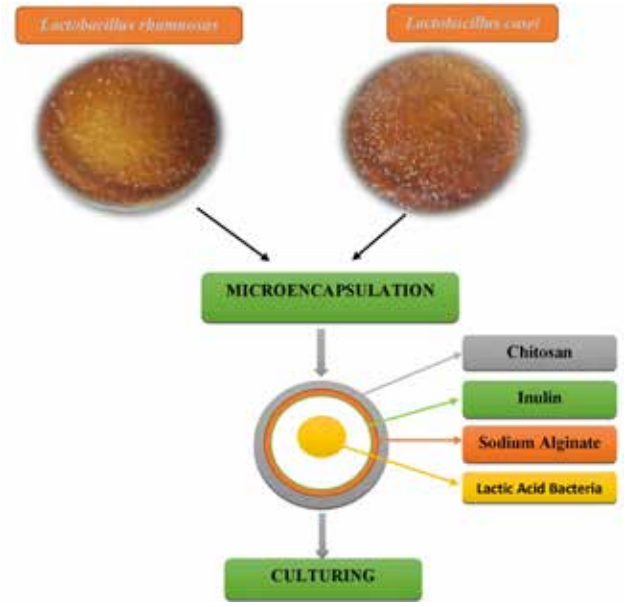

Fig. 1. Steps involved in microencapsulation of probiotics with inulin and chitosan coating.

Titratable acidity pH, total soluble solids (TSS) total and reducing sugars, pectin content, total carotenoids, total phenolic content, citrulline content (AOAC, 1; Ranganna, 9), sensory evaluation using 9-point hedonic scale (Owalade *et al.*, 7; Shobana, 12), and total plate count were observed after every 30 days. The probiotic count was observed on MRS

Fig. 2. Flow sheet for the preparation of watermelon jam variants as JR (Jam Red: Jam made from 100% red part/endocarp of watermelon); JRW (Jam Red White: Jam 50% Red part & 50 % white part/ mesocarp); JRG (Jam Red Green: Jam 50% red part & 50% green part/rind); JG (Jam Green: Jam 100% green part).

Table 1. Watermelon jam variants.

Jam variant	Flesh (endocarp): Rind (mesocarp + exocarp)	Control	Microencapsulated *Lactobacillus casie*	Microencapsulated *Lactobacillus rhamnosus*
JR	100 : 0	without added probiotic cultures	After jam preparation prior to packaging10^9 CFU/g of were added to the jam at the rate 5% w/w. After incorporation of microcapsules the jams were hot filled into sterilized glass bottles, closed and stored at room temperature (25° ± 5°C)	
JRW	50 : 50 (without exocarp)			
JRG	50 : 50 (with exocarp)			
JG	0 : 100			

agar plates after 48 h incubation at 37ºC (Garg *et al.*, 4; Zanjani *et al.*, 13) after three months.

SPSS software by General Linear Model was used to make analysis of mean separation. All measurements were conducted in triplicates and the means were reported. The means of each response variables were subjected to Bonferroni multiple comparison test at $p<0.05$ level to determine the level of significance if any, between the treatments means. The experimental design was completely randomized block design.

RESULTS AND DISCUSSION

Spherical-shaped microcapsules with a mean diameter of 128.7 µm and 145.8 µm of *L. rhamnosus* (MLR) and *L. casei* (MLC) were obtained, respectively. Extruder diameter, polymer used, and viscosity of the Na-alginate solution vary the size of microcapsules. The shape of microcapsules, however, is contingent upon the gap between the extruder and the $CaCl_2$ solution. There was a significant difference ($p<0.05$) in the probiotic count of $5.0*10^9$ CFU/g of *L. rhamnosus* and *L. casei* ($7.0*10^9$ CFU/g). The mean encapsulation yield of *L. casei* (90%) was higher than *L. rhamnosus* (87%). The difference could be due to a higher concentration of Ca^{2+} in the solution used for formulating microbeads, which may damage probiotic viability (Zanjani *et al.*, 13). Lack of proper stirring could have also contributed to low encapsulation yield; thus, during the dropping of calcium chloride solution, proper stirring should be done to ensure even distribution of cells in the sodium alginate, starch and inulin solution (Donthidi *et al.*, 2).

A higher survival rate of probiotics was observed in intestinal juices than gastric juices (Fig. 3a & 3b). Higher survival rates of encapsulated probiotic bacteria in simulated intestinal juices were observed.

Microencapsulation protects the survival of probiotics in simulated gastrointestinal juices. *L. rhamnosus* had the highest survival rate in gastric juices, recording $2.3*10^3$ CFU/g after 90 min of incubation (Fig. 3a). After 60 min, there is a considerable drop in the survival rate of probiotics, it drops even further when the incubation time is increased. When coated with chitosan, calcium alginate beads minimize calcium ions diffusion outside capsules, ensuring better protection, especially in acidic conditions of jam (Krishna and Rao, 6). Chitosan coating decreases alginate beads porosity and reduces encapsulated probiotic cell leakage, leading to better stability at low pH. Survival of *L. rhamnosus* was higher compared to *L. casei* (Fig. 3b). After 60 min, the survival of encapsulated bacteria decreased from $1.16*10^4$ to $0.23*10^4$ CFU/g (90 min) for *L. rhamnosus*. In contrast, *L. casei* decreased

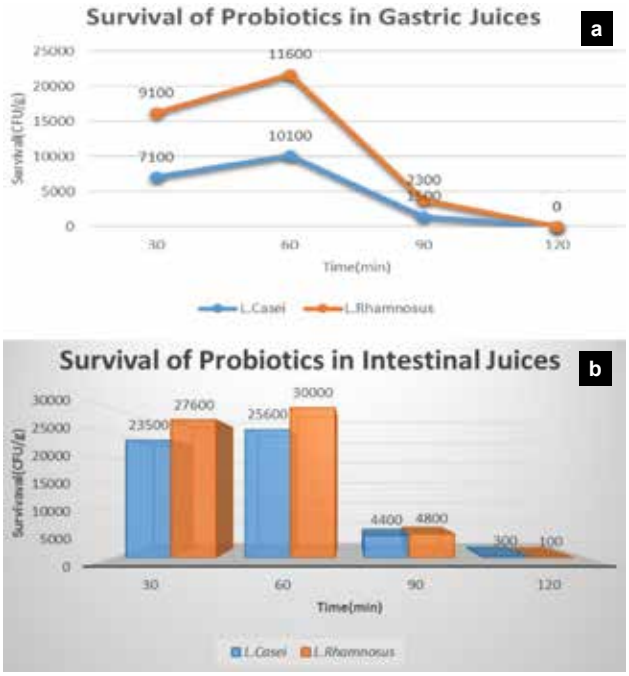

Fig. 3. (a) Survival of probiotics in simulated gastric juices and (b) survival of probiotics in intestinal juices.

from $1.01*10^4$ to $0.15*10^4$ CFU/g. It suggests that *L. rhamnosus* is more acid-resistant than *L. casei*. The different probiotic strains have different response mechanisms to tolerate high acidity. In microencapsulation, alginate reduces the detrimental effects of high acidity on probiotics (Zanjani *et al.*, 13). The protection of probiotics from high acidity using calcium alginate is better prepared with coating using polymers such as starch to increase the chances of bacterial survival due to its good binding capacity (Donthidi *et al.*, 2).

The fresh watermelon was analyzed for various physicochemical properties. The average weight of watermelon fruit, pulp and rind was 1590, 737.9 and 645.9 g, respectively. Endocarp had the highest yield (46.4%) compared to the rind (40.6%). Watermelon fruit had 0.083% acidity, 5.5 pH value and 5.63% reducing sugars. Total sugars, pulp pectin, and rind pectin were 9.83%, 0.38%, and 6.43%, respectively. Total carotenoids, phenols, citrulline and total anthocyanins were 4.62 mg/100 g, 40.2 mg/100 g, 202 mg/100 g and 9.8 mg/100 g, respectively. Similar results were reported by (Owalade *et al.*, 7) who observed a higher yield in pulp (68.97%) than in peel (30%). There was a slight increase in the acidity of all the jams during 90 days of storage. JR was significantly different from JRW, JRG and JG. JR had the highest acidity (0.71), while JG had the least acidity (0.65). Jams with probiotics have a slightly higher acidity (Table 2, 2a).

Table 2. Effect of storage time on acidity (%) of watermelon variants.

Jam variant	Time (days)											
	0			30			60			90		
	C	MLC	MLR	C	MLC	MLR	C	MLC	MLR	C	MLC	MLR
JR	0.63 ± .006	0.63 ± .005	0.63 ± .006	0.64 ± .007	0.65 ± .007	0.66 ± .007	0.64 ± .007	0.67 ± .007	0.67 ± .007	0.64 ± 0.008	0.7 ± .008	0.71 ± .008*
JRW	0.62 ± .005	0.62 ± 0.005	0.62 ± .005	0.63 ± 0.007	0.64 ± .007	0.65 ± .007	0.64 ± .007	0.68 ± .007	0.68 ± .007	0.64 ± .008	0.69 ± .008	0.7 ± .008
JRG	0.63 ± .005	0.63 ± .005	0.63 ± .005	0.63 ± .007	0.64 ± 0.007	0.64 ± .007	0.65 ± .007	0.66 ± .007	0.65 ± .007	0.65 ± .008	0.68 ± .008	0.68 ± .008
JG	0.62 ± .005	0.62 ± .005	0.62 ± .005	0.63 ± .007	0.65 ± .007	0.65 ± .007	0.64 ± .007	0.65 ± .007	0.65 ± .007	0.64 ± .008	0.65 ± .008	0.65 ± .008

Microencapsulated *L. rhamnosus* (MLR) and *L. casei* (MLC); JR (Jam Red: Jam made from 100% red part/mesocarp of watermelon); JRW (Jam Red White: Jam 50% Red part & 50 % white part/exocarp); JRG (Jam Red Green: Jam 50 % red part & 50 % green part/rind); JG (Jam Green: Jam 100 % green part); *Means significant difference ($p<0.05$).

Table 2a. Effect of watermelon jam variants on acidity (%) during storage

Bonferroni Multiple comparison test			Mean difference	p value
Acidity	JR	JRW	-0.01750*	0.012
		JRG	-.03167*	0.000
		JG	-.04417*	0.000
	JRW	JR	.01750*	0.012
		JRG	-.01417*	0.039
		JG	-.02667*	0.000
	JRG	JR	.03167*	0.000
		JRW	.01417*	0.039
		JG	-.01250	0.067
	JG	JR	.04417*	0.000
		JRW	.02667*	0.000
		JRG	.01250	0.067

Acidity in jams increases during storage because of ascorbic acid and pectin degradation. Degradation or oxidation of reducing sugars found in jams may lead to the formation of acidic compounds, which may raise the acidity of the fruit jam. Probiotics may produce organic acids, which might influence the acidity of foods (Garg *et al.*, 4). During the storage period, the pH of jam variants ranged between 3.24 and 3.5. The pH value was observed to increase with an increasing portion of rind. That is the difference in ascorbic acid and pectin composition in different parts of watermelon. Ascorbic acid degradation leads to increased acidity, which can also influence changes in the pH of fruit jams (Patras *et al.*, 8).

JR recorded the highest TSS (67.8°Brix) in control jam variants. There was an increase in the TSS of all jams. JR was significantly different from JRW and JG (Table 3, 3a). The accepted TSS range set by FAO (3) for a proper jam quality is between 65-68. As storage time progressed, there was a

Table 3. Effect of storage time on reducing sugars of watermelon jam variants.

Jam variant	Time (days)											
	0			30			60			90		
	C	MLC	MLR	C	MLC	MLR	C	MLC	MLR	C	MLC	MLR
JR	29.7 ± 1.9	29.3 ± 1.9	29.31 ± 1.9	30 ± 1.9	29.41 ± 1.9	29.5 ± 1.9	31.0 ± 1.8	31.4 ± 1.8	31.4 ± 1.8	33.6 ± 1.9	33.45 ± 1.9	33.56 ± 1.9
JRW	28.8 ± 1.9	28.78 ± 1.9	28.9 ± 1.9	29 ± 1.9	29.1 ± 1.9	29.0 ± 1.9	29.65 ± 1.8	29.67 ± 1.8	29.78 ± 1.8	30.2 ±	30 ± 1.9	30.54 ± 1.9
JRG	26.2 ± 1.7	26 ± 1.7	26.3 ± 1.7	26.6 ± 1.7	26.75 ± 1.7	26.8 ± 1.7	27.7 ± 1.8	27.7 ± 1.8	27.69 ± 1.8	28.1 ±	28 ± 1.8	28.2 ± 1.8
JG	24.75 ± 1.7	25.5 ± 1.7	25.5 ± 1.7	25.8 ± 1.7	25.75 ± 1.7	25.75 ± 1.7	26.0 ± 1.8	26.0 ± 1.7	26.12 ± 1.7	26.76 ±	26.78 ±	26.8 ± 1.8

Table 3a. Effect of watermelon jam variants on the reducing sugars during storage.

Bonferroni comparison	Multiple test		Mean difference	p value
Reducing sugar (%)	JR	JRW	-0.36833	0.669
		JRG	-1.25750	0.149
		JG	-2.24583*	0.012
	JRW	JR	0.36833	0.669
		JRG	-0.88917	0.305
		JG	-1.87750*	0.034
	JRG	JR	1.25750	0.149
		JRW	0.88917	0.305
		JG	-0.98833	0.255
	JG	JR	2.24583*	0.012
		JRW	1.87750*	0.034
		JRG	0.98833	0.255

[Microencapsulated *L. rhamnosus* (MLR) and *L. casei* (MLC)]

slight increase in reducing and total sugars. JG total sugars increased from 49.12 to 53.1% for control jam variants after 90 days. The increase in TSS might be due to the conversion of starch and other insoluble carbohydrates into sugars (Owalade *et al.*, 7).

Like TSS, the difference could be correlated to pH and acidity, which influences the conversion of insoluble polysaccharides such as pectin into reducing sugars. The reducing sugars of all the jams varied between 24.75 to 33.56%. The difference could also be because the rind has less sugar than the red portion of watermelon. The increase in reducing sugars in jam may be caused by the presence of acids such as citric and malic acid, which increases the chances of sugar conversion (Sobhana, 12). However, as storage time increased there was slight reduction in pectin content of all the jams. JG decreased from 5.4 to 4.4% in control jam samples whereas JR decreased from 0.87 to 0.78% (Fig. 4). Similar trend was observed for other jam samples. JG had highest pectin content (%) while JR recorded the lowest pectin content. Reduction in the pectin content could be due to the degradation of pectin into reducing sugars by the beta elimination process. JG had the highest pectin content, while JR recorded the lowest. Jams containing watermelon rind contain higher pectin than those with pulp (Owalade *et al.*, 7).

Variant JR had the highest amount of total carotenoids (3.6 mg/100 g) at 0 days. The second highest was JRG (3.4 mg/100 g), followed by JRW (3.2 mg/100 g) and JG (1.75 mg/100 g) respectively. During storage there was an insignificant decrease in the amount of total carotenoids during jam storage, for instance JR decreased from 3.6 mg/100 g to 2.75 mg/100 g. Other jams had similar trend. The total carotenoids ranged between 1.21 - 3.61 mg/100 g after 3 months of shelf-life (Table 4). Addition of probiotics did not influence the amount of total carotenoids in jam during storage. The red portion of watermelon contains higher total carotenoids, which generally contribute to the colour of watermelon pulp. The rind contains beta carotene and phytofluene

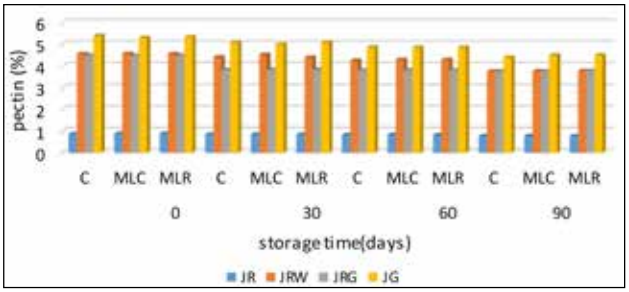

Fig. 4. Effect of storage time on pectin content (%) watermelon jam variants [Microencapsulated *L. rhamnosus* (MLR) and *L. casei* (MLC)].

Table 4. Effect of storage time on total carotenoids (mg/100 g) of watermelon jam variants.

Jam variants	Time (days)											
	0			30			60			90		
	C	MLC	MLR	C	MLC	MLR	C	MLC	MLR	C	MLC	MLR
JR	3.6 ± .02*	3.61 ± .02*	3.6 ± .02*	3.38 ± .015	3.39 ± .02	3.39 ± .02	3.21 ± .02	3.22 ± .02	3.21 ±	2.76 ± .02*	2.77 ± .02*	2.75 ± .02*
JRW	3.2 ± .02*	3.21 ± .02*	3.21 ± .02*	3.00 ± .015	3.04 ± .02	3.00 ± .02	2.86 ± .02	2.83 ± .02	2.78 ±	2.43 ± .02*	2.43 ± .02*	2.42 ± .02*
JRG	3.4 ± .02*	3.37 ± .02*	3.38 ± .02*	3.2 ± .015	3.17 ± .02	3.15 ± .02	2.93 ± .02	2.94 ± .02	2.90 ±	2.67 ± .02*	2.63 ± .02*	2.60 ± .02*
JG	1.75 ± .02*	1.73 ± .02*	1.75 ± .02*	1.63 ± .015	1.62 ± .015	1.63 ± .015	1.58 ± .017	1.55 ± .017	1.55 ±	1.22 ± .017*	1.22 ± .017*	1.21 ± .017*

compared to lycopene in the red part. It contributed to the difference in the total carotenoids content of the jams. There was a significant difference ($p<0.05$) in the number of total phenols, citrulline and total anthocyanin content in all jams, and there was a reduction with time. Watermelon pulp contains 37.9 mg/100 g of phenols, while mesocarp contains 25 mg/100 g of total phenols. Prolonged storage impacted the hydrolysis of compounds and lead to a gradual reduction in total phenols content.

Microencapsulated *L. rhamnosus* (MLR) and *L. casei* (MLC); JR (Jam Red: Jam made from 100% red part of watermelon); JRW (Jam Red White: Jam 50% Red part & 50% white part); JRG (Jam Red Green: Jam 50% red part & 50% green part); JG (Jam Green: Jam 100% green part); *Means significant difference (p<0.05).

Variant JG had the highest amount of citrulline, followed by JRG, JR and JRW, respectively. The rind is reported to contain higher citrulline content than the pulp. Jam variants may differ in the amount of phenols and citrulline depending on the part of watermelon used (Shameena Beegum *et al.*, 10). In JR, anthocyanins decreased from 7.7 to 6.4 mg/g after 90 days of jam storage. The highest anthocyanins were recorded in JR, while the lowest was in JG. The reduction might be because, during jam processing, anthocyanin equilibrium is shifted toward the colourless chalcones, which are unstable during storage.

The study shows that adding probiotics/ synbiotics affected the organoleptic properties of jams within 90 days of jam storage. Jams containing *L. casei* were the most preferred jams (Fig. 5a). However, jams containing *L. rhamnosus* were the least accepted. It is also noted that the different jam formulations affected the sensory properties. Moreover, storage time also affects the properties of synbiotic products (Kaur and Katyal, 5). (Fig. 5b).

L. casei-containing jams had better colour, taste, flavour, texture and spreadability, contributing to the higher overall acceptance of the jams. It could be due to the by-products of *L. casei.* The jams were generally accepted with a mean value of 7. After 90 days of storage, the most acceptable jam was JRW, which JR, JRG, and JG followed among jam variants (Fig. 6). The by-products of *L. casie*, dissolving of microcapsules in presences of phosphates and increase in reducing sugars during ninety day storage may improve the taste of jam.

JRW had the highest mean score in all the organoleptic properties, scoring at least a mean of 7.6. The probiotics did not alter the sensory properties of rose petal jam (Shoaei, *et al.,* 11). The jam had an overall acceptance mean of 7.7 that means like very much from a 9-point hedonic scale (Sobhana, 12). In other properties, the JRW recorded 8.17 (colour), 7.95(taste), 7.7 (texture), 7.9 (flavour) and 7.7 for

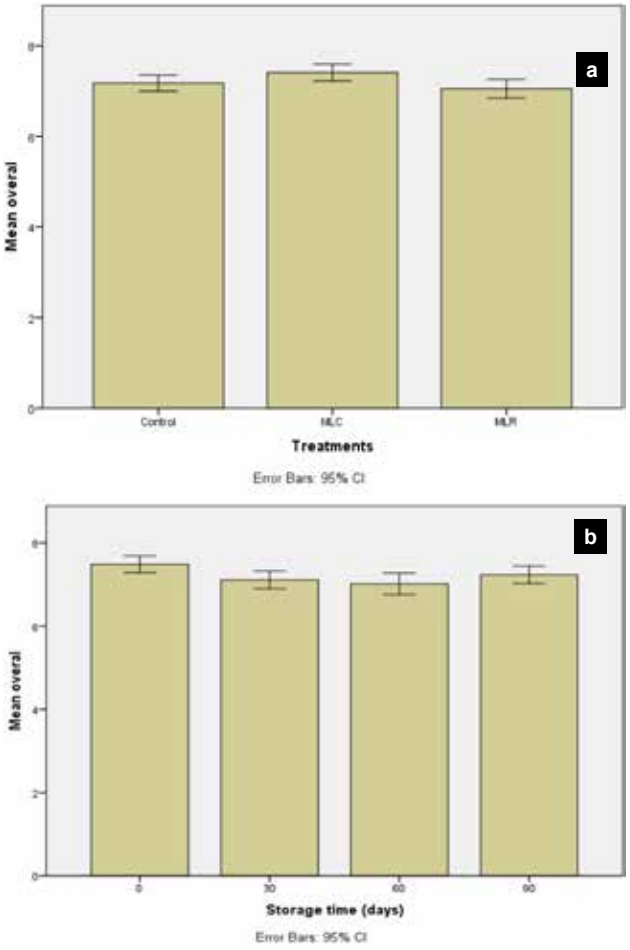

Fig. 5. (a) Effect of microencapsulated probiotics on overall acceptance and (b) Effect of storage time on overall acceptance of watermelon jam variants [Microencapsulated *L. rhamnosus* (MLR) and *L. casei* (MLC)].

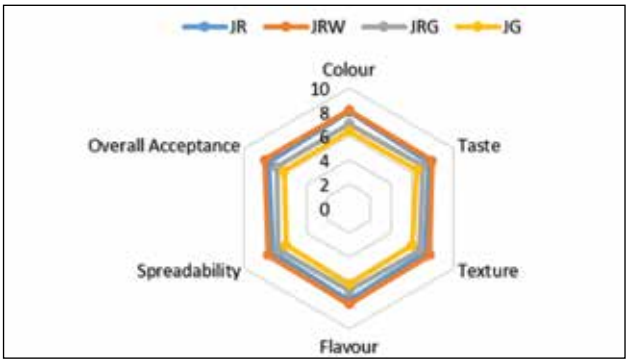

Fig. 6. Organoleptic properties of watermelon jam variants after 90 days of storage.

spreadability. Because the rind is usually disposed of as waste, it could have contributed to a lower mean score given to JRG and JG since people are not used to its taste.

TPC and probiotic count of jams containing microencapsulated *L. casei* and *L. rhamnosus* were significantly different ($p<0.05$) from control jam variants (Fig. 7). The control jam variant contained no microencapsulated bacteria. The survival of probiotics in jam is due to the protection provided by encapsulating material, which protects the organisms from the acidity of the food matrix (Shoaei, *et al.,* 11). JR samples recorded the lowest probiotic count; for instance, *L. casei* containing jams had $4.3*10^4$ CFU/g, whereas *L. rhamnosus* containing jams was $1.6*10^5$ CFU/g. Despite the reduction in the probiotic count, the highest survival rate was recorded in JG, which recorded $6.5*10^7$ CFU/g of *L. rhamnosus*. In JRG, the probiotic count was 10^6 CFU/g, followed by 10^5 CFU/g in JRW. After 90 days of jam storage, *L. rhamnosus* (MLR) had the highest survival rate in JG, which recorded $6.5*10^7$ and $4.6*10^7$ CFU/g for *L. casei* (MLC).

The probiotic count in jam variant JG met the minimal criterion of 10^7 viable cells/g. After days 90 days of jam storage, no coliform count observed. So, it is safe for human consumption. Addition of microencapsulated probiotics had a non-significant effect on the pH, TSS, total and reducing sugars, pectin content, total carotenoids, total phenols, citrulline, and anthocyanin of jam during storage.

Watermelon (cv. *Sugarbaby*) fruit rind and other parts were used to develop synbiotic jam

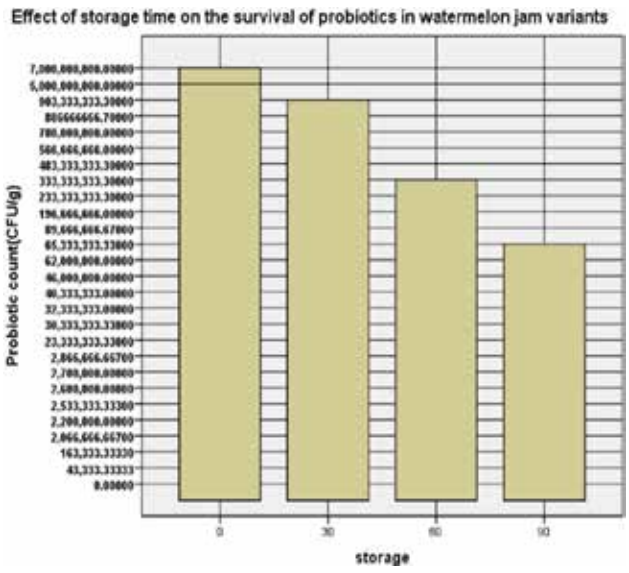

Fig. 7. Effect of storage time on survival of probiotics in watermelon jam variants.

variants. Both microencapsulated probiotic cultures, *L. rhamnosus* and *L. casei*, with prebiotic inulin, survived in simulated gastrointestinal juices. After a three-month storage period, desired probiotic count and sensory score was recorded in the novel synbiotic watermelon rind jam (JG) variant. Therefore, rind waste could be turned into a product with additional worth.

AUTHORS' CONTRIBUTION

Lab experiment, data collection and data analysis (MDS), conceptualization and experimental material (AK), proof reading (RK), editing(RG).

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DECLARATION

The authors declare that they have no conflict of interest. Publication has been approved by all co-authors.

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