Survival Pattern of Micro-Encapsulated Probiotic Yeasts and Lactic Acid Bacteria in Papaya and Carrot Beverages

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ABSTRACT

The study investigated survival pattern of micro-encapsulated probiotic yeasts and lactic acid bacteria in papaya and carrot juices. Newly isolated yeast and lactic acid bacteria along with reference strains like *Saccharomyces boulardii, Saccharomyces ellipsoideus* No. 101. *Lactobacillus acidophilus* (NCIM 2903) and *Bacillus mesentericus* (NCIM 2019) were used in the study. Micro-encapsulated strains were inoculated independently into papaya and carrot juice. Initial inoculum size was 10^7 cfu /mL and after 24 hours of fermentation, it significantly increased to 10^8 cfu /mL and their viability was assessed on a 15 day intervals for up to 45 days. Encapsulated probiotic strains (10^8 cfu /mL) survived in both juices throughout 45 day period at 4 °C of storage.

Keywords: Probiotics, Synbiotic, Papaya, Carrot

FRUITS and vegetables have been recognized as suitable media for cultivation of probiotics because they inherently contain essential nutrients, high amount of vitamins, minerals and polyphenolic compounds free from allergens. They are available with attractive appearance and taste. Studies related to non-dairy probiotic beverages such as tomato, cabbage, orange, beet root and carrot juices have been reported in conjunction with different probiotic strains (Naga *et al.,* 2016).

Fermented fruits and vegetables have an important role in feeding the world's population today. Fermentation play an important role in preservation, production of wholesome nutritious foods with a wide variety of flavor, aroma and texture that enrich human diet. Fermentation serves many benefits, including food security, improved nutrition and better social well being of people living in marginalized and vulnerable society (Swain *et al*., 2014).

Microencapsulation is one of the most important forms of controlled release of substances widely used in several fields of the industry, particularly the food industry. Encapsulated microbial cells have been used in wide range of fields for optimum activity benefiting the suitable conditions for growth and metabolism and at the same time protection from harsh environmental conditions (Cook *et al*., 2012, Wohlgemuth *et al*., 2010). Over the years, there has been increased interest in developing suitable probiotics in particular for human and animal feed (Paramera *et al*., 2011). However, nowadays probiotics are part of nutritional supplements and healthy food realms due to their established beneficial effects (Pham-Hoang *et al*., 2013).

The main objective of the study was to determine the survival pattern of probiotics in papaya and carrot juice during storage in order to assess the suitability of a juice as a new alternative functional food. Further an attempt was made to assess microencapsulation techniques for improving the survival of probiotic bacteria in papaya and carrot juices.

MATERIAL AND METHODS

Isolation of Yeasts and Lactic Acid Bacteria

Yeasts and lactic acid bacteria were isolated from papaya and carrot on yeast extract potato dextrose agar (YEPDA) and de Man, Rogossa and Sharpe (MRS) agar medium, respectively. Isolation and characterization of yeast strain was based on observations recorded by Kreger-Van Rij (1984) and lactic acid bacteria were identified using procedures of Sneath (1986). The isolated strains were used for microencapsulation and fermentation study along with reference strains *viz., Saccharomyces boulardii, S. ellipsoideus* No. 101. *Lactobacillus acidophilus* (NCIM 2903) and *Bacillus mesentericus* (NCIM 2019) from National Collection of Industrial Microorganisms, Pune.

Microencapsulation of Yeasts and Lactic Acid Bacteria

Yeast and lactic acid bacterial strains were inoculated in yeast extract potato dextrose and de Man, Rogossa and Sharpe (MRS) broth respectively, incubated at 30 ˚C for 48 hours. Broth (20 mL) was centrifuged (8000 rpm for 15 min) to harvest cells and cells were suspended in 20 mL sterile water. An equal volume of cell suspension and 4 per cent (w/v) sodium alginate were mixed properly to ensure uniform distribution of cells. The suspended mixture was suctioned in a syringe and dropped gently into 1.5 per cent (w/v) $CaCl_2$ solution (Shah and Ravula, 2000). The beads were left to harden for 45 min and then washed with distilled water to remove excess calcium deposited and then stored at 4 ˚C.

Microbiological Analysis of Synbiotic Beverages

After 24 hours of fermentation and during storage period, samples were subjected to microbiological analysis of yeast and lactic acid bacterial population by employing standard plate count method using YEPD and MRS agar medium (Pushpa Priya, 2013).

Organoleptic Evaluation

The developed fermented beverage from papaya and carrot juices were evaluated by selected five panel members with 20 point hedonic scales. This 20 point hedonic scale considers mainly appearance, color, aroma, taste and acceptability.

Statistical Analysis

The results of this study were analyzed using Web Agri. Stat Package (WASP 2.0).

Preparation of Fermented Beverage from Papaya and Carrot Juice :

1. Flow chart for preparation of fermented synbiotic beverage from papaya

Healthy papaya fruits were selected
↓
Washed with water to remove dust
↓
Cut fruit into small pecies
↓
Processed to juice using mixer
↓
Water was added (1:2.5)
↓
Honey was added (5 %)
↓
Brix adjustment was done (to 18 °brix) by adding sugar
↓
Pasteurized at 60 ˚C for 30 min
↓
Inoculated starter culture
↓
Fermented for 24 hours
↓
Synbiotic beverage was stored at 4 ˚C

2. Flow chart for preparation of fermented synbiotic beverage from carrot

Carrots were selected
↓
Washed with water to remove dust
↓
Cut into small pieces
↓
Processed to juice using mixer
↓
Water was added (1:2.5)
↓
Honey was added (5 %)
↓
Brix adjustment was done (to 18 °brix) by adding sugar
↓
Pasteurized at 60 ^{O}C for 30 min
↓
Inoculated starter culture
↓
Fermented for 24 hours
↓
Synbiotic beverage was stored at 4 ˚C

Results and Discussion

Microencapsulation and Survivability Study of Yeast and Lactic Acid Bacterial Strains during Storage

Microencapsulation has been used to improve viability of cells during storage. Yeasts and lactic acid bacterial strains were microencapsulated with sodium alginate by dropping method. The survivability of encapsulated yeast and lactic acid bacterial strains are presented in Table 1.

Initial population of yeast cells entrapped in beads ranged from 8.67 x 10^7 cfu /g to 11.67 x 10^7 cfu /g. The initial highest yeast population entrapped in beads was with PY4 isolate (11.67 x 10^7 cfu /g) followed by *Saccharomyces boulardii* (10.00 x 10^7 cfu /g) and the lowest yeast population entrapped in beads was with CY4 isolate (8.67 x 10^7 cfu /g) followed by *Saccharomyces ellipsoideus* (9.67 x 10^7 cfu /g). The population of yeast cells did not change significantly, during storage for 45 days at 4 °C. This is in conformation with the results reported by Gallo *et al.* (2014) who reported the survival of *Saccharomyces boulardii* in alginate beads for 90 days. On the other hand, viability of yeasts cells in alginate beads during storage at 4 ^{0}C for 30 days, showed non significant changes in the viable population was observed by Bevilacqua *et al.* (2020).

The initial population of lactic acid bacterial cell entrapped in alginate beads was 9.00 x 10^7 cfu /g to 11.67 x 10^7 cfu /g. The highest lactic acid bacterial cell concentration entrapped in beads was CL5 isolate (11.67 x 10^7 cfu /g) followed by *Bacillus mesentricus* (10.67 x 10^7 cfu /g) and the lowest was observed in *Lactobacillus acidophillus* (9.00 x 10^7 cfu /g) followed by PL1 isolate (10.33 x 10^7 cfu / g). During 45 days storage at 4 ^{0}C, viability of bacterial population did not change significantly. They reported survival of *Lactobacillus bulgaricus* KFRI 673 in chitosan coated alginate beads for four weeks.

Evaluation of Microencapsulated Yeast and Lactic Acid Bacterial Populations in Papaya Synbiotic Beverage

The experiment was conducted to know stability of microencapsulated yeasts and lactic acid bacterial strains for fermentation of papaya juice. The results pertaining to survivability of encapsulated yeasts and

TABLE 1

Survivability study of yeast and lactic acid bacterial isolates and reference strains in sodium alginate beads (10^7 cfu /g)

Treatments	Yeast Population (10^7 cfu /g)				Lactic Acid Bacterial Population (10^7 cfu /g)			
	1 D	15 D	30 D	45 D	1 D	15 D	30 D	45 D
T1	11.67 ^{a}	11.67 ^{a}	11.33 ^{a}	11.33 ^{a}	10.33 ^{ab}	9.67 ^{bc}	9.67 ^{bc}	8.67 ^{b}
T2	8.67 ^{c}	8.67 ^{b}	8.33 ^{b}	8.33 ^{b}	11.67 ^{a}	11.67 ^{a}	11.33 ^{a}	11.33 ^{a}
T3	10.00 ^{b}	9.67 ^{b}	9.67 ^{b}	9.33 ^{b}	9.00 ^{b}	9.00 ^{c}	8.67 ^{c}	8.67 ^{b}
T4	9.67 ^{bc}	9.67 ^{b}	9.33 ^{b}	9.33 ^{b}	10.67 ^{a}	10.33 ^{b}	10.33 ^{ab}	10.33 ^{a}

Yeast treatment details	Lactic Acid Bacterial treatment details
T1 - PY4 isolate	T1 - PL1 isolate
T2 - CY4 isolate	T2 - CL5 isolate
T3 - *Saccharomyces boulardii*	T3 - *Lactobacillus acidophilus*
T4 - *S. ellipsoideus*	T4 - *Bacillus mesentericus*

PY4- Yeast isolated from papaya, PL1- Lactic acid bacterial isolate from papaya
CY4- Yeast isolated from carrot, CL5- Lactic acid bacterial isolate from carrot

lactic acid bacterial strains in fermented papaya juice is presented in Table 2.

The viability of encapsulated probiotics after fermentation of papaya juice increased significantly from 10^7 cfu /mL to 10^8 cfu /mL. The highest yeast population was observed with PY4 isolate (12.33 x 10^8 cfu /mL) followed by *Saccharomyces boulardii* (11.33 x 10^8 cfu /mL). In case of lactic acid bacterial population CL5 isolates (12.67 x 10^8 cfu /mL) had the highest viability followed by *Lactobacillus acidophilus* (11.33 x 10^8 cfu / mL). Viability of encapsulated probiotics reduced slightly during storage period of 45 days at 4 ^{0}C. These findings are similar to results reported by Ong-Ard *et al.* (2019), where mango juice was fermented by encapsulated *Lactobacillus plantarum* and storage study was carried out for 35 days at 4 ^{0}C. Earlier studies have reported that encapsulated probiotc bacteria survived in orange and apple juices throughout six weeks of storage.

Evaluation of Microencapsulated Yeast and Lactic Acid Bacterial Populations in Carrot Synbiotic Beverages.

The experiment was conducted to know stability of microencapsulated yeasts and lactic acid bacterial strains for fermentation of carrot juice. The results pertaining to survivability of encapsulated yeasts and lactic acid bacterial strains in synbiotic carrot beverages is presented in Table 3.

Survivability of encapsulated probiotics in carrot juice was evaluated after fermentation and during storage at 4 ^{0}C for 45 days. The viability of encapsulated probiotics after fermentation of carrot juice was significantly increased from 10^7 cfu /mL to 10^8 cfu / mL. The highest yeast population was observed with PY4 (12.00 x 10^8 cfu / mL) followed by *Saccharomyces boulardii* (11.33 x 10^8 cfu / mL). In case of lactic acid bacterial populations CL5 isolate (12.33 x 10^8 cfu / mL) had the highest viability followed by *Lactobacillus acidophilus* (11.33 x 10^8 cfu / mL). Survivability of encapsulated probiotics slightly decreased during storage period of 45 days at 4 ^{0}C. Naga *et al*. (2016) reported similar findings in tomato and carrot juice fermented by encapsulated lactic acid bacteria and yeast strains during storage at 4 ^{0}C for six weeks.

Organoleptic Evaluation

The experimental results tabulated in Table 4 reveal that papaya juice with 5 per cent honey fermented by yeast isolate (PY4) recorded the highest score (16.32 out of 20) and lactic acid bacterial isolate (CL5) recorded the highest score (16.54 out of 20.00) with

TABLE 2

Survivability study of encapsulated yeast and lactic acid bacterial isolates and reference strains in synbiotic papaya beverage (10^8 cfu /mL)

Treatments	Initial Inocula (10^7 cfu /mL)	Yeast Population (10^8 cfu /mL)				Initial Inocula (10^7 cfu /mL)	Bacterial Population (10^8 cfu /mL)			
		1 D	15 D	30 D	45 D		1 D	15 D	30 D	45 D
T1	11.00	12.33 ^{a}	12.00 ^{a}	11.67 ^{a}	11.33 ^{a}	10.00	10.00 ^{c}	10.33 ^{b}	10.33 ^{b}	9.67 ^{b}
T2	8.00	9.67 ^{b}	9.33 ^{ab}	9.33 ^{b}	9.33 ^{b}	11.00	12.67 ^{a}	12.33 ^{a}	12.67 ^{a}	12.33 ^{a}
T3	10.00	11.33 ^{ab}	11.33 ^{ab}	10.67 ^{ab}	10.33 ^{ab}	9.00	11.33 ^{b}	10.67 ^{b}	10.67 ^{b}	10.33 ^{b}
T4	9.00	10.33 ^{b}	10.33 ^{b}	9.33 ^{b}	9.33 ^{b}	10.00	10.33 ^{bc}	10.33 ^{b}	9.67 ^{b}	9.67 ^{b}

Note:

Yeast treatment details

T1- Papaya juice + PY4 isolate + honey

T2- Papaya juice + CY4 isolate + honey

T3- Papaya juice + *Saccharomyces boulardii* + honey

T4- Papaya juice + *S. ellipsoideus* + honey

Lactic Acid Bacterial treatment details

T1- Papaya juice + PL1 isolate + honey

T2- Papaya juice + CL5 isolate + honey

T3- Papaya juice + *Lactobacillus acidophilus* + honey

T4- Papaya juice + *Bacillus mesentericus* + honey

TABLE 3

Survivability study of encapsulated yeast and lactic acid bacterial isolates and reference strains in synbiotic carrot beverage (10^8 cfu /mL)

Treatments	Initial Inocula (10^7 cfu /mL)	Yeast Population (10^8 cfu /mL)				Initial Inocula (10^7 cfu /mL)	Bacterial Population (10^8 cfu /mL)			
		1 D	15 D	30 D	45 D		1 D	15 D	30 D	45 D
T1	11.00	12.00 [a]	11.67 [a]	11.67 [a]	11.33 [a]	10.00	9.67 [b]	9.67 [c]	9.33 [c]	9.33 [b]
T2	8.00	9.33 [c]	9.67 [c]	9.67 [b]	9.33 [b]	11.00	12.33 [a]	12.00 [a]	11.67 [a]	11.33 [a]
T3	10.00	11.33 [ab]	11.00 [ab]	10.67 [ab]	10.33 [ab]	9.00	11.33 [a]	11.33 [ab]	10.67 [ab]	10.33 [ab]
T4	9.00	10.33 [bc]	10.33 [bc]	9.67 [b]	9.33 [b]	10.00	10.00 [b]	10.00 [bc]	9.67 [bc]	9.33 [b]

Note :

Yeast treatment details

T1- Carrot juice + PY4 isolate + honey

T2- Carrot juice + CY4 isolate + honey

T3- Carrot juice + *Saccharomyces boulardii* + honey

T4- Carrot juice + *S. ellipsoideus* + honey

Lactic Acid Bacterial treatment details

T1- Carrot juice + PL1 isolate + honey

T2- Carrot juice + CL5 isolate + honey

T3- Carrot juice + *Lactobacillus acidophilus* + honey

T4- Carrot juice + *Bacillus mesentericus* + honey

TABLE 4

Sensory evaluation of papaya and carrot beverages prepared using encapsulated yeast and lactic acid bacterial isolates and reference strains

Treatments	Papaya beverage		Carrot beverage	
	Yeast (20)	LAB (20)	Yeast (20)	LAB (20)
T1	16.32	15.45	16.15	15.57
T2	15.82	16.54	15.02	15.97
T3	15.20	14.85	15.28	15.22
T4	15.45	15.25	15.04	15.37

Note :

Yeast treatment details

T1- PY4 isolate + honey

T2- CY4 isolate + honey

T3- *Saccharomyces boulardii* + honey

T4- *S. ellipsoideus* + honey

Lactic Acid Bacterial treatment details

T1- PL1 isolate + honey

T2- CL5 isolate + honey

T3- *Lactobacillus acidophilus* + honey

T4- *Bacillus mesentericus* + honey

respect to overall acceptability compared to other strains. Carrot juice with 5 per cent honey fermented by yeast isolate (PY4) recorded the highest score (16.15 out of 20.00) and lactic acid bacterial isolate (CL5) recorded the highest score (15.97 out of 20.00) with respect to overall acceptability.

Microencapsulation has been used to improve viability of cells during storage. Microencapsulated probiotic organisms showed a much higher survival in papaya and carrot juices. The papaya and carrot juices represent suitable and alternative food matrices for the production of functional foods with probiotic organisms.

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