Int.J.Curr.Microbiol.App.Sci (2021) *10*(01): 663-674

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 10 Number 01 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1001.080

Isolation, Identification and Screening of Yeasts for Production of Papaya Synbiotic Beverage

Pampangouda[1*], Suvarna[1], V. Chavannavar[2], Mahadevaswamy[2] and K. G. Vijayalkshmi[3]

[1]Department of Agricultural Microbiology, [3]Department of Food Science and Nutrition, University of Agricultural Sciences, Bangalore, India
[2]Department of Agricultural Microbiology, University of Agricultural Sciences, Raichur, India

**Corresponding author*

Keywords

Probiotics, Synbiotic, Papaya

Article Info

Accepted:
08 December 2020
Available Online:
10 January 2021

A B S T R A C T

A study was carried out to know the probiotic properties of ten yeast isolates of papaya and carrot. Out of ten isolates, growth of PY4 and CY4 yeast isolates were more compared to other isolates at low pH (2.0, 2.5 and 3.0) and bile salt concentration (0.4, 0.5 and 0.6 %). Further these two isolates were used for fermentation of papaya juice along with reference strains *Saccharomyces boulardii and Saccharomyces ellipsoideus*. After 24 hours of fermentation, pH (from 5.8 to 3.39), TSS (from 18.00 to 15.43° brix) and total sugar (6.56 to 6.10 %) were significantly decreased, whereas in titrable acidity (from 0.32 to 0.81 %) and antioxidant activity (from 62.77 to 65.53 %) were significantly increased in papaya juice. The initial inoculums of yeast strains added to papaya juice had 4±1 x 10^7 cfu /mL. Population of yeast strains increased from 10^7 to 10^8 cfu /mL after 24 h of fermentation. The highest yeast population was recorded in papaya juice with 5 per cent honey added and fermented by PY4 isolate (13 x 10^8 cfu /mL). During the storage period of 45 days at 4 °C, all parameters of papaya beverage changed non significantly in all treatment. This study reveals that, isolate PY4 was superior to other isolates as well as reference strains in synbiotic beverage.

Introduction

Now a day's research on functional food as gaining important role related to human nutrition. Consumption of functional food results in beneficial effects on health, as well as maintaining nutritional balances (Saad *et al.*, 2011). Increasing demand for this type of food may increased health benefits and decreased some disorders associated with humans (El-Salam, 2011).

Probiotics are defined as live microbial food supplement that provide beneficial effects to consumers when administered in adequate amounts (FAO, 2002). Minimum dose of probiotics ranges from 10^7 to 10^9 CFU is generally used, despite this dose may be

different based on the different strain (Forssten *et al.,* 2011). Taking of probiotics regularly it improve regulation of intestinal function, improving immune system and inhibition of pathogens.

Most commonly available probiotics are bacteria such as *Lactobacillus* and *Bifidobacterium* (De Vrese and Schrezenmeir, 2008). Compare to bacteria probiotic potential of yeasts strains were still insupcient studied with respect to their distribution and importance in foods (Chen *et al.,* 2010). *Saccharomyces cerevisiae* var. *boulardii* yeast strain recognized and considered as probiotic (Czerucka and Rampal, 2002, Hatoum *et al.,* 2012) till date, which raises the question of whether other species of yeast might have probiotic properties as well (van der *et al.,* 2005).

In recent years, probiotic-supplemented food products plays an very important role in the food industiers and several probiotic studies have concentrating on milk based product, which presently dominate in world business of probiotics (Saad *et al.,* 2011). Milk products were used traditionally for supplementation of probiotics. Presently non-dairy probiotic products are gaining demands, because some peoples have lactose intolerance (Prado *et al.,* 2010). Regarding these reason, present studies have recommended fruit juice as another alternative vehicle and good sources of nutrients for addition of probiotics (Fonteles *et al.,* 2012, Mousavi *et al.,* 2011, Pereira *et al.,* 2017, Pereira *et al.,* 2011, Sheehan *et al.,* 2007, Yoon *et al.,* 2006). Fruit juices are commonly consumed by peoples regularly, which is help full for deliver probiotics to gut (Ding and Shah, 2008). Because of these advantages, several companies have interested in commercialization and development of probiotic beverages from fruits, such as grape, orange, mango, guava and pineapple.

Papaya has been identified as a healthy food due to the presence nutrients, antioxidants and medicinal properties. The nutrients and phyto-chemicals present in papaya help in digestion, reduce inflammation, support functioning of cardiovascular, immune and digestive systems; and also help in prevention of colon, lungs and prostate cancers.

Papaya acts as a detoxifier, activator of metabolism, rejuvenate the body and in maintaining body's homeostasis because it is rich in antioxidants, vitamin B, folate, pantothenic acid, sulphur, potassium and magnesium as well as fiber. It helps in preventing cataract and age-related macular degeneration as it has high vitamin A and carotenoids contents.

Papaya pastes are used externally for treatment of wounds and burns. Papaya is preferred for production of beverages due to its high nutritive value and reasonable price. It is known as common man’s fruit and is a rich source of nutrients like carotenoids, vitamin C, vitamin E and flavonoids (Ramachandran and Nagarajan, 2014).

The aim of this work were to isolate and screening of yeast strains for probiotic potential and select those suitable strains for preparation of a papaya fermented beverage.

Materials and Methods

Isolation and characterization of yeasts

Yeasts were isolated from papaya and carrot by standard plate count method using yeast extract peptone dextrose agar (YEPDA) medium. The isolated yeasts were further purified and characterized by employing standard procedures. Isolates were identified by studying their colony morphology, microscopic observation and sugar utilization characters.

Screening of yeast isolates by acid and bile salt tolerance

Acid tolerance was evaluated by growing isolated yeasts in YEPD broth and pH was adjusted to 2, 2.5 and 3 using 1N HCl. For bile salt tolerance bovine salt added into broth at various concentrations 0.4 %, 0.5 % and 0.6 %, then incubated at 37 °C for 24 h. Growth was measured at 6 h and 12 h using spectrophotometer, reading the optical density at 600 nm against uninoculated broth.

Preparation of papaya juice

The selected, well matured, ripe and healthy papaya fruits were washed thoroughly in water. The papaya were cut into small pieces and extracted juice using juicer.

Preparation of fermented beverage from papaya juice

Water was added to extracted papaya juice (1:2.5) and five per cent honey was added. Juice was adjusted to obtain 18 °brix by adding sugar using hand refractrometer and pasteurization done at 60 °C for 30 minute. The juice was kept in 500 mL glass bottle and closed with screw cap. The juice was inoculated with yeasts starter cultures at the rate of 6 per cent (v/v) for fermentation. Fermentation was carried out mixing of the juice for 24 h. After the fermentation juice was stored at 4 °C and samples were used for further analysis and evaluation.

Preparation of starter cultures of yeast

A loop full of yeast cultures were transferred YEPD broth and were incubated overnight at 28 °C for growth. Yeast culture was having 10^7 cfu /mL and same was used for inoculation into prepared papaya juice at 6 per cent.

Biochemical analysis of synbiotic beverages

The pH of the papaya synbiotic beverage samples was determined using a digital pH meter (Sadasivam and Manickam, 1996). Total soluble solid (TSS) was measured using a hand refractometer (Ranganna, 1995). Titrable acidity of samples was determined according to the general titration method and based on lactic acid percentage. 10 mL of sample was titrated against 0.1 N NaOH in presence of phenolphthalein. Estimation of total sugars was determined using method given by Sadasivam and Manickam (1996). Antioxidant activity of fermented beverages was determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method (Kongsuwan *et al.,* 2009).

Microbiological analysis of synbiotic beverages

After 24 h of fermentation and during storage period, samples were subjected to microbiological analysis of yeast population by employing standard dilution plate count method (Hoben and Somasegaran, 1982).

Organoleptic evaluation

The developed fermented beverage from papaya juices were evaluated by selected five panel members with 20 point hedonic scales (Amerine *et al.,* 1972).

Statistical analysis

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

Results and Discussion

Colony morphological characterization and microscopic observation of isolated yeasts

The details of isolation of yeasts are furnished in Table 1. Colonies were of medium size,

creamy white to white color and oval to round shaped. All yeast isolates exhibited budding property, cell size ranged from 2.53 x 1.52 to 5.44 x 3.17 µm and were oval in shaped.

Utilization of different sugars by yeasts isolates

The details of carbohydrates fermentation by yeast isolates from are furnished in Table 2. The change in medium color indicates sugar fermentation by isolates. The results showed that all yeast isolates grew well in different sugars *viz.* sucrose, maltose, glucose, fructose and mannose but did not grow in lactose.

Screening of yeasts and lactic acid bacterial isolates for probiotic properties

Yeast isolates were screened for probiotic properties like bile salt and pH tolerance. The bile salt tolerance test for yeast and lactic acid bacterial isolates was conducted and results are presented in Table 3. The experiment was conducted to evaluate the effect of bile salt concentrations like 0, 0.4, 0.5 and 0.6 per cent. The growth of isolates was measured in terms of OD at 600 nm. The results showed that all yeast isolates grew better at 0.4 per cent bile salt concentration, the highest OD was recorded by yeast isolate PY4 (0.29 and 0.28) followed by CY4 (0.27 and 0.24) and the least was recorded by CY1 (0.21 and 0.20) at 6 and 12 h respectively.

The acidic pH tolerance test for yeast and lactic acid bacterial isolates was assessed and is presented in Table 4. The experiment was conducted to evaluate the effect of pH at 2.0, 2.5 and 3.0. The results showed that all the strains of yeast grew better at 3.0 pH, the highest OD was recorded by yeast isolates PY4 (0.16 and 0.16) followed by CY4 (0.16 and 0.16) and the least was recorded by PY3 (0.14 and 0.13) at 6 and 12 h respectively.

Biochemical properties of papaya beverage fermented by yeast strains

The experiment was conducted to know efficiency and potential of different yeast isolates and reference strains for the fermentation of papaya juice. The results pertaining to pH, TSS, titrable acidity, total sugar and antioxidant activity content of fermented papaya juice is presented in Table 5.

pH

The initial pH of papaya juice was 5.80. After 24 h of fermentation by different yeast isolates and reference strains, pH changed from 3.39 to 3.58 in fermented papaya juice. Significantly higher pH was recorded in PY4 (3.58) inoculated with honey and it was on par with PY4 inoculated with honey (3.57) among the isolates. The papaya juice fermented by yeast strain *Saccharomyces ellipsoideus* recorded the lowest pH (3.39) followed by addition of 5 % honey with papaya juice fermented by *S. ellipsoideus* (pH 3.40). The isolated yeasts and reference strains had significantly low pH in papaya juice after 24 h of fermentation. There was slight change in pH when stored at 4 $^{\circ}$C for 45 days.

Total soluble solids (^{O}Brix)

With respect to TSS, significant differences were observed between the treatments. The initial TSS of papaya juice was adjusted to 18 $^{\circ}$brix. After 24 h of fermentation, it was noticed that TSS content varied from 15.43 to 15.90 $^{\circ}$brix. The highest TSS content was (15.90 $^{\circ}$brix) observed in papaya juice fermented with PY4 followed by (15.57 $^{\circ}$brix) on addition of 5 % honey to papaya juice. The lowest TSS content (15.43 $^{\circ}$brix) was observed in papaya juice fermented by CY4. During the storage period of 45 days at 4 $^{\circ}$C,

TSS of the papaya beverage changed slightly in all treatments.

Titarble acidity (%)

The data represents significant differences in titrable acidity of fermented papaya juice. The initial value of titrable acidity of papaya juice was 0.32 per cent. After 24 h of fermentation, the titrable acidity increased due to activity of yeast isolates and reference strains. The titrable acidity varies from 0.81 to 0.89 per cent.

Yeast isolate CY4 (0.89 and 0.86%) without and with five per cent honey was superior to other isolates followed by *S. ellipsoideus* (0.85 %) and the lowest (0.81%) was observed with yeast isolates PY4 and *S. boulardii*. The effect of cold storage on titrable acidity of papaya beverage changed slightly in all treatments.

Total sugar (%) content

Papaya juice had 6.56 per cent of total sugar initially. Synbiotic beverage contained total sugar varying from 6.10 to 6.17 per cent. Significantly higher value for total sugar was observed in treatments *Saccharomyces boulardii* with 5 per cent honey (6.17 %) followed by PY4 (6.15 %) and CY4 with 5 per cent honey (6.15 %). The lowest total sugar was observed in papaya juice with 5 per cent honey fermented by *S. ellipsoideus* (6.10 %) followed by papaya juice fermented by *S. ellipsoideus* (6.11%). Total sugar of papaya beverage changed slightly in all treatments at the time of cold storage for 45 days.

Antioxidant activity (%)

Antioxidant activity of papaya juice was 62.77 per cent before fermentation. Antioxidant activity varied from 65.53 to 69.50 per cent after fermentation in papaya juice. Among yeast isolates and reference strains, the highest antioxidant activity (69.50%) was observed in papaya juice with 5 per cent honey fermented with PY4 followed by (67.07%) papaya juice with 5 per cent honey fermented by *Saccharomyces ellipsoideus*. CY4 isolate had the lowest antioxidant activity (65.53%). During the storage period of 45 days at 4 °C, antioxidant activity of papaya beverage changed non significantly in all treatment (Table 6).

Table.1 Colony characteristics and Cell morphology of yeast isolates of papaya and carrot

Sl. No.	Source	Isolates	Colony morphological characteristics			Microscopic observation		
			Color	Size	Shape	Budding	Cell Size (L x b µm)	Cell Shape
1	Papaya	PY1	Creamy white	Medium size	Oval	+	3.29 x 2.02	Oval
2		PY2	White	Medium size	Round	+	5.06 x 2.53	Oval
3		PY3	White	Medium size	Round	+	4.82 x 2.53	Oval
4		PY4	Creamy white	Medium size	Oval	+	4.43 x 2.28	Oval
5		PY5	Creamy white	Medium size	Oval	+	4.43 x 2.25	Oval
6	Carrot	CY1	White	Medium size	Oval	+	3.17 x 2.53	Oval
7		CY2	Creamy white	Medium size	Round	+	5.44 x 3.17	Oval
8		CY3	Creamy white	Medium size	Round	+	4.43 x 4.43	Oval
9		CY4	Creamy white	Medium size	Oval	+	5.06 x 2.53	Oval
10		CY5	White	Medium size	Oval	+	2.53 x 1.52	Oval

Table.2 Utilization of carbon sources by yeasts isolates of papaya and carrot

Sl. No.	Isolates	Carbohydrates					
		Sucrose	Lactose	Maltose	Glucose	Fructose	Mannose
1	PY1	+	-	+	+	+	+
2	PY2	+	-	+	+	+	+
3	PY3	+	-	+	+	+	+
4	PY4	+	-	+	+	+	+
5	PY5	+	-	+	+	+	+
6	CY1	+	-	+	+	+	+
7	CY2	+	-	+	+	+	+
8	CY3	+	-	+	+	+	+
9	CY4	+	-	+	+	+	+
10	CY5	+	-	+	+	+	+

Table.3 Evaluation of bile salts tolerance of yeasts isolates of papaya and carrot at 600 nm

Sl. No.	Isolates	Bile salt concentrations							
		OD value at 6 h				OD value at 12 h			
		0%	0.4%	0.5%	0.6%	0%	0.4%	0.5%	0.6%
1	PY1	0.22	0.22	0.22	0.20	0.25	0.21	0.20	0.19
2	PY2	0.23	0.23	0.21	0.21	0.23	0.20	0.20	0.20
3	PY3	0.23	0.23	0.22	0.20	0.24	0.22	0.21	0.20
4	PY4	0.29	0.29	0.28	0.28	0.26	0.28	0.28	0.27
5	PY5	0.22	0.22	0.20	0.20	0.23	0.20	0.20	0.19
6	CY1	0.21	0.21	0.20	0.20	0.23	0.20	0.20	0.20
7	CY2	0.26	0.26	0.21	0.19	0.25	0.22	0.21	0.20
8	CY3	0.24	0.24	0.20	0.18	0.24	0.22	0.21	0.21
9	CY4	0.28	0.27	0.27	0.27	0.25	0.24	0.23	0.23
10	CY5	0.26	0.26	0.22	0.21	0.26	0.23	0.22	0.20

Table.4 Evaluation of pH tolerance of yeast isolates of papaya and carrot at 600 nm

Sl. No.	Isolates	pH levels					
		OD value at 6 h			OD value at 12 h		
		2.0	2.5	3.0	2.0	2.5	3.0
1	PY1	0.15	0.15	0.15	0.14	0.15	0.15
2	PY2	0.14	0.15	0.15	0.14	0.14	0.15
3	PY3	0.13	0.14	0.14	0.13	0.13	0.13
4	PY4	0.16	0.16	0.16	0.15	0.16	0.16
5	PY5	0.15	0.15	0.15	0.15	0.15	0.15
6	CY1	0.14	0.14	0.15	0.14	0.14	0.15
7	CY2	0.14	0.14	0.15	0.14	0.14	0.15
8	CY3	0.14	0.14	0.15	0.14	0.14	0.14
9	CY4	0.15	0.16	0.16	0.15	0.16	0.16
10	CY5	0.15	0.15	0.15	0.14	0.14	0.15

Table.5 pH, TSS and titrable acidity of papaya beverages prepared using yeast isolates and reference strains after fermentation and during storage period at 4 °C

Sl. No.	Treatments	pH				TSS (°Brix)				Titrable acidity (%)			
		1 D	15 D	30 D	45 D	1 D	15 D	30 D	45 D	1 D	15 D	30 D	45 D
1	T1	5.80^{a}	5.80^{a}	5.80^{a}	5.80^{a}	18.00^{a}	18.00^{a}	18.00^{a}	18.00^{a}	0.32^{d}	0.33^{c}	0.33^{d}	0.33^{c}
2	T2	3.57^{b}	3.56^{bc}	3.55^{b}	3.54^{b}	15.90^{b}	15.89^{b}	15.87^{b}	15.86^{b}	0.83^{bc}	0.84^{ab}	0.84^{bc}	0.84^{b}
3	T3	3.58^{b}	3.57^{b}	3.56^{b}	3.55^{b}	15.57^{c}	15.57^{c}	15.56^{c}	15.55^{c}	0.81^{c}	0.83^{b}	0.84^{bc}	0.85^{b}
4	T4	3.48^{de}	3.48^{de}	3.47^{cd}	3.46^{cd}	15.43^{f}	15.43^{e}	15.42^{e}	15.41^{d}	0.86^{ab}	0.86^{ab}	0.87^{ab}	0.87^{ab}
5	T5	3.46^{e}	3.46^{ef}	3.45^{de}	3.44^{cde}	15.45^{ef}	15.44^{e}	15.42^{de}	15.41^{d}	0.89^{a}	0.88^{a}	0.88^{a}	0.89^{a}
6	T6	3.52^{cd}	3.52^{cd}	3.51^{bcd}	3.50^{bc}	15.49^{de}	15.48^{de}	15.46^{de}	15.45^{d}	0.81^{c}	0.82^{b}	0.82^{c}	0.83^{b}
7	T7	3.54^{bc}	3.54^{bc}	3.53^{bc}	3.52^{bc}	15.51^{d}	15.50^{d}	15.49^{d}	15.48^{cd}	0.82^{c}	0.82^{b}	0.83^{c}	0.85^{b}
8	T8	3.39^{f}	3.39^{g}	3.38^{f}	3.37^{e}	15.45^{ef}	15.44^{e}	15.42^{de}	15.41^{d}	0.83^{bc}	0.83^{b}	0.84^{bc}	0.84^{b}
9	T9	3.41^{f}	3.41^{fg}	3.40^{ef}	3.39^{de}	15.48^{de}	15.47^{de}	15.46^{de}	15.45^{d}	0.85^{ab}	0.86^{ab}	0.86^{ab}	0.86^{ab}

Note: T1- Control papaya juice, T2- Papaya juice + PY4 isolate, T3- Papaya juice + PY4 isolate+ honey, T4- Papaya juice + CY4 isolate, T5- Papaya juice + CY4 isolate+ honey, T6-Papaya juice + *Saccharomyces boulardii*, T7- Papaya juice + *S. boulardii* + honey, T8- Papaya juice + *S. ellipsoideus*,T9- Papaya juice + *S. ellipsoideus* + honey
* Initial values of papaya juice: pH-5.80, TSS- 18 °brix and Titrable acidity-0.33 %

Table.6 Total sugar and antioxidant activity of papaya beverage prepared using yeast isolates and reference strains after fermentation and during storage period at 4 °C

Sl. No.	Treatments	Total sugar (%)				Antioxidant activity (%)			
		1 D	15 D	30 D	45 D	1 D	15 D	30 D	45 D
1	T1	6.56^{a}	6.55^{a}	6.54^{a}	6.54^{a}	62.77^{d}	62.77^{d}	62.80^{d}	62.80^{d}
2	T2	6.15^{b}	6.14^{b}	6.13^{b}	6.11^{b}	66.87^{b}	66.87^{b}	66.87^{b}	66.90^{b}
3	T3	6.12^{b}	6.10^{b}	6.09^{b}	6.08^{b}	69.50^{a}	69.53^{a}	69.53^{a}	69.60^{a}
4	T4	6.14^{b}	6.12^{b}	6.11^{b}	6.10^{b}	65.53^{c}	65.57^{c}	65.60^{c}	65.60^{c}
5	T5	6.15^{b}	6.13^{b}	6.11^{b}	6.10^{b}	66.67^{bc}	66.73^{bc}	66.73^{b}	66.77^{b}
6	T6	6.13^{b}	6.11^{b}	6.10^{b}	6.08^{b}	66.17^{bc}	66.17^{bc}	66.20^{bc}	66.20^{bc}
7	T7	6.17^{b}	6.16^{b}	6.14^{b}	6.13^{b}	66.83^{b}	66.87^{b}	66.87^{b}	66.87^{b}
8	T8	6.11^{b}	6.10^{b}	6.09^{b}	6.08^{b}	66.20^{bc}	66.17^{bc}	66.17^{bc}	66.17^{bc}
9	T9	6.10^{b}	6.09^{b}	6.08^{b}	6.07^{b}	67.07^{b}	67.10^{b}	67.10^{b}	67.13^{b}

Note: T1- Control papaya juice, T2- Papaya juice + PY4 isolate, T3- Papaya juice + PY4 isolate+ honey, T4- Papaya juice + CY4 isolate, T5- Papaya juice + CY4 isolate+ honey, T6-Papaya juice + *Saccharomyces boulardii*, T7- Papaya juice + *S. boulardii* + honey, T8- Papaya juice + *S. ellipsoideus*,T9- Papaya juice + *S. ellipsoideus* + honey * Initial values of papaya juice: Total sugar-6.56 % and Antioxidant activity – 62.77 %

Table.7 Population of yeasts in the papaya beverages (10^8 cfu /mL)

Sl. No.	Treatments		Days			
		Initial Inocula (10^7 cfu /mL)	1 D	15 D	30 D	45 D
1	T1	0.00	0.00^{e}	0.00^{e}	0.00^{f}	0.00^{e}
2	T2	4.0	7.00^{d}	7.33^{d}	7.33^{e}	7.00^{d}
3	T3	4.0	13.00^{a}	13.00^{a}	12.67^{a}	12.33^{a}
4	T4	4.0	7.67^{d}	7.33^{d}	7.00^{e}	6.67^{d}
5	T5	4.0	10.00^{bc}	10.00^{bc}	9.67^{cd}	9.33^{bc}
6	T6	3.0	9.00^{cd}	8.67^{cd}	8.33^{de}	8.00^{cd}
7	T7	3.0	12.33^{a}	12.33^{a}	11.67^{ad}	11.33^{ab}
8	T8	4.0	8.33^{cd}	8.00^{cd}	8.00^{de}	8.00^{cd}
9	T9	4.0	11.33^{ab}	11.00^{ad}	10.33^{bc}	10.33^{ab}

Note: T1- Control papaya juice, T2- Papaya juice + PY4 isolate, T3- Papaya juice + PY4 isolate+ honey, T4- Papaya juice + CY4 isolate, T5- Papaya juice + CY4 isolate+ honey, T6-Papaya juice + *Saccharomyces boulardii*, T7- Papaya juice + *S. boulardii* + honey, T8- Papaya juice + *S. ellipsoideus*,T9- Papaya juice + *S. ellipsoideus* + honey

Table.8 Sensory evaluation of papaya beverages prepared with yeast isolates and reference strains

Sl. No.	Treatments	Parameters											
		Appearance	Colour	Aroma	Bouquet	Vinegar	Total acidity	Sweetness	Body	Flavour	Astringency	Overall quality	Total score
		(0-2)	(0-2)	(0-2)	(0-2)	(0-2)	(0-2)	(0-1)	(0-1)	(0-2)	(0-2)	(0-2)	(20)
1	T1	1.50	1.50	1.60	1.80	1.00	1.50	1.00	1.00	1.40	1.40	1.50	15.20
2	T2	1.50	1.46	1.56	1.82	1.20	1.56	0.90	0.90	1.70	1.52	1.70	15.82
3	T3	1.50	1.56	1.70	1.88	1.20	1.58	0.90	0.80	1.75	1.60	1.85	16.32
4	T4	1.50	1.45	1.52	1.72	1.30	1.56	0.80	0.80	1.62	1.54	1.64	15.45
5	T5	1.40	1.52	1.54	1.74	1.20	1.55	0.90	0.80	1.64	1.56	1.68	15.53
6	T6	1.50	1.48	1.52	1.70	1.10	1.54	0.90	0.80	1.64	1.50	1.70	15.38
7	T7	1.50	1.56	1.56	1.72	1.20	1.55	0.80	0.80	1.66	1.54	1.75	15.64
8	T8	1.60	1.48	1.58	1.65	1.20	1.52	0.80	0.80	1.64	1.60	1.70	15.57
9	T9	1.50	1.54	1.62	1.66	1.10	1.54	0.80	0.80	1.70	1.62	1.72	15.60

Note: T1- Control papaya juice, T2- Papaya juice + PY4 isolate, T3- Papaya juice + PY4 isolate+ honey, T4- Papaya juice + CY4 isolate, T5- Papaya juice + CY4 isolate+ honey, T6-Papaya juice + *Saccharomyces boulardii*, T7- Papaya juice + *S. boulardii* + honey, T8- Papaya juice + *S. ellipsoideus*,T9- Papaya juice + *S. ellipsoideus* + honey

Population of yeasts in synbiotic papaya beverages

The data related to yeast population in synbiotic papaya beverages is presented in Table 7. The initial inoculum of yeast strains added to papaya juice had 4±1 x 10^7 cfu /mL. Population of yeast strains increased from 10^7 to 10^8 cfu /mL after 24 h of fermentation. The highest yeast population was recorded in papaya juice with 5 per cent honey added and fermented by PY4 isolate (13 x 10^8 cfu /mL) followed by *Saccharomyces boulardii* (12.33 x 10^8 cfu / mL).

The lowest yeast population was recorded in papaya juice fermented with PY4 isolate (7 x 10^8 cfu / mL). The effect of cold storage on cell viability of yeast cultures in fermented papaya juice decreased slightly in all treatments. It is observed that yeast strains were capable of surviving in papaya beverage for 45 days at 4 $^{\circ}$C.

Organoleptic evaluation of synbiotic papaya beverage

The experimental results clearly indicate that papaya juice with 5 per cent honey fermented by yeast isolate (PY4) recorded the highest score (16.32 out of 20) taking into consideration of all factors. The least score (15.20 out of 20) was recorded with uninoculated papaya juice (Table 8).

Yeasts were commonly used for food fermentation since ancient time. Milk based products contain certain yeast strains and are occurring as a part of gastrointestinal tract. Yeast strain *Saccharomyces boulardii* commonly used as probiotic agents among the strains of the genus *Saccharomyces*. In this study, ten yeast strains were isolated from papaya and carrot, which were then purified. Further, these isolates were screened for probiotic characteristics.

Probiotics need to withstand in high bile salt and low acidic condition in gut. Besides the strong acid condition in the stomach, the probiotic microorganisms taken orally have to defend against the bile salt in the gastrointestinal tract. Hence, bile tolerance is also considered to be one of the important properties required for high survival of the probiotic organism. There is no consensus about the precise concentration to which the selected strains should be tolerant. The physiological concentration of bile salts in the small intestine is between 0.2 and 2.0% (Gunn, 2000). In this investigation, PY4 and CY4 exhibited high acid and bile salt tolerance. Similar results have been reported by several *in vitro* studies which indicate that yeasts belonging to *Saccharomyces*, *Debaryomyces*, and *Kluyveromyces* species are extremely tolerant low pH and high bile salt concentration (upto 1% oxgall) (Kourelis *et al.,* 2010).

In the present study, pH of juice decreased after fermentation compared to fresh juice and pH decreased due to accumulation of H ions released during fermentation. This observation is in concurrence with kokum was fermented using *Saccharomyces ellipsoideus* (Girish, 2008). pH of fermented orange juice was 3.81 for *Saccharomyces ellipsoideus* and 3.17 for *Saccharomyces cerevisiae* (Wahab *et al.,* 2005).

TSS of fermented beverage decreased and varied after fermentation by different yeast strains. This could be due to yeast strains posse's different capacities of fermentation efficacy and sugar utilization pattern. These results are in conformation with results reported with kokum juice fermented using yeast strains (Girish, 2008).

In the present study, titrable acidity of beverage was significantly increased compared to the unfermented papaya juice.

The increases of titrable acidity in fermented papaya beverages is due to accumulation of organic acids during fermentation period.

Total sugar of papaya synbiotic beverage decreased after fermentation of papaya juice. This is because of utilization of sugar like fructose present in fruits by yeast strains.

Antioxidant activity of papaya synbiotic beverage increased after 24 h of fermentation compared to unfermented juice in the present investigation. The similar trend in antioxidant activity was reported in papaya juice fermented with lactic acid bacteria (Chen *et al.*, 2018).

The probiotic populations are very important in synbiotic beverage preparation. The probiotic yeast population increased significantly after 24 hours of fermentation, it may be due to availability of nutrients in papaya juice and addition of honey. These findings are similar with kokum juice fermentation using yeast strains (Latha, 2012).

The combination of papaya juice with 5 per cent honey fermented by yeast isolate recorded the highest score and had the best acceptability due to addition of honey to papaya juice. These evaluations are similar to results of fermented guava juice using yeast strains (Nausheen, 2017).

References

Amerine MA, Berg HW and Cruess WV 1972. The technology of wine making 3rd Ed. Publ: AVI Co. West Port, Connecticut. pp: 126-132.

Chen LS, Ma Y, Maubois J L, He SH, Chen LJ and Li HM 2010. Screening for the potential probiotic yeast strains from raw milk to assimilate cholesterol. Dairy Science & Technology, 90(5), 537–548.

Chen R, Chen W, Chen H, Zhang G and Chen W 2018. Comparative evaluation of the antioxidant capacities, organic acids, and volatiles of papaya juices fermented by *Lactobacillus acidophilus* and *Lactobacillus plantarum. J. Food Quality*, 4:1-12

Czerucka D and Rampal P 2002. Experimental effects of *Saccharomyces boulardii* on diarrheal pathogens. Microbes and Infection, 4(7), 733–739.

De Vrese M and Schrezenmeir J 2008. Probiotics, prebiotics, and synbiotics *Advances in Biochemical Engineering/Biotechnology*, 111, 1-66.

Ding WK. and Shah NP 2008. Survival of Free and Microencapsulated Probiotic Bacteria in Orange and Apple Juices. International Food Research Journal, 15(2), 219–232.

El-Salam MHA 2011. Preparation and properties of probiotic concentrated yoghurt (labneh) fortified with conjugated linoleic acid. International Journal of Food Science and Technology, 46, 2103-2110.

FAO 2002. *Food and Agriculture Organization / World Health Organization*, (April), 1–11. Retrieved from http://www.fao.org/es/ESN/Probio/probio.htm.

Fonteles TV, Costa MGM, de Jesus ALT and Rodrigues S 2012. Optimization of the fermentation of cantaloupe juice by *Lactobacillus casei* NRRL B-442. Food and Bioprocess Technology, 5(7), 2819–2826.

Forssten SD, Sindelar CW and Ouwehand A C 2011. Probiotics from an industrial perspective. Anaerobe, *17*(6),410–413.

Girish DN 2008, Development of microbial consortium for fermentation of kokum

(*Garcinia indica choisy*) juice. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Bengaluru.

Gunn JS 2000. Mechanisms of bacterial resistance and response to bile. *Microbes and Infection*, 2, 907-913.

Hatoum R, Labrie S and Fliss I 2012. Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. Frontiers in Microbiology, 3, 1–12.

Hoben HJ and Somasegaran P 1982. Composition of the pour, spread and drop plate methods for enumeration of *Rhizobium spp*. In: Inoculants made from pre-sterilized peat. *Appl. Environ. Microbiol.*, 14(5): 1246-1247.

Kongsuwan A, Suthiluk P, Theppakorn T, Srilaong V and Setha S 2009. Bioactive compounds and antioxidant capacities of phulae and nanglae pineapple. *Asian J. Agr. Food sci*., pp: 44-50.

Kourelis A, Kotzamanidis C, Litopoulou-Tzanetaki E, Scouras ZG, Tzanetakis N and Yiangou M 2010. Preliminary probiotic selection of dairy and human yeast strains. *Journal of Biological Research.* 13, 93-10.

Latha B 2012. Fermentation of blended kokum (*Garcinia indica* cv. *Choisy*) juice for value addition. M. Sc., thesis, University of Agricultural Sciences Bengaluru-560 065.

Mousavi ZE, Mousavi SM, Razavi SH, Emam-Djomeh Z and Kiani H 2011. Fermentation of pomegranate juice by probiotic lactic acid bacteria. World Journal of Microbiology and Biotechnology, 27(1), 123–128.

Nausheen S 2017. Bioenrichment of guava juice with prebiotic and probiotics. M. Sc., thesis, University of Agricultural Sciences Bengaluru-560 065.

Pereira ALF, Feitosa WSC, Abreu VKG, Lemos TO, Gomes WF, Narain N and Rodrigues S 2017. Impact of fermentation conditions on the quality and sensory properties of a probiotic cupuassu (Theobroma grandiflorum) beverage. Food Research International, 100, 603–611.

Pereira ALF, Maciel TC and Rodrigues S 2011. Probiotic beverage from cashew apple juice fermented with *Lactobacillus casei.* Food Research International, 44(5), 1276–1283.

Prado S, Romalde JL and Barja JL 2010. Review of probiotics for use in bivalve hatcheries. Veterinary Microbiology, 1-11.

Ramachandran p and Nagarajan S 2014. Quality characteristics, nutraceutical profile and storage stability of aloe gel-papaya functional beverage blend. *Int. J. Food Sci.*, 3: 460-461.

Ranganna S 1995. Hand book of analysis and quality control for fruit andvegetable products (2nd Ed.). Tata McGraw Hill publishing Co, Ltd., New Delhi.

Saad SMI, Cruz AG and Faria JAF 2011. Probiotics and Prebiotics an Aliments: Fundamentals of Application Tecnologies. (1. ed.). Sao Paulo: Livraria Varela.

Sadasivam S and Manickam A 1996. Biochemical methods. New Age International (P) Limited, Publishers.

Sheehan V M, Ross P and Fitzgerald GF 2007. Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. Innovative Food Science and Emerging Technologies, 8(2), 279–284.

van der A A, Skovgaard K and Jespersen L 2005. In vitro screening of probiotic properties of *Saccharomyces cerevisiae* var. boulardii and food-borne Saccharomyces cerevisiae

strains. International Journal of Food Microbiology, 101(1), 29–39.

Wahab O, Okunowo, Okotore RO and Osuntoki AA 2005. The alcoholic fermentative efficiency of indigenous yeast strain of different origin on orange juice. *African J. Biotechnol.*, 4(11): 1290-1296.

Yoon KY, Woodams EE and Hang YD (2006), Production of probiotic cabbage juice by lactic acid bacteria. Bioresource Technology, 97(12), 1427–1430.

How to cite this article:

Pampangouda, Suvarna, V. Chavannavar, Mahadevaswamy and Vijayalkshmi, K. G. 2021. Isolation, Identification and Screening of Yeasts for Production of Papaya Synbiotic Beverage. *Int.J.Curr.Microbiol.App.Sci.* 10(01): 663-674. doi: https://doi.org/10.20546/ijcmas.2021.1001.080