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REGULAR ARTICLE

Optimization, production and scale up of debittered kinnow beverage by α-L-rhamnosidase producing yeast

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ABSTRACT

The α-L-Rhamnosidase is used for debittering the citrus juice by hydrolyzing bitter naringin to nonbitter prunin and rhamnose. The present study was carried out on fermentative production of debittered kinnow (*Citrus reticulata* Blanco) beverage using α-L-rhamnosidase producing yeast in order to utilize its immense potentiality in processed kinnow juice industry. The effect of percent yeast inoculum concentration (0.25-1.25 v/v), total soluble solids (12-16 °B), temperature (15-35 °C) and incubation time (12-60 h) were studied to optimize the production of α-L- rhamnosidase enzyme from *Clavispora lusitaniae* in kinnow juice. Results indicated that yeast showing maximum rhamnosidase activity (0.056 IU mL^{-1}) in presence of yeast inoculum concentration (0.75% v/v), brix (13 °B), temperature (30 ± 5 °C) and incubation time (48 h). Further, these optimized conditions were used in upscale production of debittered kinnow beverage. The physicochemical parameters of freshly prepared beverage TSS 13.00 ± 0.20 °B, acidity 0.14 ± 0.03%, pH 3.40 ± 0.10, brix acid ratio 92.85 ± 0.00, limonin 6.90 ± 0.10 ppm, naringin 443.00 ± 10.00 ppm, total sugars 12.90 ± 0.30%, reducing sugars 2.42 ± 0.20 and ascorbic acid 27.80 ± 1.00 mg/100 mL. All the physicochemical parameters did not change significantly during storage. The decrease of naringin with storage was 443.00 ± 10.00 to 143.70 ± 4.00 ppm due to the α-L-rhamnosidase activity of yeast. The percentage of ethanol and CO_2 were 0.89 ± 0.05% and 1.46 ± 0.06 bar after three months of storage. All the sensory parameters like taste, color, aroma, bouquet, flavor and astringency of kinnow beverage were stable at storage period of 90 days with almost no change in organoleptic sensation. Thus the technology presented here, a very less time consuming and safe for production of debittered kinnow beverage on large scale in citrus food industries.

Keywords: Kinnow beverage; *Clavispara lusitaniae*; α-L-rhamnosidase; Debittered

INTRODUCTION

India is the second largest producer of fruits, with a production of 44.04 million tonnes of fruits from an area of 3.72 million hectares and holds third rank in respect of production of citrus fruits in the world. Kinnow, a hybrid of *Citrus nobilis* and *Citrus delicosa* is a prevalent citrus fruit in Punjab covering an area of 46,000 hectares with the production of 9.88 lakh tones NHM (2014).

Kinnow mandarin juice has high therapeutic value as antispasmodic, sedative, cytophylactic, digestive, anti carcinogenic, anti inflammatory and anti allergic. The health benefits of citrus fruit juices have been attributed due to the presence of bioactive and antioxidant compounds. A total of 150 g edible portion of orange provides 0.3 g fiber and 17 g of carbohydrates that can supply upto 73 kilocalories.

Kinnow juice turns bitter after extraction due to chemical naringin (flavanoid) and limonin (limonoid). Naringin is the major component in citrus fruit with very bitter taste and a threshold of 20 $mgKg^{-1}$ in water and detectable limit less than 1.5 $mgKg^{-1}$ (Chen et al., 2010). The presence of limonin and naringin in excess of 6 ppm and 600 ppm respectively has been established as an objectionable level of bitterness in processed citrus products such as juice, wine and vinegar (Guadagni et al., 1973).

Numerous techniques are used to reduce naringin such as adsorptive debittering (Fayoux et al., 2007), enzymatic hydrolysis (Puri and Kalra, 2005), poly-styrene divinyl

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benzene styrene resin treatment and β-cyclodextrin treatment (Mongkolkul et al., 2006). These techniques have limitations in altering nutrient composition either through chemical reactions or removal of nutrients, flavor and color etc. Another suitable debittering procedure is the stepwise hydrolysis of naringin by naringinase (Chen et al., 2010). The enzyme naringinase is composed of *α*-L-rhamnosidase (EC 3.2.1.40) and *β*-D-glucosidase (EC 3.2.1.21). Naringin (4',-5,7'-trihydroxyflavonone-7-rhamnoglucoside) is first hydrolyzed by α-L-rhamnosidase activity of naringinase to rhamnose and prunin (one third of the bitterness of naringin) which can be further hydrolyzed into glucose and naringenin by the β-D-glucosidase component of naringinase. The potential application of rhamnosidase is used in the debittering of citrus fruit juices (Busto et al., 2007), manufacture of prunin from naringin, manufacture of L-rhamnose by hydrolysis of natural glycosides containing terminal L-rhamnose, enhancement of wine aromas by enzymatic hydrolysis of terpenyl glycosides containing L-rhamnose, elimination of hesperidin crystals from orange juices, conversion of chloropolysporin B to chloropolysporin C, the derhamnosylation of many L-rhamnose containing steroids for example, diosgene, desglucoruscin, ginsenosides-Rg2, etc. whose derhamnosylated products have their clinical importance (Feng et al., 2005).

The nutritional and therapeutic value of kinnow provides ample scope for processing into a value added fermented product with retention of organoleptic properties, nutritional attributes, characteristics sensory properties, flavor, aroma, texture and long shelf life. So, the aim of this work is to optimize and produce debittered kinnow beverage using α-L-rhamnosidase producing yeast *Clavispora lusitaniae* KF633446.

MATERIALS AND METHODS

Yeast culture

Yeast strain producing rhamnosidase enzyme was isolated from whey beverage and identified as *Clavispora lusitaniae* (accession number- KF633446) on the basis of morphological, biochemical and 18S rDNA sequence analysis.

Screening of juice components for optimized α-L-rhamnosidase production

The physical and nutritional conditions were optimized following 'one-at-a-time' approach to enhance the yield of α-L- rhamnosidase enzyme in diluted kinnow juice (juice:water; 1:1.5). The effect of percent inoculum concentration (0.25, 0.5, 0.75, 1 and 1.25 v/v), total soluble solids in (12, 13, 14, 15 and 16 °B), incubation time (12, 24, 36, 48 and 60 h) and temperature (15, 20, 25, 30 and 35 °C) on enzyme activity were evaluated. For each parameter optimization, three sets of independent experiments were carried out and the average value was reported.

α-L-Rhamnosidase enzyme assay

50 mL of the diluted kinnow juice in Erlenmeyer flask (100 mL) was aerobically cultured at 30±5 °C for 48 h on a rotary shaker (150 rpm). After centrifugation (12,000 × *g* for 10 min), the supernatant was collected for measurement of rhamnosidase activity**.** The α-L-rhamnosidase activity (RA) was determined using p-nitrophenyl-α-L-rhamnoside (p-NPR, Sigma) as the substrate (Romero et al., 1985). The reaction mixture consisted of 0.1 mL of 4.8 mM p-NPR solution, plus 0.19 mL of 50 mM citric acid/Na citrate buffer, pH 5.0 and 10 µl of enzyme or buffer (for the blank) and was incubated at 50 °C. Aliquots of 50 µL from the reaction mixture were removed every 2 min and placed into 1.5 mL of 0.5 M NaOH. These aliquots were kept in an ice bath until the absorbance was measured at 400 nm (Rajal et al., 2009). One unit (U) of enzyme activity was defined as the amount of enzyme required to release 1 µmol of p-nitrophenol per minute.

Preparation of debittered kinnow beverage

A debittered kinnow beverage was prepared under optimized conditions of inoculum concentration, TSS, temperature and incubation time.

Extraction of juice

Kinnow (*Citrus reticulata* Blanco) was procured from Department of Fruit Science, PAU, Ludhiana, Punjab, India. Fruits were washed in chlorinated water and then used for the extraction of juice. Juice was extracted aseptically under hygienic conditions by kinnow juice extractor.

Preparation of sugar solution

The sugar solution was prepared by boiling (500 g) granulated sucrose in one litre of water for 10 min and then allowed to cool at room temperature and stored aseptically in sterilized glass bottles.

Inoculum preparation

The inoculum was prepared in diluted juice with brix adjusted to (13°B). A loopful culture of 24 h old yeast (*Clavispora lusitaniae* KF633446) was inoculated in 100 mL diluted kinnow juice in 250 mL Erlenmeyer flask and incubated at 30±5 °C for 24 h to achieve concentration of 10^5- 10^6 cells mL^{-1}.

Fermentation, bottling and storage

The physico-chemical analysis (pH, % acidity, TSS, brix acid ratio, naringin, limonin and juice yield) of fresh kinnow juice was performed. Juice was diluted in the ratio 1:1.5 with water. Diluted juice was pasteurized at 82 °C for

15 sec, cooled and brix adjusted to 13 °B by adding sugar solution followed by inoculation of yeast i.e. 0.75% (v/v). It was incubated for 48 h at 30±5 °C. The beverage was refrigerated for 24 h, siphoned, bottled and stored in refrigerated conditions.

Shelf life determination of kinnow beverage

Shelf life fermented debittered kinnow beverage, stored at refrigerated temperature (4°C) was studied and evaluated fortnightly for physicochemical, microbiological and sensory qualities.

Physiochemical and microbiological analysis of kinnow juice and beverage

The total soluble solids and pH of kinnow juice and beverage were determined by using Erma hand refractometer of 0-32 °B (Erma, Tokyo, Japan) and pH meter (ECIL, Hyderabad, type 101; Electronic Corporarion of India Ltd., Hyderbad, India). Total acidity expressed as citric acid was estimated following the procedure of AOAC (1999). Brix-acid ratio was calculated through dividing TSS value by total acidity of the juice and carbonated beverage. Total sugars were estimated by phenol sulphuric acid method (Dubois et al., 1956). Reducing sugars were estimated by the method of Miller (1959). The titration method using 2, 6-dichlorophenol indophenol dye was used to estimate ascorbic acid (AOVC 1996). The total phenolic content (TPC) was determined by spectrophotometry, using gallic acid as a standard, according the method described by Singleton and Rossi (1965). Limonin content was estimated by colorimetric method (Vaks and Lifshitz, 1981) and naringin content was estimated by Davis method (1947). Carbon dioxide volumes in beverage bottles were determined by Zahm and Nagel piercing device (CO_2 tester, Zahm and Nagel Co., Inc., Holland, New York, USA) and percent alcohol (v/v) was calculated by spectrophotometric determination method of ethanol (Caputi et al., 1968). Total yeast count was enumerated on GYE agar by serial plate dilution method.

Sensory evaluation

The organoleptic evaluation of kinnow beverages was done on the basis of appearance, taste, color, aroma, bouquet, body, flavor, astringency and overall acceptability by a panel of judges. Consumer acceptance for the products was evaluated on a nine point "Hedonic scale" (Amerine et al., 1965).

Statistical analysis

Statistical analysis was done by using CPCS1 software. Standard errors were calculated for all mean values. Differences were considered significant at the $p \leq 0.05$ level.

RESULTS AND DISCUSSION

Screening of juice components for optimization of α-L-rhamnosidase production

Effect of percent inoculum concentration on α-L-rhamnosidase

Five different concentration of inoculum, 0.25%, 0.5%, 0.75%, 1% and 1.25% (v/v) of the standard stock inoculum was added in the juice and incubated for 24 h at room temperature. A differential response in rhamnosidase activity was obtained which showed that the 0.75% inoculum concentration exhibited maximum enzyme activity i.e. 0.057 IU mL^{-1} and 0.25% exhibited minimum rhamnosidase activity i.e. 0.023 IU mL^{-1} in kinnow juice (Fig. 1). Increase in inoculum size resulted in lesser enzyme production, due to the nutrient exhaustion and oxygen limitation. Similar results were also reported in *Bacillus methylotrophicus* (Mukund et al., 2014) and *Staphylococcus xylosus* MAK2 (Puri and Kalra, 2005) for naringinase production. Puri et al., (2005) studied the inoculum level of 3-15% (v/v) in the salt medium with naringenin as an inducer to establish the effect of inoculum size on the naringinase production by *A. niger*. They observed that 10% (v/v) inoculum was optimal for growth as well as naringinase production and the lag phase was also minimal.

Effect of brix (°B) on α-L-rhamnosidase production

In juice, brix was adjusted to 12, 13, 14, 15 and 16 °B by adding sugar solution followed by inoculation of yeast i.e. 0.75% (v/v). It was incubated for 24 h at room temperature. The effect of different °B on yeast rhamnosidase activity was tested and best °B for maximum rhamnosidase activity (0.05 $IUmL^{-1}$) was 13 in juice (Fig. 1). Further, with the increase in the initial sucrose concentration the rhamnosidase production was decreased which indicated that the higher sucrose concentration had an adverse effect on the enzyme production efficiency of the yeast. Naringinase activity was repressed by glucose, sucrose, citrate and lactose although these carbon sources supported excellent growth (Puri et al., 2005; Bram and Solomons, 1965). Production of α-L-rhamnosidase by *A. nidulans* is mediated by carbon catabolite repression, which appears to be CreA-independent (Orejas et al., 1999). Further, it has been reported that the enzyme was not produced when *A. kawachii* was grown on 0.5% glucose as the sole carbon source (Koseki et al., 2008).

Effect of incubation time on α-L-rhamnosidase production

Effect of different incubation time on enzyme activity was studied. Kinnow juice (brix 13 °B and inoculum concentration 0.75% v/v) was incubated for different time periods (12, 24, 36, 48 and 60 h) at room temperature. Fig. 1 shows that the maximum enzyme activity in juice

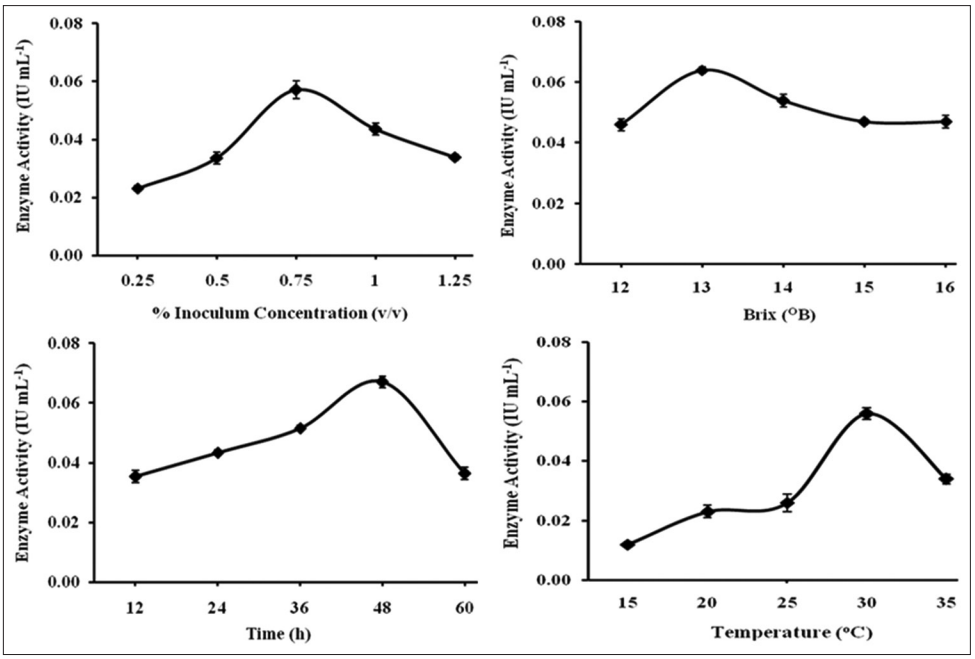

Fig 1. Effect of percent inoculum concentrations, brix (°B), incubation time and temperature on α-L-rhamnosidase production in kinnow juice.

was observed after 48 h of fermentation. Maximum naringinase production (12.05 UL^{-1}) was observed at 34 h of fermentation, which corresponds to a stationary phase of growth (Mukund et al., 2014). In the batch reactor, the maximum α-rhamnosidase activity was obtained after 10 days from *Penicillium ulaiense* (Rajal et al., 2009). The reduction in production time is important because it decreases the fermentation costs and contamination with opportunistic microorganisms in scale up process.

Effect of temperature on α-L-rhamnosidase production

Five different levels of temperature were also studied. Kinnow juice (brix 13 °B and inoculum concentration 0.75% v/v) was incubated at different temperatures (15, 20, 25, 30 and 35 °C) for 48 h. Data in graph in Fig. 1 show the effect of changing temperature on enzyme activity. As the temperature increases, initially the enzyme activity increased while it decreased at higher temperatures at the same time. The decrease in enzyme activity may be the deactivation of enzyme due to the weakening of non covalent interactions that stabilize the protein structure. The optimum temperature for *Pichia angusta* rhamnosidase was observed at 40 °C (Yanai and Sato, 2000). The reported temperature optima of α-L-rhamnosidases are in the range of 40-80 °C though one bacterial α-L-rhamnosidase active at 4 °C is reported (Orrillo et al., 2007).

Physicochemical characteristics of kinnow juice (*Citrus Reticulata* Blanco)

The physicochemical characteristics of fresh kinnow juice was evaluated on the basis of chemical analysis. The results are presented in Table 1, showing TSS- 8.00±1.00 °B, titrable acidity- 0.29±0.02%, pH- 3.50±0.10, brix acid ratio- 27.58±1.00, total sugars- 8.90±0.10%, reducing sugars- 2.00±0.50%, ascorbic acid- 30.40±1.00 mg/100 g, total polyphenol content- 58.40±2.00 mg GAE/100 mL, limonin- 7.40±0.20 ppm, naringin- 421.80±10.00 ppm, juice yield- 55.00±5.00% and peel and pomace- 39.00±4.00%. In kinnow fruit, the juice content was found to be in the range of 36.00 % to 62.00 % (Jagjiwan, 2001) and ascorbic acid content in the range of 13.30 to 46.90 mg/100 mL (Pruthi et al., 1983; Singh et al., 1978). The acidity, pH and TSS of kinnow juice were reported in the range of 0.28-0.51%, 4.20-4.28 and 8.00-15.75% by Kaur (2002). The amount of reducing and nonreducing sugars has been found around 3.95% and 3.65% (Veldihus, 1971). The acceptability and higher sensory score of beverages is very much dependent on its physicochemical properties including appearance, flavor, acidity and TSS. There may

Table 1: Physicochemical characteristics of kinnow (*Citrus reticulata* Blanco)

Parameters	Kinnow juice
TSS (°B)	8.00±1.00
Acidity (%)	0.29±0.02
pH	3.50±0.10
Brix-acid ratio	27.58±1.00
Total sugars (%)	8.90±0.10
Reducing sugars (%)	2.00±0.50
Ascorbic acid (mg/100 mL)	30.40±1.00
Total polyphenol content (mg GAE/100 mL)	58.40±2.00
Limonin (ppm)	7.40±0.20
Naringin (ppm)	421.80±10.00
Juice yield (%)	55.00±5.00

Mean values±standard error of three independent experiments

be changes in the physicochemical characteristics and loss of some compounds that impart flavour and aroma to the beverages during pasteurization and storage (Jairath et al., 2012).

Scale up of the optimized process in the laboratory bench scale fermenter

The optimized process parameters (brix 13 °B, inoculum concentration 0.75% v/v, temperature 30 °C and incubation time 48 h) for production of maximum rhamnosidase enzyme was used for preparation of fermented debittered beverage. The experiment was conducted in laboratory fermenter (capacity- 10L) at Department of Microbiology.

Evaluation of microbiological and physicochemical properties of kinnow beverage during storage

The results of microbiological and physicochemical properties of kinnow beverage during storage period of 90 days are summarized in Table 2. The results showed a significant decrease in brix from 13.00±0.20 °B to 11.20±0.30 °B and brix acid ratio from 92.85±0.00 to 19.31±0.00. Similar results have been reported by Sarolia and Mukherjee, 2002 in their studies on lime juice, Khandelwal et al., 2006 during the fermentation of kinnow sera, cane and kinnow cane juice and Ahmed et al., 2008 in preparation of ready to serve mandarin (*Citrus reticulata*) diet drink. The increase in TSS content of juice during storage might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides.

The pH also decreased from 3.40±0.10 to 3.00±0.10, while acidity increased from 0.14±0.03% to 0.56±0.01% after fermentation. pH is inversely proportional to the acidity of any medium. This decrease in pH and increase in acidity was attributed to formation of acidic compounds by degradation of reducing sugars (Zia, 1987; Akhtar et al., 2010). A similar result of decreasing pH was also reported by Saleem, 1980 and Ahmed, 2008.

The percentage decrease in total sugars was from 12.90±0.30% to 9.50±0.40% and percentage decrease in reducing sugars was from 2.42±0.20% to 1.32±0.10% at the end of 90 days. The sugars in citrus are mainly glucose, laevulose and sucrose. Similar results were also observed by Jairath, 2012 for preparation of amla beverage. The increase in total sugar content of juice during storage might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides. Ascorbic acid (vitamin C) content was reduced from the initial concentration 27.80±1.00 mg/100 mL to 6.48±0.40 mg/100 mL after 90 days.

Table 2: Effect of storage time on microbiological and physicochemical properties of kinnow beverage

Parameters	Days									
	Fresh	10	20	30	40	50	60	70	80	90
TSS (°B)	13.00±0.20	12.80±0.10	12.50±0.20	12.40±0.30	12.20±0.30	12.00±0.30	11.80±0.30	11.50±0.20	11.40±0.20	11.20±0.30
Acidity (%)	0.14±0.03	0.21±0.02	0.32±0.04	0.36±0.02	0.40±0.04	0.45±0.03	0.49±0.02	0.51±0.03	0.56±0.01	0.58±0.01
pH	3.40±0.10	3.30±0.20	3.30±0.10	3.20±0.20	3.20±0.20	3.20±0.10	3.10±0.10	3.10±0.10	3.10±0.10	3.00±0.10
Brix-acid ratio	92.85±0.00	60.95±0.00	39.06±0.00	34.44±0.00	30.50±0.00	26.66±0.00	24.08±0.00	22.54±0.00	20.35±0.00	19.31±0.00
Total sugars (%)	12.90±0.30	12.40±0.40	12.30±0.40	11.90±0.40	11.70±0.20	11.20±0.20	10.90±0.50	10.10±0.30	9.80±0.30	9.50±0.40
Reducing sugars (%)	2.42±0.20	2.26±0.10	2.10±0.20	1.58±0.40	1.56±0.10	1.54±0.50	1.49±0.10	1.37±0.10	1.35±0.20	1.32±0.10
Ascorbic acid (mg/100 mL)	27.80±1.00	26.40±1.00	16.80±0.20	14.50±0.50	10.50±0.50	8.50±0.50	7.72±0.20	7.54±0.10	6.78±0.20	6.48±0.40
Total polyphenol contents (mg GAE/100 mL)	53.48±3.00	51.70±1.00	50.10±2.00	48.70±3.00	47.90±1.00	45.79±4.00	44.50±2.00	43.90±3.00	42.10±1.00	40.80±2.00
Limonin (ppm)	6.90±0.10	6.20±0.20	5.70±0.20	5.40±0.20	5.10±0.10	4.70±0.20	4.48±0.20	4.15±0.10	3.80±0.10	3.52±0.10
Naringin (ppm)	443.00±10.00	420.50±5.00	376.40±4.00	284.50±6.00	213.00±7.00	178.00±6.00	160.60±8.00	155.40±5.00	148.90±2.00	143.70±4.00
Alcohol (%, v/v)	0.00±0.00	0.11±0.01	0.35±0.03	0.54±0.02	0.63±0.03	0.72±0.30	0.75±0.40	0.80±0.20	0.82±0.30	0.89±0.05
CO_2 (bar)	0.00±0.00	0.65±0.05	0.72±0.02	0.82±0.02	1.16±0.04	1.19±0.01	1.21±0.03	1.25±0.05	1.33±0.03	1.46±0.06
Total yeast count (cfu mL^{-1})	$58\times10^5\pm10.00$	$64\times10^5\pm20.00$	$49\times10^6\pm10.00$	$52\times10^6\pm10.00$	$65\times10^6\pm20.00$	$23\times10^7\pm10.00$	$33\times10^7\pm20.00$	$46\times10^7\pm20.00$	$52\times10^8\pm10.00$	$58\times10^8\pm20.00$

Mean values±standard error of three independent experiments

The decrease of ascorbic acid (vitamin C) in beverage during storage results from oxidation of ascorbic acid by ascorbic acid oxidase due to a combined effect of oxygen and light (Bhardwaj and Mukherjee, 2011; Jairath et al., 2012; Bhardwaj, 2013). The mean polyphenol contents of kinnow beverage were significantly decreased from 53.48±3.00 mg GAE/100 m L to 40.80±2.00 mg GAE/100 mL during storage.

The polyphenol contents of commercial fruit juices in the case of pineapple, orange and mango juices were higher than those of Thai beverages, reported by Abdullakasim et al., (2007). Different factors such as processing techniques, clarification and pasteurization can affect polyphenol contents of commercial juices. According to Ritter et al. (1992) and Karadeniz and Eksi (2001) reports, clarification also decrease the polyphenolic contents of commercial fruit juices. Polyphenol contents decreases constantly with the progress of the ripening, while in red coloured varieties it increases during the last ripening stage due to the maximal accumulation of anthocyanidines and flavonols (Marinova et al., 2005).

The decrease of limonin from 6.90±0.10 to 3.52±0.10 ppm might be due to production of CO_2 during storage. Carbon dioxide at pressures of 21 to 41 MPA at 30°C-60°C for 1h resulted in an average removal of 25% of the limonin from navel orange juice (Kimball, 1987). A gradual increase in limonin content in juice blends with storage period might be due to conversion of a chemical compound limonate-a-ring lactone (non-bitter) in to limonin (bitter) in the juice (Premi et al., 1994). The decrease of naringin with storage was 443.00±10.00 to 143.70±4.00 ppm due to hydrolysis of naringin into rhamnose and prunin by α-L-rhamnosidase activity of yeast. The alcohol production starts after 10 days and gradually increased from 0.11±0.01% to 0.89±0.05% after 90 days. The CO_2 pressure 0.65±0.05 bar starts building after 10 days and reached up to 1.46±0.06 bar after 90 days. Sensitivity of yeast cells to ethanol marginally increased on decreasing the pH from 6.00-3.00. During fermentation process, CO_2, alcohol and glycerol produced is proportional to the amount of sugar fermented.

The yeast strain produced large amount of glycerol at the expense of ethanol represent an advantageous alternative for the development of beverages with low ethanol content versus physical process which alter the organoleptic properties of the final product (Jairath et al., 2012). Total yeast count was increased from $58x10^5$±10.00 to $58x10^8$±20.00 cfu mL^{-1} at the end of 90 days. This study indicated that the shelf life of beverage was 90 days.

Evaluation of sensory attributes of beverage during storage

The changes in sensory attributes like taste, color, aroma, bouquet, flavor and astringency of kinnow beverage were analyzed once every 10 days. All the sensory parameters were stable at storage period (90 days) with almost no change in organoleptic sensation (Table 3). Beverage was found to be acceptable up to 3 months of storage. The storage temperature can greatly affect the beverage tastes and smells. Lower temperatures will emphasize acidity and tannins while muting the aromatics. Higher temperatures will minimize acidity and tannins while increasing the aromatics. The presence of yeast in beverage gave a desirable freshness to the fermented beverage due to production of carbon dioxide and ethanol. Bhardwaj (2013) also reported that the low temperature and high relative humidity did not cause any change in qualitative characters and palatability of stored juice and helped in maintaining juice flavor, colour, TSS: acid ratio and sugars in balanced form than the ambient storage condition. In ambient condition change in colour of kinnow juice might be attributed to oxidation of phenolic compounds present in juice and maillard reaction between sugars and amino acids (Gonzalez, 2000). A gradual decrese in flavour and taste which may be due to the degradation of ascorbic acid and furfural production (Kausar et al., 2012) and may also be due to heat treatment applied during processing (Pruthi et al., 1984).

CONCLUSION

On the basis of results it can be concluded that the strain *Clavispora lusitaniae* is capable for producing debittered kinnow beverage using the optimized process parameters. The beverage can be stored for a period of 3 months at refrigeration temperature with minimum changes of all physico-chemical characters. Thus the technology presented here can also redress the problem of bitterness in food industries by reducing the naringin content of citrus juices.

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Author contributions

P. S. involved in all the experiments; to measure α-L rhamnosidase activity, optimization of parameters for beverage preparation and upscale production of kinnow beverage. P. P. S. was involved in overall planning and supervision. F. B. was involved in experiment; upscale production of kinnow beverage. R. K. S. was involved

Table 3: Effect of storage time on organoleptic properties of kinnow beverage

Sensory attributes	Days									
	0	10	20	30	40	50	60	70	80	90
Appearance	7.40±0.10	7.40±0.20	7.30±0.10	7.30±0.20	7.40±0.20	7.30±0.20	7.30±0.10	7.40±0.20	7.30±0.20	7.30±0.10
Taste	7.90±0.40	8.10±0.30	8.20±0.20	8.10±0.20	8.10±0.30	7.80±0.20	7.80±0.10	7.80±0.40	7.90±0.50	7.90±0.40
Colour	8.00±0.20	8.00±0.10	8.00±0.30	8.00±0.20	7.90±0.40	7.80±0.10	7.80±0.10	7.90±0.10	7.80±0.20	7.80±0.20
Aroma	8.60±0.30	8.70±0.20	8.75±0.10	8.73±0.20	8.70±0.20	8.50±0.20	8.70±0.10	8.60±0.20	8.40±0.20	8.40±0.10
Bouquet	7.24±0.20	7.25±0.10	7.35±0.20	7.38±0.10	7.30±0.20	7.36±0.20	7.38±0.10	7.35±0.20	7.28±0.10	7.28±0.20
Body	7.40±0.20	7.45±0.30	7.45±0.20	7.48±0.30	7.42±0.20	7.45±0.30	7.45±0.10	7.42±0.10	7.40±0.20	7.48±0.10
Flavor	8.40±0.30	8.50±0.10	8.55±0.20	8.60±0.20	8.60±0.30	8.00±0.20	8.00±0.10	7.50±0.10	7.40±0.20	7.20±0.10
Astringency	7.50±0.10	7.60±0.20	8.00±0.30	8.40±0.10	8.50±0.20	8.50±0.30	8.50±0.10	8.20±0.20	8.30±0.20	8.20±0.20
Overall acceptability	8.20±0.10	8.30±0.20	8.40±0.10	8.40±0.20	8.20±0.10	8.00±0.30	7.90±0.30	7.80±0.20	7.80±0.10	7.80±0.05

Mean value of three replicates. Like extremely 9, Like very much 8, Like moderately 7, Like slightly 6, Neither like/Dislike 5, Dislike slightly 4, Dislike moderately 3, Dislike very much 2, Dislike extremely 1

in planning and writing of experiment; optimization of parameters for beverage preparation.

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