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RESEARCH PAPER

Optimization and Preparation of Debittered Kinnow Beverage

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ABSTRACT

The development of delayed bitterness in citrus products is a major problem to citrus producers and juice processors worldwide due to limonin and naringin, the bitter components, thereby affecting its consumer acceptability. The present study was carried out on fermentative production of debittered kinnow (*Citrus reticulate* Blanco) beverage using inducible enzymes naringinase, limonin dehydrogenase and α-L-rhamnosidase producing fermentative yeast *Clavispora lusitaniae*. The two process optimized are: bioprocess for low alcoholic naturally carbonated beverage and debittering of juice by partially purified enzymes. The physico-chemical characteristics of freshly prepared beverage were; 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. The significant reduction of limonin and naringin content observed in T11 (beverage) during storage was 42.53% and 45.82%, respectively.

Keywords: Kinnow, *Clavispora lusitaniae,* limonin, naringin, citrus products

Citrus is the main fruit crop in the world, with a total production of 122 million tons in 2008. World production of citrus fruits in the period 2009-2010 was 123 million tonnes which includes grape fruit, lemons, limes, oranges, tangerines and other citrus fruits. India ranked 5th in citrus production (5% of worlds production) with 6,286,000 tones. These fruits are well endowed with a variety of phytonutrients, vitamin C, folate, dietary fibres and minerals as well as many antioxidant phytophenolics, including flavonoids, amino acids, triterpenes, phenolic acids and carotenoids that can potentially protect health of the consumers. These are utilized primarily or juice recovery, where about half of the processed citrus including peels, segment membrane and seeds are generated as waste. These solid residues are referred to as citrus wastes. It is made up of pulp (juice sac residue), the peel (albedo and flavedo), rag (membranes and cores), seeds(sugars (glucose, fructose, sucrose), lipids (linoleic, oleic, phyosterol, stearic acid, palmitic, glycerol and linolenic), acids (malic and citric acid and benzoic acid, succinic and tartaric acid), enzymes (pectinesterase, phosphatase, peroxidase), insoluble carbohydrates (pectin, cellulose), bitter principle (limonin, isolimonin), flavonoids (naringin, hesperidin), peel oil (D-limonene), vitamins (ascorbic acid, carotenoids, vitamin B complex), volatile constituents (esters, aldehydes, alcohols, hydrochloric acids, ketones), pigments (xanthophylls, carotenes), and minerals (potassium and calcium) (Bambidis and Robinson, 2006).

The processing of citrus fruits has faced many commercial restrictions due to bitterness caused by

naringin, limonin and nomilin (Thammawat *et al.* 2008). The threshold have been established (Sahota *et al.* 2015) and excess level of these compounds causes intolerable bitterness in processed citrus products such as juice, wine and vinegar.

Many techniques were used to reduce bitterness by naringin such as adsorptive debittering (Fayoux *et al.* 2007), enzymatic hydrolysis (Puri and Kalra, 2005), poly-styrene divinyl benzene styrene resin treatment and β-cyclodextrin treatment (Mongkolkul *et al.* 2006). But these techniques alter the nutrient composition of juice and the nutrients, flavor and color.

To control the quality and improve the commercial value of the citrus juices, maintaining the health properties and increasing the acceptance by the consumer, the reduction of bitter component by enzymatic hydrolysis appears as one promising technique with industrial application due to its high specificity, efficiency and convenience on commercial scale.

The microbial enzymes are of paramount importance due to their cost efficient production, therefore replacing the chemical methods for debittering. Enzyme concentration, temperature, pH variation, and inducer concentration are important parameters responsible for stability and activity of an enzyme action. The yeast *Clavispora lusitaniae* (KF 633446) strain has the potential to produce fermentative debittering enzymes, naringinase, limonin dehydrogenase and α-L-rhamnosidase thereby, used for the debittering of citrus juices. The debittering enzymes can offer advantages such as cost effectiveness, single step hydrolysis, short incubation, preservation of flavour, retention of color, vitamins and organoleptic components of juice.

In the agro-industrial sector the use of enzymes led to a process optimization, reducing process-related energy costs, improving nutritional safety and quality of food, the development of new products. 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. Naringinase has potential biotechnological applications in citrus processing and bioprocess industries such as wine industries for aroma enhancement, glycolipids production, biotransformation of antibiotics, steroids. The enzyme limonin dehydrogenase eliminate the production of bitter component limonin by catalyzing the oxidation of Limonate A-Ring lactone (LARL), to the corresponding 17-dehydroxylimonate a non-bitter derivative. The enzyme rhamnosidase has many applications in industries as : debittering of citrus fruit juices (Busto *et al.* 2007), manufacture of prunin from naringin, aroma enhancement by enzymatic hydrolysis of terpenyl glycosides containing L-rhamnose, elimination of hesperidin crystals from orange juices, conversion of chloropolysporin B to chloropolysporin C (Feng *et al.* 2005).

In this work, optimization of minimal media for maximum enzyme production using fermentative yeast *Clavispora lusitaniae* was carried out by supplementation of kinnow, lemon and grapefruit peels for the low cost value aided substrate.

MATERIALS AND METHODS

Yeast culture

The yeast *Clavispora lusitaniae* (KF 633446) strain producing fermentative debittering enzymes, naringinase, limonin dehydrogenase, α-L-rhamnosidase enzymes was obtained from the Department of Microbiology, Punjab Agricultural University, Ludhiana.

Raw material

Fresh, fully ripened kinnow (*Citrus reticulata* Blanco) fruits were procured from the Department of Fruit Science, Punjab Agricultural University, Ludhiana. The selected fruits were washed in the chlorinated water and then used for the extraction of juice. The juice was extracted aseptically under hygienic conditions with a 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 yeast 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}.

Preparation of debittered citrus beverage

A debittered beverage was prepared under optimized conditions of inoculum concentration- (0.75% v/v), TSS (13 °B), incubation temperature (30±5 °C) and incubation time (48 h). The diluted juice was inoculated @ 0.75% v/v with freshly prepared inoculum and incubated at 30±5°C for 48 h in batch scale glass digester.

Fermentation

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).

Bottling and storage

It was incubated for 48 h at 30±5 °C. The beverage was refrigerated for 24 h, siphoned, bottled and stored in refrigerated conditions.

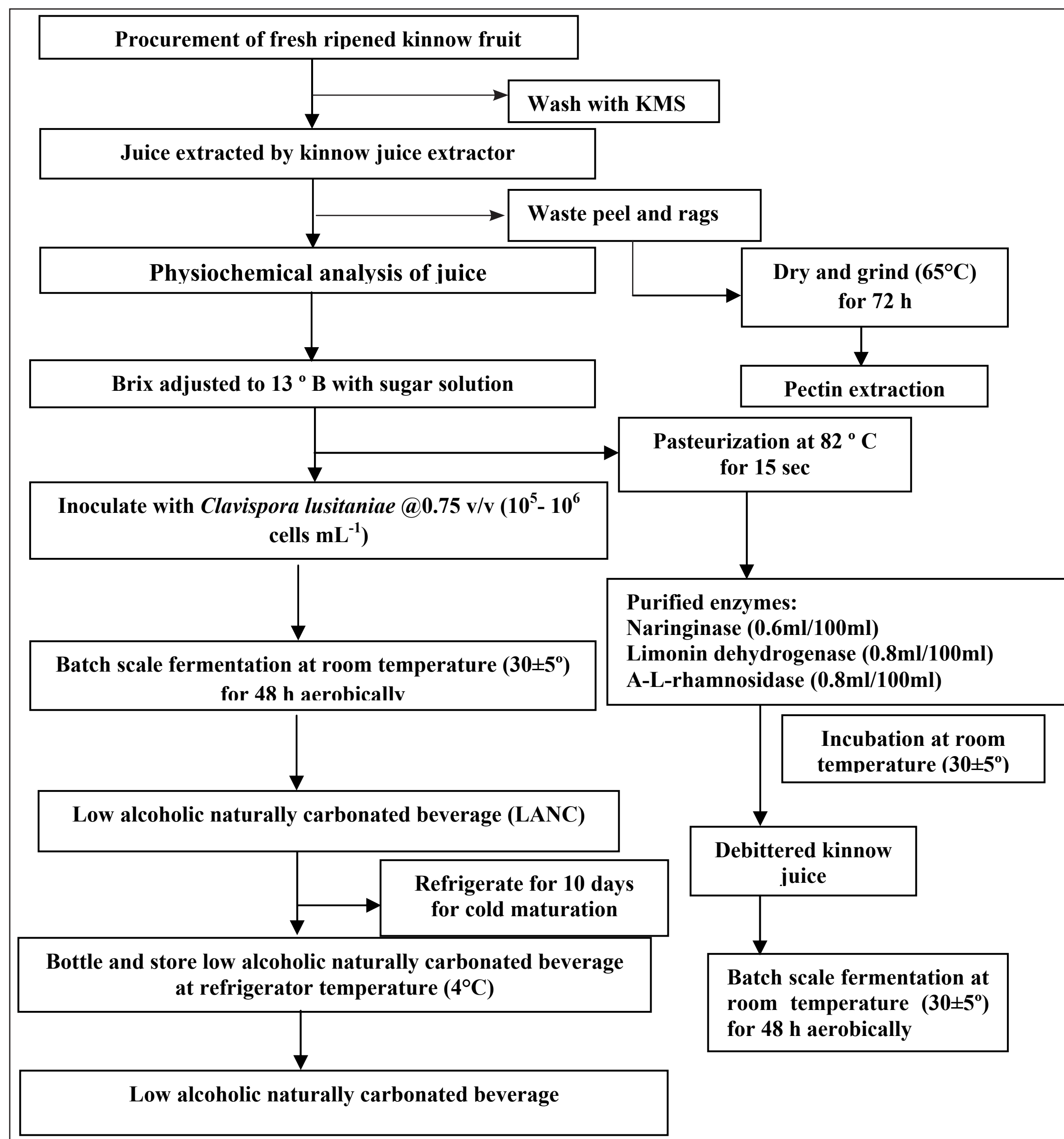
Enzymatic debittering of citrus juice

Technology for the debittering of kinnow juice under optimized fermentation conditions were developed by yeast *Clavispora lusitaniae* (Kaur 2017). The concentration and incubation time of purified debittering enzymes for debittering of kinnow juice has been optimized. The purified enzymes Naringinase (0.6 mL), limonin dehydrogenase (0.8mL) and α-L-rhamnosidase (0.6mL) was added the diluted kinnow juice (13 °B) and kept for 10 h at room temperature.

The samples were drawn after regular interval of two hours for estimation of naringin and limonin. (**T0**= control, **T2**= 0.8ml Limonin dehydrogenase, **T4**= T1 (0.6ml Naringinase) +T2, **T6**= T2+T3 (0.8ml α-L-rhamnosidase), **T7**= T1+T2+T3, **T8**=control (Beverage), **T9=**T8+T1+T2+T3, **T10**= Beverage, **T11**= T10+T1+T2+T3).

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 Corporation of India Ltd., Hyderabad, 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.



Flow diagram: Production of debittered kinnow juice and fermented low alcoholic naturally carbonated beverage

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

A bioprocess optimized for the preparation of

low alcoholic naturally carbonated beverage from kinnow juice under optimized conditions: inoculum concentration (0.75% v/v), inoculum temperature (30±5°C) and time (36h).

The yeast *Clavispora lusitaniae* is capable of fermenting acidic fruit juices to debittered low alcoholic naturally carbonated beverage (LANC).The fresh juice was diluted (1:3) with TSS 13°B, 0.75 % @ *C. lusitaniae* yeast inoculum, incubation temperature (30±5°C) and time (36hrs). The debittering process technology was optimized by two processes:

- Juice + enzymes (Naringinase, limonin dehydrogenase and α-L-rhamnosidase)
- Juice + *Clavispora lusitaniae* (0.75%, v/v)

Shelf-life studies of kinnow beverage

Shelf-life of fermented debittered kinnow beverage stored at refrigerated temperature (4 °C) and room temperature was studied and evaluated fortnightly for physico-chemical, microbiological and organoleptic qualities.

Evaluation of microbiological and physico-chemical properties of kinnow beverage during storage at refrigerator

The microbiological and physiochemical parameters of beverage as TSS (°B), Acidity (%), pH , Brix-acid ratio, total sugar (%), Reducing sugars, Ascorbic acid (mg/100mL), Total polyphenol contents (mg GAE/100mL), Limonin (ppm), Naringin (ppm), Alcohol (%,v/v), CO_2 (bar) and total Yeast count (cfu mL^{-1}) were analysed.

The results showed a significant decrease in Brix from 13±0.2 °B to 11.2±0.3 °B at refrigerated temperature. The brix acid ratio also decreased from 92.85±0.0 to 19.31±0.0. Similar results have been reported in lime juice (Sarolia and Mukherjee 2002.The increase in TSS content of juice during storage might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides.

The pH decreased from 3.4±0.1 to 3.0±0.1 while acidity increased from 0.14±0.03% to 0.56±0.01% during storage. pH is inversely proportional to the acidity of any medium. This decrease in pH and increase in acidity was attributed due to formation of acidic compounds by degradation of reducing sugars (Zia 1987; Akhtar *et al.* 2010). Our results are in accordance with Saleem (1980) and Ahmed (2008).

The total sugars decreased from 12.9±0.3% to 9.5±0.4%. The reducing sugars also decreased from 2.42±0.2% to 1.32±0.1%. 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 reduced from 27.8±1 mg/100 mL to 6.48±0.4 mg/100 mL. 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). But the decrease was significantly less under refrigerated storage condition which may be attributed to low temperature and high relative humidity in storage, which inhibited the conversion of acid in sugars and decreased rate of ascorbic acid oxidation.

The mean polyphenol contents of kinnow beverage decreased significantly from 53.48±3 mg GAE/100 mL to 40.8±2 mg GAE/100 mL. According to Ritter *et al.* (1992) and Karadeniz and Eksi (2001) reports, clarification also decreased the polyphenolic contents of commercial fruit juices. Polyphenol contents decreased constantly with the progress of the ripening, while in red coloured varieties it increased during the last ripening stage due to the maximal accumulation of anthocyanidines and flavonols (Marinova *et al* 2005).The decrease of limonin from 6.9±0.1 to 3.52±0.1 ppm might be due to production of CO_2 during storage. Similar results were reported by Singh *et al.* (2015). Sahota *et al.* (2015) reported that limonin content decreased from 7.6 ppm to 4.0 ppm in kinnow beverage. Naringin content was decreased from 443±10 to 143.7±4 ppm .The decrease in naringin

content occurreddue to hydrolysis of naringin into rhamnose and prunin by α-L-rhamnosidase activity of yeast *Clavispora lusitaniae*.

The alcohol production started 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 started building after 10 days and reached up to 1.46±0.06 bar after 90 days.

Total yeast count was increased from $58\times10^5\pm10$ to $58\text{x}10^8\pm20$ cfu mL^{-1} at the end of 90 days at refrigerated temperature.

Effect of enzymatic treatments on limonin content in kinnow juice

The yeast *Clavispora lusitaniae* has the efficiency to degrade limonin content in juice. The limonin concentration of freshly drawn juice was 7.5 ppm. During 30 days storage at ambient temperature, the limonin content was recorded, the decreasing trend of limonin concentration was observed in treatments containing debittering enzymes viz. **T2** (0.8ml Limonin dehydrogenase), **T4** [T1 (0.6ml Naringinase) +T2], **T6** [T2+T3 (0.8ml α-L-rhamnosidase)], **T7** (T1+T2+T3), (Beverage), **T9** **(**T8+T1+T2+T3), **T10** (Beverage), **T11** (T10+T1+T2+T3).

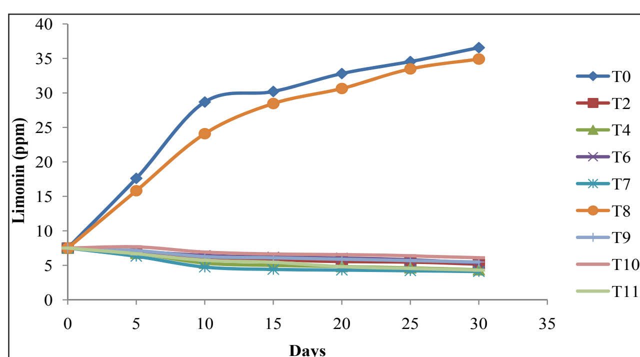


Fig. 1: Effect of enzymatic treatments on limonin content in kinnow juice

T0= *control*, **T2**= *0.8ml Limonin dehydrogenase*, **T4**= *T1 (0.6ml Naringinase) + T2*, **T6** = *T2 + T3 (0.8ml α-L-rhamnosidase)*, **T7** = *T1 + T2 + T3*, **T8** = *control (Beverage)*, **T9 =** *T8 + T1 + T2 + T3*, **T10** = *Beverage*, **T11** = *T10 + T1 + T2 + T3*

The limonin content in **T2** decreased from 7.5 ppm to 5.18 by 17.6%, 26% and 30% and in **T4** limonin concentration reduced from 7.5 ppm to 4.36 ppm by 29%, 35.6%, and 41.9% over the period of 10, 20 and 30 days respectively (Fig. 1).

Maximum reduction was observed in **T6** by 14.2%, 19.2% and 28.8% from 7.5 to 5.34 ppm; Significant reduction was observed in both **T7** and **T11**i.e 15.86%, 42.53%, 45.33% and 24%, 35.86% 42.53% respectively. The moderate reduction was also observed in **T10** by 7.73% on 10th of storage and with the increase in storage time, limonin reduced by 12.4%, 18.53% on 20 and 30 days period.

In T10 (beverage), the limonin content was decreased to 18.53% due to efficiency of *C. lusitaniae* to metabolize and eliminate the bitter component by the production of limonin dehydrogenase. In T11, the limonin content reduced by 42.53% during the storage period of 30 days due to synergistic effect of yeast strain with the combination of debittering enzymes. The limonin content decreased significantly in T4, T7, T11 whereas limonin content in T6, T9 were found to be non-significant.

The increased limonin content was recorded in T0 (control) and T8 control (beverage). The percent yield in T0 (control) increased from 73.84%, 75.16%, 77.47% (7.5ppm to 36.55) and in T8 (control beverage) i.e 68.86%, 73.64% and 77.59% (from 7.5ppm to 34.91) over the period of 10, 20 and 30 days respectively. This increase in limonin content in control (T0, T8) occurred due to conversion of Limonoate-A-ring lactone (non-bitter) into limonin (bitter) component.

These results are in agreement with Singh *et al.* (2015) who showed decrease in limonin content from 6.90 ppm to 3.53 ppm in kinnow beverage. Puri *et al.* (2002) also purified limonin dehydrogenase from the cell culture of *Rhodococcus fascians* which degrades limonin by 66% kinnow juice. Hasegawa *et al.* (1982) showed 70% reduction in limonin content in naval orange juice serum with *Arthrobacter globiformis* cells in acrylamide gel.

The addition of 0.8 mL/100mL concentration of partially purified limonin dehydrogenase enzyme is an optimized concentration in debittering of citrus juices as maximum reduction in limonin content was

Table 1: 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 (ºB)	13±0.2	12.8±0.1	12.5±0.2	12.4±0.3	12.2±0.3	12±0.3	11.8±0.3	11.5±0.2	11.4±0.2	11.2±0.3
Acidity (%)	0.14±0.03	0.21±0.02	0.32±0.04	0.36±0.02	0.4±0.04	0.45±0.03	0.49±0.02	0.51±0.03	0.56±0.01	0.58±0.01
pH	3.4±0.1	3.3±0.2	3.3±0.1	3.2±0.2	3.2±0.2	3.2±0.1	3.1±0.1	3.1±0.1	3.1±0.1	3.0±0.1
Brix-acid ratio	92.85±0.0	60.95±0.0	39.06±0.0	34.44±0.0	30.5±0.0	26.66±0.0	24.08±0.0	22.54±0.0	20.35±0.0	19.31±0.0
Total sugars (%)	12.9±0.3	12.4±0.4	12.3±0.4	11.9±0.4	11.7±0.2	11.2±0.2	10.9±0.5	10.1±0.3	9.8±0.3	9.5±0.4
Reducing sugars (%)	2.42±0.2	2.26±0.1	2.1±0.2	1.58±0.4	1.56±0.1	1.54±0.5	1.49±0.1	1.37±0.1	1.35±0.2	1.32±0.1
Ascorbic acid (mg/100 mL)	27.8±1	26.4±1	16.8±0.2	14.5±0.5	10.5±0.5	8.5±0.5	7.72±0.2	7.54±0.1	6.78±0.2	6.48±0.4
Total polyphenol contents (mg GAE/100 mL)	53.48±3	51.7±1	50.1±2	48.7±3	47.9±1	45.79±4	44.5±2	43.9±3	42.1±1	40.8±2
Limonin (ppm)	6.9±0.1	6.2±0.2	5.7±0.2	5.4±0.2	5.1±0.1	4.7±0.2	4.48±0.2	4.15±0.1	3.8±0.1	3.52±0.1
Naringin (ppm)	443±10	420.5±5	376.4±4	284.5±6	213±7	178±6	160.6±8	155.4±5	148.9±2	143.7±4
Alcohol (%,v/v)	0±0.0	0.11±0.01	0.35±0.03	0.54±0.02	0.63±0.03	0.72±0.3	0.75±0.4	0.8±0.2	0.82±0.3	0.89±0.05
CO_2 (bar)	0±0.0	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})	$58\times10^5\pm10$	$64\times10^5\pm20$	$49\times10^6\pm10$	$52\times10^6\pm10$	$65\times10^6\pm20$	$23\times10^7\pm10$	$33\times10^7\pm20$	$46\times10^7\pm20$	$52\times10^8\pm10$	$58\times10^8\pm20$

recorded in T7 (45.33%). This reduction in limonin content is due to the combination of debittering enzymes and their stability during period of 30 days.

Effect of enzymatic treatment on naringin content of kinnow juice

The Naringinase enzyme produced by *Clavispora lusitaniae* is efficient in eliminating the bitter component naringin. The initial naringin content in fresh juice was 249.75 ppm. During storage period of 30 days, the naringin concentration was recorded, the declining trend was observed in treatments containing debittering enzymes viz. **T1**= 0.6 ml Naringinase, **T4**= T1 (0.6ml Naringinase) + T2, **T5**= T1+T3 (0.8ml α-L-rhamnosidase), **T7**= T1+T2+T3, **T9=**T8+T1+T2+T3, **T10**=Beverage, **T11**=T10+T1+T2+T3 due to conversion of naringin to naringenin by the catalytic action of naringinase enzyme. The naringin content in **T1** was decreased (249.75to 145.22 ppm) by 25.71%, 32.46% and 41.85% and in **T4** naringin concentration declined (249.75 to 135.91 ppm) to 26.6%, 37.18% and 45.58% over period of 10, 20 and 30 days; marginal reduction was recorded in **T5** i.e. 22.67% 32.22% and 42.69% ; moderately decreased in T7 naringin from 249.72 ppm to 153.57ppm (38.91%) while slight reduction was recoded in **T9** on 10th day 30.49% and further decreased to36.25%, 40.58% till 30th day and content reduced to 148.38 ppm. Significant reduction was observed in **T10** and **T11** to 128.18ppm, 135.2ppm by 48.67% and 45.82% on 30th day of storage respectively Fig. 2).

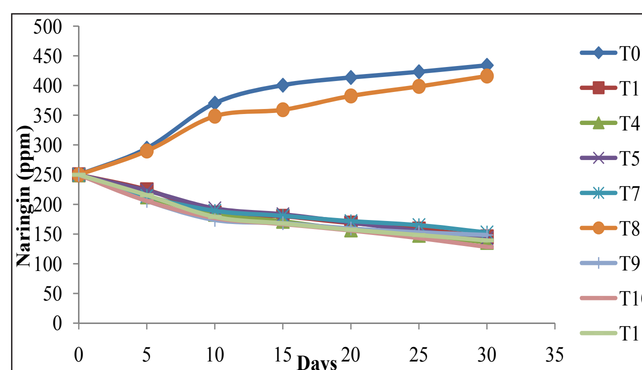


Fig. 2: Effect of enzymatic treatment on naringin content in kinnow juice

T0= *control*, **T1**= *0.6 ml Naringinase*, **T4**= *T1 (0.6ml Naringinase) +T2*, **T5**= *T1+T3 (0.8ml α-L-rhamnosidase)*, **T7**= *T1+T2+T3*,

T8=*control (Beverage),* **T9=***T8+T1+T2+T3,* **T10**= *Beverage,* **T11**= *T10+T1+T2+T3*

During the storage period of 30 days naringin content was increased (434.12, 416.02 ppm) in T0 (control) and in T8 (control beverage) respectively. The percent yield of naringin in T0 increased to 32.55%, 32.36%, 42.46% and in T8 i.e. 25.71%, 32.56% and 41.845.

The maximum decrease of naringin content was observed in **T10** i.e. 48.67%**.** The decrease in naringin content in beverage is due to hydrolysis of naringin into rhamnose and prunin by α-L-rhamnosidase. In T4, the naringin content reduced to during storage period of 30 days is due to stability of partially purified naringinase enzyme. The naringin content reduced significantly ($p<0.05$) in T4, T5, T10, T11.

The results are in accordance with Prakash *et al.* (2002) who showed that naringin content reduced to 75% in Indian grapefruit juice. Yalim *et al.* (2004) reported the naringin elimination upto 30-40% in various citrus products. Patil *et al.* (2014) showed that maximum decrease of naringin (74%) was obtained at naringinase concentration of 100U/100mL with incubation at 40°C for 4hrs.

It is concluded from the study that the optimized concentration of naringinase enzyme is 0.6mL/100mL that is highly able to reduce the naringin content in citrus juices. The low alcoholic naturally carbonated beverage obtained was found to be fresh, safe, stable, more natural, minimally processed free from additives, contaminants, adulterants, and harmful pathogenic bacteria. It offers more nutrients, effervescence, longer shelf life and other physiological benefits. CO_2 act as a critical solvent which forms carbonic acid and hence responsible for enhancing shelf life of the beverage. The acceptability and higher sensory score of beverage is dependent upon its physiochemical characteristics and loss of some compounds that imparts flavor and aroma of the beverages during storage (Sahota *et al.* 2009). The presence of alcohol with <1% (v/v) is also responsible for enhancing the shelf life of the beverage.

CONCLUSION

On the basis of results it can be concluded that the strain *Clavispora lusitaniae* is capable of 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.

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