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TECHNOLOGY DEVELOPMENT FOR THE PRODUCTION OF SELF CARBONATED LESS ALCOHOLIC LEMON BEVERAGE

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Abstract

Baramasi lemon (Citrus limon), a variety of lemon is well acclimatized to agro-climatic parameters of the Punjab. The higly nutritious lemon var. Citrus limon beverage was prepared with very low alcohol content and high natural carbonation in lemon juice and fermenting with Clavispora lusitaniae under standardized Brix 16 oB, acidity 0.2-0.3 % and temperature 30±5 oC for 36 h aerobically. The quality parameters of Citrus limon beverage were of pH 2.6, TSS 14.1oB, acidity 0.41%, ascorbic acid 2.00 mg/100ml, alcohol 0.803 (% v/v), and CO_2 (Bar) 1.50 and Total Plate Count 2.3x108 cfu/ml and ranked between "liked very much to moderate" and had a shelf life durability of three months. All the components were retained in all the beverages during storage period of three months and the final yielded beverage product was healthy souce for body.

Keywords: Baramasi lemon; shelf life studies; clarification; carbonated beverages; less alcohol.

Introduction

The different types of citrus fruits either of genus *Citrus* are categorized under the family of *Rutaceae* including very common fruits like lemon, lime, orange and grapes. These citrus fruits, more specifically lime and lemon have indegenious origin inhabitating mostly in the tropical and sub-tropical regions (Gupta *et al*., 2014; Davinder *et al*., 2017; Chhikara *et al*., 2018; Raut *et al*., 2018; Kumar *et al*., 2018; Haldar *et al*., 2019). About approximately 17.35 million tons of lime and lemon fruit were propagated world-wide by inhabitating 1.08 million hectare of cultivated field with maximum producers being countries like India, Mexico, China, Argentina and Brazil all-together account for 61.32% of gross annual production (FOASTAT, 2018) and energy-trasportation engagement (Duran *et al.,* 2015; Chauhan *et al.,* 2015; Kotia and Ghosh, 2015; Kotia *et al*., 2016a-d; Patel *et al.,* 2017; Kotia and Ghosh, 2017a-b; Kotia *et al.,* 2017;Jha *et al.,* 2019; Kotia *et al.* 2018a-b; Priyadarshi *et al.,* 2019; Kotia *et al.,* 2019; Vyas *et al.,* 2010 . Baramasi lemon *(Citrus limon),* a variety of lemon is well acclimatized to agro-climatic parameters of the northern region of India, especially found abundantly in Punjab.Lemon fruits were subjected to various evaluations for preparation and yield of new products such as juice concentrated form, carbonated beverages with low/no alcohol and powdered cocktails (Kaur *et al.*,2014; Sangma *et al.*, 2019; Sogi *et al.*, 2010; Tanay *et al.*, 2014; Pramanik *et al.*, 2015; Pramanik and Padan, 2016a; Pramanik and Padan, 2016b; Dhaka *et al.*, 2016). This lemon variety is known to be susceptible to low temperature stress conditions such as chilling inury that freezes the plant transport system and disrupts the plasma membrane of lemon so it demands for ambient conditions for storage and for improved shelf-life durability (Shahnah *et al.*, 2007; Yadav *et al.*, 2011; Jilte *et al.*, 2019). Citrus fruits are highly rich with compounds like flavonoids glycosides, coumarins, essential oils and bioactive compounds like polyphenols, ascorbic acid (vitamin C) (Duthie *et al.*, 2000; Chowdary *et al.*, 2019; Gupta *et al.,* 2013; Kumar *et al.,* 2013; Sharma *et al.*2018; Vyas *et al.* 2018). The flavonoids present in citrus acts as antioxidants that can regulate catalytic activities of enzymes and inhibits proliferation of cell (Ortuno *et al.*, 2006). Flavonoid covers a broad spectrum of biological properties beneficial for health such as antimicrobial (against bacteria and fungi), antidiabetic, anticancer and antiviral properties. Being such a great source of many important bioactive compounds, lemon is consumed world-wide in many forms but the most common is lemon juice accepted universally which provide all those benefits along with keeping hydrated and fresh by removing toxic compounds from our body (Sharma and Kumar, 2015; Mehta *et al.,* 2016; Sharma, 2016; Sharma and Manhas, 2017). Due to enormous health and nutritional qualities, there is a great opportunity for upgradation of lemon juice by processing and treatment using various techniques with outcome into a desired and value added yield enhancing shelf-life, health benefits and organoleptic properties. Presence of various nutrients and vitamins protects cell membrane and its different structures from any damage by neutralizing the free radicals that gets incorporated into the system (Sonali and Geeta, 2014). Consumption of fresh fruits and vegetables in form of juices, smoothies and fermented beverages with natural carbonation promotes a healthy lifestyle to human welfare (Wootton-Beard and Ryan, 2011; Corbo *et al.*, 2014 ; Marsh *et al.*, 2014; Hurtado *et al.*, 2015; Chand and Kapoor *et al.*, 2014; Arora *et al.*, 2015; Panghal *et al.*, 2017; Kumar *et al.*, 2017).Processing the juice with fermentation adds more value to the beverage by preserving essential nutrients, lowers bitterness level replacing with aromatic flavor in the beverage and production of CO_2 due to carbonation gives natural fizzines to the final product making it more appealing to the consumers (Kumar *et al.,* 2018) Carbonation is a crucial step as its enhances the palatability and preservation period (Sameen *et al.*, 2013). As carbonated drinks when consumed creates soft, fizzy and tickling sensation to the tongue makes them popular universally (https://www. unesda.eu/lexikon, 2019). A beverage is a form of drink formulated for consumptions of human worldwide with variations in taste and appearances including sparkling water, carbonated water and drinks, diet drinks, energy drinks, and organic drinks (Appleton *et al.*, 2018; Arora *et al.*, 2015; Chilana *et al.*, 2015; Kaur *et al.*, 2017; Singh 2018; Precuieue *et al.*, 2018; Kumar *et al.*, 2020; Kaur *et al.*, 2014). The main objective of the study is the preparation of highly nutritious,

clarified and tasty lemon beverage by extracting juice from the Baramasi lemon variety, processing by fermentation and physico-chemical analysis with sensory parameters thereby attaining least alcoholic, highly natural carbonated, transparent, healthy and flavory lemon beverage product.

Materials and methods

Fruits

Lemon var. Baramasi (*Citrus limon*) was procured from the Horticulture Department, PAU, Ludhiana.

Juice extraction

After manual screening of good fruits, undesirable fruits were discarded off from the selection criteria. These fruits were then subjected to washing with chlorinated water, peeled, washed again and then processed for juice extraction aseptically under hygienic conditions.

Lemon

Fruits were leached off with water and juice was extracted using lemon squeezer.

Preparation of sugar solution

The sugar was collected from the nearby market of Ludhiana city and a solution was was attained by vigorous boiling of 500 g in 1 L water for about 10 minutes, further subjected to cooling and storage under aseptic conditions.

Physico-chemical study of extracted lemon juice

The physico-chemical scrutiny (TSS, pH, Brix acid ratio, juice yield and % acidity) of extracted lemon juice was done and diluted with water to achieve the palatable % acidity (0.32-0.40 %). The diluted extracted lemon juice was further pasteurized at temperature 82^0C for time period of 15 s, further subjected to cooling and brix adjustment to 16 ºB by sugar solution addition to the juice.

Inoculum preparation

Preparation of inoculum proceeded by boiling juice for 5 minutes and adjusting the brix to 16 ^{0}B. Once boiled, 24 h grown old yeast culture of *Clavispora lusitaniae* was inoculated using inoculationg loop in 100 ml of diluted juice and incubated at 30 ^{0}C for 24 h till a final concentration of 10^6-10^7 cells/ml was obtained.

Fermentation

The diluted juice was inoculated using inoculum volume of 0.5 % v/v at 30±5 ^{0}C for 36 h under aerobic conditions.

Bottling and Storage

This prepared lemon beverage was subjected to siphoning and bottling under refrigerated conditions and then stored under same conditions.

Clarification of the beverage with fining agents

Bentonite solution 4.5 % (w/v) was prepared in distilled water and added to the beverage at a concentration of 1 % (v/v) and 2 % (v/v) concentration and kept under refrigerated conditions for 24 h followed by siphoning and bottling and kept for storage under refrigerated conditions. Gelatin solution 4.5 % (w/v) was prepared in distilled water and added to the beverage at a concentration of 1 % (v/v) and 2 % (v/v) concentration and kept under refrigerated conditions for 24 h, subjected to siphoning and bottling and kept for storage under refrigerated conditions.

Results and Discussion

Studies on shelf life durability of Lemon beverage

Shelf life of naturally carbonated less-alcoholic lemon beverage of lemon variety Baramasi (summer crop) stored under refrigerated conditions was observed and analysed after every 15 days for microbiological, biochemical and organoleptic properties.

Physicochemical properties

Before fermentation, raw juice was analysed for its physicochemical properties (Table 1). The factors which control the fermentation process include pH, sugars, availability of oxygen and temperature. The results of Baramasi (summer crop) beverage (Table 2, Fig.1) showed significant reduction in brix from 16.0 ^{0}B to 14.1 ^{0}B and Brix acid ratio decreased from 57.85 to 34.59. The acidity rose from 0.28 % to 0.41 % and pH of the beverage reduced from 2.8 to 2.6 and at the end of 90 days. The decline in pH level and increase in acidity % was very much significant and accounted due to production of CO_2 which forms weak acid on dissolution. According to results of Kitabatake *et al.* (2003), reported pH decline while acidity percent increment in traditional non- alcoholic beverages. As per reports of Ogiehor *et al.* (2008), there was decrease in pH from 5.10 to 2.90 while increase in titratable acidity (TA) from 0.021 to 0.060 during storage of zobo beverage produced from *Hibiscus sabdarifa* for 21 days.

The % reduction in total sugars is 23.14 % as it decreased from 14.48 % to 13.68 % after 30 days and 11.13 % after 90 days, respectively. The percentage decrease in reducing sugars is 33.16 % as it decreased from 7.69 % to 7.08 % after 30 days and gradually decreased to 5.14 % after 90 days. Because ripened fruits are great source of sugars (2.0-2.05 %), sucrose breakdown occurs in early onset of fermentation declining glucose level with respect to fructose level, thereby, denoting rapid utilization of glucose because sucrose hydrolysis results in balanced amount of fructose and glucose levels, hence, decreasing total and reducing sugars level with increased fermentation period.

The ethanol concentration at the end of 15 days was 0.187 (% v/v) and progressively increased to 0.517 (% v/v) after 45 days and finally touched upto 0.803 (% v/v) after 90 days. The CO_2 pressure of 0.50 bar was observed after 30 days which maximized to 1.20 bar after 60 days and reached upto 1.50 bar at the end of 90 days of storage. Higher CO_2 pressure of about 3000 KPa is required to stop the fermentation and increased CO_2 pressure enhances the fermentation lag-time and maximum specific growth rate of yeasts (Cahill *et al.*, 1980). Flocculation of yeast cells with particulates brings about an entrapment of CO_2 gas, agitating the medium with a stimulation of fermentation (Ough and Grout, 1978). In the secondary fermentation, the CO_2 production is proportional to the sugar fermentation (Amerine *et al.*, 1980). Impact of the incubation period reveals that with increased fermentation time, yeast utilizes the fermentable sugars (glucose and fructose) and converts into alcohol and CO_2.The viable cell count increased upto $2.3x10^8$ during the storage period of 90 days. Ascorbic acid contents of lemon var. Baramasi (summer crop) was 38.05 mg/100ml. In Baramasi (summer crop) beverage, ascorbic acid reduced from 14.00 to 9.40 mg/100ml after 30 days of

storage and gradually decreased to 5.87 after 60 days and 2.00 mg/100ml after 90 days of storage of beverage, because photo-oxidation of ascorbic acid (Lehninger, 1975). Loss of ascorbic acid can be compensated by the use of colored bottles for storage of the beverages to prevent photooxidation and by creating anaerobic conditions under high carbon-dioxide pressure. Obire *et al.* (2015) reported the results of fermented waste fruit juice which reveals that after 22 days of fermentation there is reduction in ascorbic acid in pineapple juice from 16.7 to 5.2 mg/100ml, 35.6 to 25.0 mg/100ml for banana juice, while from 163 to 25.0 ml/100ml for pawpaw juice.

Sensory attributes

The mean sensory scores for color, bouquet, appearance, flavor, astringency, aroma, body, and overall acceptability of Baramasi (summer crop) beverage ranked accordingly between liked very much to moderately liked after 90 days storage (Table 3). In Baramasi beverage (summer crop), the mean sensory scores for appearance, color, body, flavor, astringency and bouquet varied non-significantly throughout the storage period. Aroma scores enhanced consecutively from 7.6 to 8.0 after 45 days. Flavour scores increased from 7.4 to 8.0 upto 45 days while for astringency there is increase in scores from 7.6 to 8.4 due to carbonation (1.5 bar) and increase in acidic content after storage.

Effect of clarification on organoleptic qualities of the beverage

Bentonite and gelatin 4.5% (w/v) solution each were used to clarify Baramasi with a concentration of 1 % and 2 % (v/v) concentrations each. Bentonite is used to remove heat unstable proteins and yeast cells. It settles out well, and can be easily filtered (Jackson, 2016). All the sensory attributes varied significantly among bentonite (1% and 2%) treatments in Baramasi beverage. Bentonite 1 % scored better than 2 % clarified beverage as its overall acceptability was 8.0 with 1 % as compared to 5.2 with 2 % as evidenced from Table 4. It may be due to deterioration of the color and clarity due to brownish bentonite clay. Similar results were obtained by (Rai *et al*., 2007).

Gelatin binds with negatively charged proteins, thereby, forming large aggregates and aids in rapid sedimentation. The results of sensory evaluation of gelatin treatment showed that overall acceptability of 1 % concentration scored higher than 2 % concentration. In Baramasi (summer crop), scores of astringency in 1 % was 8.6 respectively (Table 4). The results are in accordance with Vardin and Fenercioglu (2003) who showed that clarification with gelatin was effective to clarify pomegranate juice.

Thus, on the basis of sensory evaluation 1 % (v/v) of 4.5 % (w/v) concentration both for bentonite and gelatin treatments were found to be effective for the clarification of Baramasi (summer crop) beverages. Out of these, gelatin had superior results than bentonite treatment.

Table 1: Physicochemical characteristics of raw lemon juice7

Parameters	Fresh	15d	30d	45d	60d	75d	90d	CD (5%)
pH	2.8	2.8	2.7	2.6	2.6	2.7	2.6	0.076
TSS ^{o}B	*16.0	16.0	15.7	15.3	14.9	14.5	14.1	0.230
Acidity %	0.28	0.28	0.35	0.37	0.38	0.41	0.41	0.012
Brix-acid ratio	57.85	59.43	44.55	41.45	39.56	35.37	34.59	2.920
Total sugars %	14.48	14.39	13.68	13.14	12.31	11.81	11.13	0.070
Reducing sugars %	7.69	7.49	7.08	6.68	6.28	5.81	5.14	0.059
Alcohol (%,v/v)	-	0.187	0.467	0.517	0.607	0.760	0.803	0.016
Ascorbic acid (mg/100ml)	14.00	11.65	9.40	7.85	5.87	2.41	2.00	0.233
CO_2 (Bar)	-	-	0.50	0.93	1.20	1.37	1.50	0.108
Total Plate count (Yeast) (cfu/ml)	-	2.5×10^6	4.8×10^7	6.4×10^7	5.9×10^7	8.4×10^7	2.3×10^8	-

Table 2: Physicochemical and microbiological analysis of beverage

Sensory attributes	Fresh	15 d	30 d	45 d	60 d	75 d	90 d	CD (5%)
Color	7.4	7.4	7.4	8.2	8.2	7.6	7.6	NS
Appearance	7.4	7.4	7.4	7.4	7.4	7.4	7.4	NS
Bouquet	7.4	7.4	7.8	7.8	7.6	7.6	7.6	NS
Aroma	7.6	7.8	7.8	8.0	8.0	7.0	7.0	0.639
Flavor	7.4	7.4	7.6	8.0	7.4	7.4	7.0	NS
Astringency	7.6	7.6	8.0	8.2	8.4	7.8	7.8	NS
Body	7.0	7.4	8.0	7.8	7.6	7.6	7.6	NS
Overall acceptability	7.0	7.6	7.6	8.2	7.6	7.6	7.6	0.639

Table 3: Sensory analysis of beverage

Parameters	Lemon var. *Baramasi* (summer crop)
pH	2.2
TSS ^{o}B	7.0
Acidity %	5.12
Reducing sugars %	3.12
Brix-acid ratio	1.36
Total sugars %	4.39
Juice yield %	37.5
Ascorbic acid (mg/100ml)	38.05

Table 4: Effect of clarification on sensory attributes
*Mean value of five replicates

Sensory attributes	Bentonite		Gelatin		CD (5%)
	1%	2%	1%	2%	
Appearance	7.4	5.0	7.8	7.0	0.670
Aroma	7.6	5.2	6.4	6.2	0.670
Color	7.8	5.4	7.8	7.2	0.636
Body	6.8	5.8	8.2	7.2	0.899
Bouquet	8.0	4.6	7.6	7.4	0.793
Astringency	7.6	6.2	8.6	7.2	0.821
Flavor	7.8	5.0	7.4	6.8	0.994
Overall Acceptability	8.0	5.2	8.8	8.0	0.636

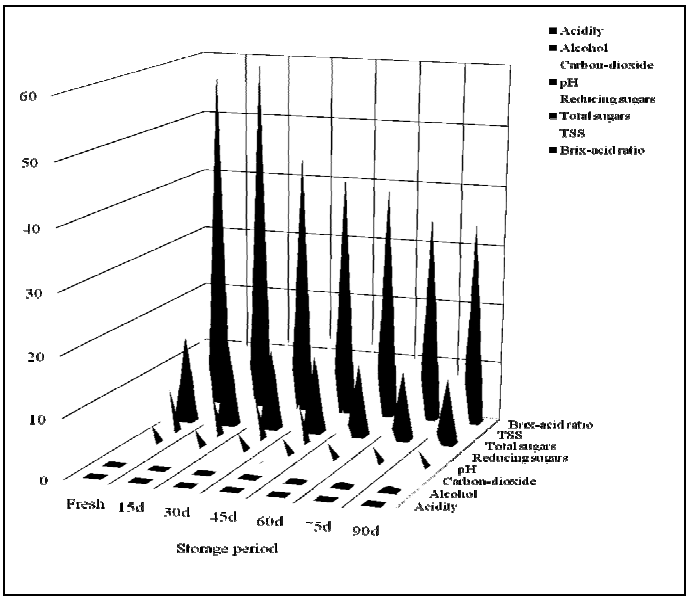

Fig. 1 : Physicochemical properties during storage period

Conclusions

The final yield beverage product of *Citrus limon* has pH 2.6, TSS 14.1^{0}B, per cent acidity 0.41, ascorbic acid 2.00 mg/100ml, alcohol % (v/v) 0.803 and CO_2 (Bar) 1.50. The average sensory scores for astringency shoot up from 7.6 to 8.4, while that of aroma augmented from 7.6 to 8.0 after a period of 60 days. The beverage was found to be stable for a period of three months.

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