

Manufacturing of a novel naturally carbonated fruit beverage

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ABSTRACT

Around 70% of the total plum production in Iran is exported or consumed locally. The rest is not marketable due to its low quality or its extra offer. Novel yeast owning peculiar property of producing high carbon dioxide concentration without alcohol production was isolated from Iranian white cheese whey. The yeast *Candida colliculosa* was identified morphologically and biochemically and its desired property of natural carbonation was exploited to produce a non-alcoholic naturally carbonated plum beverage. The fruit beverages were prepared using two plum varieties; yellow Golden Drop (*Prunus domestica* ssp. *Syriaca/Mirabelle*) and red Ghermez (*Prunus divaricata var. caspica*). The prepared beverages had acceptable sensory quality, high carbonation (~1.6 bar) and physico-chemical properties at the end of 90th day of storage under refrigerated condition.

KEYWORDS: *Candida colliculosa*, plum, physicochemical properties, sensory quality.

INTRODUCTION

There are about 75 native varieties of plum and prune in Iran [1]. These varieties, with diverse color (purple, green, bright to dark yellow, orange or red) and shape (within a round, oval, conical, or heart-like shape) are cultivated well in Iran. The yield of plum and prune in the world and Iran in 2007 has been about 4 and 10 mt/ha, respectively [2]. Plums are rich in nutritional substances such as vitamins, minerals and antioxidants and have several health benefits. The latest provide protection from the superoxide anion radical and prevent damage to our neurons and the fats that are part of our cell membranes. However, more than 30% of the total quantity of plum produced in Iran is wasted annually [3].

The major problem arising in the food industry for fruits is mainly reported as the long term storage. Different methods have been adapted to extend fruit shelf life, but most of them are including chemical preservation which may cause toxic and adverse effects, while some of these methods may not be efficient [4]. Such inadequate conditions during storage lead to spoilage, nutrient loss and structural defects in fruits. The fermented beverage retains nutrients, and additionally produced CO₂ is anti microbial and adds tangy taste, fizz and sparkle to the beverage [5]. The natural antimicrobial property of CO₂ produced during fermentation has been exploited for extending the shelf life. CO₂ is effective against gram-negative aerobic spoilage bacteria and psychotropic bacteria. In fact, it has been established that CO₂ penetrates cells by diffusion across the membrane, and thus interferes with cytoplasmic enzymes and influence cellular metabolism [6].

The aim of the present investigation was to exploit the peculiar property of yeast isolate, *Candida colliculosa*, for production of an acceptable non-alcoholic naturally carbonated plum beverage from two major Iranian plum varieties, i.e. Golden drop (*Prunus domestica* ssp. *Syriaca/Mirabelle*) and Ghermez plum (*Prunus divaricata var. caspica*), respectively.

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MATERIALS AND METHODS

Isolation and Identification of yeast isolates

Iranian white cheese was prepared by inoculating starter mesophilic culture (CHOOZIT 230, Bulk cultures, Danisco, Germany) containing *Lactococcuslactis* subsp. *Lactis* and *Lactococcuslactis* subsp. *Cremoris* and thermophilic yoghurt culture (YO-MIX 532, Bulk cultures, Danisco, Germany) containing *Streptococcus thermophiles* and *Lactobacillus delbruckii* subsp. *Bulgaricus* [17]. Whey collected during preparation of cheese was heated at 84°C for 15 min, cooled to 35°C and inoculated with 2% of the same mixed starter cultures of mesophile and thermophile (1:1) used in preparation of cheese. After 8 hrs fermentation at 35°C, the pH of fermented whey was reduced to 4.5 and precipitate was separated from whey filtrate.

This precipitate usually contained more than 4% protein. This fermented whey protein concentrate was used in fermented whey beverage making after adjustment of its total solids to 6%. The whey beverage was again incubated for 12 hrs at 30°C and kept at refrigerator for one week for natural carbon dioxide production. The yeasts were isolated on spread plates of yeast extract glucose chloramphenicol agar after making serial dilutions [18]. The plates were incubated at 25°C for 72 hrs. Colonies with distinct morphological differences were selected and purified by streaking on potato-dextrose agar (PDA). The yeast isolates were maintained at 4 °C on PDA slants and subcultured fortnightly.

A total of ten morphologically identical yeast colonies were screened and isolated from whey beverage which on streak purification revealed one distinct colony type. The data from the taxonomic researches (colonial and cell morphology and physiological characteristics) were analyzed by using the Manuals of Kreger van Rij, Kurtzman and Fell, and Barnett et al. [19-21]. The yeast isolate was selected which able to produce less alcohol (< 0.1%, w/v) and high CO₂ (1.2-1.6 bar). This isolate was exploited to produce non-alcoholic naturally carbonated beverages.

Preparation of yeast inoculums

A loopful of 24 hrs old activity growing yeast culture was inoculated in 500 ml of Erlenmeyer flasks containing 250 ml Glucose yeast extract (GYE) broth. It was incubated at 25 °C for 24 hrs to prepare inoculums [5].

Fruit juice preparation

Fresh matured and healthy red and yellow plums were purchased from the local retail market and were brought in wooden crates to the Central Food Chemical Laboratory of the Department of Food Science and Technology, Ramin Agricultural and Natural Resources University (Khuzestan), Mollasani where research work was carried out. Plums were thoroughly washed with running tap water and any fruit with signs of defect and immaturity sorted out. The juices were extracted from fruits by food processor and filtered with muslin cloth.

Fruit beverage preparation

Fruit juices were diluted in the ratio 1:2 with water, pasteurized at 82 °C for 15 s [22] and rapidly cooled to ambient temperature (25 °C). Brix of fruit beverages were adjusted to 14 °Bx by adding pasteurized sugar solution.

Sugar solution

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

Fruit beverage fermentation

The yeast inoculum was added to the yellow and red plum beverages (0.5%, v/v) individually. The inoculated beverages incubated at ambient temperature (25 ±1°C) for 24 hrs. The beverage was then cooled, bottled (300 ml glass bottles) and stored in refrigerated conditions, i.e. 7 °C.

Study of yeast growth in beverage

The yeast kinetics was studied and employed for carrying out fermentation. The yeast concentration was determined spectrophotometrically by measuring the absorbance (optical density or OD) of the beverage at 600 nm during fermentation and storage period. Cell biomass was also measured gravimetrically as dry cell weight after cell separation through centrifugation at 4000 rpm [23].

Physico-chemical analysis

Changes in various parameters including pH, titrable acidity, TSS, brix/acid ratio, dry weight, alcohol and CO₂ concentration was also studied periodically from beginning of beverage fermentation up to end of 90 days of storage. The pH of the juice was determined using a digital pH meter (Genway Company, Model 3505, UK). Acidity was determined by titrating with 0.1 N NaOH using phenolphthalein as indicator [24]. Total acidity expressed as % anhydrous citric acid. Percent total soluble solids (%TSS) determined by using digital refractometer of 0-53°Bx (ATAGO Pal-1). The direct colorimetric method was used for the measurement of vitamin C (L-ascorbic acid) which is based on the measurement of the extent to which a 2, 6-dichlorophenol-indophenol solution is decolorized by ascorbic acid in sample extracts and in standard ascorbic acid solutions [25]. Reducing sugars was determined according to Ranganna [26] by employing Lane–Eynon method. Percentage of alcohol (v/v) in beverage was estimated by spectrophotometric method [27]. Carbon dioxide Pressure was measured with a manometer supplied with pin and special apparatus to fit at the mouth of bottles. All chemicals were purchased in analytical and purified grade from Sigma Chemical Co. (St. Louis, MO) and Merck (Darmstadt, Germany).

Microbial evaluation

Total yeast count was enumerated on yeast extract glucose chloramphenicol agar by serial plate dilution and pour plate method. A standard curve of optical density versus yeast count that covered the appropriate range of concentrations was made with a series of five prepared suspensions.

Sensory evaluation

A panel of trained 5 judges evaluated the samples for color, appearance, texture, taste, aroma and overall acceptability using a 9 score scale through hedonic test, where 0 indicates dislike extremely and 9 like extremely [28].

Statistical analysis

All analyses were performed in triplicate. The data collected from studies were analyzed using SPSS program version 16.0.2 [29]. Analysis of variance (ANOVA) was carried out to determine significant differences. Duncan's multiple-comparison test was used as a guide for pair comparisons of the treatment means. The level of significance for all analysis was done at $p \leq 5\%$.

RESULTS

Physico-chemical changes during fermentation and storage

The results presented in Table 3 indicate that significant changes occurred in physical and chemical parameter during the fermentation of juice under optimized fermentation condition.

Sensory evaluation

Statistical analysis revealed that the kind of plum beverages and storage periods have not significant effect on all the sensory attributes of naturally carbonated beverages. However, yellow plum beverage samples, except for color, had higher sensory scores than red plum beverages (Table 4). Furthermore, there was a declining trend towards all sensory parameters after 30 days of storage at refrigerated condition.

Table 1. Physico-chemical changes during fermentation and storage of two naturally carbonated plum (red and yellow) beverages

Parameters	<i>Red plum (P. divaricatavarsaspica)</i>		<i>Yellow plum (P. domestica ssp. Syriaca)</i>	
	Initial value	Final value	Initial value	Final value
pH	3.2	2.9	3.4	3.2
TSS ¹ (°Bx)	14	13.1	14	13.4
Titration acidity (%) ²	0.59	0.71	0.44	0.53
Brix/acid ratio	23.73	18.45	31.82	25.28
Reducing sugar (%)	7.83	6.09	7.21	5.64
Vitamin C (mg/100g)	16.4	9.8	17.4	10.0
Alcohol (%w/v)	0	0.1	0	0.1
CO ₂ (bar)	0	1.59	0	1.52

¹TSS: Total soluble solids; ²Calculation based on citric acid; values are means of three replicates

Table 2. Sensory score of fermented beverages obtained from two plum varieties

Sensory attributes	<i>Red plum (P. divaricatavarsaspica)</i>		<i>Yellow plum (P. domestica ssp. Syriaca)</i>	
	After one week	After 90 days	After one week	After 90 days
Appearance	8.4	7.7	8.4	7.9
Color	8.6	7.4	8.2	7.3
Texture	8.3	8.0	8.5	8.1
Aroma	8.6	8.0	8.7	8.0
Taste	8.5	7.8	8.4	7.9
Overall acceptability	8.2	7.9	8.5	8.0

Values are means of three replicates

The mean values of overall acceptability for red and yellow plum beverages after one week maturation under refrigerator temperature were determined as 8.2 and 8.5, and after 90 days of storage reached to 7.9 and 8.0, respectively.

DISCUSSION

Whey microflora

High numbers of yeasts were present in Iranian white cheese whey beverage ranging from 3.3 to 6.9 cfu/ml, with a mean value of 6.1 cfu/ml. Kaminarides and Laskos[30] suggested that yeasts may play a significant role in the preservation of Feta cheese and attributes to chemical and organoleptic qualities. The considerable amount of yeasts is probably due to nutritious substances (water soluble protein, inorganic salts, lactose, casein particles, etc.) existing in whey that arise by continuous migration from cheese to whey/brine [7].

Physico-chemical changes during fermentation and storage

Decrease in pH, TSS (°Bx) and increase in CO₂ concentration and titration acidity were observed during 90th day of storage. Sahuta *et al.* [22] also reported constant decrease in brix (15.0 to 12.1°Bx) and pH (2.9 to 2.6) till the end of 90 days storage of naturally carbonated blended fruit beverages. Furthermore, Sirohi *et al.* [35] observed an increase in acidity with decline in pH throughout storage of whey based mango beverage. The decrease in pH and the increase in acidity are attributed to the production of CO₂ that forms weak acid on dissolution. Ocloo and Ayernor [36] also reported decrease in pH values with increased total acidity with concomitant increase in yeast growth and alcohol contents of the fermenting sugars syrup.

The decrease in soluble solid contents and reducing sugar content was also observed owing to disappearance of carbohydrates in the fermenting medium and rapid multiplication of yeast cells. However, final alcohol concentration in beverage was less than 0.1% due to non-alcoholic nature of fermentation by the yeast *Candida colliculosa* and also due to very less utilization of sugars during

fermentation as evident in reduction of TSS from 14 to 13.1 and 14 to 13.4 for red and yellow plum varieties, respectively. As it shown in Table 3, the titratable acidity, expressed as citric acid, ranged from 0.59 to 0.71% for the red plum juice beverage, and from 0.44 to 0.53% for the yellow variety. The reducing sugar of red and yellow plum beverages ranged from 7.83 to 6.09% and from 7.21 to 5.64%, respectively. After 90 days of storage at 7 °C, the overall loss of ascorbic acid was 40.24 and 42.53% in the red and yellow plum beverage samples, respectively. The decrease of ascorbic acid concentration during storage can be attributed to vitamin C oxidation [37].

Sensory evaluation

The acceptability and sensory quality of beverages is to a great extent dependent on its physicochemical properties particularly its acidity, TSS and brix/acid ratio. The brix/acid ratio is a commonly used quality index in much type of fruits [38] to measure fruit maturity and palatability. However, it may also be used as a sensory index for evaluation of fruit beverages [39]. According to the results, the brix/acid ratio ranged from 23.73 to 18.45 for red plum variety and from 31.82 to 25.28 for yellow plum beverages. The decrease in brix/acid ratio during storage period may adversely influence the sensory quality of beverages. However, the decrease in sensory quality mainly could be attributed to the physicochemical deterioration and loss of some compounds which may impart flavor and aroma to the final beverages during storage.

Conclusion

Increased interest in the beneficial health effects from consuming plum fruit resulted in the current research aimed to produce a high nutritious fruit juice beverage. The beverage was naturally carbonated by fermentation; an operation which enhances the original flavor of the fruit juices yet leaves not the slightest after-taste of yeast nor reduces the natural acidity of the juices. A pure yeast isolate *Candida colliculosa* from whey beverage, morphologically and physiologically characterized, was used to develop a reliable, controllable and reproducible technology for preparation of low alcoholic naturally carbonated beverage from yellow plum (*Prunus domestica* ssp. *Syriaca/Mirabelle*) and red (*Prunus divaricata var. caspica*) varieties. The results from this study showed that plum beverages with low alcohol and high CO₂ concentration can produce using isolated yeast from fermented whey beverage. Both plum beverages scored 'liked very much' to 'liked moderately' due to naturally produced CO₂ during fermentation that added effervescence, sparkle and tangy taste to the beverage.

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