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Production of Non-Alcoholic Fermented Plum Beverages

Hossein Jooyandeh

Department of Food Science and Technology,
Ramin Agricultural and Natural Resources University, Ahvaz (Mollasani), Iran

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. A 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.

Key words: Naturally carbonated beverage · Plum · Candida colliculosa · Physico-chemical property · Isolation

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 CO_2 is anti microbial and adds tangy taste, fizz and sparkle to the beverage [5]. The natural antimicrobial property of CO_2 produced during fermentation has been exploited for extending the shelf life. CO_2 is effective against gram-negative aerobic spoilage bacteria and psychotropic bacteria. In fact, it has been established that CO_2 penetrates cells by diffusion across the membrane and thus interferes with cytoplasmic enzymes and influence cellular metabolism [6].

The incidence of yeast in Feta [7, 8] and white-brined cheeses like Iranian [9], Turkish [10] and Sudanese white cheeses [11] is usually high. Yeasts are not added as a part of starter culture during cheese manufacture; however, relatively high counts of them are frequently observed in many soft, semisoft and surface ripened cheeses, probably originating from the processing equipment and the dairy environment [12]. According to Tamime and Kirkegaard [7], permissible limit of the mold count in Feta cheese is 100 cfu/g. However, there is no specific standard for the count of yeast in Feta and similar cheeses. Although yeasts are not predominant microflora in Feta [13, 14] or in white-brined cheeses [15], as a typical

Corresponding Author: Hossein Jooyandeh, Department of Food Science and Technology,
Ramin Agricultural and Natural Resources University, Ahvaz (Mollasani), Iran.
Tel: +98-612322-4341, Fax: +98-612322-2110.

yeast cell has a volume at least 50 times the volume of the largest bacterial cell, the larger biomass of the yeasts might indicate that their role in the maturation of cheese is more important than the counts would suggest [16].

Particular conditions of Iranian white cheese whey/brine such as low pH, abundant water soluble components and long refrigerating storage period influence the existence of its different yeast strains. Therefore, presence of halophilic, psychrotrophic and thermophilic yeasts along with their divers and peculiar properties such as ability to produce excessive carbonation is not unexpected.

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.

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 *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *Cremoris* and thermophilic yoghurt culture (YO-MIX 532, Bulk cultures, Danisco, Germany) containing *Streptococcus thermophilus* 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 sugars used for biochemical analysis were glucose, galactose, maltose, sucrose, lactose, ribose, xylose, L-arabinose, sucrose, D-arabinose, trehalose, cellobiose, melibiose, salicin, raffinose, inulin, manitol and melezitose. The yeast isolate was selected which able to produce less alcohol (< 0.1%, w/v) and high CO_2 (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].

Chemicals: All chemicals were purchased in analytical and purified grade from Sigma Chemical Co. (St. Louis, MO) and Merck (Darmstadt, Germany).

Physico-Chemical Analysis: Changes in various parameters including pH, titrable acidity, TSS, brix/acid ratio, dry weight, alcohol and CO_2 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.

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 AND 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. They also isolated and identified high numbers of yeasts (128 different yeast colonies), with the most common species found to be *Saccharomyces* (59%), *Candida* (17%) and *Pichia* (12%). 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 continiouse migration from cheese to whey/brine [7].

Identification of Yeast: The physiological and biochemical researches of the yeasts strains were performed, by using over 30 tests for assimilation of carbon and nitrogen sources. The results are shown in Table 1. Of the total yeast, the genera *Saccharomyces* and *Candida* were the predominant of yeasts isolated from all naturally carbonated whey beverage samples. The yeast isolates were identified as *Saccharomyces cerevisiae var. ellipsoideus* (No. 1), *Candida colliculosa* (No. 2), *Candida crusei* (No. 3), *Kluyveromyces lactis var. lactis* (No. 4) and *Mycotorula rugosa* (No. 5) base on physiological and biochemical criteria.

The isolate *Candida colliculosa*, which come across a unique character of producing infinitesimal alcohol and elevated CO_2 concentration, was exploited to produce non-alcoholic naturally carbonated fruit beverage from two red and yellow plum varieties.

Kinetics of Yeast in Beverages: Growth kinetics of yeast *Candida colliculosa* was studied in juices of two plum varieties: yellow (*Prunus domestica ssp. Syriaca/Mirabelle*) and red plum (*Prunus divaricata var caspica*) to understand the growth and metabolic activities of yeast throughout fermentation and its

Table 1: Biochemical and morphological characteristics of yeast isolates from whey beverage

	Yeast isolates.				
Characteristics/ Assimilation	1	2	3	4	5
Color	cream-dull	cream-shiny	cream-yellow	cream-dull	white, semi-dull
Surface	smooth	smooth	rough-acicular	smooth	wrinkled verrucose
Elevation	slightly convex	convex	convex	slightly convex	convex
Cell shape	RO-OV	RO-EL	EL, CY, FL	EL	EL, FL
D-Glucose	+	+	+	+	+
D-Galactose	+	-	-	+	+
Maltose	+	+	-	+	-
Sucrose	+	+	-	+	+
Lactose	-	-	-	+	-
Trehalose	+	±	-	±	-
Melibiose	-	-	-	-	-
D-Xylose	+	±	±	±	-
L-arabinose	-	±	-	-	-
D-arabinose	-	±	-	-	+
Raffinose	+	+	-	+	-
Inulin	-	+	-	-	+
Dulcitol	-	-	-	-	-
Inositol	-	-	-	-	-
L-Sorbose	±	±	-	+	+
D-Mannitol	±	+	-	+	+
D-Ribose	±	±	-	-	-
L-Rhamnose	+	-	-	-	-
Cellobiose	+	±	-	+	-
Melezitose	+	±	-	+	-
Xylitol	+	+	-	+	+
Galactiol	+	+	-	+	+
DL-Lactate	+	+	+	+	+
Ethanol	+	±	+	+	+
Methanol	-	-	-	-	-
Nitrate	-	-	-	-	-
Citrate	+	±	+	-	-
Succinate	±	+	+	+	+
Glycerol	±	±	±	+	+
Salicin	-	±	-	+	-
D-Glucosamine	-	-	-	-	-

+: fermentation; -: no fermentation; ±: slow fermentation; R: Round; O: Oval; E: Elliptical; C: Cylindrical; F: Filamentous

interaction during fermentation with respect to optical density (OD), viable cell count, cell biomass, CO_2 production, ethanol production and its effect on pH, titrable acidity (% citric acid), TSS (°Bx), brix/acid ratio. Results showed no significant differences between two plum beverages varieties in terms of experimental growth parameters, therefore only results related to red plum beverage are shown in Table 2.

The growth curves of optical density, viable cell count and cell biomass were found to be show normal patterns with first a short lag period of 6 hrs followed by exponential growth up to 24 hrs. Results in Table 2 indicate sharp increase in optical density, viable cell count and cell biomass during this time. The corresponding increase in values optical density, viable cell count and cell biomass were 0.19-0.49, 6.64-9.23 log cfu/ml and 0.11-0.64 respectively (Table 2). After 15th day of storage, viable count and OD were decreased, showing death phase. Decrease in yield of growth and acceleration of yeast death may be due to inadequate supply of nitrogenous substances, vitamins, concentration of dissolved oxygen and concentration of insoluble solids.

In similarity to these results, Sahuta *et al*. [22] found high concentration of yeast (8.5×10^9 cfu/ml) in fermented mixed guava and lemon juice beverages after 90 days of storage, though they reported constant increase of yeast population throughout of storage period. Giovanelli *et al*. [31] also investigated kinetics of grape juice fermentation under aerobic and anaerobic condition and found that under anaerobic condition sugars were exhausted within 200 hrs and number of cells reaches to 8 log cfu/ml.

Table 2: Growth of yeast in red plum beverage in terms of optical density (OD), cell count, cell biomass and its effect on production of alcohol and CO_2

Time after*	OD at 600 nm	Viable count (log cfu/ml)	Cell biomass (g)	Alcohol (%w/v)	CO_2 (Bar pressure)
(hrs)					
0	0.00^{e}	6.11^{f}	0.00^{e}	0.00^{c}	0.00^{f}
3	0.11^{de}	6.20^{f}	0.00^{e}	0.00^{c}	0.00^{f}
6	0.19^{cd}	6.64^{ef}	0.11^{e}	0.00^{c}	0.00^{f}
12	0.30^{c}	7.82^{d}	0.49^{d}	0.02^{c}	0.14^{f}
24	0.49^{b}	9.23^{c}	0.64^{c}	0.06^{b}	0.51^{e}
48	0.58^{ab}	10.38^{ab}	0.77^{b}	0.07^{ab}	0.86^{d}
Day(s)					
3	0.63^{a}	10.41^{ab}	0.83^{ab}	0.09^{a}	1.25^{c}
7	0.66^{a}	10.71^{a}	0.86^{ab}	0.10^{a}	1.30^{bc}
15	0.65^{a}	10.59^{ab}	0.89^{a}	0.10^{a}	1.44^{abc}
30	0.62^{a}	10.50^{ab}	0.90^{a}	0.10^{a}	1.50^{ab}
60	0.61^{ab}	10.03^{b}	0.92^{a}	0.09^{a}	1.60^{a}
90	0.57^{ab}	9.95^{b}	0.92^{a}	0.09^{a}	1.56^{a}

^{a,b,c} Means in the same column having different letters are significantly different ($P<0.05$); * 24 hrs incubation at 25°C and then storage at refrigerator temperature; values are means of three replicates; OD: optical density

Table 3: Physico-chemical changes during fermentation and storage of two naturally carbonated plum (red and yellow) beverages

	Red plum (*P. divaricata var caspica*)		Yellow plum (*P. domestica ssp. Syriaca*)	
Parameters	Initial valu	Final value	Initial valu	Final value
pH	3.2	2.9	3.4	3.2
TSS[1] (°Bx)	14	13.1	14	13.4
Titrable 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

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

Physico-chemical analysis during fermentation showed that low amount of ethanol (<0.1% w/v) and high concentration of CO_2 (1.6 bar) was produced at the stationary phase of cell (Table 2). The alcohol content after 7 days reached up to constant level 0.1 percent v/v and finally reached up to 0.09 percent v/v after 90 days. The low concentration of alcohol in final product may be due to the high concentration of CO_2 which has adverse effect on alcohol production. Cahill *et al*. [32] reported fourfold inhibition of yeast growth rate and alcohol production with increase in CO_2 pressure. The fermentation time of the beverage depends, among other things, on the yeast which is used and the level of alcohol which is not to be exceeded in the end product. The level of alcohol in food and beverage in Islamic countries like Iran and Pakistan should be less than 0.1%, since alcohol is forbidden. Consumer products with added ingredients that contain alcohol must have less than 0.1% ethanol, including both added and any natural ethanol, to qualify as halal. At this level, one cannot taste, smell, or see the alcohol, a criterion generally applied for impurities [33]. Therefore the time for fermentation and subsequent storage temperature was so adjusted that lower amount of alcohol and higher concentration of CO_2 was obtained. The CO_2 pressure of 0.51 bar starts after one day and increased to 1.44 bars after 15 days and reached up to maximum level 1.60 bars after 60 days and thereafter was slightly decreased at the end of 90 days of storage and reached to 1.56 bars. Ilamaran and Amutha [34] also reported gradual decrease in pressure content of carbonated banana beverage during storage.

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.

Decrease in pH, TSS (°Bx) and increase in CO_2 concentration and titrable 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,

Table 4: Sensory score of fermented beverages obtained from two plum varieties

	Red plum (*P. divaricata var caspica*)		Yellow plum (*P. domestica ssp. Syriaca*)	
Sensory attributes	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

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 CO_2 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: 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. 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.

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.

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 CO_2 concentration can produce using isolated yeast from fermented whey beverage. Both plum beverages scored 'liked very much' to 'liked moderatly' due to naturally produced CO_2 during fermentation that added effervescence, sparkle and tangy taste to the beverage.

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