## Investigation of the Effects of Carbonation and Orange Juice on the Physical, Chemical and Microbial Characteristics of Pasteurized Carrot Juice

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#### Abstract

The effect of  $CO_2$  (0.5 l min<sup>-1</sup> for 15 min) and orange juice (30% v/v carrot juice) on phisico-chemical characteristics as well as microbial flora of carrot juice within the three months of storage was investigated. Both orange juice and  $CO_2$  caused a decrease in pH value of carrot juice but pH reduction was higher when orange juice (30 percent) was added. Samples of carrotorange juice had higher value of acidity and lower pH. Blending orange juice with carrot juice caused higher percentage of precipitation, decrease in a\* and b\* values and increase in L\* value. The results of this experiment showed that sugar decreases the percentage of precipitation in pasteurized carrot juice. No microbial growth was detected in either of the treatments.

Keywords: Carrot-orange juice, Carbonation, Physico-chemical, Storage time, Microbial, Sugar content

### **1-Introduction**

Fruit and vegetable juices are recommended for a healthy diet and various health benefits [1]. Fruit juices are rich sources of vitamin C, E, phenolic compounds, carotenoids and can prevent type of certain diseases (cancer and cardiovascular) [2-6].

There are different treatments which makes juices more acceptable for consumers. Beverages containing carbon dioxide are very popular products nowadays [7-8] and carbonated carrot juice can be appreciable as one of these products. Carbon dioxide enhances the taste of beverages and their appearance [9]. In addition , CO<sub>2</sub> can cause a decrease in pH and at high pressures

has an inhibitory effects on microbial growth [10] but high levels of gas spoil the flavour of fruit and vegetable juices [11]. Blending with orange juice can be considered as another treatment of carrot juice that in addition to induce the special sensory and nutritive characteristics, it provides a good source of functional compounds, vitamins and antioxidants that contribute to the health of the consumers [12]. The growth of some bacteria, mold and yeast flora in mixed orange and carrot juice [13-15] and the effect of PEF (pulsed electric field) and heat pasteurization on some physical- chemical characteristics of carrot-orange juice [16] have been

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investigated up to now.

The objective of this study was to evaluate the influence of CO<sub>2</sub>, orange juice and sugar addition on some chemical, physical, sensorial and microbial characteristics of pasteurized carrot juice.

## 2-Materials and Methods 2-1-Treatment of Carrot Juice

Fresh carrots (*Daucus carrota* L. cv. Alpha) of good quality were purchased from a local market and stored at 5 °C. Juices were extracted and prepared as follows:

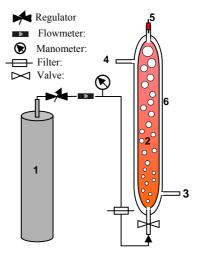
After peeling and washing with water (manual), the carrots were grinded with grinding mill (Kenwood FP/690, England). As acidification before juice extraction can prevent clarification of carrot juice, citric acid (0.5 g kg<sup>-1</sup>) (BBCA company, Arhui, China) was added and then the mash was heated (70 °C, 10 min) in water bath (Memmert, WB14, Germany) for blanching. To increase the yield of juice extraction, the enzymes of pectinase (Rohapect PTE) and cellulase (Rohament CL) prepared from AB-Enzyme Company (Darmstdat, Germany) were added and mash incubation was performed at 40 °C for 45 minutes in incubator (FOC 225I, Europe). After manual juice extraction, clarification was done by centrifuge at 3000 rpm for 10 minutes (Kubota 6900, Japan). Pomace was separated and the juice was pasteurized (95 °C, 1 min) in rotary (Heidolph 4001, Germany) [17-18].

Concentrated orange juice with °Brix of 70 was purchased from Ramsar Gardeners Factory (Ramsar, Iran) and reconstituted with water to obtain a regular orange juice. To prepare carrot-orange juice blend, carrot (70%), orange (30%) and sugar (4%) were mixed.

For treating pasteurized carrot juice with  $CO_2$ , a glass instrument with a cold water jacket was designed (Fig. 1).

Since  $CO_2$ , dissolves better at low temperatures [7] the jacket was designed.  $CO_2$  was bubbled through carrot juice from a gas cylinder via a plastic tube at a rate of approximately 0.5 1 min<sup>-1</sup> for 15 minutes.

The juice was then bottled in sterilized glass bottles with screw caps and they were stored at 4  $^{\circ}$ C for three months.



**Fig. 1**. Carbonation unit: (1) CO<sub>2</sub> cylinder; (2) carrot juice; (3) water inlet; (4) water outlet; (5) gas outlet; (6) glass tank.

A total of four juice treatments were produced to compare with carrot juice alone (C) as control. Treatments were: the pasteurized carrot juice with 4% sugar (CS), the pasteurized carrot juice treated with CO<sub>2</sub> (CC), the pasteurized carrot juice with 4% sugar and the samples treated with CO<sub>2</sub> (CSC) and the mixed carrot orange juice including 4% sugar (COS). All the samples were stored at 4 °C and their physicochemical and microbiological characteristics were investigated at the first of storage and during three months of storage with one week intervals.

#### **2-2-Physico-chemical Analysis**

pH measurements were performed using a Metrohm model 744 pН meter (Switzerland). Titrable acidity was determined potentiometrically using 0.1 N NaOH and phenolphthalein (indicator). The results were expressed as g 100 ml<sup>-1</sup> on the basis of citric acid. Total soluble solids (TSS) was measured by the refractive index in terms of °Brix with an Atago N1 refractometer (Japan) [19]. Percentage of precipitation was determined after calculating the height of sediment that accumulated at the bottom of the test tubes. Color of the juices was measured by Hunter Lab model Colorflex 40/0 (USA) in mode of Daylight color. Letters  $L^*$ ,  $a^*$ , and  $b^*$ represent luminosity or lightness, redness and yellowness, respectively [20].

Each sample was assessed at the first of storage and for three months of storage with one week intervals except color which was measured with one month intervals.

#### 2-3-Microbial Analysis

To evaluate the microbiological efficiency of the treatments, one ml of each sample without dilution was cultured (1ml/plate) to determine the number of colony forming units (CFU/ml) using pour plate technique. Media of plate count agar (Merck) for aerobic mesophyl bacteria, orange serum agar (Merck) for acidophyl bacteria and yeast extract glucose chloramphenicol agar (Merck) for yeasts and moulds were used [21].

#### **2-4-Sensory Analysis**

25 untrained volunteers were selected for sensory analysis. The samples of carbonated pasteurized carrot juice with 4% sugar (CSC), carrot orange juice blend with 4% sugar (COS) along with carrot juice alone (C) as control were coded and after ranking the samples by assessors, preferred sample was determined [22-23].

#### **2-5-Statistical Analysis**

Experimental data were analyzed by analysis of variance (ANOVA) with significance defined at P < 0.05, and significance differences between the means were determined by Duncan's multiple range test. SPSS software version 12.0 was performed for the statistical analysis. All the reported values are the means of triplicate determinations.

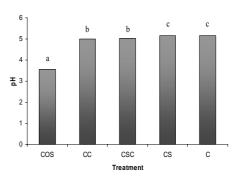
### **3-Results and Discussion**

The effect of the treatments on chemical (pH, acidity, °Brix, percentage of precipitation, color) and microbial flora were as follows:

#### **3-1-Effect on pH**

Results showed that sample of blended carrot and orange juice (COS) had the lowest pH value because of lower pH of orange juice and the samples treated with  $CO_2$  (with and without sugar) were in the same group without any significant difference (Fig. 2). These treatments with significant difference had lower pH value than the control sample and carrot juice with 4% sugar. Alklint *et al.* (2004), also reported that adding  $CO_2$  bubbles decreases the pH of carrot juice [24].

There was no pH variation during storage period which is in good agreement with the results reported for blended orange and carrot juice (80% orange and 20% carrot) [16], pasteurized carrot juice [25], and orange juice treated with Pulsed Electric Fields (PEF) [26].



**Fig. 2**. Effects of different treatments on pH. Different letters show significant difference between the treatments (p < 0.05) by duncan's multiple range test.

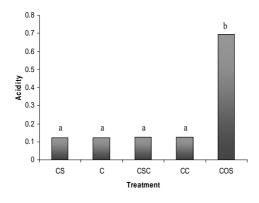
#### **3-2-Effect on Titrable Acidity**

With reference to our findings (Fig. 3) all the samples were located in two groups. Among the samples, the highest acidity allocated to treatment of blended carrot and orange juice (COS) which is because of higher acidity nature of orange. In another word, this sample had the lowest pH and the highest acidity.

Although the titrable acidity of CSC and CC treatments were slightly higher than the

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CS and C samples but there was no significant difference between them. statistically. According to Woodroof and (1981)findings. Philips carbonation increased the sour flavor and titrable acidity of treatments including grape juice and blended grape and apple juice [27]. The acidity of samples did not vary (p < 0.05) during storage but Rivas et al. 2006 recorded that during storage, the sample of blended orange and carrot juice treated with PEF showed an increase in total acidity after 7.5 weeks of storage at 12 °C [16].

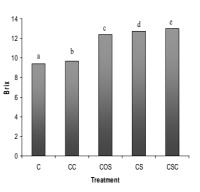


**Fig. 3**. Effects of different treatments on titrable acidity. Different letters show significant difference between the treatments (p<0.05) by duncan's multiple range test.

# **3-3-Effect on Total Soluble Solids**

With reference to information of variance analysis (not shown) and Fig. 4 all treatments had significant difference. It can be mentioned that because of higher content of soluble solids, especially sugars of carrot juice, treatments which contain carrot and sugar showed the greatest °brix. As orange juice has less soluble solids than carrot juice, with participation of orange in a mixed juice, the °brix decreased.

In fruit and vegetable juices, fermentation of sugars by microorganisms can cause spoilage. Therefore, °brix will change. Due to absence of microbial spoilage in our treatments, there were no significant changes in °brix. These findings are similar to the ones reported by Rivas *et al.* 2006 and Yoem *et al.* 2000 [16, 26].



**Fig. 4.** Effects of different treatments on °brix. Different letters show significant difference between the treatments (p<0.05) by duncan's multiple range test.

# **3-4-Effect on Percentage of Precipitation**

As it can be seen (Fig. 5), treatments of carrot juice which contained 4% sugar and carbonated carrot juice with 4% sugar had lower percentage of precipitation. This behavior seems to reflect the thickening effect of sugar in solutions such as juices [28]. But the sample of COS had the greatest percentage of precipitation. As orange juice has a high acidity, after mixing it with carrot juice, the proteins were coagulated and their tendency to precipitation increased.

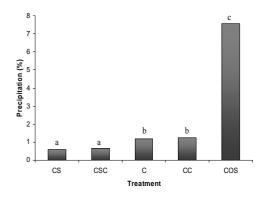
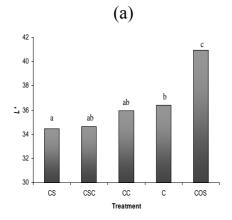
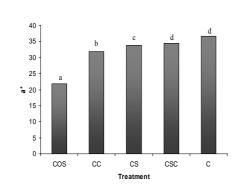


Fig. 5. Effects of different treatments on percentage of precipitation. Different letters show significant difference between the treatments (p<0.05) by duncan's multiple range test.

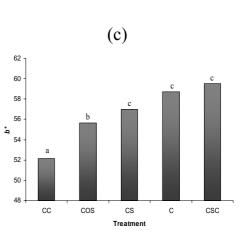
#### **3-5-Effect on Color**

To evaluate of color three parameters  $(L^*)$ .  $a^*$  and  $b^*$ ) that represent lightness, redness and yellowness, respectively were measured (Fig. 6). The highest  $L^*$  and lowest  $a^*$  was related to COS treatment. Citrus have great lightness [29], as a result the  $L^*$  of mixture of orange-carrot juice was higher than other treatments. Since  $\beta$ -caroten is the major carotenoid (60-80%) of carrot pigments which is responsible for orange-red color, with decreasing of carrot in mixed carrot orange juice, the  $a^*$  value decreased [30]. With regard to the effect of storage at 4 °C for 3 months on color parameters, no significant variation was found. It needs to be mentioned that also Rivas et al. 2006 found the same result for  $L^*$  of the PEFtreated samples and opposite result for  $L^*$ of pasteurized blended orange and carrot juice in which it diminished (at 12°C) [16].





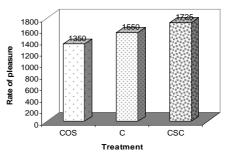
(b)



**Fig. 6**. Effects of different treatments on color parameters:  $L^*$  (a),  $a^*$  (b) and  $b^*$  (c). Different letters show significant difference between the treatments (p<0.05) by duncan's multiple range test

#### **3-6-Sensory Analysis**

As it shown in Fig. 7, the preferred sample devoted to carbonated carrot juice with 4% sugar. It may be worth to say that this results can be different in different region because of people appetite.



**Fig. 7**. Effect of different treatment of pasteurized carrot orange blend including 4% sugar (COS), pasteurized carrot juice (C) and carbonated pasteurized carrot juice including 4% sugar (CSC) on pleasuring factor.

#### **3-7-Microbial Analysis**

During the time of this experiment no microbial growth including aerophil bacteria, acidophil bacteria, yeast and mold was observed.

## **4-Conclusions**

On the basis of the results presented it may be concluded that both orange juice and CO<sub>2</sub> cause decrease in pH value and increase in acidity of carrot juice. The highest precipitation related to mixture of carrot and orange juice and in contrast samples treated with sugar and sugar along with CO<sub>2</sub> had the lowest precipitation. It can be mentioned that as the in prolonged shelf life, the precipitation also will increase. The results of this experiment showed that addition of sugar decreases the precipitation percentage of pasteurized carrot juice and addition of orange juice increases the  $L^*$  value and decreases the  $a^*$  and  $b^*$  values. Most probably it is due to the content of pigments in orange juice. During the time of this experiment no microbial growth was observed.

### 5-Acknowledgement

The authors would like to thank the Tarbiat Modares University for financial support.

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## بررسی تاثیرات کربناته کردن و آب پرتقال بر خصوصیات فیزیکی، شیمیایی و میکروبی آب هویج پاستوریزه

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چکيده

تاثیر دی اکسید کربن (۰/۰ لیتر بر دقیقه به مدت ۵ دقیقه) و آب پرتقال (۳۰٪ حجمی آب هویج) بر روی مشخصات فیزیکو شیمیایی و میکروبی آب هویج در طول نگهداری سه ماهه بررسی شد. CO<sub>2</sub> و آب پرتقال باعث کاهش pH آب هویج شدند، لیکن وقتی آب پرتقال (۳۰٪) اضافه شد کاهش pH بیشتر بود. نمونه های مخلوط آب پرتقال و آب هویج اسیدیته بیشتر و pH کمتری داشتند. اختلاط آب پرتقال با آب هویج موجب افزایش رسوب، کاهش در مقادیر \*a و \*b و افزایش \*L شد. نتایج این تحقیق نشان داد که کاهش شکر، درصد رسوب آب هویج پاستوریزه شده را افزایش می دهد. در هیچکدام از تیمارها رشد میکروبی مشاهده نشد.

**کلید واژگان**: آب هویج-پرتقال، کربناته کردن، فیزیکو شیمیایی، زمان نگهداری، مقدار شکر