

## Production of Noncarbonated Lemon Orange Juice

Afshin Salahvarzi, Toktam Mostaghim\*, Mohammad Reza Khani

Department of Food Science and Technology, Shahr-e-Qods Branch,  
Islamic Azad University, Tehran, Iran

\*E-mail: toktammostaghim@yahoo.com

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### Abstract

Citrus fruits are rich in nutrients and the phytochemicals needed for a healthy life. The present study is an attempt to examine production of noncarbonated orange juice with lemon. To this end, 11 juice formulations with different percentages of orange concentrate and natural lemon flavors were prepared and physicochemical (pH, acidity, density, brix, dry mass, and ethanol), microbial (lactic acid bacteria, acid-fast bacteria, alcohol fast bacteria, mold, and yeast), and sensory properties were examined. It was found that increase of percentages of orange concentrate and lemon natural flavor in the formulation led to significant increase in total dry mass, acidity, and ethanol level. On the other hand, as result of this increase, density remained almost unchanged and pH level significantly decreased. The results of microbial tests showed that number of acid lactic bacteria, acid-fast bacteria, mold, and yeast decreased in all treatments and there was no significant difference among the treatments in this regard. The treatments, regarding physicochemical and microbial properties, were at standard range and all the samples were drinkable. Regarding sensory properties, treatment No. 6 (4.6% orange concentrate and 0.08% lemon natural flavor) was better than other treatments as to taste, color, aroma, appearance, and general acceptability. Overall results indicated that 4.6% of orange concentrate and 0.08% of lemon natural flavor was the best formulation of a noncarbonated orange juice

**Keywords:** Lemon natural flavor, orange concentrate, noncarbonated juice

### Introduction

Fruit juices and side-products enjoy a market of millions consumers all around the world. Citrus trees grow in about 50 countries and the fruits are very popular thanks to their good taste and quality. Fruit juice industry appeared when production of citrus fruit exceeded consumption. Citrus fruit industry uses citrus fruits such as orange, tangerine, grapefruit, sweet lemon, and lemon for producing juice and other side products (Antil, 2011). Orange is one of the main citrus fruits and it is produced in different regions of the world and orange produced in each region has different and unique specifications. Mandarin orange (tangerine) is one of main type of orange with soft peel. Satsumas, which mainly grows in Japan, is a key member of Mediterranean region citrus fruits. Another type of citrus fruit is Tangor, which is something between orange and tangerine. Bitter orange is another largely grown type of orange, which is mostly used in South Europe for producing marmalade (Boeing, 2012). Two groups of bitter chemical ingredients found in citrus juice are limonoids and flavonoids. Limonin is mostly found in seeds and tissue of the fruit. Bitterness caused by these combinations in citrus juice is very evident in orange juice. Namilin is another limonoid with bitter taste like limonin and plays a role in general bitterness. Another source of bitterness is flavonoid naringin, which like limonin, is mostly found in the tissue and pulp compounds of the juice with a non-bitter isomer. Naringin and disaccharide neohesperidose (glucose + Rhamnose) that have a role to play in bitterness and yield ester are products of naringine hesperidose hydrolysis,

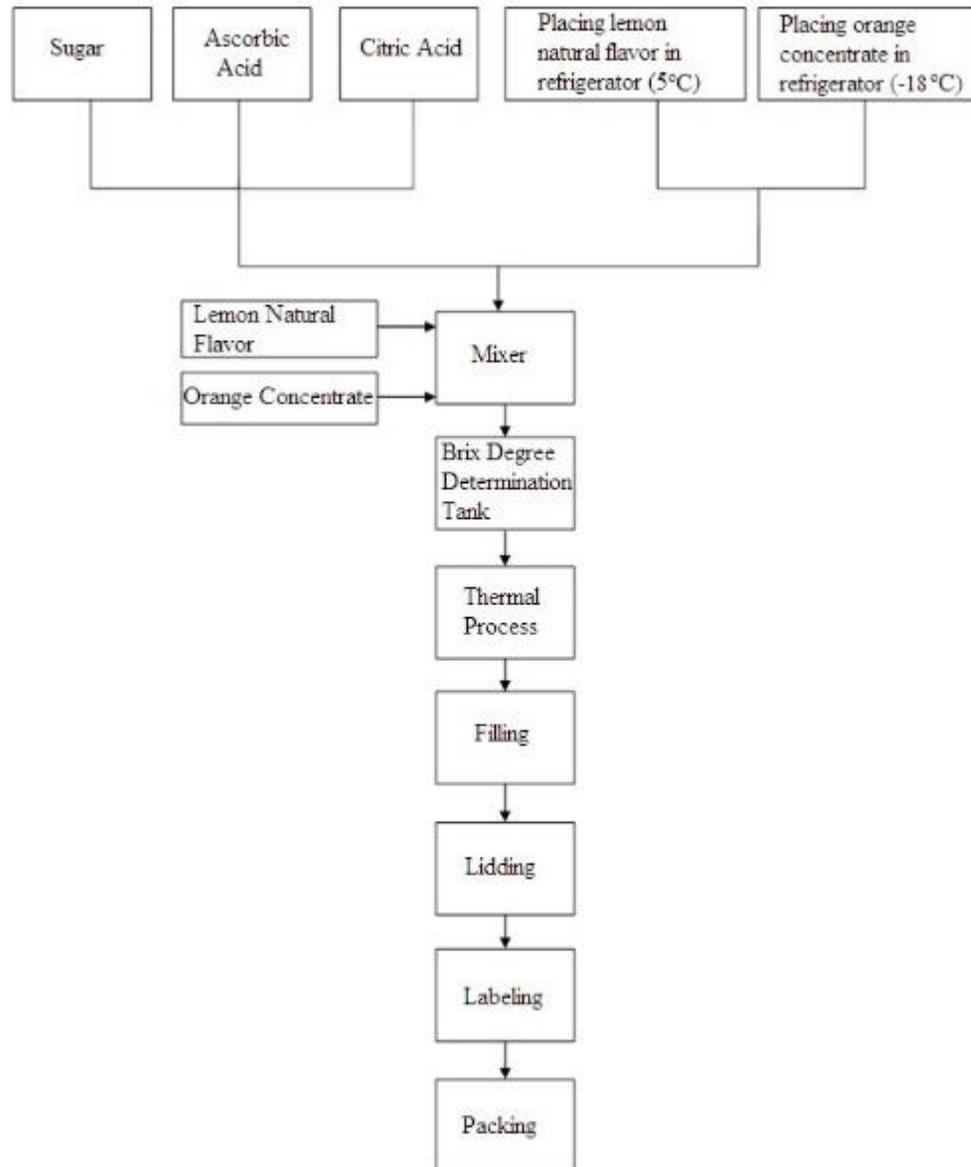
which is a less bitter compound (Caladia, 2010). Yellow sour lemon is mostly produced in Italy and some of Mediterranean countries. Lemon is also produced at commercial scale in the USA. In addition to cooking, the yellow oval shape fruit is also used widely in making variety of fruit juices. Green lemon needs humid and warm climate and Egypt, Africa, Mexico, and West of India are main producers of the lemon. Mexico and India are two major producers of green lemon. In comparison with other types of lemon, green lemon is smaller (8-10cm), more spherical, and greenish-yellow in color with fresh and strong taste (Ecomos, 2012).

Noncarbonated drink is a non-fermented and fermentable drink that are produced by mixing condensed fruit juice with edible parts of one or more fruits and vegetables in the form of pulp, slices, mash and other permitted additives, sweeteners, and water, which is kept in containers (Iran National Standard, No. 2837). Fruit concentrate or content of the juice influence sensory properties of the product. Therefore, achieving desirable mouth feel and sensory properties such as color, taste, aroma, and appearance needs the right formulation, which is achieved through an accurate and scientific process. Using different additives such as flavors is a critical and risky process as they have considerable effects on the sensory properties. Finding the right formulation is achievable through preparing examining several treatments and dilution tests (Ladania, 2008). Velazquez et al. (2013) evaluated the effects of homogenize process by imposing extremely high pressure on active bio-combinations along with the antioxidants' effect on orange juice. They concluded that changes in ascorbic acid and carotenoid acid is mainly a function of pressure of the process; although, ascorbic acid remainder in the sample remained higher than that of thermal pasteurization process. On the other hand, flavonoid content increases along with homogenize process at high pressure, while antioxidants content remains unchanged between processed and fresh orange juice. This is in contrast with thermal pasteurization process where content of the antioxidants decreases considerably. Leahu et al. (2013) examined physicochemical properties changes of orange, kiwi, and apple juice and their mixture on shelf and found that shelf-time had significant effects on general acceptability of the samples regarding color, taste, and aroma. Given this introduction, purpose of the present paper is to introduce a method to produce mixed citrus fruits juice (natural concentrate of orange, natural lemon flavor, and other natural citrus flavors). The final product is a quality product (28% fruit juice), which can hopefully gain good market share.

## Materials and Methods

**Table 1: List of material used**

Material type	Company and country	Material type	Company and country
Sugar	Shadianeh – Iran	OSA cultivation environment	Liofilchem - Italy
Orange concentrate	Noosh Mazandaran – Iran	MRS Agar cultivation environment	Quelab – Canada
Citric acid	Hang – China	Orange extract	Hagsan – Iran
Ascorbic acid	Hang – China	DG18 cultivation environment	Micromedia – Spain
Natural lemon	Masrou – Iran	Peptone water	Merk – Germany
Natural lemon flavor	Hagsan – Iran	DRBCA cultivation environment	Merk – Germany



**Figure 1: Schematic view of preparation process of noncarbonated orange juice with lemon flavor**

Following public awareness about health risks of carbonated drinks with preservatives in recent years, fruit juice and drinks without chemical preservatives have become the target and requirements of fruit juice companies in Iran. Doubtlessly, promoting the new products and improving public awareness in this regard will lead to decrease of unhealthy drinks' share of the market. Physicochemical, microbial, and sensory properties of the final product were examined as well.

The present study is aimed to examining production of noncarbonated orange juice with lemon. To achieve the best formulation, 11 treatments with different percentages of orange concentrates and lemon natural flavor were prepared and physicochemical (pH: Iran National Standard No.2685, acidity: Iran National Standard No.2685, density: Iran National Standard No.2685, brix: Iran National Standard No.2685, total dry may: Iran National Standard No.2685, and

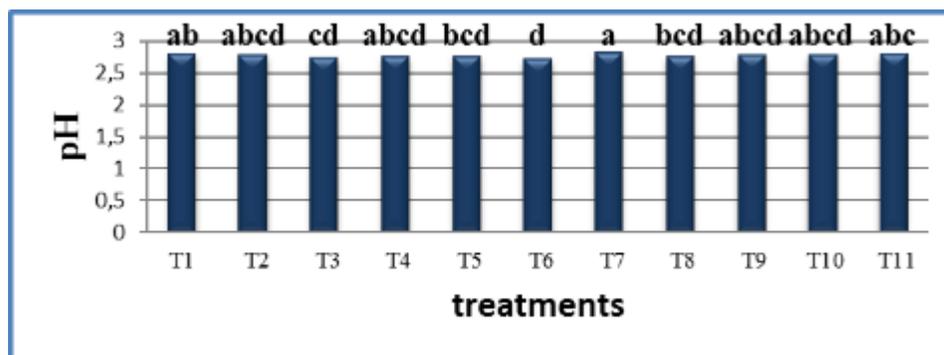
ethanol: Iran National Standard No.2685), microbial (lactic acid bacterial: Iran National Standard No.3414, acid-fast bacteria: Iran National Standard No.2685, mold, and yeast: Iran National Standard No.10899-1), and sensory (color, taste, aroma, appearance, and general acceptability) properties were examined. Dilution process was performed in preparation of orange concentrate, lemon natural flavor, and the formulations. At first, 4gr-orange concentrate samples were prepared using accurate lab level and mixed with distilled water up to 100ml in volumetric flask. Afterward, required amount of the concentrate was added to the treatments. The process was followed by preparing lemon flavor so that 8gr-samples of natural lemon flavor were prepared and then mixed with distilled water up to 100ml in volumetric flask and then added to the treatments. Natural lemon and orange concentrates were used in the formulation. The concentrates were first moved to refrigerator – orange concentrate at  $-18^{\circ}\text{C}$  and lemon concentrate at  $5^{\circ}\text{C}$ . Sugar, citric acid, and ascorbic acid were mixed and added to the weighed materials in a mixer. Afterward, lemon natural flavor and orange concentrate were added to the mixture and thoroughly mixed before being transferred to brix measurement container. After the measurement, the samples underwent thermal process ( $90^{\circ}\text{C}$ , 20-40s) and, then, cooled down to  $70^{\circ}\text{C}$ . Afterward, the samples were poured into lidded polyethylene terephthalate containers, and labeled. (Fig. 1)

### Results

The exact percentage of the materials in the formulations was selected through test and trail process and since the experiments were random, there was no ascending or descending orders. Error risk rate is expectedly high in test and trail random process, while all the treatments could have ascending or descending trends. The experiments were repeated three times and the collected data were analyzed using ANOVA in SPSS 16.0. To compare mean value at 95% ( $p < 0.05$ ), Duncan's multiple test was used. Independent variables were different percentages of lemon natural flavor and orange concentrate; and dependent variables were all physicochemical properties (brix, density, dry mass content, total acidity, pH, ethanol, and microbial tests). The diagrams were produced in Excel Microsoft.

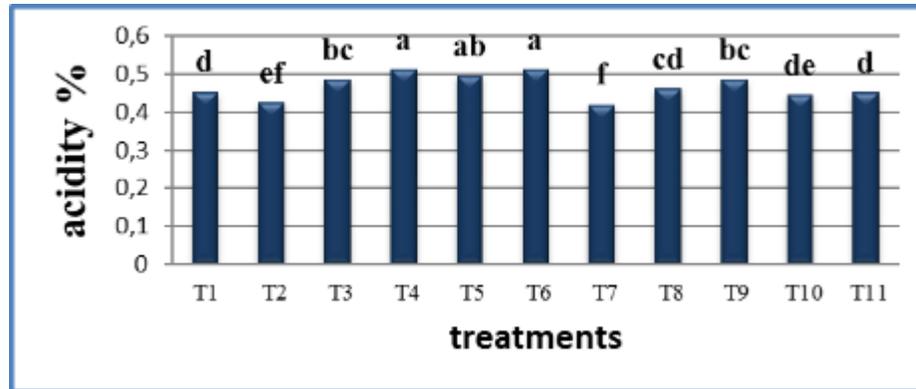
**Table 2: Physicochemical properties of orange concentrate**

	pH	Brix	Ash (5)	Acidity (%)	Formalin
Orange concentrate	3.42	64	2.3	5.16	100



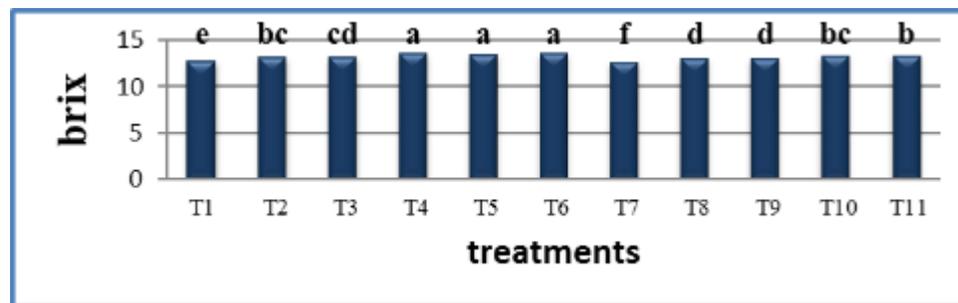
**Figure 2: Mean pH values of different treatments**

Mean pH values of different treatments of the juice are pictured in the figure above. Clearly, treatment No.7 with minimum level of orange and lemon natural flavor concentrates has the highest pH (2.93), and treatment No.6 with maximum level of orange and lemon natural flavor concentrates has the lowest pH level (2.72). There is no significant difference between treatments No.2, 4, 9, and 10. ( $p > 0.05$ )



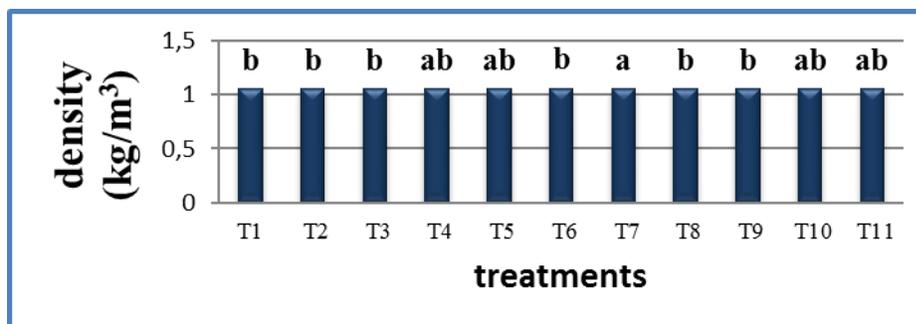
**Figure 3: Mean acidity values of different treatments**

Mean acidity values of different treatments of the juice are pictured in the figure above. Clearly, treatment No.6 has the highest acidity (0.513%). There is no significant difference between treatments No. 6 and 4 regarding acidity level ( $p>0.05$ ). Treatment No.7 with minimum level of orange and lemon natural flavor concentrates has the lowest acidity level (0.42). There is no significant difference between treatments No.7 and 2. ( $p>0.05$ )



**Figure 4: Mean brix values of different treatments**

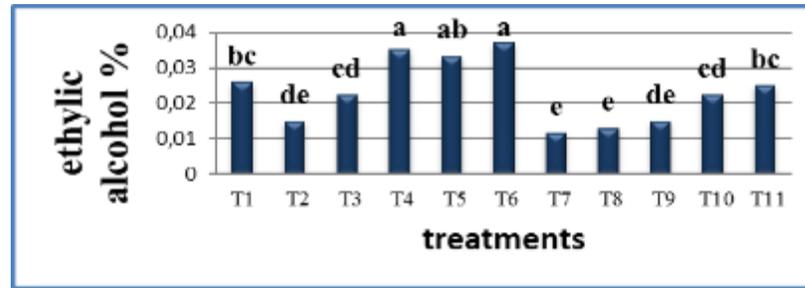
Solid content of the treatments was measured based on brix level. The results of statistical analysis showed significant effect of the different formulation on brix of the treatments ( $p<0.05$ ). Figure 4 illustrates mean value of brix of the treatments under study. Clearly, treatment No.6 has the highest brix value (13.63%); however, it is not significantly different from treatments 4 and 5 ( $p>0.05$ ). Treatment No.7 has the lowest brix level (12.64), followed by treatment No.11 (12.64).



**Figure 5: Mean density (kg/m<sup>3</sup>) of different treatments**

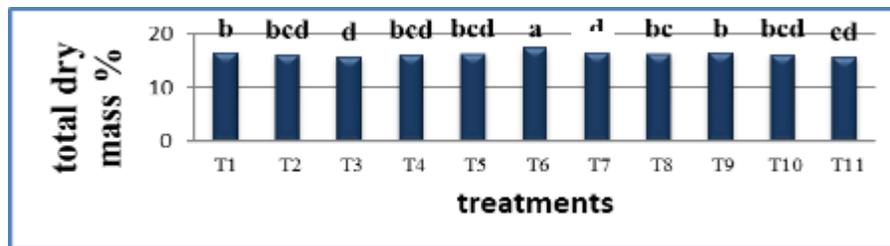
Mean density of the treatments of the juice are pictured in the figure above. Clearly, treatment No.6, 5, and 4 have the highest density (1.055) followed by treatments No.2, 10, and 11.

However, there is no significant difference between the treatments No.6, 5 and 4 and treatments No. 2, 10, and 11 ( $p>0.05$ ). Minimum density level is observed in treatments 1, 3, 7, 8, and 9; and there is no significant difference among them. ( $p<0.05$ )



**Figure 6: Mean values of ethanol percentage of all juice treatments**

Data analysis showed that the effect of the treatments on ethanol content of the produced juices was significant ( $p<0.05$ ). Figure 6 pictures ethanol level of different treatments. Highest ethanol levels are observed in treatments No.6 (0.037%), No. 4 (0.035%), No. 5 (0.033%); however, there is no significant difference between these treatments ( $p >0.05$ ). In addition, treatments No. 7 (0.012%) and No. 8 (0.013%) have the lowest ethanol levels.



**Figure 7: Mean total dry mass percentage of all juice treatments**

Mean total dry mass of different treatments is pictured in figure 7. Statistical analyses show that there is a significant difference between the treatments regarding total dry mass of the juice ( $p<0.05$ ). Clearly, treatment No.6 has the highest total dry mass value (17.25%) followed by treatments No. 1, 7, 9; which are not significantly different however ( $p>0.05$ ). Treatment No.3 has the lowest dry mass value (15.74).

**Table 3: Results of microbial analysis of lactic acid bacteria**

Lactic acid bacteria	Treatments
Negative	T1, T2, ... T11

The results of microbial tests on lactic acid bacteria are listed in Table 3. Clearly, number of lactic acid bacteria in all treatments is negative and there is no negative difference between the treatments. ( $p>0.05$ )

**Table 4: Results of microbial analysis of acid-fast bacteria**

Acid fast bacteria	Treatments
Negative	T1, T2, ... T11

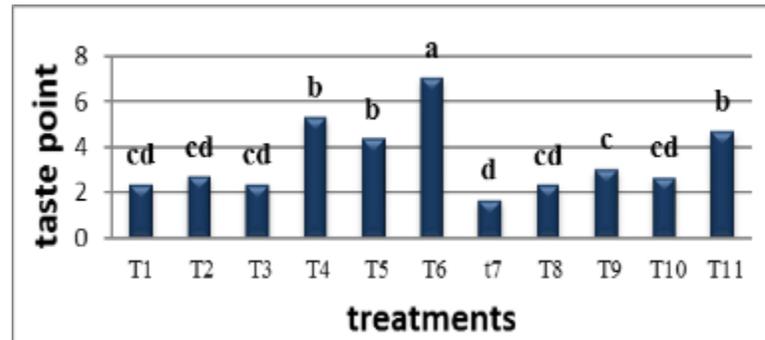
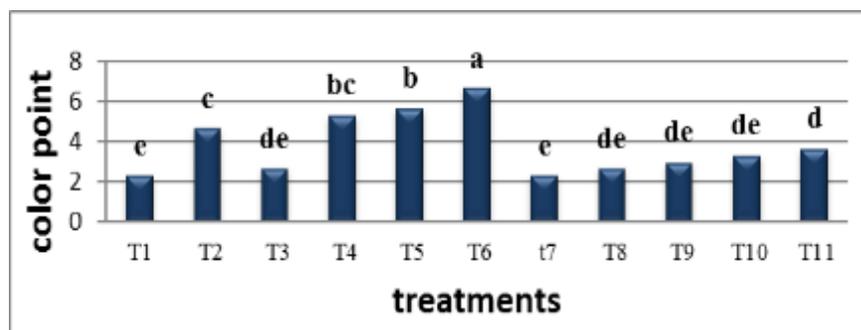
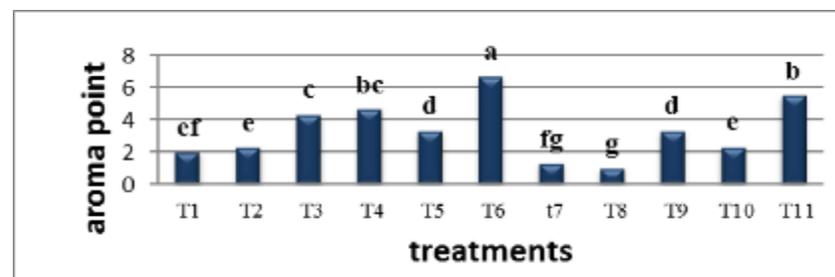
The results of microbial tests on acid-fast bacteria are listed in Table 4. Clearly, number of acid-fast bacteria in all treatments is negative and there is no negative difference between the treatments. ( $p>0.05$ )

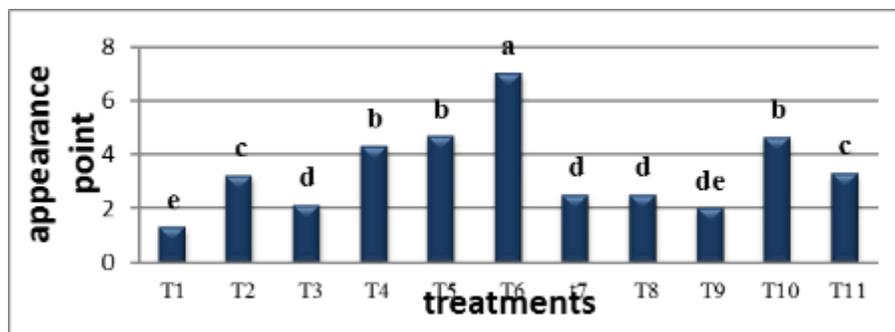
**Table 5: Results of microbial analysis of mold and yeast of the treatments**

Mold and yeast	Treatments
Negative	T1, T2, ... T11

The results of microbial tests on mold and yeast of the treatments are listed in Table 5. Clearly, number of mold and yeast in all treatments is negative and there is no negative difference between the treatments. ( $p>0.05$ )

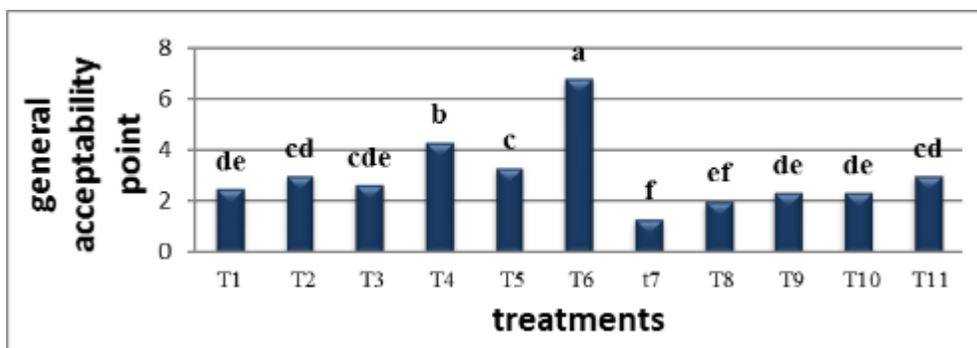
The results of statistical analysis supported significant effects of the treatments on sensory parameters of the product juice ( $p<0.05$ ). Mean point of taste, color, aroma, appearance, and general acceptability of juices are pictured in figures 8-11. As to taste, treatment No. 6 obtained the highest point followed by treatments No. 4, 5, 11, and treatment No. 7 obtained the lowest point (1.65). Regarding color, treatment No. 6 obtained the highest point (6.65) followed by treatment No. 5 (5.67) and treatment No. 1 obtained lowest point (2.33). With respect to aroma, treatment No. 6 had the best aroma followed by treatment No.11 and treatment No.7 & 8 obtained the lowest point. Finally, as to appearance, treatment No. 6 had the highest point (7) followed by treatments No. 4, 5, and 11.

**Figure 8: Mean points of taste****Figure 9: Mean points of color****Figure 10: Mean points of aroma**



**Figure 11: Mean point of appearance**

According to Figure 12 and mean points of acceptance of all treatments, treatment No.6 obtained the highest general point of acceptance comparing with other treatments ( $p < 0.05$ ) followed by treatment No.4; and treatment No. 7 obtained lowest point of acceptance.



**Figure 12: Mean point of general acceptance**

### Discussion and Conclusions

Production of noncarbonated orange–lemon juice was discussed. To find the best possible formulation, 11 treatments with different ratios of orange concentrate and natural lemon flavor were prepared and physicochemical (pH, acidity, brix, total dry mas, and ethanol), microbial (lactic acid bacteria, acid fast bacteria, mold, and yeast), and sensory (color, aroma, taste, appearance, and general acceptability) tests were performed.

Physicochemical properties assessments indicated that lowest and highest pH and acidity levels were obtained by treatments No. 6 and 7 respectively. Acidity and pH are the main antimicrobial hurdles in drinks (Mentz, et al. 2010). In general, risk of contamination and growth of pathogens increases along with increase of pH - food pathogens rarely growth in  $pH < 4.6$  (Lawlor et al., 2009). In light of this, pH of noncarbonated and alcoholic drinks is usually kept below this level (Sperber, 2009). Ali Khan et al. (2009) argued that thymus essence had significant effects on total acidity level of pear juice. Raeisi et al. (2013) used rice husk essence to enrich orange juice and found that pH of the final product increases with increase of the essence portion in the formulation.

Increase of orange concentrate and lemon natural flavor percentages in the formulation increased solved solid content of the juice (brix) and total solid content so that with highest percentages of orange concentrate and lemon flavor, treatment No. 6 had highest brix and total solid mass values. The lowest brix degree and total dry mass value was observed in treatment No.7 with minimum orange concentrate and lemon natural flavor. According to Iran National Standard, brix degree of noncarbonated fruit juice must be at least 11. Therefore, all the formulations were standard as to brix degree (12.64-13.64%). Alikhani et al. (2009) examined the effects of thymus essence on

different properties of pear juice and reached results consistent with the present study. They found that increase of percentage of the essence in the juice formulation led to significant increase in brix degree of the juice. Yusefi (2011) produced pomegranate and lemon essence drink and consistently found that increase of lemon ratio in the drink increased its brix degree.

Orange concentrate level had significant positive effect on ethanol percentage of the treatments. Consistently, treatment No. 6 with highest orange concentrate and lemon natural flavor values had the highest ethanol value and lowest level of ethanol was found in treatments No.7 & 8 with lowest orange concentrate and lemon flavor levels. Iran National Standard requires ethanol level in noncarbonated drinks below 0.15% and thereby, all the treatment met the standard in this regard (0.012-0.037%).

As to density, treatment No.6 with the highest level of orange concentrate and lemon natural flavor had highest density and treatment No. 7 had the lowest density among the other treatments. However, there was no significant difference between the treatments regarding density ( $p>0.05$ ). Iran National Standard states that minimum density of non-carbonated fruit juice must be at least 1.035kg/m<sup>3</sup>. Therefore, all the formulations met the standard regarding density (1.049-1.55). Raiesi et al. (2012) reported that adding rice husk essence to orange juice had no significant effect on density of the final product.

Microbial analyses showed that microbial load of all the treatments was negative and regarding physicochemical and microbial properties, all the treatment met Iran National Standard codes. According to the standard, number of lactic acid bacteria, acid-fast bacteria, mold, and yeast in the samples should be negative and as our results showed, all samples met the requirements.

Regarding sensory evaluation, the findings showed that the highest sensory parameters point was obtained by treatment No. 6, which makes the treatment the best formulation for producing orange –lemon noncarbonated drink. Results of sensory evaluation indicated that treatment No.7 with lowest level of orange concentrate and lemon natural flavor had the lowest acceptability point. Our results in this regard are inconsistent with Altonkaia et al. (2013). They found that increase of percentage of pomegranate peel essence in apple juice had no significant effect on the sensory parameters.

## References

- Al-Ani, W.N., Al-Haliem, S.M., & Tawfik, N.O. (2010). Evaluation of the Antibacterial Activity of Citrus Juices: An In Vitro Study. *Al-Rafidain Dent Journal*, 10(2): 376-382.
- Altunkaya, A., Rikke, V., Hedegaard, Harholt, J., Brimer, L., Vural, G. & Leif, H. (2013). Palatability and chemical safety of apple juice fortified with pomegranate peel extract. *Food and Function*, 4: 1468–1473.
- Anitei, S. (2007). Where did citrus fruits originate from? *Softpedia*. Available from: <http://news.softpedia.com/news/Where-Did-Citrus-Fruits-Originate-From-67365.shtml>. Accessed 2011 June.
- Boeing, H., Bechthold, A., Bub, A., Ellinger, S., Haller, D., Kroke, A., Leschik-Bonnet, E., Mueller, M.J., Oberritter, H., Schulze, M., Stehle, P., & Watzl, B. (2012). Critical review: Vegetables and fruit in the prevention of chronic diseases. *European Journal of Nutrition*, 51: 637–663.
- Claudia, A., Spinelli, N.F., Anderson, S., Cristiana, P.P., & Pilar, R.M. (2010). Influence of the hot – fill water – spray – cooling process after continuous pasteurization on the number of decimal reductions and on *Alicyclobacillus acidoterrestris* growth in orange juice stored at 35.c. *International journal of food science and emerging*, 137: 295 – 298.
- Dietary Reference Intakes (2006). *The Essential Guide to Nutrient Requirements*; The National Academies Press: Washington, DC, USA, 2006.

- Economos, C., & Clay, W. (2012). Nutritional and health benefits of citrus fruits. Food and Agriculture Organization. Available online: <ftp://ftp.fao.org/docrep/fao/X2650T/X2650t03.pdf> (accessed on 1 October 2012).
- Fraser, P.D., & Bramley, P.M. (2004). The biosynthesis and nutritional uses of carotenoids. *Prog.Lipid Research*, 43: 228–265.
- Gallicchio, L., Boyd, K., Matanoski, G., Tao, X., Chen, L., Lam, T.K., Shiels, M., Hammond, E., Robinson, K.A., Caulfield, L.E., Herman, J.G., Guallar, E., & Alberg, A.J. (2008). Carotenoids and the risk of developing lung cancer: A systematic review. *American Journal of Clinical Nutrition*, 88: 372–383.
- Iran National Standard Org. (2006). Standard No. 10889-1, Published by Iran Standard and Industrial Research Org. microbiology of foods and husbandry animal feed- a comprehensive method to count mold and yeasts – part one: colon counting method in the products with aqua activity more than 0.95, 1st ed., p.8-16
- Iran National Standard Org. (2006). Standard No. 2685, Published by Iran Standard and Industrial Research Org. – Fruit Juice- test methods, 1st ed., p. 7-22
- Iran National Standard Org. (2006). Standard No. 2837, Published by Iran Standard and Industrial Research Org. noncarbonated fruit juice – specifications. 2nd ed., p. 6, 14, 15.
- Iran National Standard Org. (2006). Standard No. 3414, Published by Iran Standard and Industrial Research Org. drinks- juice and products- microbiologic test methods, 1st ed., p. 9-17
- Iran National Standard Org. (2006). Standard No. 4083, Published by Iran Standard and Industrial Research Org. Condensed orange juice, specifications, 1st revision, p. 9.
- Katsuura, S., Imamura, T., Bando, N., & Yamanishi, R. (2009). Beta-carotene and beta-cryptoxanthin but not lutein evoke redox and immune changes in RAW264 murine macrophages. *Molecule Nutrition Food Research*, 53: 1396–1405.
- Ladaniya, M.S. (2008). *Citrus fruit: biology, technology and evaluation*. San Diego, CA: Academic Press.
- Lawlor, K., Schuman, J., Simpson, P. & Taormina, J. (2009). Compendium of the Microbiological Spoilage of Foods and Beverages, In Sperber, W.H. and Doyle, M.P. (eds.) *Food Microbiology and Safety*, pp. 245–283, Springer, New York.
- Leahu, A., Damian, C., Oroian, M. & Ropciuc, S. (2013). Physico-chemical parameters of fruit juices evolution during storage. *Lucrări Științifice-Seria Zootehnie*, 59: 213-217.
- Mentz, G., Vriesekoop, Zarei, M., Zhu, B. & Aldred, P. (2010). The growth and survival of food-borne pathogens in sweet and fermenting brewers' wort. *International Journal of Food Microbiology*, 140: 19–25.
- Nazarian A, Mortazavi S. A, Bolandi M, & Armin M. (2011). Producing and surveying physicochemical properties of soybean-milk drink based on sour cherry-barberry, *Food Technology Journal*, 4(3):35-45.
- Raiesi F, Razavi S.H, Hojatolslami M. V., & Keramat J (2012). Functional orange juice production using rice husk essence. *Journal of Nutrition and Food Industries of Iran*, 7 (4): 45-53.
- Yusefi H. (2011). Pomegranate and lemon essence drink, Pomegranate National Conference, Ferdous.