Original Article

Functional drink formulation containing orange juice and tropical almond leaf extract: physicochemical, sensory and microbial properties

Tayebeh Nakhaei Moghaddam[1], Amir Hossein Elhamirad[2*], Mohammad Reza Saeidi Asl[2], Mostafa Shahidi Noghabi[3]

[1] PhD Student in Food Science and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran. [2]Department of Food Science and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran. [3]Department of Food Chemistry, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran.

Abstract

In this study, the production of orange juice containing tropical almond's red leaf extract and optimization of its formulation based on physicochemical, sensory and microbial characteristics have been investigated. For this purpose, the extracts of tropical almonds were added to the orange juice at 4 levels (0, 5, 10, 15 and 20%) and physicochemical, sensory and microbial properties were evaluated for 3 months after production at 4 and 25 °. Increasing the concentration of the tropical almond's red leaf extract increased the brix and color intensity but did not significantly affect the turbidity of the orange juice samples. Microbial count in the orange juice samples after one month of storage at 25 °C revealed that the increase in the number of molds, yeast, and aerobic. In regard to the general acceptability characteristic, the orange juice sample that contained the 20 % extract received the highest score.

Keywords: Functional, Beverage, Orange Juice, Tropical Almond Leaves Extract

Introduction

Orange juice is one of the most popular and recognized juices to be marketed and, with a yearly production rate of over 70 million tons, comprises roughly half of the world's total juice production. The high commercial value of orange juice is due to its delicious sensory properties (taste and color), high level of vitamin C, and the fact that it contains natural antioxidants. One of the major obstacles to the sale of orange juice is its short shelf life. Microbial spoilage, taste loss and ascorbic acid degradation are three important factors in the product's loss of quality during storage. Today, consumer demand is rising for orange juice with high quality, natural flavor, optimal texture, minimal additives and least thermal processing [1]. Although conventional thermal processing methods improve orange juice's storage life and satisfy health expectations, they decrease the product's qualitative and quantitative indices [2]. The use of antioxidants is crucial for preventing the oxidation of food compounds and increasing shelf-life. Nowadays, scientists and nutritionists are constantly seeking natural compounds with antioxidant properties. Plants are one of the most important sources of natural antioxidants. In addition to being used in foods for augmenting quality and nutritional value, herbal extracts are also used in other products such as beverages, colors, cosmetics and pharmaceuticals [3]. Although synthetic antioxidants are currently used extensively in the food industry, many of these compounds have harmful effects on human health. Given this fact, natural antioxidants (derived mostly from plant extracts) have become popular among consumers and seem to be important in preventing a number of diseases [4].

Terminalia catappa, named Garam Zangi in Persian, belongs to the Combretaceae family (Indian almonds). This plant widely grows in the hot regions of the world, including the countries of East Asia [5], and is native to countries such as India, Malaysia, Cameroon and Madagascar [5-7]. The main objective of the present study was to investigate the chemical and microbial characteristics of orange juice containing the

Address for correspondence: Mr. Amir Hossein Elhamirad, Department of Food Science and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran.
E-mail: ah_elhami@iaus.ac.ir

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tropical almond's red leaf extract in order to arrive at the optimal formulation for the related functional beverage.

Materials and Methods

Red leaves of the tropical almond and orange concentrate was purchased from the Chabahar market. citric acid, ethanol, DPPH solution, butylated hydroxyanisole (BHA) synthetic antioxidant, gallic acid, folic acid reagent, phenolphthalein reagent, sodium hydroxide, chloramphenicol agar growth medium, and plate count agar growth medium and solvents used were of analytical grade, having been purchased from Merck and Sigma.

Sample preparation and storage conditions

In the present study, three different leaf samples (green, yellow and red leaves) were collected from the tropical almond of the city of Chabahar. The leaf samples were evaluated and confirmed in terms of their identification. In order to extract the leaves using pulsed electric fields, the samples were transferred to the Institute of Food Science and Technology (Mashhad, Iran) and stored at a temperature of -18° C until the extraction was carried out. Dried leaves were turned into fine powder by being milled for 20 seconds and then passed through a 40 mesh sieve. 5 g of the powder of each sample was mixed with a mixture of ethanol-water solvent (7:3 ratio) and stirred for 12 hours at room temperature. The extracts were then filtered through Whatman No. 1 filter paper and centrifuged at 2500 rpm for 10 minutes with a Hettich EBA 20 centrifuge. The mixture of extract and solvent was concentrated using a rotary evaporator (Heidolph, Germany) at 40 °C under vacuum. The remaining solvent was then removed using a UNB40 vacuum oven (Memmert, Germany) at 40 °C under vacuum. The extracts of tropical almonds were added to the orange juice at 4 levels (0, 5, 10, 15 and 20%) and physicochemical, sensory and microbial properties were evaluated for 3 months after production at 4 and 25 °. Then, the amount of soluble solids (degree of brix), pH, acidity, ash, turbidity, color, microbial flora and sensory characteristics of beverage were studied.

Physicochemical properties

Soluble solids (degrees of Brix) and pH of samples were measured at room temperature (20° C) by refractometer (RX5000-α – ATAGO company -Tokyo Japan) and pH meter (Metrohm company- Germany), respectively [8, 9].

For measuring the turbidity of beverage samples, a turbidity meter (2100 HACH – USA) was used and the turbidity of samples was reported in terms of NTU units. The titration method was used to measure acidity according to Iran's national standard (No.2685). To determine the percentage of ash, the electric furnace (ELF 11/6B – Carbolit Company - England) was used at 550 ° C for 6 to 8 hours [10].

It was used to measure the color of the samples from the digital camera (Canon PowerShot SX240 HS) under the conditions described by Yam & Papadakis In 2004. Then the data was analyzed using Image j software and parameters L*, a* and b* were obtained. Also, the total color difference (ΔE) was calculated according to Equation (1) [11]:

$$\Delta E = [(L^* - L^*_{Ref})^2 + (a^* - a^*_{Ref})^2 + (b^* - b^*_{Ref})^2]^{1/2} \quad (1)$$

Microbiology tests

In this study, the total count (number of aerobic mesophilic bacteria), mold and yeast were measured. For this purpose, the method used in the study of Ali et al. In 2000 was used [12].

Microbial tests were performed at 4 different times (0, 1, 2 and 3 months after production), 2 dilutions (1-10, 2-10) and 3 replications.

Sensory evaluations

A five-point Hedonic method was used to measure the sensory properties of beverage samples. Also, a simple scoring method was used to rate the samples. For this purpose, after the initial training, 10 people were selected as evaluators. The highest score was 5 and the lowest score was 1. The sensory properties of the samples were evaluated for color, taste, appearance, odor, mouse feel and total acceptance. Then, the average of the obtained scores for each sensory trait was calculated and using the Duncan multiplication comparison method, the significance of the differences between the samples was determined [13].

Statistical analysis

The present study was conducted in a completely randomized design. Data was collected and stored in Excel software. ANOVA was used to analyze the data using SPSS software. Significant differences between the samples were predicted using the Duncan test with a 95% confidence level. All calculations were based on the average value of 3 repetitions per process.

Results and Discussion

Physicochemical properties

- pH

The results of the analysis of variance demonstrates that in all samples, the pH of the orange juice significantly increased (P <0.05) over time. However, the pH increased at a slower rate in the orange juice containing 20 % red leaf extract. pH changes were of greater magnitude in samples stored at 25 °C. Some reasons for the increase in pH across all samples may be the growth of aerobic mesophilic microorganisms and the destruction of vitamin C during storage. However, the antimicrobial effects of the tropical almond's red leaf extract at 10 and 20 % concentrations caused a decrease in pH changes.

- Soluble solids

The comparison of the soluble solids content of the samples shows that the amount of brix obviously depends on the percentage of added solids, such that the control sample had the least amount of brix (12.60±0.08); in contrast to the other samples that had different percentages of added extract. Increasing the concentration of the leaf extract caused a significant increase (P<0.05) in the brix of the orange juice (12.94±0.07).

- ### Turbidity

The addition of the tropical almond's red leaf extract did not significantly affect the turbidity of the orange juice samples (P>0.05). However, storage time significantly (P<0.05) reduced the sample turbidities, and this reduction was amplified by increased storage temperature. In citrus juices, the pectin methyl esterase enzyme is naturally present as its originates from the cell walls of the fruit. This enzyme possesses high thermal stability but is deactivated at a temperature higher than the temperature that kills microbes. Over time, this enzyme separates methoxyl groups from the pectin chain, resulting in the production of the free radicals of carboxylic acid. Divalent cations, including calcium, are able to produce macro-polymers by making cross-links between pectin chains through these groups. These large molecules are deposited over time and reduce the turbidity of the product [14, 15].

- ### Color intensity

The difference in color intensity parameter (ΔE), which is calculated from the main color indices (a*, b* and L*), is the relative color variation of each sample compared to the control sample. Orange juice samples containing 10 and 20 % tropical almond extracts had more color intensity variation than the control sample and the sample containing the 5% extract, which is due to reactions that bring about color changes in orange juice. One of these reactions is the oxidation of carotenoid pigments, which can be attributed to the presence of carotenoid oxidizing compounds, including hydrogen peroxide, and other active oxygen species in the environment. The presence of phenolic compounds in the extracts of tropical almond leaves is a major factor in preventing or delaying oxidation. On the one hand, phenolic compounds act as an initial antioxidant by donating hydrogen and terminating the reactions of the oxidative chain. On the other hand, phenolic compounds act as inhibitors by inhibiting free oxygen radicals [16]. By increasing the concentration of the tropical almond leaf extract, the color intensity of the orange juice also increased, which was due to the heightened antioxidant properties of the concentrated extracts. In fact, the antioxidant ability was concentration dependent. In general, increasing the concentration of phenolic compounds directly increases the ability of various extracts to inhibit free radicals. Due to an increasing number of hydroxyl groups in the reaction environment at higher concentrations of phenolic compounds, the probability of hydrogen donation to free radicals increases, and consequently, the inhibitory power of the extract increases.

By increasing the duration of storage, the intensity of color decreased in orange juice containing 10 and 20 % extracts of tropical almond leaves. Antioxidants present in the extracts remained active for a certain period of time, but their effect gradually decreased. As a result, the oxidation process of the pigments gradually accelerated and the samples lost color intensity.

- ### Mold, yeast, and aerobic mesophilic bacterial count

The microbial quality of fresh orange juice was investigated by total mold, yeast, and aerobic mesophilic bacterial (total bacterial count) counts, and the results are presented in tables 1 and 2. Immediately after production, the average logarithm of the number of colonies per milliliter (log Cfu /mL) of total mold and yeast was 4.13, compared to 4.22 for total bacteria. The results of microbial count in orange juice after one month of storage at 25 °C demonstrated that the number of molds, yeast, and aerobic mesophilic bacteria increased to above 6 (the maximum permitted microbial population in fresh orange juice) in all samples [12]. The amount of mold, yeast and aerobic mesophilic bacteria after one month of storage at 4° C was less than the permitted limit only in samples containing 10 and 20 % red leaf extract. After two months, the number of the microorganisms was greater than the permitted limit in all samples (at both storage temperatures). In other studies, the storage life of fresh orange juice in refrigerated conditions over 14 days has been investigated [17]. In the present study, orange juice containing 20% of the red leaf extract of tropical almonds had the least microbial growth after one month of storage compared to the other samples. However, after two months, the total microbial count of this sample also passed the permitted limit.

Table 1. Total mold and yeast count in orange juice samples during storage at 4 and 25 °C.

Treatments Number		Storage time (days) Percentage			
Extract concentration (%)	Storage Temp.(°C)	0	30	60	90
0 (control)	4	0/05^{a,A} 4.13±	0/05^{b,EF} 6.32±	0/06^{c,D} 6.59±	0/09^{d,D} 7.18±
	25	0/05^{a,A} 4.13±	0/07^{bG} 6.47±	0/09^{c,E} 6.82±	0/08^{d,E} 7.87±
5	5	0/06^{a,A} 4.13±	0/03^{b,E} 6.26±	0/07^{c,D} 6.54±	0/11^{d,D} 7.12±
	25	0/06^{a,A} 4.14±	0/06^{b,FG} 6.40±	0/08^{c,E} 6.77±	0/08^{d,E} 7.75±
10	4	0/03^{a,A} 4.14±	0/03^{bB} 5.74±	0/06^{c,AB} 6.22±	0/09^{d,BC} 6.79±
	25	0/03^{a,A} 4.14±	0/06^{b,D} 6.12±	0/04^{c,C} 6.39±	0/07$^{d, C}$ 6.93±
20	4	0/05^{a,A} 4.13±	0/04^{b,A} 5.43±	0/07^{c,A} 6.14±	0/10^{d,A} 6.54±
	25	0/05^{a,A} 4.13±	0/06^{b,C} 6.02±	0/08^{c,BC} 6.28±	0/09^{d,B} 6.74±

* In each row, dissimilar lower case letters indicate significant differences (P<0.05) based on the Duncan test.

** In each column, dissimilar upper case letters indicate significant differences (P<0.05) based on the Duncan test.

Table 2. Total bacterial count in orange juice samples during storage at 4 and 25 °C.

Treatments		Storage time (days)			
Extract concentration (%)	Storage Temp.(°C)	0	30	60	90
0 (control)	4	0/07^{a,A} 4.22±	0/06^{bd} 6.11±	0/12^{hc,B} 6.42±	0/13^{d,C} 6.93±
	25	0/07^{a,A} 4.22±	0/02^{be} 6.36±	0/08^{cC} 6.71±	012$^{d, D}$ 7.35±
5	4	0/03^{a,A} 4.23±	0/05^{bD} 6.08±	0/06^{c,AB} 6.33±	010^{d,C} 6.90±
	25	0/03^{a,A} 4.23±	0/05^{bE} 6.31±	0/10^{cC} 6.68±	0/09^{dD} 7.45±
10	4	0/05^{a,A} 4.22±	0/08^{bB} 5.51±	0/07^{cA} 6.18±	0/12^{d,AB} 6.64±
	0/05^{a,A} ±4.22	0/08^{bD} 6.19±	0/08^{cA} 6.27±	0/02^{d,BC} 6.81±	0/05^{a,A} 4.22±
20	4	0/05^{a,A} 4.23±	0/05^{bA} 5.26±	0/04^{be} 6.18±	0/09^{dA} 6.52±
	25	0/05^{a,A} 4.23±	0/08^{bC} 5.93±	0/05^{cA} 6.211±	0/11^{d,BC} 6.81±

* In each row, dissimilar lower case letters indicate significant differences (P<0.05) based on the Duncan test.
** In each column, dissimilar upper case letters indicate significant differences (P<0.05) based on the Duncan test.

The antimicrobial activity of the tropical almond leaf extract can be attributed to the phytochemical compounds that it contains. By binding with bacterial proteins and cell walls, these compounds can destroy the bacteria [18]. Phenolic compounds break the cell wall, perforate the cytoplasmic membrane, damage the membrane proteins, impair the functioning of membrane enzymes, and cause cell death [19]. Although the antimicrobial activity of many herbal extracts has been studied, no detailed report regarding the antimicrobial mechanism has been published to date, which is due to differences in the structure of bacterial cell walls and the types of antimicrobial compounds present in plant extracts [20]. However, the main mechanism by which the phenolic compounds of plant extracts destroy bacteria involves cell wall destruction, damage to the cytoplasmic membrane and membrane proteins, cytoplasmic coagulation and intracellular content leakage [21]. The use of natural products as antibacterial agents is a suitable method for controlling the presence of pathogenic bacteria and increasing the shelf life of processed foods [22]. However, by decreasing the pH and keeping the samples in cold temperatures, it seems that the solubility of the phenolic compounds gradually decreases, and their inhibitory ability against bacteria is consequently diminished [23].

- ## Sensory properties

According to the literature, the storage life of refrigerated fresh orange juice is up to 14 days [17]. Hence, the sensory properties of the samples of orange juice stored at refrigerated temperatures were evaluated after two weeks. The sensory properties of the samples included color, flavor, fragrance and general acceptability, as shown in Fig. 1. According to the results of sensory analysis, there was no significant difference in orange juice characteristics between the samples (P<0.05). The evaluators did not differ significantly between the color intensities of the different samples, although the orange juice lacking extract (control) obtained a relatively lower score than the other samples. In the case of orange juice flavor, samples containing 10 and 20 % extracts of tropical almond leaves scored higher than the remaining samples. The sensory characteristic of orange juice fragrance is largely influenced by microbial growth and the consequent production of metabolites derived from microbial fermentation [24]. Figure 1 also shows a higher fragrance score in samples containing 10 and 20 % extracts, which is inversely proportional to the microbial growth of these samples. In regard to the general acceptability characteristic, orange juice containing 20 % extract obtained the highest score. With the exception of the color characteristic, the orange juice lacking extract (control) received the lowest sensory scores amongst the samples. As it can be observed, the acceptability of the orange juice containing the extract was consistent with the changes in antimicrobial ability and the magnitude of microbial growth. This was such that the sample containing 20 % red leaf extract had the lowest microbial count and was selected by the evaluators as the best sample in terms of maintaining the orange juice fragrance.

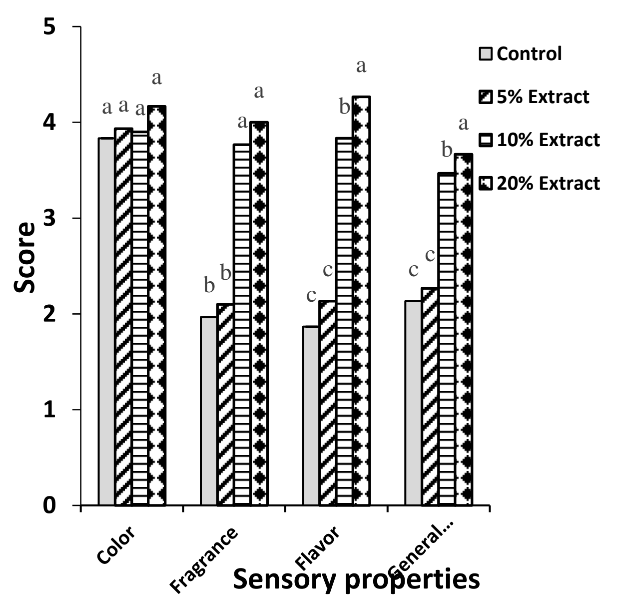

Figure 1: Sensory properties of orange juice containing the red leaf extract of the tropical almond.

Different concentrations of the tropical almond's red leaf extract were used to prepare orange juice. Investigation of the chemical and microbial properties of orange juice containing the mentioned extract showed that in all samples, the pH of orange juice increased over time. However, this process was slower in orange juice containing 20% leaf extract. Increasing the concentration of the red leaf extract increased the brix of the orange juice samples. However, neither storage time nor temperature affected the soluble solids content of the samples. The addition of the tropical almond's red leaf extract had no significant effect on orange juice turbidity, but the storage time of the samples significantly reduced the amount of turbidity, and this phenomenon was amplified by increased storage temperature. As the concentration of the extract increased, the intensity of orange juice color increased, such that the orange juice containing 10 and 20 % extracts showed higher color intensity than other samples due to the antioxidant properties of the added extract. The results of microbial count in orange juice after one month of storage at 25 °C indicated that the number of molds, yeast, and aerobic mesophilic bacteria was above 6 (the permitted limit of microbial population in fresh orange juice) in all samples. The number of mold, yeast and aerobic mesophilic bacteria present after one month of storage at 4 °C was only lower than the permitted limit in samples containing 10 and 20 % red leaf extracts. However, the microbial count was above the permitted limit in all samples after two months of storage. With regard to the characteristics of orange juice flavor and fragrance, samples containing 10 and 20 % extracts achieved higher scores than the remaining samples. For the general acceptability characteristic, orange juice containing 20 % extract received the highest score. Overall, the results of the present study indicate that the extract of tropical almond leaves can have useful applications as an economically viable additive and a phenolic compound rich source of natural antioxidants.

Considering the antioxidant effects of the tropical almond's leaf extract, it is recommended for use in the prolongation of the shelf life of meat products. It is also suggested that other researchers should study the enrichment of other juices and the production of functional beverages using extracts of other native Iranian plants in the future.

Conflict of interest

There is no conflict to declare.

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Author Contributions

Dr. Elhamirad and Saeidi Asl designed the study and interpreted the results. Dr. Saeidi Asl, also drafted the manuscript. Nakhaei Moghaddam performed the experiment and collected test data and helped to draft the manuscript. Dr. Shahidi Noghabi helped and consulted in performing the experiment and also writing and editing manuscript.

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