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ORIGINAL ARTICLE

Licorice, Clove, Nutmeg, Vanilla and Malt Extracts Beverage Production

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ABSTRACT
There is a growing interest in the use of herbal extracts in soft drinks because of their health effects. Although the market is currently small and dedicated, it becomes increasingly important. In addition to its usual function, which is retaining body water in an appropriate level or hydration, a beverage has other benefits as well. In this study, functional beverages based on malt, cinnamon, licorice, clove, nutmeg and vanilla extracts with different ratios of malt extract (31, 30, 29, 28, 27 and 26 grams per liter), Licorice extract (0.025, 0.030, 0.035, 0.040, 0.045, 0.050 grams per liter), cinnamon (0.025, 0.030, 0.035, 0.040, 0.045, 0.050 grams per liter), nutmeg (0.025 grams per liter), cloves (0.025 grams per liter) and vanilla (0.218 grams per liter) produced and physicochemical tests including total acidity, pH, density, total sugar was conducted within 30 days. Data analysis was performed using mean data comparison by Duncan test in a completely randomized design for 5 treatments and the control, in 3 replicates. Total acidity, sugar and ash significantly increased during storage (p<0.01). The analysis of microbiological results showed that the population of acid resistant microorganisms of the beverage within thirty day storage, did not show significant difference (p <0.05). There was no mesophilic aerobic population within thirty days of storage (p <0.05). Microbiological evaluation within 30 days showed no significant difference in the number of molds and yeasts (p<0.05) and their number was fixed within thirty days.
***Key words**: malt extract, licorice extract, cinnamon extract, extract nutmeg, clove extract, vanilla extract, soft drink*

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INTRODUCTION

Malt extract, mainly a concentrated barley malt syrup, is produced with a sugar concentration of 70 to 80% [10] . Barley, scientific name of *Hordeum vulgare* contains about 30 species, the most important species of which are: 1. six-row barley, which has 2 subspecies: *Hordeum hexastichum* and *Hordum vulgare*, the grain is uniform and more suitable for livestock feed. 2. two-row barley, which is of two types:
H. zeocrition and *H. distichum*. Most species of two-row barley have thinner crusts, more symmetry and the germination in the process of malting is more uniform. Malting is the most important food application of barely [10, 18]. Malting is the restricted and controlled germination of cereals that after drying produces a product with desired nutritional properties. This is among the oldest biotechnology operations [23]. Barley malting process produces superior product with higher quality due to the fact that barely seeds have often the same size and uniformity and they almost germinate simultaneously. The extract prepared from malt dough contains of 90-92% carbohydrates in addition to a range of nitrogenous substances, polyphenols, salts and many other materials. Major part of malt extract comprises of fermented sugars that make up 61-65% of the total extract. Factors such as cluster morphology, crust thickness, grain size and cell wall polysaccharides are the main factors affecting the

amount of malt extract [24]. Malt extract has numerous applications in all over the world due to its enzyme characteristics, flavor, color and nutritional value. Malt is used in various food industries such as confectionary, biscuits, cracker fermentation, different types of caramel, confetti, vinegar, alcohol, brewery, many breakfast cereals, beverages, drinking milks including chocolate milk, bread, baby food as supplement [16].

Cinnamon (*Cinamomu mverrum*) belongs to the laurel family and is native to Sri Lanka [12]. Chinese cinnamon in the history of this plant is a type of Cinnamon called *Cinnamon aromaticum* or Cassia which is native to China. It is a tree with a height of 20 to 30 meters, the bark of which is used as cinnamon [25]. Cinnamon essential oil is used in the pharmaceutical industry as a good aroma and flavoring for bad smell drugs. Also it is used in toothpaste and mouthwash formulations to improve the taste and smell [12].

The main use of licorice (in the West) is sweetening food products, because it is fifty times sweeter than sucrose, and in addition, has medicinal properties. Dried root of licorice can be chewed as a seasoning [12, 14]. By having a daily intake of more than 20 grams, there would be possible incidence of adverse effects. The most important property of licorice is its impact on digestive system [11].

Cloves, the scientific name is *Syzygiuma romaticum*. This tree is native to the islands of Indonesia and Oceania and due to its special beauty is currently grown and used as an ornamental tree in most parts of the world [4]. The main ingredient of clove is eugenol which has sedative and antiseptic effects. It is used in dentistry to soothe toothaches [9].

In the late 15th century, Portugal brought a large amounts of the plant to Europe. Due to its high value, Spain began to trade the plant through the Indian Ocean and later they could take the business from the Portuguese. Its trade was dominated by the Dutch in the 17th century [4].

Nutmeg: *Myristica fragrans* is the scientific name for nutmeg tree. Nutmeg tree is an evergreen perennial plant of [7].Nutmeg grows in hot and humid climate. Aromatic compounds including propanale, benzoxide, safranol and eugenol are detected in Nutmeg.

This plant is used as a medicine to cure some diseases such as diarrhea, mouth ulcers, insomnia. Moreover, there is some evidence of its therapeutic effects on hallucinogenic or psychotropic properties and also to some extent sedative effects have been observed [19].

Vanilla is a climbing perennial plant that produces the vanilla pod. Among different types of vanilla, there is planifolia which was most found in the 19th century in Indian Ocean and Indonesia. The main component of vanilla is vanillin which is prepared synthetically. It is derived of lignin contained in sulfite liquor of whey and synthetically obtained from eugenol and guaiacol. Apart from industrial uses such as ice cream, chocolate, cakes, pastries, biscuits, soft drinks, vanilla has also health benefits such antioxidant, anticancer, antipyretic, sedative, sexual stamina enhancer [17]. According to the national standard of Iran, vanilla dosage is 10 milligrams per kilogram of body weight per individual [1].

MATERIALS AND METHODS

Material:

Malt extract syrup was prepared from Zamzam (West) Co. and other extracts were provided from Etolcelje Co. which made in Slovenia.

Sample preparation (beverage formulation)

Malt, licorice, cinnamon, nutmeg, cloves, vanilla extracts along with sugar and carbon dioxide gas were mixed in specified ratios as demonstrated in Table 1.

Table 1. The treatments used in the study

Citric acid (gr)	sugar (gr)	vanilla (gr)	(gr) clove	nutmeg (gr)	cinnamon (gr)	Licorice (gr)	Malt (gr)extract	
0.4	70	0.218	0.025	0.025	0.025	0.025	31	F1
0.4	70	0.218	0.025	0.025	0.030	0.030	30	F2
0.4	70	0.218	0.025	0.025	0.035	0.035	29	F3
0.4	70	0.218	0.025	0.025	0.040	0.040	28	F4
0.4	70	0.218	0.025	0.025	0.045	0.045	27	F5
0.4	70	0.218	0.025	0.025	0.050	0.050	26	F6
0.4	70	0.218	0.025	0.025	-	-	-	C

Measurement of sample characteristics:

Physical test:

Density measurement: the density was measured by densitometry [3].

pH measurement: the pH of the samples was determined by the pH meter model WTW [3].

Measurement of foreign particles: detection method of sediments and foreign particles was according to the national standard of Iran No. 1249 [2].
Chemical tests:
Determination of titratable acidity: Acidity determination method was based on NaOH 0.1 normal titration of 10 g of the sample mixed with 250 ml of boiled and cooled distilled water in the presence of phenolphthalein reagent. The final acidity was reported in grams of lactic acid per 100 g of sample.
Total sugar measurement: Fehling test was used to measure the total sugar. In this method, sucrose is converted to reducing sugars by using acid hydrolysis, then reducing sugars convert divalent copper ions of Fehling solution to monovalent copper ions in an alkaline environment. Finally, based on the amount of consumed solution, total sugar content was calculated from the following equation.

Reducing sugar content after hydrolysis with total sugar in gram per ml $= \frac{F \times 100 \times 100 \times 100}{25 \times 25 \times V}$

F = Fehling factor
V = Consumption of the neutral solution A.
Total solid measurement (dry residue): Measurement of dry residue of beverage was performed using an oven (memmert, model UFE500 made in Germany) according to the national standard of Iran No. 2280.

Microbiological test:

Microbiological test was performed after removing the gas from the sample under sterile conditions, according to Table 2. After a given time, the plates were examined and the mean value of total number of two plates was reported as number of microorganism for per ml of sample [1].

Table 2 - Guidelines for carbonated malt beverages microbiological tests

incubation time	incubation temperature	method / specific culture media	**Microbiological test**	Row
48 hr	37 ºC	plate count agar / pour plate	Mesophilic aerobic bacteria	**1**
5 days	30ºC	Pour Plate/Orange-Serum Agar	microorganisms resistant to acid	**2**
5 days	25ºC	Pour / Dichloran Rose-Bengal (DRB) Plate	Mold and yeasts	**3**

Statistical analysis: the experiment was conducted in a completely randomized design with 5 treatments and a control in 3 replications. The results were examined by the software SPSS 20. The data was analyzed by ANOVA and Duncan's test.

RESULTS AND DISCUSSION

Specific gravity (density) Measurement: Figure 1 shows the effect of storage time on the specific weight. In this work, the specific gravity (density) had quite similar trends on the first, 15th and 30th day. Minimum and maximum specific gravity (density) on the three days of examination were associated to the control and the treatment F6, respectively. The specific gravity decreased on the 30th day compared to the first day, but a slight increase was observed compared to the15th day.

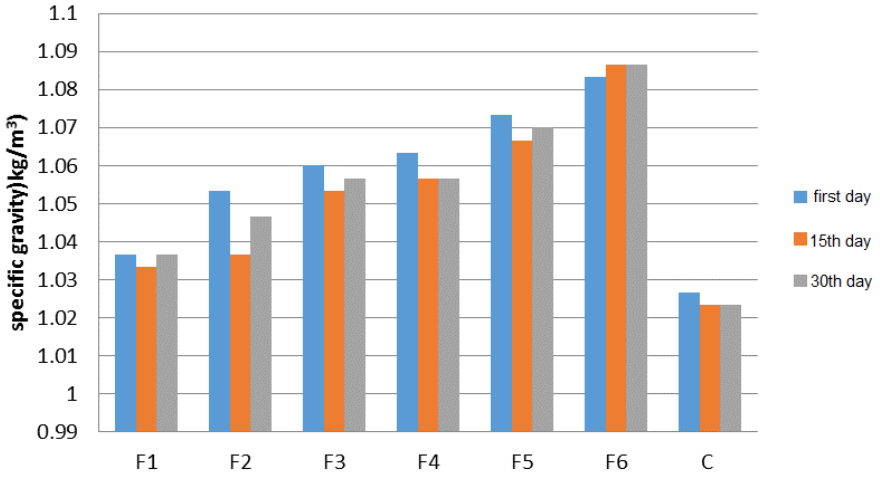

Figure 1 - Effect of 30 day storage on the specific gravity

pH: Figure 2 shows the effect of storage on pH. It was found that the lowest pH on the first day of storage at ambient temperature had been related to the control. As observed in the diagram, the lowest pH value was related to the control and pH changes was quite similar on the evaluation days. Generally, the pH increased within the thirty days of storage at room temperature compared to the first day, probably due to a small increase in hydrogen ion concentration in the environment.

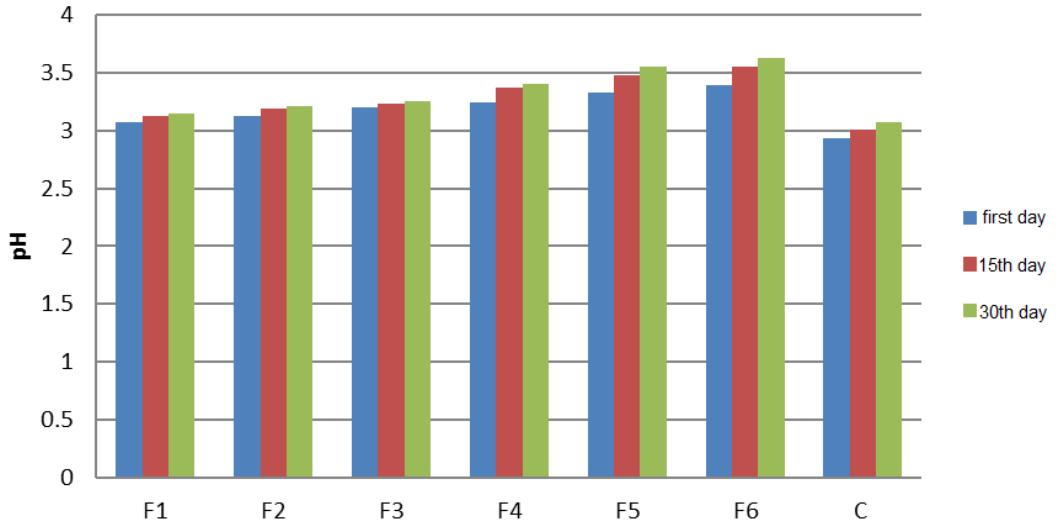

Figure 2 - Effect of 30 day storage on the pH

Determination of titratable acidity: Figure 3 shows the effect of storage time on the titratable acidity. The lowest acidity at ambient temperature was related to the control. As can be seen in Figure 3, the acidity had an increasing trend during the 30-day storage time. However, this increase was greater in the first day compared to the 15th day and the changes between the day 15 and 30 were less. In fact, total acidity determination shows acid content of food. These acids frequently are organic acids. Organic acids are found naturally in foods, added to foods or produced during processes such as fermentation. Taste, color and microbial stability of foods are affected by these acids.

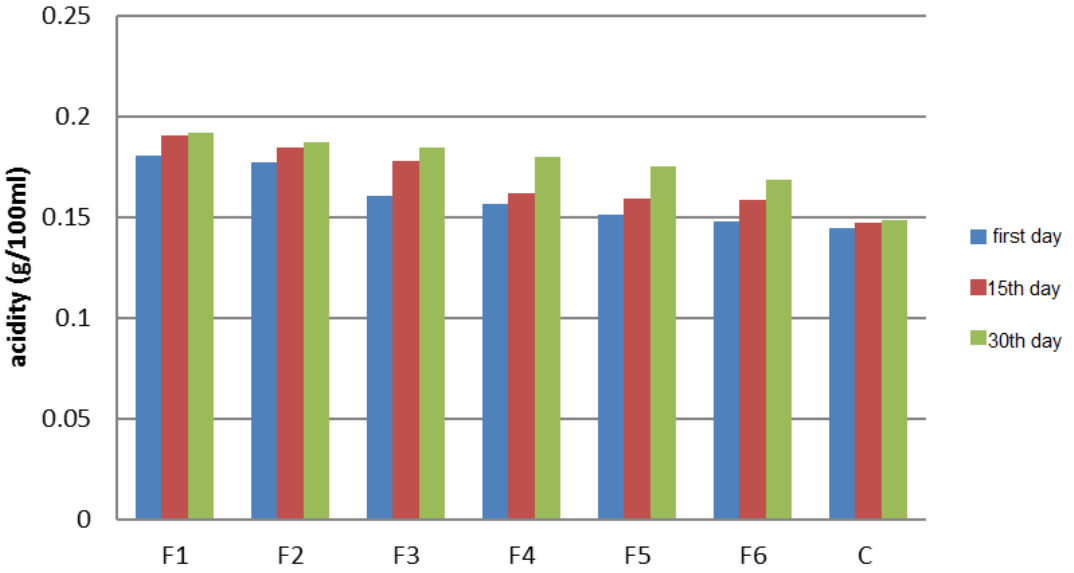

Figure 3. Effect of 30 day sorage on titratable acidity

Total sugar measurement: Figure 4 shows the effect of storage time on the total sugar. The lowest sugar content was related to the control and the highest belonged to the treatment F6. The total sugar changes on the first, 15th and 30th days had quite similar trend and generally the total sugar increasingly changed

during the 30 days. The reason is probably breakdown and hydrolysis of the s1ugar bonds during the storage.

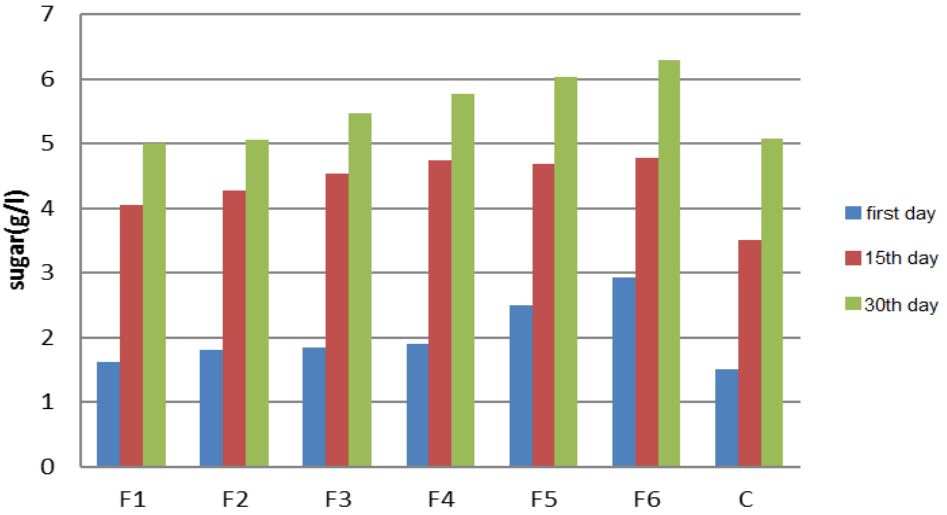

Figure 4 - Effect of 30 days on the total sugar

Measurement of total solids (dry residue): Figure 5 shows the effect of storage on the total solids (dry residue). The lowest and the highest contents of dry solids were related to the control and F6 on the first day, respectively. Similar trends were achieved for the total solids on the first and 30thday, the only difference in changes was between the control and F6. Generally, the total solid contents (dry residue) had a decreasing trend during the 30 days of storage.

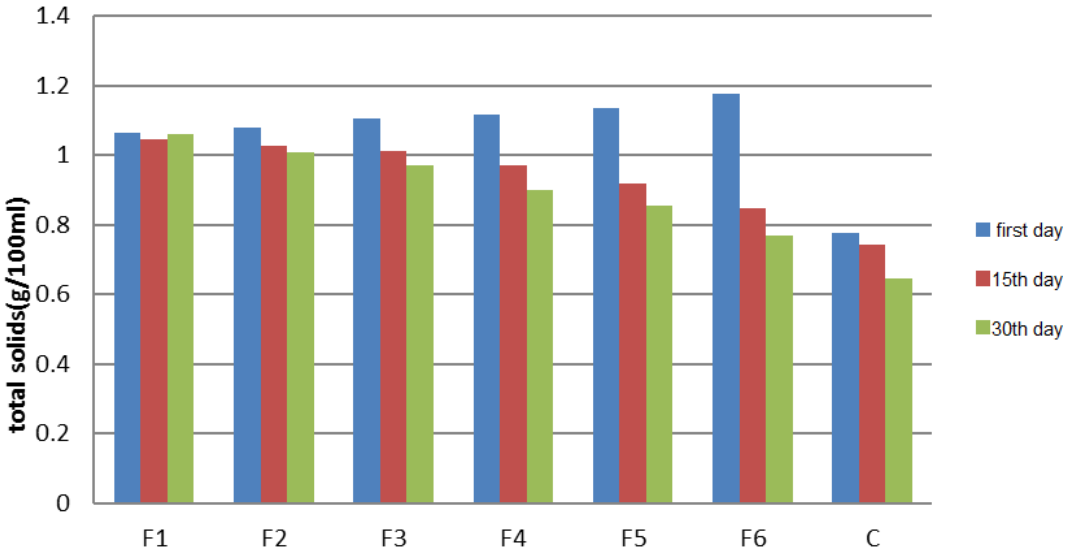

Figure 5 - Effect of 30 day storage on total solids

Microbiological tests: these tests showed that no acid resistant micro-organisms, mesophilic aerobic bacteria, molds and yeasts were found in the beverage and so their use is allowed without any microbial problems.

DISCUSSION AND CONCLUSION

The results showed that in all the treatments, pH increased and acidity decreased during the 30-day storage, however, these changes were not significant. Vesaltalab and Qolami in [15] investigated the effects of the essential oil and clove extract on some qualitative characteristics of grape during the storage and demonstrated that natural ingredients such as essential oils and extracts of clove can control rottenness of white seedless grape during storage. Clove essential oil at different concentrations also decreased water loss, soluble solids concentration, shrinkage and browning of the grape during the

storage. Also it can delay the total phenol content loss, acidity decrease and softening of berries and increase the shelf life of the fruit. The results of this work were in good agreement with our results. Karami et al [11] evaluated antimicrobial activity of methanol extract of licorice root in orange beverage and found that licorice root extract could improve durability within 90 days of storage. Licorice is one of the most important herbs containing phytochemical ingredients with good health effects. Among these compounds, there are glycyrrhizin and phenolic compounds as antioxidants and reducing oxidative cell damage leading to cancer, heart disease. Evaluation of phenolic compounds during storage showed that licorice root extract in the acidic pH of orange beverage could retain the products' stability for 3 months. The results were consistent with the present work.

In all the treatments, the specific gravity (density) significantly changed. Reisi et al [6]examined the production of functional orange drinks using rice bran. Physicochemical characteristics evaluation of beverage containing rice bran extract suggests that addition of rice bran extract to orange beverage may result in specific gravityvariation. The results showed that an increase in rice bran extract in formulation would increase the specific gravity due to the compounds of the extract. The results obtained were consistent with the present study.

The total sugar increased within 30 days in all treatments due to the hydrolysis of sucrose. The total sugar in the treatment F1 containing 31 grams malt extract was higher than the other treatments. Because it has the highest sugar content. In The treatments with less malt extract, the total sugar content was lower. The minimum and maximum amounts of the total solids were related to the control and F1 containing 31 g of malt extract, 0.025 licorice, 0.025 g of cinnamon, 0.025 g of nutmeg, 0.025 g of cloves, 0.218 g vanilla, 70 sugar, respectively. In other words, by increasing the content of malt extract, soluble solids increased due to the high amount of water soluble solids in malt extract. Hossieni et al (2012) studied physicochemical properties and stability of soft drinks containing malt barley and oats. In this study, malt based drinks with different ratios of oat and barley malt produced and physicochemical tests were performed during 6 months of storage. The results showed that by increasing oat malt in the beverage formulation, the reducing sugar and total sugar content increased. The results also showed that the beverages with higher amount of oat malt, had higher sugar content because of α-amylase activity and partial hydrolysis of starch contained in the beverage. The results were consistent with the results of the present work.

The results demonstrated that the total solids content had a slight decrease during the 30-day storage. Generally, the amount of soluble solids in all the treatments is directly correlated with the amount of malt extract. Microbiological analysis showed that the final product contains no harmful microorganisms or byproduct.

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