

## Simultaneous Effects of Sucrose and Stevia Sweetener on the Sensory and Physicochemical Properties of Apple Beverage

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### **ABSTRACT:**

The present study investigates the simultaneous effects of sucrose and stevia sweetener on the sensory and physicochemical properties of apple beverages. To examine and optimize the formulation of the functional apple beverage, three factors—apple concentrate, sugar, and stevia—were selected at three different levels. Using a partial factorial design within a D-optimal framework, 18 different treatments were identified based on the variable levels under study. The beverages were then produced according to these treatments. The results from the physicochemical, microbiological, and organoleptic tests were analyzed within a completely randomized design, and Duncan's test was used at a 5% probability level to compare the means of the treatments. Data analysis was conducted using SPSS software. The results indicated that changes in sucrose percentage and the amount of stevia sweetener affected the moisture content. Specifically, treatments containing a lower percentage of sucrose and a higher percentage of stevia had higher moisture content compared to treatments with higher sucrose and lower stevia levels. In sensory evaluation, an increase in sucrose concentration resulted in higher beverage consistency, but with higher amounts of stevia, panelists gave lower scores. Despite this, all the samples received high sensory scores ranging from 4 to 5 from the panelists. Overall, it can be concluded that the formulated beverage, based on sensory evaluation and economic assessments, can be introduced to the industry as a low-calorie, functional beverage without harmful effects.

**Keywords:** Apple beverage, sucrose, stevia sweetener, mold, and yeast growth.

### **INTRODUCTION:**

Apple, scientifically known as *Malus domestica*, is considered one of the healthiest foods around the world. Since ancient times, people across the world have had a strong affinity for fruits. According to historians, the original home of the apple was the cold regions of northern Europe, from where this valuable and beneficial fruit spread to other parts of the world. Apple was one of the first fruits to be known to humanity. Human civilization has long been connected with apples, symbolizing love, fertility, friendship, beauty, happiness, health, wisdom, and strength, among other things. There are many different varieties of apples (Nazarian et al., 2011).

In recent decades, the consumption of low-calorie foods containing sugar substitutes has become popular to reduce energy intake, control body weight, and manage diseases like diabetes and blood sugar levels. As a result, the increased use of low-calorie sweeteners in food production has led to a higher demand for various sweeteners. Many synthetic sweeteners pose risks to consumers, such as phenylketonuria sensitivity and potential carcinogenicity. Therefore, significant attention has been given to natural sweeteners, including stevia-based sweeteners, which offer high-quality alternatives (Montazer et al., 2012).

The benefits of beverages based on fruit juices, vegetable juices, and plant and herb extracts lie in their ease of digestion and absorption. These beverages allow for maximum nutrient absorption without the body expending unnecessary energy for digestion and absorption. These products effectively remove toxins and waste from the body and are beneficial in treating diseases like cancer and diabetes. Considering these advantages, natural fruit and vegetable juices are among the best alternatives to carbonated soft drinks and are also nutritionally superior (Torabi et al., 2012). Concentration refers to a process of compression, focusing, and thickening. Fruit concentrate is produced through a process that includes fruit purchasing, washing, juicing, filtration, concentration, and storage in large tanks. To produce what is commonly known as fruit juice, six times the amount of water, flavor, aroma, essence, and vitamins are added, and the product is then packaged. A wide variety of beverages such as fruit juices, sodas, herbal drinks, distillates, and essences have entered the industrial food and pharmaceutical market and are widely consumed (Torabi et al., 2012).

Stevia is a naturally derived sweetener that has gained significant popularity in many countries. It is a non-caloric sweetener and a suitable alternative to artificial sweeteners like aspartame, saccharin, and cyclamate,

without the harmful effects associated with their overconsumption. Stevia is an herbaceous plant sensitive to cold. The sweet compound derived from it is known as steviol glycoside, which is extracted from the *Stevia rebaudiana* Bertoni plant. This small shrub belongs to the Asteraceae family and is native to the border regions of Brazil and Paraguay. Four main steviol glycosides have been identified in stevia: rebaudioside A, stevioside, rebaudioside C, and dulcoside A. These compounds are 250-400 times sweeter than sucrose. Stevia is approximately 200-300 times sweeter than sugar, with rebaudioside A currently offering the best sweetness quality among the varieties.

In a study by Aryaei et al. (2009), a fruit beverage was produced using melon seeds. Due to the neutral pH of melon seeds, long-term storage was not possible, so they used three treatments—orange concentrate, orange saccharose, and citric acid—to lower the beverage's pH to 4.3. Their findings showed that the melon seed beverage containing orange concentrate had the highest overall acceptance, and its flavor score remained above 3 throughout the storage period.

In a study conducted by Aryaei et al. (2015), the effect of some additives on the properties of apricot concentrate and peach juice under cold storage conditions was investigated. The growth of mold, yeast, and total bacterial count in peach juice and apricot concentrate containing 0.2% ascorbic acid and 0.0505% EDTA along with 0.2% ascorbic acid in separate treatments increased compared to the control group (juice and concentrate without additives). In another study by Emami Far et al. (2010), *Stevia rebaudiana* Bertoni, a low-calorie natural sweetener from native Paraguayan plants, was examined. Baghaei (2011) produced a milk-fruit juice beverage as a new product, indicating that milk and fruit juice blends, due to their carotenoid, vitamin C, and phenolic compound content, can be considered functional foods.

Parvaneh et al. (1997) optimized the production of low-calorie quince jam using the artificial sweetener stevia. Their results showed that using stevia powder enabled the production of low-calorie quince jam by reducing sugar content by 50% without altering the product's organoleptic properties. Taqinejad Kafshgari et al. (2013) produced a beneficial orange beverage using rice bran extract. Their findings indicated that adding rice bran extract to the orange beverage formulation altered the physical and chemical properties of the samples.

Araqi et al. (2013) reported that stevia is a natural sweetener that is about 300 times sweeter than sucrose. As a low-calorie, non-toxic compound, it serves as a suitable alternative to sucrose and certain sweeteners like aspartame for diabetic, heart disease, phenylketonuric, obese, and hypertensive patients. Aryaei et al. (2009) optimized the parameters for the production of raisin concentrate, with the best sample achieved using a 1:2 solvent-to-raisin ratio, an extraction temperature of 60°C, and a concentration

temperature of 70°C. Jabarouti et al. (2015) produced a fruit drink from a blend of orange-tangerine concentrate, sugar, water, and pectin in yogurt water (30-60). After ultra-pasteurization and packaging in Tetra Pak cartons, the drink was stored for 12 weeks in both refrigerated and room temperature conditions. Over time, the product's flavor, aroma, texture, overall acceptance, and pH scores decreased, though no significant change in mouthfeel was observed.

Studies suggest that daily consumption of apple juice (without sucrose and preservatives) or one or more apples per day can help prevent colorectal or lung cancer. The presence of a fiber called pectin induces a feeling of fullness. Pectin also reduces the absorption of fats and sugars during digestion, promoting better elimination and reducing fat accumulation. Apple juice improves brain function, enhancing alertness and cognitive abilities. By using stevia instead of sucrose, we aim to improve flavor and health properties while preventing issues like high blood pressure, blood sugar spikes, and other problems caused by sucrose consumption. The production of this beverage can contribute to health and support diabetic patients. Given the points mentioned, the aim of the present study is to examine the simultaneous effects of sucrose and stevia sweeteners on the sensory and physicochemical properties of apple beverages.

## **MATERIALS AND METHODS:**

### **1. Sample Preparation:**

**Stevia Concentrate:** First, the dried stevia leaves were ground using a mixer. Then, distilled water was added to the mixture, enough to absorb twice its weight, as stevia has a high water absorption rate. The solution was then placed in a water bath (Bain-marie) at 70°C for 3.5 hours. Afterward, the solution was filtered once with a clean cloth, and then again using filter paper. The filtered solution was placed in a vacuum evaporator at 80°C with a rotation speed of 4 rpm until the Brix level reached 65 (Aryaei et al., 2009).

**Formulation of Functional Apple Beverage:** First, apples were peeled, de-stemmed, de-seeded, washed, and dried. The apples were then chopped and mixed with water in a 1:1 ratio, along with 1% citric acid. The mixture was heated until the Brix level stabilized at 70%. In the next step, the solution was transferred to an evaporator, where the apple concentrate was produced. For the optimization experiments, a response surface methodology was employed to design the tests. To investigate and optimize the formulation of the functional apple beverage, three factors were selected at three different levels, as outlined in Table 1.

**Table 1: Factors and Measurement Levels**

Factors	Levels		
Concentrate (%)	10	10	10
Stevia (%)	0.3	0.2	0.1
Sucrose (%)	20	25	30

Using a partial factorial design within the framework of the D-optimal design, 18 different treatments were determined based on the levels of the variables under investigation (Table 2). The beverages were then produced based on these treatments.

**Table 2: Selected treatments based on D-optimal design**

Observations	Stevia	Sucrose	Concentrate
1	0.2	30	10
2	0.2	20	10
3	0.3	20	10
4	0.1	25	10
5	0.2	20	10
6	0.3	20	10
7	0.2	30	10
8	0.11	20	10
9	0.2	20	10
10	0.1	25	10
11	0.1	30	10
12	0.1	25	10
13	0.3	30	10
14	0.3	25	10
15	0.2	30	10
16	0.3	30	10
17	0.1	25	10
18	0.3	20	10

## 2. Chemical Analysis:

**Moisture Measurement:** A drying oven was used to measure the moisture content. First, the sample was heated in a water bath (Bain-marie) to evaporate the water, then placed in an oven at 103°C to dry completely. The following formula was used to calculate the moisture content (according to the National Iranian Standard No. 2527):

$$\text{Moisture (\%)} = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample}} \times 100$$

**Total Acidity Measurement:** The total acidity was measured according to the Iranian standard method (No. 1527).

To measure the reducing sugar in the samples, the **\*\*Lane-Eynon\*\*** volumetric method was used. The reducing sugar (n) and total sugar (N) were measured before and after hydrolysis in two stages, respectively. It is always expected that  $N > n$ , with hydrolysis being performed using hydrochloric acid (HCl) and heat (Emami Far et al., 2010). Then, the percentage of sucrose was calculated using the following formula:

$$\text{Sucrose (\%)} = (N - n) \times 0.9$$

The amount of ascorbic acid was determined by a specific method. The concentration of ascorbic acid in the samples was expressed in milligrams of ascorbic acid per 100 milliliters or 100 grams. Five ccs of the solution was filtered using filter paper. Then, it was mixed with 25 cc of distilled water. Two milliliters of the starch indicator were added, followed by titration with 0.01N iodine until a light blue color appeared.

The amount of ascorbic acid was calculated using the following formula (Parvaneh, 1997).

$$\text{Amount of ascorbic acid} = \frac{V \times 0.88}{S}$$

To determine the Brix of the samples, an Abbe refractometer was used.

For measuring the viscosity of the beverage, a capillary viscometer was employed. The procedure involved measuring the time required for a standard volume of liquid to flow through a capillary tube of known length. The viscosity of the beverage was determined within the temperature range of 30 to 80 degrees Celsius, with 10-degree intervals. Then, the sample viscosity was calculated using the following equations (Yousefi Asli et al., 2012).

$$v = \frac{\mu}{\rho} = \alpha t$$

$$\frac{\mu}{\mu_{ref}} = \frac{\rho}{\rho_{ref}} \cdot \frac{t}{t_{ref}}$$

In these equations:

- $\alpha$  is the calibration constant of the viscometer,
- $\mu$  is the viscosity of the sample (Pa.s),
- $\mu_{ref}$  is the viscosity of the reference fluid (distilled water) (Pa.s),
- $t$  is the flow time of the sample (s),
- $t_{ref}$  is the flow time of the reference fluid (s),
- $\rho$  is the density of the sample (kg/m<sup>3</sup>),
- $\rho_{ref}$  is the density of the reference fluid (kg/m<sup>3</sup>),
- and  $v$  is the kinematic viscosity (m<sup>2</sup>/s).

## 3. Color Analysis:

The color of the fruit juice was measured using a Hunter Lab colorimeter, made in the USA.

## 4. Microbial Analysis:

**Total Microorganism Count:** Dilution to 10<sup>-3</sup> was performed, and 1 ml of the sample was plated on PCA media, followed by pouring 15 ml of PCA agar into the plates. The plates were swirled to ensure uniform distribution and then incubated at 32°C for 48 hours. Colonies were counted and multiplied by the dilution factor (Iran National Standard No. 5272).

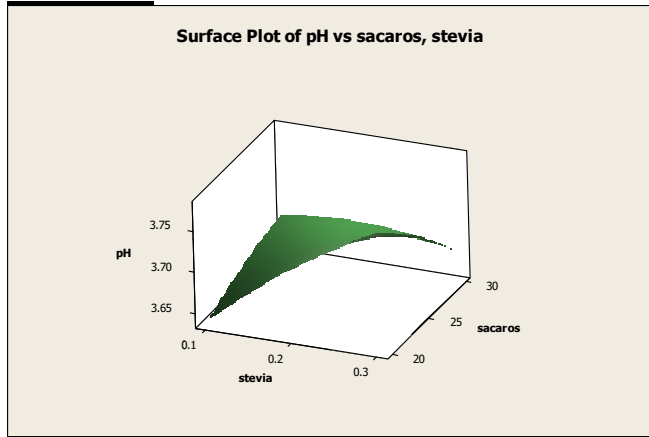
**Mold and Yeast Count:** Similarly, a 10<sup>-3</sup> dilution was prepared, and 1 ml of the sample was plated on PDA media. The plates were incubated at 30°C for 3-5 days, after which colonies were counted (Iran National Standard No. 997)

## 5. Sensory Evaluation:

Twelve evaluators assessed the sensory properties of the product. The data from the sensory tests were analyzed using a completely randomized block design, with a 95% confidence level using SPSS software.

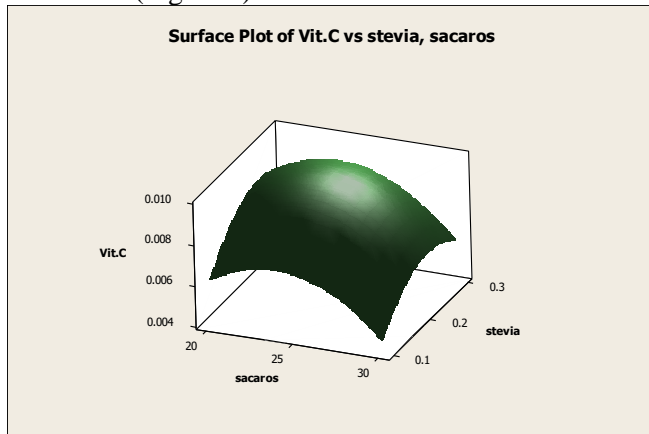
The analysis of the results obtained from the physicochemical, microbial, and organoleptic tests was conducted using a completely randomized design. To compare the means of the treatments, Duncan's test at a 5% significance level was employed. Data analysis was performed using SPSS software, and the charts were created using Excel software.

## RESULTS:



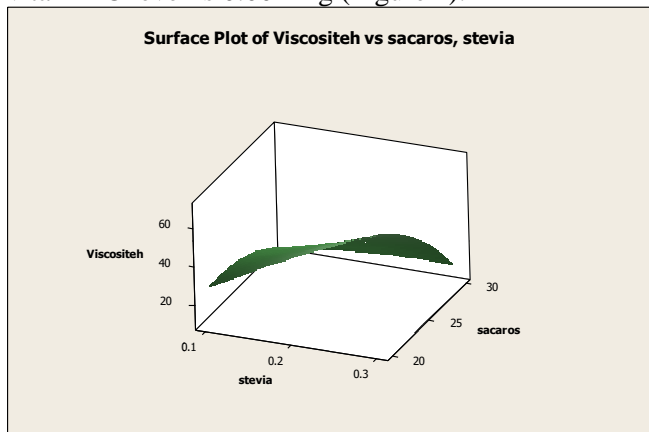
**Figure 1.** The interactive effect of stevia and sucrose on pH changes.

The highest pH level is observed with 0.3 grams of stevia and 20 to 25 grams of sucrose, while the lowest pH level is seen with 0.1 grams of stevia and 20 grams of sucrose (Figure 1).



**Figure 2.** The interactive effect of stevia and sucrose on ascorbic acid changes.

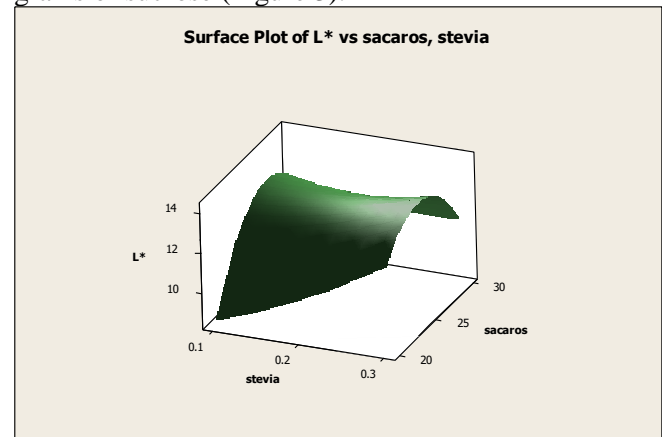
Results show that with 0.1 grams of stevia and 30 grams of sweetness, there is the greatest reduction in vitamin C. However, with 25 grams of sugar and 0.2 grams of stevia, the highest vitamin C level is achieved, measured at 0.010 mg, and the lowest vitamin C level is 0.004 mg (Figure 2).



**Figure 3.** The interactive effect of stevia and sucrose on viscosity changes.

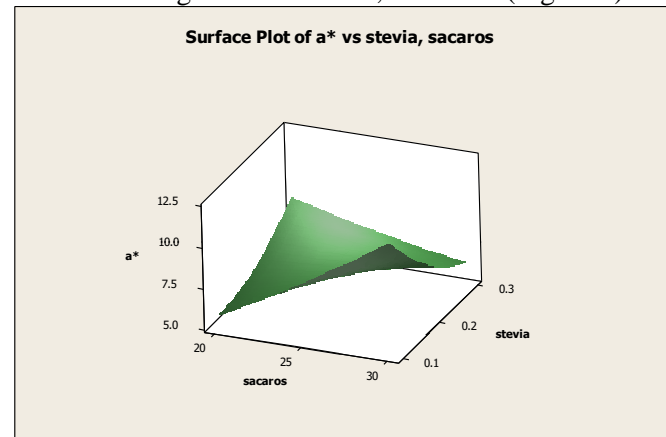
The highest viscosity is found in the combination of 30 grams of sucrose and 0.3 grams of stevia, while the

lowest viscosity is with 0.1 grams of stevia and 20 grams of sucrose (Figure 3).



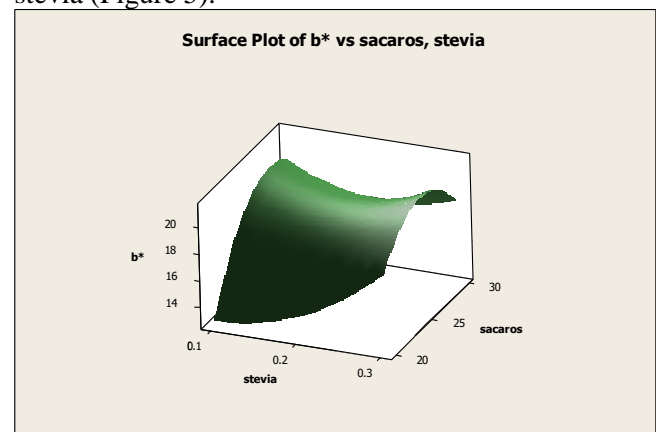
**Figure 4.** The interactive effect of stevia and sucrose on L\* changes.

The highest L color value is associated with 30 grams of sucrose and 0.3 grams of stevia. It is also worth noting that 25 grams of sucrose with 0.3 grams of stevia show a high color level, in the range of 12 to 14. The lowest L color value is seen with 0.1 grams of stevia and 20 grams of sucrose, below 10 (Figure 4).



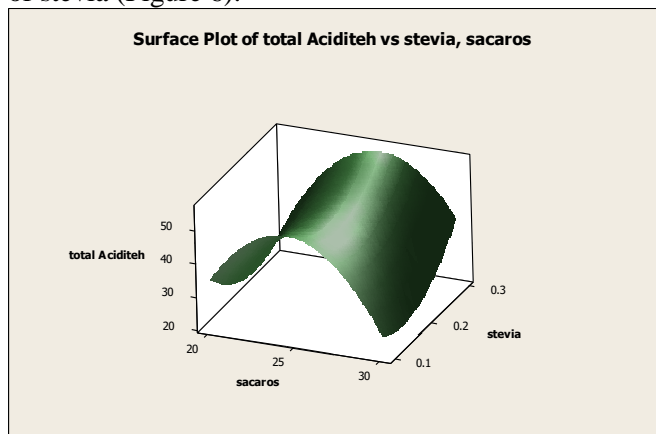
**Figure 5.** The interactive effect of Stevia and Sucrose on A\* changes.

The highest color value is observed with 30 grams of sucrose and 0.1 grams of stevia, while the lowest value is with 20 grams of sucrose and 0.1 to 0.15 grams of stevia (Figure 5).



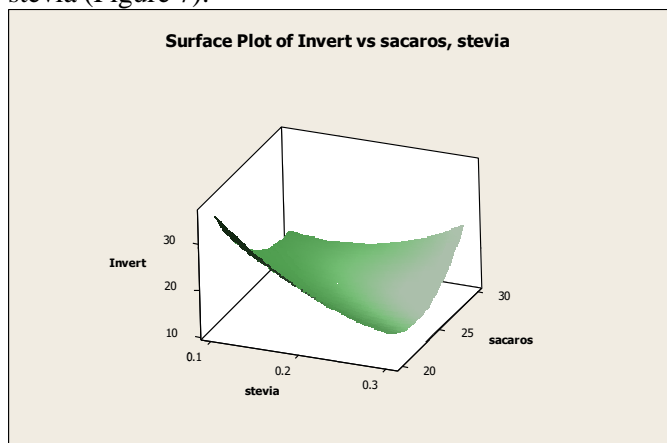
**Figure 6.** The interactive effect of stevia and sucrose on B\* changes.

The lowest b color value is found with 0.01 grams of stevia and 20 grams of sucrose, while the highest b\* value is seen with 30 grams of sucrose and 0.3 grams of stevia (Figure 6).



**Figure 7.** The interactive effect of stevia and sucrose on acidity changes.

The lowest acidity is observed with 0.1 grams of stevia and 30 grams of sucrose, while the highest acidity is associated with 25 grams of sucrose and 0.2 grams of stevia (Figure 7).



**Figure 8.** The interactive effect of stevia and sucrose on invert sugar changes.

The highest amount of invert sugar is found with 20 grams of sucrose and 0.1 grams of stevia, exceeding 35. The lowest level of invert sugar is associated with 25 grams of sucrose and 0.3 grams of stevia. It's worth mentioning that 30 grams of sucrose and 0.3 grams of stevia also approaches the lowest level (Figure 8).

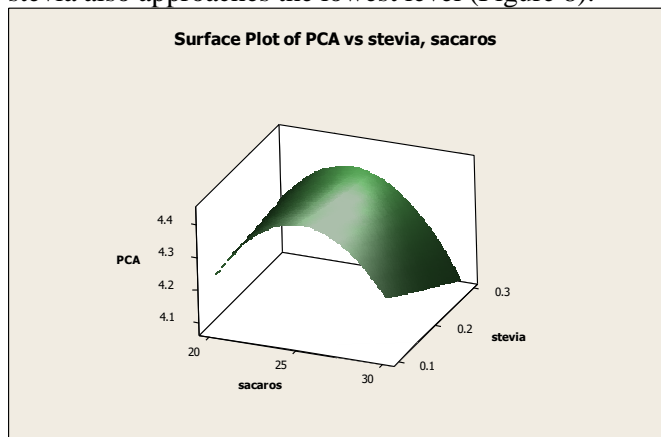
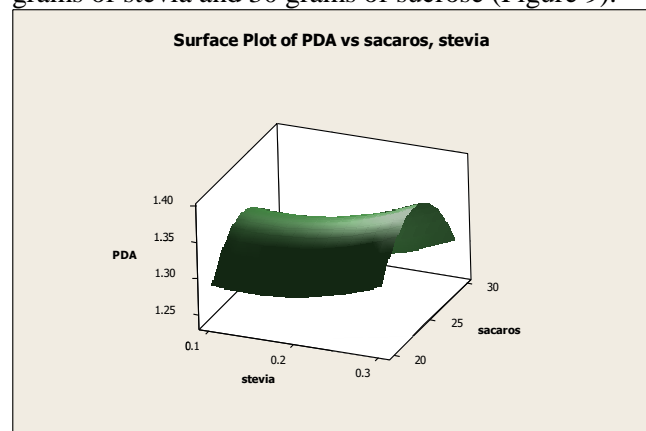
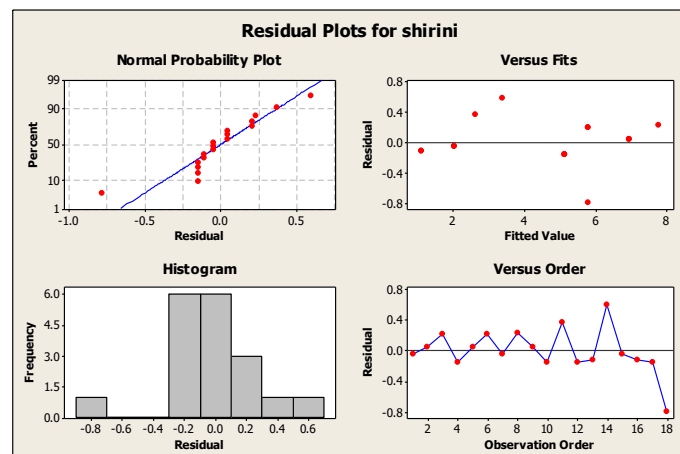
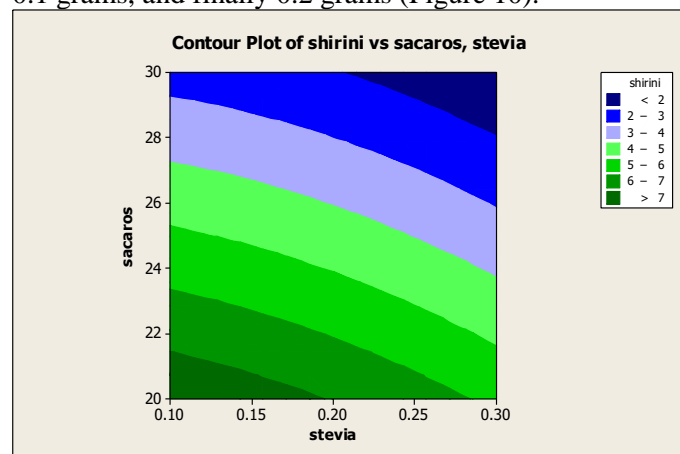


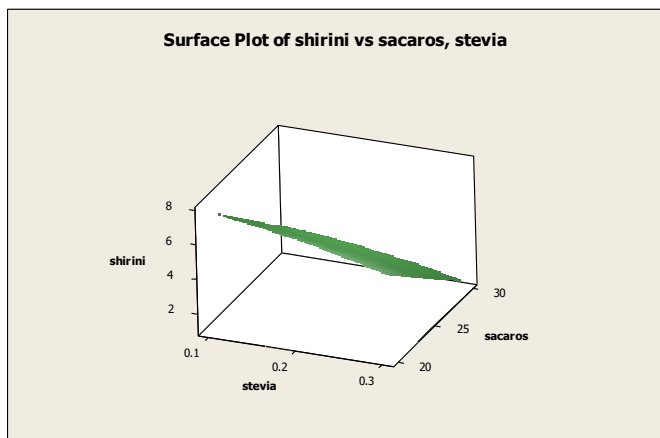
Figure 9. The interactive effect of stevia and sucrose on mold and yeast changes.

In the mold and yeast test using the PCA medium, the highest amount is seen with 0.3 grams of stevia and 25 grams of sucrose. The levels of 25 grams of sucrose with 0.2 and 0.1 grams of stevia are also close to this maximum. The lowest value is observed with 0.3 grams of stevia and 30 grams of sucrose (Figure 9).



The lowest levels in the PDA medium are with 0.1 grams of stevia and 30 grams, as well as 20 grams of sucrose. The highest levels are associated with 25 grams of sucrose in three amounts: 0.1 grams, 0.2 grams, and 0.3 grams of stevia. It is worth noting that 0.3 grams shows the highest level, followed closely by 0.1 grams, and finally 0.2 grams (Figure 10).





**Figure 10. The interactive effect of stevia and sucrose on sensory evaluation changes.**

## **DISCUSSION:**

The changes in acidity levels in various treatments of the apple functional beverage showed significant differences over time ( $p < 0.05$ ). During the storage period, the acidity levels decreased in all treatments. Al-Haddad et al. (2013) also reported that pH and acidity in fruit juices are inversely related. The results of this study are consistent with those of Jazayeri et al. (2009) and Al-Haddad et al. (2013).

The ascorbic acid content in different treatments of the apple functional beverage showed significant changes over time ( $p \leq 0.05$ ). Ascorbic acid levels decreased in all treatments during the storage period. Ascorbic acid breaks down during storage or fruit ripening due to enzymatic activity, leading to a reduction in its concentration. The highest ascorbic acid content was observed in all treatments with a 10% apple concentrate, likely due to the high antioxidant capacity of the concentrate, which helps reduce the degradation of ascorbic acid. In a study by Polana et al. (2011) on the effects of processing and storage on the antioxidant and color properties of some low-sugar fruit jams, a decrease in ascorbic acid levels was also observed with increasing storage time (three months at room temperature).

The pH levels in different treatments of the apple functional beverage also showed significant changes over time ( $p \leq 0.05$ ). In general, pH levels increased during the storage period in all treatments. A comparison of pH levels across treatments during different storage periods revealed that pH was influenced by the amount of stevia, sugar, and apple concentrate ( $p \leq 0.05$ ). In a study by Baghaei et al. (2011) on the production and storage stability of a melon seed-based fruit beverage, it was found that the pH level in beverages containing separate treatments of blood orange concentrate, blood orange sugar, and acetic acid increased until the 28th day of storage, but decreased from day 28 to day 42.

Changes in sucrose levels in different treatments of the apple functional beverage showed significant differences over time ( $p < 0.05$ ). Sucrose levels decreased in all treatments during the storage period. It was found that with an increase in stevia, sucrose, and

concentrate concentration, the sucrose levels in the beverage samples also increased. This can be attributed to the sucrose content present in these two ingredients. The results of this study are consistent with those of Dakhani and Beheshti (2003).

The changes in invert sugar levels in various treatments of the apple functional beverage showed significant differences over time ( $p < 0.05$ ). The levels of invert sugar increased in all treatments during the storage period.

Moisture content in different treatments of the apple functional beverage also showed significant differences over time ( $p < 0.05$ ). During the storage period, moisture levels decreased in all treatments. Comparison of moisture content across treatments during different storage periods indicated that moisture was influenced by the percentage of added stevia sweetener and sucrose ( $p \leq 0.05$ ). The results of this study align with those of other researchers. In a study by Ali Farhadi et al. (2010) on the effects of adding ascorbic acid, salt solution, lemon juice, and honey on the drying and sensory characteristics of dried mango, it was found that moisture loss during drying was lower in treatments containing sugar compared to other treatments.

The changes in the L index in different treatments of the blood orange functional beverage showed significant differences over time ( $p < 0.05$ ), with a decrease in L values in all treatments during the storage period. Comparison of L values across different storage periods indicated that L was affected by the type of additive and the concentration of apple concentrate ( $p < 0.05$ ). In all treatments, the color of the juice darkened over time. The changes in an index in different treatments of the apple functional beverage also showed significant differences over time ( $p < 0.05$ ), with an increase in value during the storage period. The production of by-products from browning reactions, such as melanoidin and furfural, contributed to the increase in the b index in the beverage samples. A positive b value indicates a yellow color in the beverage samples. The maximum L value during the period was 46.21, the minimum was 14.21, and the optimal value was 20.52. These results are consistent with the findings of Goli et al. (2011) and Al-Haddad et al. (2013).

Sensory analysis of different treatments of the apple concentrates functional beverage showed significant changes over time ( $p \leq 0.05$ ). The data indicated that, at lower concentrations, increasing the concentration and reducing the stevia concentration improved the aroma of the beverage samples. However, at higher concentrations, increasing the concentrate led to a decrease in the market acceptability of the product, with panelists giving lower scores to the samples. In a study by Mohammadi Hosseini et al. (2014), the use of stevia, a natural sweetener, instead of sugar in the production of quince jam was evaluated. The results showed that using stevia powder made it possible to produce low-calorie quince jam, where reducing sugar

by 50% did not alter the organoleptic properties, including color, taste, and texture.

No growth of mold, yeast, or bacteria was observed in any of the treatments. The reason for this could be the strong antibacterial and antifungal activity of stevia, and sugar, as well as the presence of phenolic compounds and ascorbic acid in apple concentrate, due to their ability to chelate metal ions. In a study conducted by Al-Haddad et al. (2013), the growth of mold, yeast, and total bacterial count in peach juice and apricot concentrate containing 0.2% ascorbic acid and 0.05% EDTA along with 0.2% ascorbic acid in separate treatments was reduced compared to the control (juice and concentrate without additives).

Brix measures the concentration of soluble solids in water. Iraqi et al. (2013) studied the effect of replacing sucrose with stevia in saffron diet syrup. They reported that with an increase in sucrose concentration, Brix increased, but stevia concentration negatively affected Brix, reducing it. The negative effect of stevia concentration on Brix was also reported by Shokrallahpour et al. (2010) and Nasiri (2013).

### **CONCLUSION:**

Overall, it can be concluded that the formulation of the produced beverage can be introduced to the industry as a low-calorie functional drink without adverse effects based on sensory evaluation and economic assessments. The best formulation was found to be 10% apple concentrate combined with sucrose and stevia in a 50:50 ratio.

Given the very suitable antioxidant properties of apples, it is recommended to investigate their antioxidant activity in a beverage combined with sugar and stevia. If the results are confirmed, producing a functional beverage containing apple concentrate combined with stevia and sucrose, with a 50:50 ratio while considering economic factors, is suggested.

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