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Optimizing the formulation of aloe vera-based vegetable diet drink

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ABSTRACT

The aim of this research was to produce a diet beverage based on aloe vera and investigate its physicochemical, microbiological, antioxidant, and sensory properties. To achieve this goal, aloe vera gel at concentrations of 5%, 5.7%, and 10% underwent heat treatment at 80°C for 30 minutes (Treatment A) and 90°C for 3 minutes (Treatment B). Aspartame was added at concentrations of 100, 150, and 200 ppm, and the beverages were stored for a period of 31 days. The study was conducted using a fractional factorial design with 34 samples under investigation. Optimization was performed based on a maximum amount of 10 gr of aloe vera, 200 ppm of aspartame, and a minimum amount of 5 gr of aloe vera and 100 ppm of aspartame. The use of the sweetener aspartame was highly effective in improving the taste of the beverage, as it provides a much sweeter sensation compared to saccharide sugars and is beneficial for diabetic patients. Based on the microbiological test results, the heat treatment (pasteurization) was successful. Furthermore, sensory evaluation showed improvements in taste, aroma, texture, and mouthfeel with the addition of aloe vera gel to the samples with no undesirable characteristics observed. After determining the models and variable combinations using the Design Expert software, an optimized sample was introduced, containing 200 ppm of aspartame, 10 gr of aloe vera, and the optimized pasteurization process, which was Treatment B for 30 minutes. The results of this study indicate that a vegetable-based diet beverage containing aloe vera can be successfully produced as a beneficial product with desirable sensory characteristics that are acceptable to consumers.

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1-1- Introduction

Recent economic and social developments have raised numerous health concerns for humans. The high levels of stress and tension in modern life have led to the development of diseases such as heart attack, high blood pressure, intestinal disorders, and various types of cancers [1]. Vegetable drinks, often known as "green smoothies," are gaining popularity as an important part of a healthy diet. These beverages are typically made using fresh vegetables and fruits and can be a rich source of vitamins, minerals, and fiber [2, 3].

Drinks are becoming increasingly popular worldwide due to their exceptional taste and enhanced nutritional properties. Quality evaluation of a beverage involves not only the measurement of oxygenated compounds (aldehyde, alcohol, ester, and ketone) but also the assessment of soluble solids, acids, and vitamin C [4]. Over the past few decades, the average consumption of natural beverages has also increased, driven by growing public awareness of health and wellness. In every country, the production of water from natural raw materials is a crucial industry within the food processing sector. In this industry, fruit juice or beverages are pasteurized to extend their shelf life, achieve an ideal visual appearance, and ensure safety. This process, in addition to creating a desirable appearance, also considers the chemical and microbial characteristics of the final product [5]. Pasteurization is a key process that facilitates the consumption of fruit juice products by enhancing their visual appeal and promoting their hygienic standards. However, this process also presents challenges. For instance, during pasteurization, the product is exposed to heat, which can lead to a reduction in certain nutrients. One such nutrient is vitamin C, which is known to be heat and light sensitive. Therefore, despite the benefits of pasteurization, this process may also eliminate some of the product's nutritional value [6, 7].

A significant challenge in the commercialization of certain fruit juice products, particularly vegetable juices, lies in the limitations associated with their shelf life. During the storage period of fruit juices, several factors contribute to their quality deterioration, including microbial spoilage, the development of off-flavors, and ascorbic acid breakdown [7, 8].

In today's world, many nutritionists recommend the consumption of antioxidant-rich plants, fruits, and vegetables to meet the body's antioxidant needs. This recommendation is based on the fact that these natural sources typically have fewer side effects while promoting better health. However, most fresh fruit juices are susceptible to spoilage reactions such as browning and oxidation. Therefore, the use of appropriate additives is essential to preserve flavor, extend product shelf life, and reduce waste. These additives must be able to protect the product from the negative effects of spoilage factors without altering the organoleptic properties of the fruit juice. Additionally, these additives should be cost-effective while increasing the nutritional value of the product.

Given the risks associated with the use of chemical preservatives, natural and plant-based preservatives are now widely used. Aloe vera is a perennial plant with swollen green leaves that are attached to the stem in a corn pattern. These leaves are covered by a thick epidermal layer consisting of a cuticle and enclosing the mesophyll. This epidermal layer is attached to parenchymal cells that contain a transparent mucilaginous gel, known as aloe vera gel [9-11]. The wide range of benefits of aloe vera is primarily attributed to the polysaccharides present in the gel of its leaves. This miraculous plant contains over 75 nutrients and 200 active compounds, including a wide range of vitamins, enzymes, minerals, sugars, lignin, anthraquinones, saponins, salicylic acid, and amino acids [11]. Similar research in the scientific field shows that aloe vera has a clear inhibitory ability against the bacteria Mycobacterium smegmatis, Micrococcus luteus, Enterococcus faecalis, Bacillus sphaericus, and Klebsiella pneumoniae [11-13].

Considering the importance of consuming beverages for maintaining health and the numerous benefits of aloe vera in combination with food in terms of health effects, the aim of this research was to optimize the formulation of a vegetable-based diet drink using aloe vera.

2-1- Materials and Methods

1-2-1- Aloe vera gel preparation

Fresh and mature aloe vera leaves were purchased from the local market. After washing, the tips, ends, and edges of the leaves were carefully trimmed. Then, using a hand knife, the middle part of the leaf was longitudinally cut and the skin and leaves were separated from the middle flesh of the leaf (fillet), which contains the gel. To remove bitterness, the separated gel was mixed with distilled water for half an hour and then separated. The fillets were then finely chopped and blended for 5 minutes using a blender. The final mixture was collected after passing through a cloth strainer, in order to obtain the pure gel. A 25% (w/w) concentration of glycerin was prepared by adding sterile distilled water to the pure gel [11].

1-2-2- Beverage production method

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To produce the beverage, the samples were first prepared in different weights and then the gel and syrup were fully mixed in a blender. Then, the desired beverage was prepared using water, sugar, citric acid, and the selected high-intensity aspartame sweetener. The sample was subjected to homogenization and pasteurization in a high-speed mixer-homogenizer at 50 bar for 10 minutes until the pH reached 3 to 5.3 and the Brix reached 8. The homogenization was performed at temperatures of 80°C and 90°C for 30 minutes, respectively. The samples were then packaged in polyethylene packages and immediately cooled to 80°C using cold water. They were stored in refrigerators and rooms at 4-6°C and 25°C, respectively, until analysis. The samples were evaluated at intervals of 1, 15, and 31 days [14].

1-2-3- Product test methods

1-2-4- pH

The pH of the aloe vera samples was measured using a pH meter (Jenway, model PH 3510 meter, made in England) [15].

1-2-5- Acidity

To measure the acidity of the samples based on citric acid, 10 ml of the sample was titrated with 0.1 N NaOH in the presence of phenol phthalein indicator (Merck Germany). The amount of this indicator was calculated in degrees Dornic using formula number 1 [16].

(1) Total acidity= 100 × Acidity index × ml of 0.1 N NaOH consumed = Sample weight

Each ml of 0.1 N NaOH is equivalent to 0.0064 g of citric acid.

1-2-6- Viscosity measurement

A Brookfield (USA) model RVDV rotary viscometer was used to measure viscosity. The viscosity of the samples was measured using spindle number 500 in the speed range of 5 to 200 rpm at 25°C and expressed in centipoise units [17].

1-2-7- Brix measurement

The measurement of soluble solids was carried out by refractometer in degrees Brix at 20°C according to Iranian National Standard (No. 2685) [18].

1-2-8- Measurement of phenolic compounds

The Folin-Ciocalteu reagent method was used to measure the total polyphenol content. In this test, the absorbance of the samples was measured at a wavelength of 765 nm using a UV spectrophotometer (model 50CARY, made in Australia). The total phenolic content of the samples was calculated using the gallic acid standard curve. The equation was y=211/+0/075x with R2=0/89. The total phenolic content was expressed as milligram gallic acid equivalent per gram of dry sample [19].

1-2-9- Measurement of antioxidant activity

The antioxidant effect of the produced beverage samples was evaluated using the DPPH radical scavenging capacity (RSA) assay. Antioxidant activity was measured using a UV spectrophotometer at a wavelength of 517 nm (model 50 CARY, made in Australia) [20]. The percentage of RSA was calculated using formula number 2:

% RSA =[1 - (AControl – A (1) sample) / AControl) × 100] (2)

Sample absorbance =Asample

Control absorbance = AControl

RSA = DPPH radical scavenging activity

1-2-10- Turbidity measurement

Since a beverage should generally have a more desirable clear appearance, turbidity is considered a negative factor. To measure turbidity, a HANNA Micro Turbidity Meter, HI 93703 model, processor Turbidity meter) from HANNA (USA) was used. The turbidity was reported in NTU units [21].

1-2-11- Total microbial count

The total microbial count was determined using Plate Count Agar (PCA) medium according to Iranian National Standard No. 5484 on the days of production, 15, and 31. The plates containing the samples were incubated at 31°C for 72 hours in an incubator (model zn 1434, made in Iran) and the number of colonies was counted after this period [20].

1-2-12- Sensory evaluation methods

In this study, the evaluators were trained on how to perform sensory evaluation before the test. To evaluate the quality and sensory characteristics, a sensory evaluation test was conducted by 30 trained evaluators (from among the experts of the food industry production unit). For this purpose, sensory evaluation forms were prepared and samples of aloe vera-based vegetable beverages were provided to the evaluators. For this purpose, a 9-point hedonic test was used for each of the characteristics of color, texture, taste, mouthfeel, and overall acceptability (hedonic sensory evaluation).

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1-2-13- Statistical analysis of data

A statistical design specifically for formulation and optimization was used. The beverage had a fixed and variable formulation. The fixed formulation included sugar, citric acid, and water, and the variable formulation process components (aloe vera, aspartame, and pasteurization at 80°C for 30 minutes (treatment A) and 90°C for 3 minutes (treatment B)) were designed in 34 different treatments according to Table 1 using Design Expert software version 13.

Table 1: The values of the variables used in the designed formulas.

	Factor 1	Factor 2	Factor 3	Factor 4
Run	A: Aloe vera	B: Aspartame	C: Time	D: Pasteurization
	%	ppm	Day	
1	10	200	15	A
2	10	100	15	A
3	7.5	200	1	B
4	10	200	15	B
5	7.5	150	15	B
6	5	150	31	B
7	5	150	1	A
8	7.5	100	31	A
9	7.5	200	31	B
10	7.5	150	15	B
11	5	100	15	A
12	5	200	15	B
13	7.5	150	15	A
14	7.5	150	15	A
15	7.5	100	1	A
16	10	150	1	B
17	10	150	1	A
18	7.5	150	15	A
19	7.5	200	31	A
20	10	100	15	B
21	7.5	150	15	B
22	5	100	15	B
23	7.5	100	31	B
24	7.5	200	1	A
25	10	150	31	A
26	5	200	15	A
27	7.5	150	15	B
28	7.5	100	1	B
29	7.5	150	15	A
30	5	150	31	A
31	5	150	1	B
32	10	150	31	B
33	10	200	31	A
34	10	200	31	B

(B: 90C-3min - A: 80C-30min)

2-2- Results and Discussion

2-2-1- Investigation of acidity and pH results

According to Figure 1 and the results of the analysis of variance, among the studied factors, the single and interaction effects of the factors on the acidity and pH of the samples, the two factors of aloe vera concentration and time had a significant effect on the increase in pH and decrease in acidity over time and the percentage increase in the amount of aloe vera additive ($p>0.05$).

The linear equation showed a good fit to the experimental data, the equation with R2 and adj-R2 values of 0.378 and 0.284, respectively, and the non-significance of the regression non-conformity ($p>0.05$) is as follows:

$pH = 3.27 + 0.003\ T + 0.013\ AV + 0.00006\ T \times AV$
($R2 = 0.378$, $adj\text{-}R2 = 0.284$)

As can be seen, the pH of the aloe vera-containing samples changed during storage. The pH of the samples increased steadily over time until day 31 due to chemical and biochemical reactions. This is probably due to the reduced degradation rate of bioactive compounds in the vegetable beverage samples and the increased antimicrobial effect of these compounds at high temperatures [22].

Also, the reason for the significant increase in pH with increasing percentage of aloe vera gel substitution can be attributed to the decrease in hydrogen ion concentration due to the pH effect of aloe vera gel (5.4) or the increase in the aqueous phase, which is consistent with the results of Mashau et al. (2020), Arianfar et al. (1396), and Mir Ghafouri and Rahimi (1395) [11, 23]. As a result, adding aloe vera increases pH and decreases acidity.

It should be noted that although the effect of storage time on the pH of the beverage samples was statistically significant, these changes are very minor and in the order of 0.05 in terms of

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technology. In other words, the dependence of pH on storage time is significant, but the resulting changes in the pH of the product are minor and negligible. Aloe vera gel can reduce the microorganisms present in the samples and lower the acidity due to the antimicrobial and antioxidant properties of its compounds [24].

A-Aloe vera (%)
B-Aspartame
(ppm)
C-Time (Day)
D-Pasteurization

pH = 0.023A + 0.021

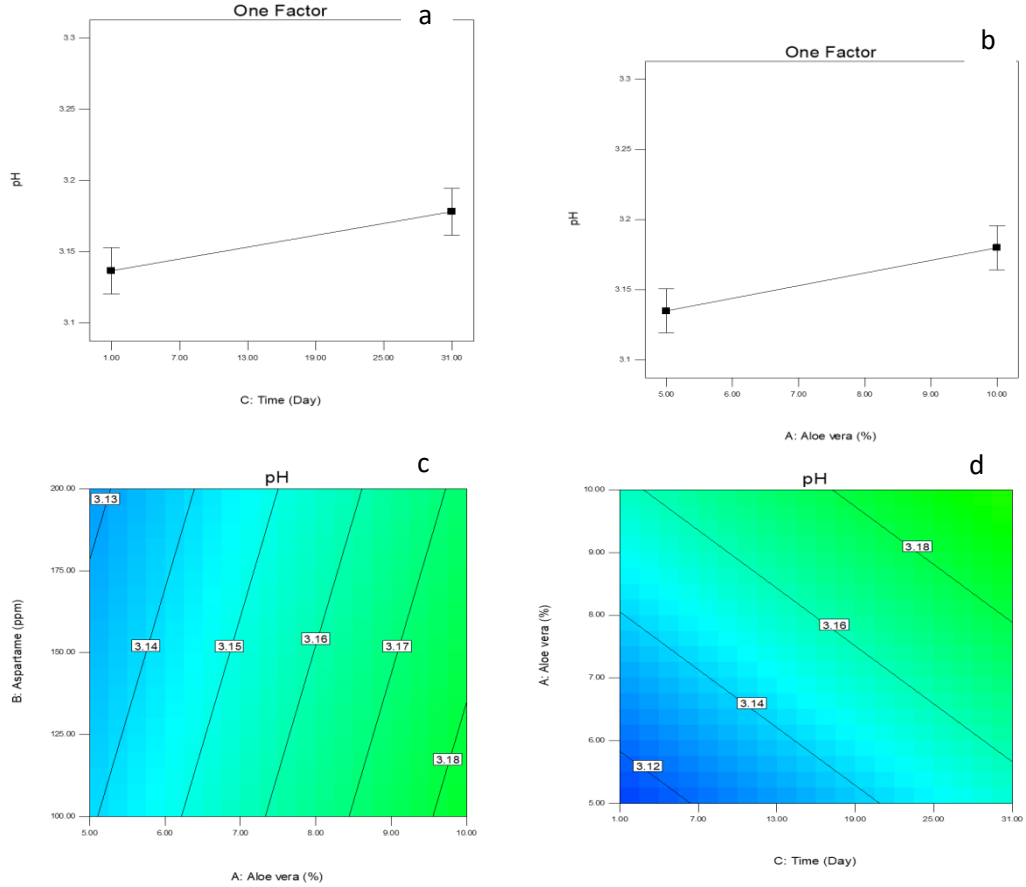

Figure 1: The Changes of acidity in produced drink with respect to the variables of aloe vera, aspartame sweetener, pasteurization and time

2-2-2- Viscosity measurement

According to the results of the analysis of variance, for the beverage containing 5, 5.7, and 10% aloe vera and pasteurized at 80°C and 90°C for 30 and 3 minutes, respectively, on days 1, 15, and 31, the single effect of the factors and the interaction effect of the four factors of aloe vera level, aspartame sweetener, pasteurization, and time on the viscosity of the samples were significant ($p>0.05$). As can be seen in Figure 2, the viscosity of the samples increased both individually and with aspartame with increasing aloe vera substitution and time, but the individual effect of aloe vera was more pronounced over time when used in conjunction with aspartame. The linear equation showed a good fit to the

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experimental data, the equation with R2 and adj-R2 values of 0.423 and 0.331, respectively, and the non-significance of the regression non-conformity (p>0.05) is as follows:

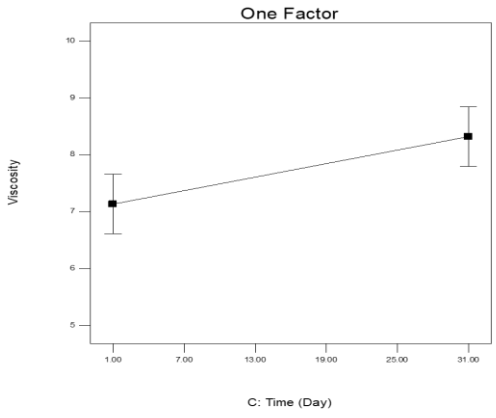
viscosity= 0.64A- 0.4B- 0.59C- 0.34C

The viscosity of the samples increased with increasing aloe vera ratio. Based on the results obtained, the addition of aloe vera gel, due to its ability to create bonds with free water in the tissue, leads to an increase in the viscosity of the product. The highest viscosity was observed in the samples that had 10% aloe vera, probably due to the increase in the amount of solids caused by the increase in the concentration of aloe vera gel powder and the increase in temperature during the pasteurization process. In other words, this may be due to the optimal balance between the amount of aloe vera and the other ingredients in the mixture. Above this concentration, the mixture may become too thick or gel-like, which may not be desirable for a beverage product [25, 26]. In fact, the gel can form hydrogen bonds with free water molecules in the mixture, which increases the overall viscosity of the product. This is a common phenomenon in food science and is often used to adjust the texture and mouthfeel of a product [27]. The increase in viscosity with temperature during pasteurization is probably due to the thermal denaturation of proteins present in the aloe vera gel. When proteins are heated, they unfold and can interact with each other to form a network that increases the viscosity of the solution [28]. In addition, the increase in aloe vera powder concentration may also contribute to the increase in viscosity, as there are more solids in the mixture [29]. This can be attributed to the difference in the amount of aloe vera gel used and the different gel-forming ability of this material.

In addition, the viscosity of beverages changes with the type of sweetener used. Disaccharides, due to their hydrophilic nature and ability to form hydrogen bonds with water molecules, create a higher viscosity in the medium. In addition, molecular size is also a factor in hydrogen bonding with water. Aloe vera, with its low molecular weight and high tendency to absorb water, leads to an increase in the viscosity of the beverage [26, 30].

viscosity= 0.64A- 0.4B- 0.59C- 0.34C

a



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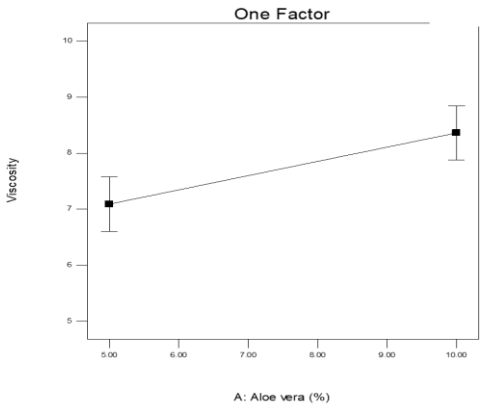

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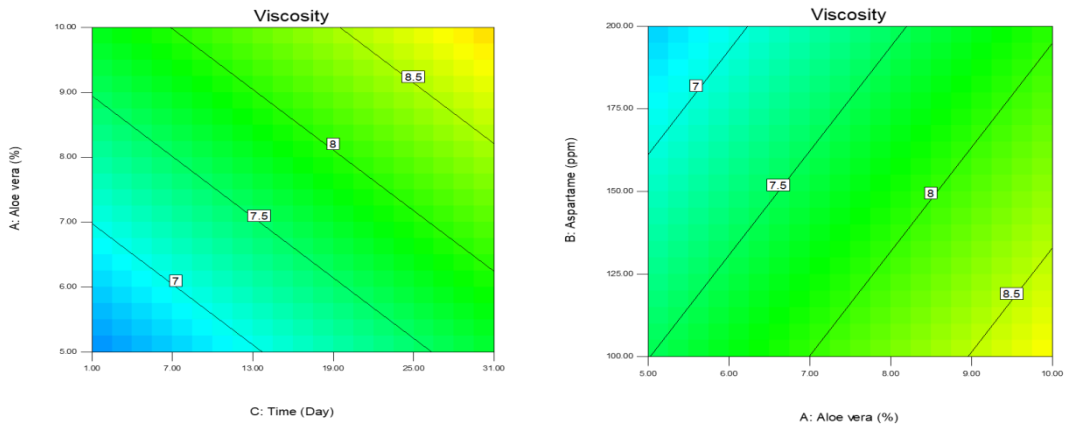

Figure 2: Changes in viscosity of the produced beverage relative to variables of aloevera extract, aspartame sweetener, pasteurization, and time.

2-2-3- Brix Measurement

According to the analysis of variance, for the beverage containing 5, 5.7, and 10% aloe vera and pasteurized at 80°C and 90°C for 30 and 3 minutes, respectively, on days 1, 15, and 31, the single effect of the factors and the interaction effect of the four factors of aloe vera level, aspartame sweetener, pasteurization, and time on the Brix of the samples had no significant effect, and the Brix of the samples was 8 throughout the time period ($p>0.05$). This is due to the lack of effect of adding aloe vera gel on the Brix of the samples.

Brix value is a primary measure of sugar concentration in beverages and fruit juices. Therefore, this result could mean that aloe vera gel, while it can increase the viscosity of the beverage (as shown by previous research), does not appear to affect the Brix value as well [11]. This may be because aloe vera gel itself contains amounts of water-soluble compounds or, due to its hydrophilic properties, affects the sugar present in the solution [31, 32]. As a scientific phenomenon, this result is consistent with previous studies on the effect of aloe vera gel on the physical and chemical properties of beverages [30, 33]. On the other hand, the Brix of the product changes due to the consumption of sugars by bacteria, mold, and yeast. The lack of change in Brix during storage is a result of the effective parameters in the product's shelf life that have prevented reactions affecting Brix [34].

2-2-4- Total Phenolic Compounds Measurement

According to the analysis of variance, for the beverage containing 5, 5.7, and 10% aloe vera and pasteurized at 80°C and 90°C for 30 and 3 minutes, respectively, on days 1, 15, and 31, the single effect of the factors and the interaction effect of the four factors of aloe vera level, aspartame sweetener, pasteurization, and time on the total phenolic compounds of the samples were significant ($p>0.05$). As can be seen in Figure 3, the total phenol content of the samples increased with increasing aloe vera substitution level, but the increase was lower over time than before. This increase ranged from 57.7 to 98.14 ppm total phenolic content. The quadratic equation showed a good fit to the experimental data, the equation with R2 and adj-R2 values of 0.693 and 0.619, respectively, and the significance of the lack of fit ($p>0.05$), regression is as follows:

Total phenolic compounds = 36.83 + 6.69 A + 0.24 B - 1.86 C - 0.04 D + 0.03 AB - 0.02 AC + 0.01 AD + 0.005 BC + 0.001 BD + 0.0005 CD - 0.004 A2 - 0.002 B2 - 0.001 C2 - 0.0001 D2 (R2 = 0.693, adj-R2 = 0.619)

The results of this study, based on analysis of variance (ANOVA), show that aloe vera level, aspartame sweetener, pasteurization, and time all had a significant effect on the total phenolic compounds in the beverage samples. These effects can also occur individually or in interaction with each other. The presence of phenols in beverages is important because these compounds have strong antioxidant properties and can help protect the body from oxidative damage. This study shows that as the aloe vera level increases, the total phenol content of the samples increases. However, over time, the rate of increase in phenols decreases, which may indicate the stable nature of phenolic compounds in the beverage environment. The quadratic regression equation obtained shows a good ability to describe the experimental data. The R2 and adj-R2 values are 0.693 and 0.619, respectively, indicating a strong regression model. These values indicate that the model was able to explain approximately 69.3% and 61.9% of the variation in total phenol content. The non-significant lack of fit ($p > 0.05$) indicates that

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the model adequately interprets the experimental data and that there is no significant statistical difference between the experimental and predicted data.

On the other hand, this difference or significant effect may be due to the fact that the total phenolic compound content in beverages can be influenced by several factors. Some materials, like aloe vera, contain high levels of phenolic compounds. Therefore, as the amount of these materials increases in the beverage, the phenol content also increases [35]. Additionally, some thermal processes, such as pasteurization, can alter the amount of phenolic compounds. Furthermore, over time, some phenolic compounds may break down or react with other substances, consequently changing the overall phenol content. Storage conditions can also impact the amount of phenolic compounds. For example, light, heat, and humidity can cause phenolic compounds to degrade [36].

In essence, aloe vera gel, which contains numerous phenolic compounds, has gained scientific attention for its antioxidant, anti-inflammatory, and antimicrobial properties. These chemical compounds include a hydroxyl group attached to an aromatic hydrocarbon group [37].

Aloin [1] or barbaloin, one of the primary phenolic compounds in aloe vera gel, is an anthraquinone glycoside found mostly in the outer leaf of the plant and is removed during processing due to its potential to cause side effects [38]. Aloe-emodin [2], another phenolic compound in aloe vera, is known for its medicinal properties including antiviral and anticancer activity [37]. Catechins, a type of phenolic flavonoid found in aloe vera and other substances like green tea, possess antioxidant properties [37, 39]. Aloe vera gel also contains other flavonoids like aloe resins and aloin which contribute to its antioxidant, anti-inflammatory, and antimicrobial properties [40].

Phenolic acids such as p-coumaric, ferulic, and cinnamic acid. The presence of these phenolic compounds in aloe vera makes it a valuable component in medicinal, cosmetic, and food products. However

Total phenol= -8.24E A+ 7.89E B- 4.27E C- 2.21E D- 4.38E AC - 0.01A2

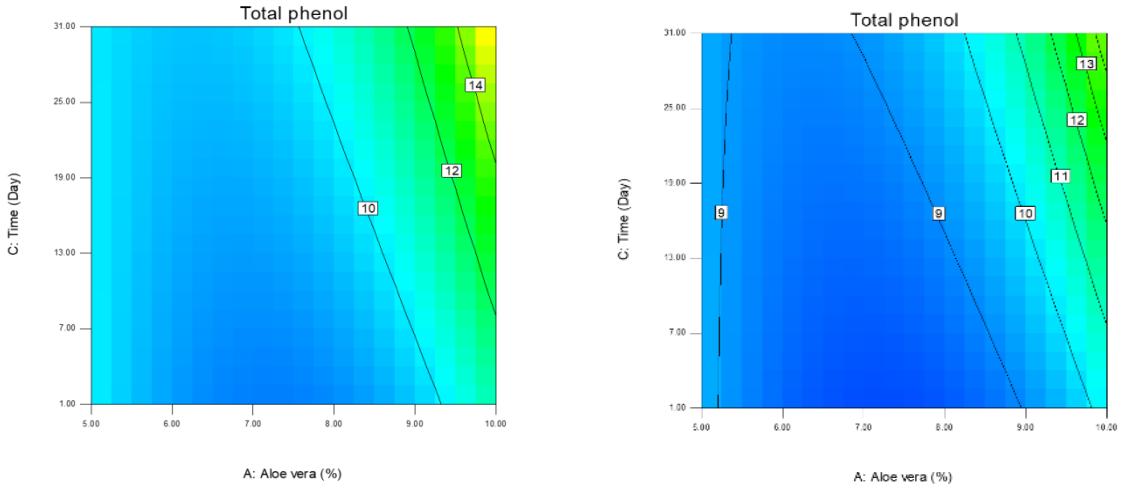

Figure 3: Changes in total phenolic content of the produced beverage relative to variables of aloevera extract, aspartame sweetener, pasteurization

, and time.

2-2-5- Antioxidant Activity Measurement

According to the analysis of variance, the single effect of the factors and the interaction effect of the four factors of aloe vera level, aspartame sweetener, pasteurization, and time on the antioxidant activity of the samples were significant ($p>0.05$). As can be seen in Figure 4, the antioxidant activity of the samples increased both individually with increasing aloe vera level and time, and this increase was significant from low to high levels (direct relationship with increasing aloe vera and time). While with increasing aloe vera to 5% and simultaneous increase in aspartame and time, the antioxidant activity of the samples also increased. The quadratic equation did not show a good fit to the

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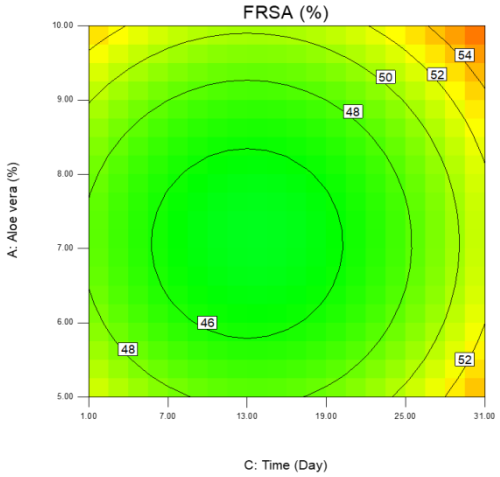
experimental data, the equation with R2 and adj-R2 values of 0.390 and 0.231, respectively, and the non-significance of the lack of fit ($p<0.05$), regression is as follows:

Antioxidant activity = -12.97 + 1.96 A + 0.23 B + 0.04 C + 0.03 D + 0.01 AB + 0.02 AC + 0.02 AD - 0.01 BC + 0.001 BD + 0.0005 CD - 0.01 A2 - 0.01 B2 - 0.002 C2 - 0.0001 D2 (R2 = 0.390, adj-R2 = 0.231)

The antioxidant activity of extracts is due to the presence of various active groups such as hydroxyl and carbonyl groups. As the amount of polyphenols increases, the antioxidant capacity of extracts also increases significantly. It is important to note that beverages containing aloe vera gel have a higher polyphenol content. Heating these samples increases their polyphenol content. This increase is due to the breakdown of high molecular weight polyphenols into lower molecular weight compounds. Therefore, it can be said that heating has a positive effect on increasing the polyphenol content. In addition, with increasing aloe vera gel content, the antioxidant activity and polyphenol content of beverage samples increase significantly. Aloe vera gel is a valuable source of antioxidants, which is due to its polyphenolic compounds [10, 43]. During storage, the antioxidant activity and polyphenol content of the samples increased significantly. The increase in pH increases the amount of total phenolic compounds (due to increased solubility of some of them under acidic conditions and, as a result, their non-precipitation), which in turn increases antioxidant activity [44].

%FRSA = 1.35 A- 0.91 B+ 1.75 C- 0.79 D+ 3.91 A2 + 4.38C2



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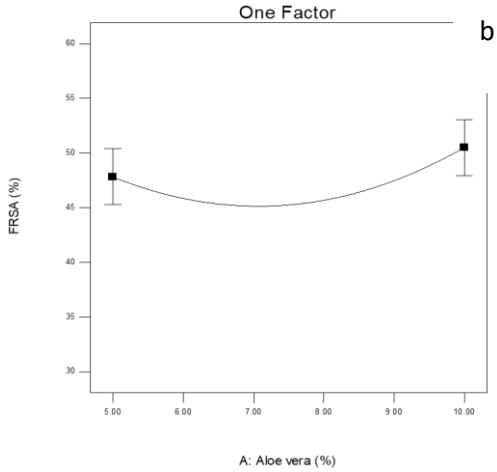

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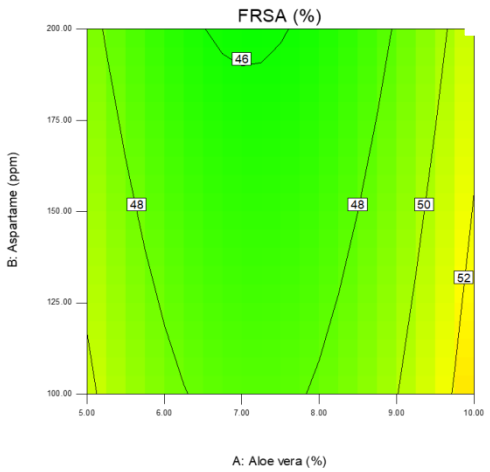

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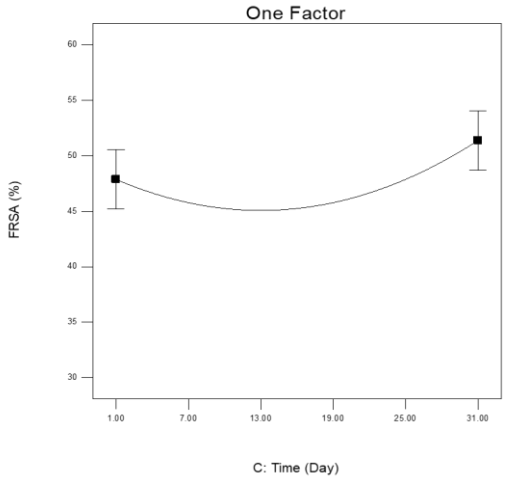

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Figure 4: Variations in the antioxidant properties of the produced drink.

2-2-6- Turbidity Measurement

According to the analysis of variance, the single effect of the factors and the interaction effect of the four factors of aloe vera level, aspartame sweetener, pasteurization, and time on the turbidity of the samples were significant (p>0.05). As can be seen in Figure 4-5, the turbidity of the samples increased with increasing aloe vera substitution level, and this increase ranged from 7 to 12 turbidity content. The linear equation did not show a good fit to the experimental data, the equation with R2 and adj-R2 values of 0.530 and 0.460, respectively, and the non-significance of the lack of fit (p<0.05), regression is as follows:

Turbidity = 2.09 + 0.29 A + 0.02 B + 0.03 C + 0.01 D + 0.005 AB - 0.003 AC + 0.002 AD - 0.001 BC + 0.0005 BD + 0.0001 CD - 0.004 A2 - 0.001 B2 - 0.001 C2 - 0.0001 D2 (R2 = 0.530, adj-R2 = 0.460)

Samples packaged in opaque bottles have lower turbidity than samples packaged in transparent bottles. This is because light is the main cause of turbidity. Overall, it can be concluded that increasing temperature and time accelerate the formation of turbidity in the samples. Phenolic and pectin compounds, proteins and polysaccharides are the most important factors causing turbidity, which can act together or alone. This is probably because our sample is rich in phenolic compounds, both in the vegetable beverage formulation itself and in the added aloe vera gel. However, it also depends on the composition of the raw materials and on microbial activity [11, 47].

$$NTU = 1.25\ A - 0.4\ B + 1.71\ C + 0.13\ D + 3.91\ A2 + 4.38\ C2$$

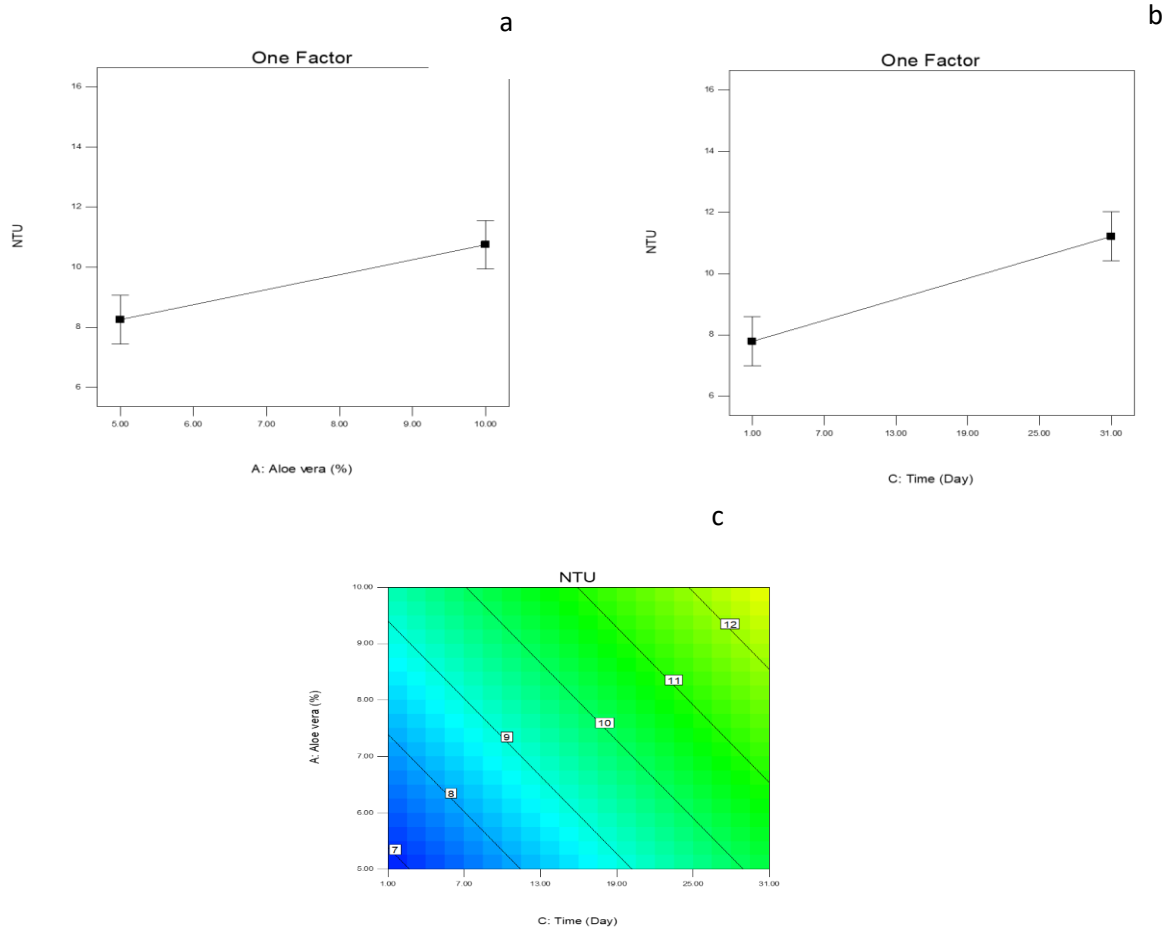

Figure 5- Variations in the turbidity of the produced drink

2-2-7- Microbial Count

The issue of antibiotic resistance has become a serious concern. The bacteria studied in this research can cause diseases as primary or opportunistic pathogens and can also become resistant to various antibiotics [48]. Therefore, it seems necessary to conduct research to obtain antimicrobial agents from other sources such as plants. One of these plants is aloe vera, the beneficial effects of its extract have

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been known for a long time, but there is limited research on its antimicrobial effects [49].

The results of the microbial count in terms of the presence of total microbial count showed that among the factors studied, the single effect of the factors and the interaction effect of the four factors of aloe vera level, aspartame sweetener, pasteurization, and time on the changes in microbial count were only positive in 10% aloe vera, 200 ppm aspartame and 15 days storage and also in 5% aloe vera, 150 ppm aspartame and 1 day storage. In other cases and samples, negative microbial counts were observed. In other words, this study confirmed the antimicrobial effect of aloe vera gel.

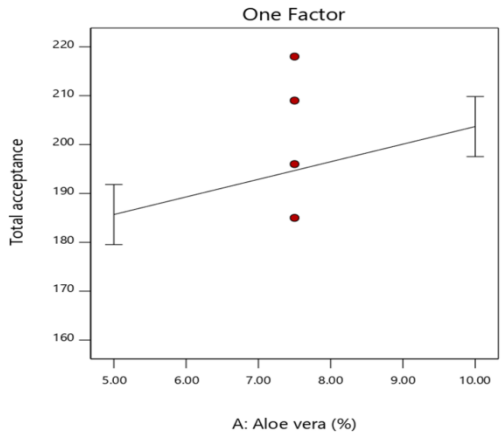
In Iran, despite various studies on the different properties of aloe vera, limited studies have been conducted on the antimicrobial properties of this plant on clinical bacteria [11, 50]. The results of this study also showed that the total bacterial count was sensitive to aloe vera gel and their growth was almost stopped. The difference in the reported concentration values is due to the different extraction methods and different strains used in the experiments. In various studies conducted on the effective compounds of this plant based on different extraction methods, the presence of numerous compounds has been proven [51, 52].

The antimicrobial activity of aloe vera gel may be due to the presence of a wide range of antibiotic compounds, of which phenols and tannins have been identified as the most important active components in this field [36]. Phenolic substances, along with high molecular weight proteins, form complex complexes and thus, after absorption, can react with cellular enzymes (oxidases and reductases) present in the cytoplasm and cell wall. On the other hand, these substances can prevent microorganisms from accessing surface cell receptors [53].

2-2-8- Sensory Evaluation

According to the results obtained, as shown in Figure 6, among the factors studied, the single effect of the factors and the interaction effect of the four factors, the level of aloe vera with increasing effect had a significant effect on increasing overall acceptance, the aspartame sweetener with increasing effect had a significant effect on decreasing overall acceptance, pasteurization and time did not show much difference in satisfaction with the beverage, although they had less effects. As a result, this subject proves that the use of aloe vera gel probably due to its own special taste derived from the presence of various compounds [54] and also having a filling property, it increased the oral feel and also the overall acceptance of the beverage sample ($p<0.05$).

a



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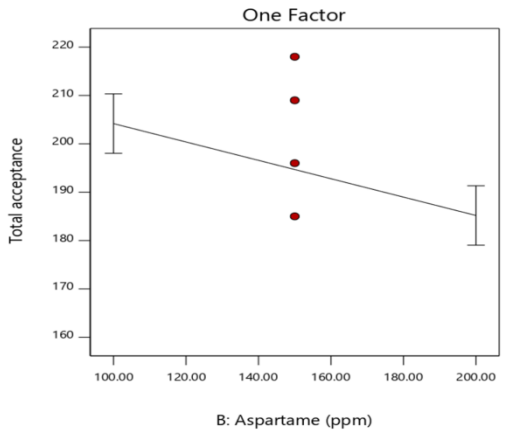

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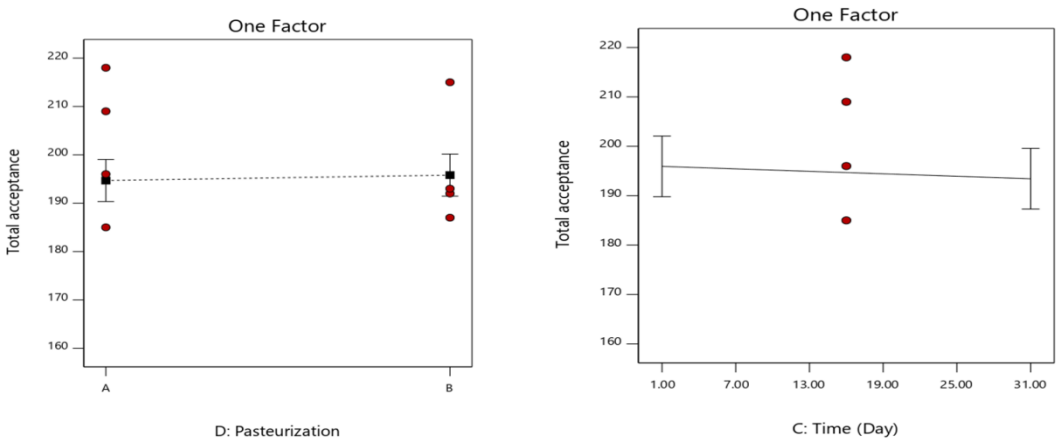

Figure 6: Changes in the sensory evaluation of the produced beverage with respect to the variables of aloe vera, aspartane sweetener, pasteurization and time

2-2-9- Optimization Evaluation and Selection of Optimal Formulas

Formulation optimization of products was performed using the statistical response surface method (RSM) as RSM can reduce the number of experiments required, which saves time and money. In this method, each variable is coded and placed in the range of 1- to 1+, which simplifies the regression analysis. The vegetable beverage included a specific formula. To produce the product, the amount of aspartame sweetener in the samples was reduced and aloe vera gel was added to the samples to provide the lost viscosity. In the RSM method, three factors were evaluated at three different levels to investigate and optimize the formulation of this product. Based on preliminary experiments, the minimum and maximum levels for the three factors were obtained. After producing these treatments, the products were evaluated for responses such as viscosity and Brix. Subsequently, with the determination of a suitable model for viscosity and Brix between aloe vera-containing formulas, sensory evaluation was performed.

After determining the models, optimization was performed to achieve the best formulation in terms of viscosity and Brix, etc. Initially, the lower and upper levels and the desired level were determined for each response, and then based on the described models and the specified levels, a suitable combination of variables (X1, X2, and X3) was introduced by the software. The software introduced several desirable points (formulas) with maximum Brix and had little difference from the control sample in terms of these characteristics. From these points, 34 formulas were selected for the product and then these formulas were prepared and tested for viscosity, Brix, and ... Based on the obtained and predicted responses, it can be understood that the determined models can be used to produce this product.

Next, a flavor evaluation test was performed between the produced treatments. According to the results obtained, there was no significant difference in terms of taste between the aloe vera gel-containing treatments. As a result, the best treatment with the desired formulation was selected.

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2-2-10- Conclusion

Consumer awareness of the link between diet and health and the use of low-calorie foods is increasing day by day. In this context, vegetable-based beverages are becoming increasingly popular and accepted as one of the derivatives of the beverage family. A vegetable-based beverage is a product that is prepared according to a defined standard by mixing vegetable water, permitted food flavor or natural extract, permitted food acids and other components. In vegetable-based beverages, thickeners and sweeteners such as aspartame sugars are used. These thickeners are mostly limited in Iran and must be ordered and prepared from abroad, which means they are imported and expensive. Aloe vera gel was considered as a thickener, antioxidant and antimicrobial in the production of vegetable-based beverages in this study. The addition and increase of aloe vera gel technologically increased the pH, viscosity, phenolic compounds and antioxidant activity. Considering its domestic production and the antimicrobial and antioxidant properties of aloe vera gel compared to other imported thickeners commonly used in beverage production, it has greater preference and efficiency and higher nutritional value. Also, the existence of high amounts of aloe vera in Iran can be a good source for the production of this product.

From a sensory evaluation point of view, no undesirable observation was made with the addition of aloe vera gel in the samples in terms of improving taste, smell, improving texture and mouthfeel. However, with the evaluation of overall acceptance, with the increase of aspartame, its acceptance decreased. This could be due to its specific taste and the specific taste of the evaluators. Making desirable changes in technological properties and improving nutritional properties without making undesirable changes in sensory properties showed that aloe vera gel can be used as a suitable additive and thickener in the production of vegetable-based beverages. The desirable nutritional properties of aloe vera gel can be used to produce functional products by improving the level of antioxidant and antimicrobial properties.

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چکیده

هدف از این پژوهش، تولید نوشیدنی رژیمی بر پایه آلوئه‌ورا و بررسی ویژگی‌های فیزیکوشیمیایی، میکروبی، آنتی اکسیدانی و حسی آن بود. بدین منظور، ژل آلوئه‌ورا در غلظت‌های ۷/۵،۵ و ۱۰٪ طی فرآیند حرارتی با دمای ۸۰ به مدت ۳۰ دقیقه (تیمار **A**) و ۹۰ به مدت ۳ دقیقه (تیمار **B**)، اسپارتام در مقادیر ۱۵۰،۱۰۰ و ppm ۲۰۰ و مدت زمان نگهداری در محدوده ۳۱ روزه با استفاده از طرح آزمایشات فاکتورهای کسری در ۳٤ نمونه مورد مطالعه قرار گرفت. ضمن بررسی ویژگی‌های فیزیکی و شیمیایی و میکروبی و آنتی اکسیدانی و حسی نوشیدنی‌های تولید شده، بهینه‌سازی بر اساس بیشینه مقدار آلوئه ورا ۱۰ گرم، آسپارتام ppm۲۰۰ و کمینه مقدار آلوورا به میزان ۵ گرم و آسپارتام ppm ۱۰۰ انجام گردید. با توجه به نتایج آزمون میکروبی، تیمار حرارتی (پاستوریزاسیون) موفق بوده است. همچنین یافته‌های ارزیابی حسی نشان داد بهبود طعم، بو، بهبود بافت و احساس دهانی با افزودن ژل الوئه ورا در نمونه‌ها، هیچ عدم مطلوبی مشاهده نشد که می‌تواند مورد توجه باشد. پس از تعیین مدل‌ها و ترکیب متغییرها توسط نرم‌افزار دیزاین اکسپرت، نمونه بهینه معرفی شد که حاوی ppm ۲۰۰ آسپارتام و ۱۰ گرم آلوئه ورا و بهینه فرآیند پاستوریزاسیون، تیمار دوم به مدت ۳۰ دقیقه انتخاب گردید. نتایج حاصل از این تحقیق نشان داد، نوشیدنی رژیمی سبزیجات بر پایه آلوئه ورا می‌تواند به طور موفقیت‌آمیزی به عنوان یک محصول فراسودمند با ویژگی‌های حسی مطلوب و قابل پذیرش برای مصرف‌کنندگان تولید گردد.

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