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Scientific Research

Notice to optimizing the use of medicinal plants by producing soft drinks can be a suitable solution in order to encourage consumers to use food with natural source that have beneficial effects on the health. The purpose of this research is optimizing the extraction process of Hibiscus Tea and providing the best way to preserve the nutritional value of this plant during extraction and applying this extract in diet soft drinks formulation by using the natural sweetener Stevia and evaluation of physico-chemical changes during storage. Thus, extracts of Hibiscus Tea was done in three temperatures of 60, 75 and 90 ° C in the range of 10 to 20 minutes and to select the best extraction temperature and time in Hibiscus Tea samples, terms of PH, antioxidant activity, anthocyanins and phenolic compounds were studied. Then by using extraction of Hibiscus Tea, soda with four different formulations were prepared. To consider the durability of soda, in 90 days, every 30 days lasting soft drink samples in terms of antioxidant activity, anthocyanins and phenolic compounds were evaluated. Finally it was determined that temperature $41/71$ ° C for $18/81$ minutes is the best temperature and time for extracting Hibiscus Tea, that In this optimal conditions, the antioxidant activity 62/4062%, the amount of anthocyanins 72/394 mg/l and total phenolic compounds 65/2564 mg / 100 ml was determined. Also it was detrmined that antioxidant activity, anthocyanins and phenolic compounds at the end of storage time than the first day of manufacturing soft drinks decreased. **DOR:20.1001.1.20088787.1402.20.142.9.9**

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1- Introduction

Sour tea medicinal plant is a one-year plant species with the scientific name Hibiscus sabdariffa¹ From the Malwaseh family² It is known as sour tea in Iran. Different parts of this plant including its flowers, leaves and seeds can be used in food industry and pharmaceutical industry. The original home of this plant is West Africa and today it is widely cultivated in West Asia, America, Australia and many other countries. This plant is cultivated in Sistan and Baluchistan, Fars and Golestan provinces in Iran. This plant is rich in anthocyanins, flavonoids, ascorbic acid and many other valuable substances and surprisingly has high antioxidant and antibacterial activity. It has a special place in traditional medicine and from the point of view of pharmacists, the extract of this plant is recommended for blood pressure. Therefore, it has anti-hypertensive, anti-carcinogenic, anti-proliferation and proliferation of cancer cells (especially in breast, uterine and ovarian cancers), anti-spasmodic and antiparasitic [1,2,3].]

Undoubtedly, the highest consumption of soft drinks in the world belongs to developed countries and countries with high per capita national income. The highest consumption of industrial soft drinks was reported between 1970 and 1985. From the early 90s, the composition of these soft drinks gradually changed to so that laxatives, sedatives, a group of vitamins and some addictive substances were added to these compounds because with the increase in the level of awareness of people in European countries and the United States, the consumption of carbonated soft drinks had decreased and consumers generally turned to soft drinks Natural products such as fruit juice and natural syrups prepared from fruits and medicinal plants became popular. Considering the problems of producing industrial soft drinks in Iran, such as technical production and management problems, raw material problems, consumption and export markets, as well as the harms attributed to industrial soft drinks from the point of view of consumer health, many researchers are looking for solutions. They are aimed at optimizing the consumption of traditional and natural drinks, because many of the ingredients used in cola drinks are made of synthetic materials. For this reason, in recent years, a lot of research has been done to produce carbonated fruit drinks with fruit flavor, carbonated fermented drinks such as beer, and also drinks that are produced from the juice of dairy products such as whey and butter. Medicinal plants are also among the cases where some of them have been used traditionally for more than a few thousand years,

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so the production of carbonated soft drinks from medicinal plants can be considered as a suitable alternative to industrial soft drinks. [4,5,6,7,8,9,10,11,12,13]

Stevia is also a natural sweetener that is about 300 times sweeter than sucrose, which, as a low-calorie and non-toxic combination, has led to a suitable alternative to sucrose and some sweeteners such as aspartame in diabetic patients, heart patients, ketonic phenol and Have obesity and blood pressure. Drinks are among the products that can use stevia to replace sugar. Stevia is now cultivated in different countries of Asia and Europe. Its leaves contain many different components such as flavonoids, organic acids, chlorophyll, labdense-sterol, mono-disaccharide, tripterpenoid and mineral salts. [14,15,16,17,18,19]

Considering the society's tendency to use carbonated drinks like cola and their adverse effects on society's health due to their synthetic compounds and the use of unhealthy sweeteners that cause an increase in diseases such as cancer, diabetes, etc., it is necessary Research on the use of plants Medicines in the food industry to reduce the mentioned problems and realize the above goals, the present research is presented, in which the formulation of the production of carbonated soft drinks based on sour tea using the natural sweetener stevia is investigated. In this research toOptimization of sour tea extract extraction process by response surface method, production of diet soft drink from sour tea using stevia natural sweetener, and evaluation of physicochemical changes of soft drink during the storage period were discussed.

2- materials and methods

2-1- Sample preparation

500 grams of dried sour tea petals were purchased from Zahedan city located in Sistan and Baluchistan province, which is one of the major sour tea production centers in Iran. Then, all the frills and impurities were separated as much as possible, and after that, washing with distilled water was done, and after drying again, the sour tea petals were crushed by a grinder.

2-2- Sour tea extract extraction

Extraction from sour tea by hot bath method*Hot water bath)*It was done at three temperatures of 60, 75 and 90 degrees Celsius in the time range of 10 to 20 minutes with 13 treatments and 5 repetitions at the central point. In this way, 20 grams of crushed petals of sour tea were weighed (by digital scale AND-FX300GD) and transferred to Erlen along with 500 ml of distilled water and its lid was covered with foil. For each of the temperatures (60, 75, 90 degrees Celsius), three samples were prepared in this way and one of the

¹ - *Hibiscus Sabdariffa*

²- Malvaceae

times (10, 15, 20 minutes) was applied on each of the samples with the same temperature. Then, each of the samples was placed in a bain-marie (model LAUDA E200) to apply the desired temperature and time on it. Considering that the temperature of 75°C and the time of 15 minutes were considered as the central temperature and time, extraction was done at this temperature and time with five repetitions. After leaving the samples from the bain-marie, they were darkened for one hour, so that the samples were kept in a dark place for one hour. Then, each sample was passed through a filter paper by a vacuum pump. And in this way, the extracts were filtered. Immediately after filtering, the extracts were transferred to dark glasses and placed in a freezer with a temperature of - 18 degrees Celsius to perform physical and chemical tests. In this way, a total of 13 samples of sour tea extract were obtained [20].

2-3- Formulation of carbonated soft drink using sour tea extract

In order to formulate the soft drink, re-extraction was done from sour tea at a temperature of 75 degrees Celsius for 15 minutes. Carbonated water was obtained from a soft drink factory. Stevia and sugar were also used as sweeteners. Four formulas were used to prepare soft drinks:

Formula*A* : 0.1 gram of stevia + 30 ml of sour tea extract + 50 ml of carbonated water

Formula*B* : 0.02 grams of stevia $+1.5$ grams of sugar + 30 ml of sour tea extract + 50 ml of carbonated water

Formula C : 5 grams of sugar + 30 ml of sour tea extract + 50 ml of carbonated water

Formula $D \n10.02$ grams of stevia + 3 grams of sugar + 30 ml of extract + 50 ml of carbonated water

The soft drinks prepared were poured into dark bottles and sealed. Then the bottles for 15 minutes at a temperature of 70 °C were pasteurized by hot water and then quickly cooled to 30 °C.

By doing the initial sensory test of the formula*D* (0.02 grams of stevia $+3$ grams of sugar $+30$ ml of extract + 50 ml of carbonated water) was recognized as the best formula. Then, the produced soft drinks were stored in the refrigerator at 4 degrees Celsius for further tests for three months. was transferred

2-4- The investigated parameters on sour tea extract and soft drink

In order to examine the extracts produced from sour tea with different temperatures and times, the tests of measuring anthocyanin, measuring antioxidant activity and measuring phenolic compounds were performed on each of the samples with three repetitions. Also, in order to check the physicochemical characteristics of the soft drink produced from sour tea during 90 days of storage, the mentioned tests in addition to the test to

measure the amount*pH* It was done on all four soft drink formulas every 30 days with three repetitions.

2-4-1- Physical and chemical tests used measurement*pH*

To determine*pH* from device*pH* meter model*SUNTEX Sp – 701* was used so that after Fine adjustment of the device by buffer 4 and 7,*pH* It was measured at 20 degrees Celsius. For the accuracy of the test and to reduce the amount of error, the test was performed in three repetitions [21].

Measurement of antioxidant activity

Measurement of antioxidant activity by the method*DPPH* (Burits et al. in 2000) was carried out. [23] In order to measure the amount of antioxidant activity and phenolic compounds of sour tea extract and soft drink, first of all, special ethanol extracts of sour tea extract and four soft drink formulas were used to do this. Two tests were prepared and performed as follows. To prepare the ethanol extract from the extract, first, 10 ml of sour tea extract was placed on a shaker with 40 ml of 96% ethanol for two hours with a magnet, then the resulting solution was filtered through filter paper. After that, the residues on the strainer were washed with 40 ml of ethanol plus 10 ml of water, and the solution under the strainer was again placed on the shaker for one hour and then strained. The solution obtained from re-filtering was used to measure the amount of antioxidants. To prepare the ethanol extract from the soft drink, the above steps were carried out exactly, with the difference that after the first straining, the solution was used to perform the antioxidant test. $DPPH$ It was prepared with a It was prepared with a concentration of 0.008 (0.02 g*DPPH* dissolved in 250 ml of 80% ethanol).

Measurement of antioxidant activity

The amount of 0.25 ml of the ethanol extract that was prepared from the sour tea extract to measure the antioxidant activity in the previous step with 3.5 ml of solution*DPPH*It was mixed and stirred for 30 seconds by a vortex machine, and after 30 minutes of darkening, these samples were poured into the cell of a spectrophotometer (UV-VIS Shimadzu model) and the absorbance was measured at a wavelength of 517 nm. The absorption of the control sample was also determined in the same way, with the difference that instead of 0.25 ml of the produced ethanolic extract, 0.25 ml of 80% ethanol was used for the antioxidant activity measurement test. Then, using formula (1), the amount of antioxidants in sour tea extract was calculated [25,24,23].

Formula 1): (Ablank- Asample / Ablank) x 100% antioxidant A_{blank} : the absorption rate of the control sample (methanol. water. *DPPH*. water, *DPPH*.

Asample: the absorption rate of the sample containing the ethanolic extract (extract,*(DPPH*.

In order to measure the antioxidant activity of the soft drink, all steps were carried out in the same way as the measurement of the antioxidant activity of the extract, with the difference that in the zero and first month of keeping the soft drink, from 0.5 ml and in the second and third months from 2 ml of the ethanol extract of the soft drink. was used

Measurement of phenolic compounds

In order to perform this test, Folin Ciocalto method (*ordinance* et al. in 2006) [26]. In short, Folin's reagent diluted ten times was prepared first (1 ml of Folinciocalto reached the volume of 10 ml of distilled water) then sodium carbonate solution was prepared. 2.5 ml We added Folin solution to 0.25 ml of ethanol extract prepared from sour tea extract. It was kept stationary for 3 minutes, and then 2 ml of sodium carbonate reagent was added to this sample and it was stirred for 30 seconds, and after 30 minutes of darkness, the absorption of this sample was read by a spectrophotometer at 765 nm. The control sample was also prepared according to the above steps, with the difference that instead of 0.25 ml ethanolic extract, distilled water was used. This experiment was done with three repetitions for each of the extract samples. Finally, the amount of phenolic compounds of each of the sour tea extract samples was calculated by putting the numbers obtained from reading the absorbance of the samples in the equation of the line obtained from drawing the gallic acid diagram [26].

Y=0.0103X-0.2712

 $=$ Y absorbance read by spectrophotometer

 $X =$ concentration of total phenolic compounds in mgGAL/100ml

In order to perform this test, all the steps mentioned in the measurement of the phenolic compounds of the extract were performed, with the difference that 0.5 ml of the ethanolic extract of soft drink was used. Y=0.003X-0.434

Measurement of anthocyanin by method*pH* **differential**

Many methods have been reported to measure and quantitatively evaluate anthocyanins in foods. The way that Francis and Fulki³ used in 1968, it is still the most appropriate method. In this method, absorption of samples prepared by buffer $1pH =$ and $4/5 = pH$ It was measured by a spectrophotometer at a wavelength of 517 nm. The main pigment of sour tea is cyanidin-3-glycoside, which shows the highest absorption at the wavelength of 517 nm.[20] .

First buffer = $1pH$, including 125 milliliters of 0.2M potassium chloride, 335 milliliters of 0.2M \overrightarrow{A} = \in CL formula (2)

C mg/100ml = $\triangle A / \triangle E \times M \times D$

A: Absorption shown by the device

L: Tube length in centimeters

D : The dilution factor is (sample volume**/** Buffer $sizeD=$)

△*A* : The difference between the two absorptions in*pH* $= 1$ and $4/5 = pH$

M : Molecular mass of main anthocyanin of sour tea*g/mol*It is 445.

€ : Molar absorption, molar absorption index for pure pigments. Mol absorption of cyanidin-3-glycoside, the main anthocyanin in sour tea*l/mol.cm*It is 29/600.

The preparation of the samples to measure the anthocyanin of the soft drink was done in the same way as the anthocyanin measurement of the extract, with the difference that 5 ml of soft drink and 5 ml of each of buffers 1 and 4.5 were used. Finally, the absorption numbers were placed in formula (2) and the amount of anthocyanin was calculated.

3- Results and discussion

In this research, to investigate the effects of different temperature and time conditions on the parametersActivityAntioxidant ،Phenolic compounds and the amount of anthocyanin have been studied to optimize the extraction process from the sour tea medicinal plant. Also, the effect of storage time on the parameters*pH*, antioxidant activity, phenolic compounds and anthocyanin levelIt has also been evaluated in soft drinks produced from sour tea extract.In this research, the experimental data were fitted with the help of the following linear model:

 $Y = b0 + b_1 A + b_2 B$

The response values in the sour tea extract extraction process are given in table (1) and the values predicted by the model in table (2). According to the above results, it can be seen that there is a close relationship between the values obtained from the experiments and the values predicted by the model.

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hydrochloric acid, and buffer = 5.4*pH* including 400 ml of 1 M sodium acetate, 240 ml of 1 M hydrochloric acid and 360 ml of distilled water. Then 5 ml of buffer 1*pH*= was added to 0.7 ml of sour tea extract and after 15 minutes, it was placed in the spectrophotometer and its absorption rate was obtained. Also, 5 ml buffer = 4.5*pH* Also, 0.7 ml of extract was added and absorption was measured after 5 minutes [20]. Using the following formula, the anthocyanin content of each sample was obtained:

³ - Fransis & Fuleki

Time	Temp	Anthocyanins (mg/l)	Phenolic content(mgGAL/100ml)	DPPH%
10.00	60.00	57.2	60.89	58.53
15.00	75.00	79.96	67.49	65.37
15.00	75.00	83.61	66.23	59.06
15.00	75.00	63.75	65.62	59.82
15.00	90.00	78.94	70.42	66.31
20.00	90.00	77.91	71.47	69.33
20.00	75.00	83.6	68.56	65.48
10.00	75.00	62.47	61.57	64.46
15.00	75.00	63.76	65.94	60.09
10.00	90.00	79.18	69.72	67.24
15.00	60.00	51.76	59.04	59.72
20.00	60.00	64.4	59.18	60.88
15.00	75.00	83.62	67.42	58.27

Table 1- Values of answers in the process of extracting sour tea extract

Table 2- Values predicted by the model in the extraction process of sour tea extract

Time	Temp	Anthocyanins (mg/l)	Phenolic content(mgGAL/100ml)	DPPH%
10.00	60.00	56.60	59.07	57.79
15.00	75.00	77.49	69.90	65.71
15.00	75.00	65.62	61.41	59.61
15.00	75.00	86.51	72.25	67.53
15.00	90.00	61.11	60.24	58.70
20.00	90.00	82.00	71.07	66.62
20.00	75.00	67.04	64.49	61.75
10.00	75.00	76.06	66.83	63.57
15.00	75.00	71.55	65.66	62.66
10.00	90.00	71.55	65.66	62.66
15.00	60.00	71.55	65.66	62.66
20.00	60.00	71.55	65.66	62.66
15.00	75.00	71.55	65.66	62.66

3-1- Examining the amount of anthocyanin extracted from sour tea

As can be seen in table (3), the linear model for the amount of anthocyanin extracted was statistically significant (0.05*p*<), but the test of poor fit was not significant (≤ 0.05) .*p*) which indicates the fit of the fitted model. The significant term of the model includes temperature (0.05). *p*<) was Time had no significant effect on the increase of anthocyanin. Figure (1-a) shows that the amount of anthocyanin increased significantly with the increase in extraction temperature. The effect of temperature in increasing the efficiency of anthocyanin extraction has been more obvious according to Figure (1-b). The effect observed in increasing the amount of anthocyanin can be attributed to the increase in the duration of mass transfer [27].

Tang et al. (2011) investigated the effect of blackberry anthocyanins extraction with the help of ultrasound in 20 to 100 minutes. It is proven that due to stabilization of balance

It was extracted between the solvent and the material, and the maximum amount of anthocyanin extraction was 56.14 mg/g at time 40. They also investigated the effect of extraction at temperatures of 20-60 degrees Celsius, and with the increase in temperature from 20 to 40 degrees Celsius, the amount of anthocyanin increased and reached from 54.09 to 62.58 mg/g, and then with increasing The temperature decreased from 40 to 60 degrees due to the decomposition of anthocyanins, and the best temperature was reported as 40 degrees Celsius.[28] .

compounds

As can be seen in table (4), the linear model of the amount of total phenolic compounds is statistically significant ($p<0.01$), but the lack of fit test is not significant (≤ 0.05) .*p*) which indicates the fit of the fitted model. The significant terms of the model include temperature $(0.01 \, p<)$ was The results obtained from table (4) for*0.8633=R-Squared* و ∞ = \cdot , \wedge \uparrow \cdot \wedge \downarrow R *-SquaredIt indicates a very good* matching of the calculation model with the tested points and high accuracy of the model. Figures (1-c) and (1-t) show that the amount of extraction of phenolic compounds has increased with the increase in temperature, and the effect of temperature in this increase is more obvious. Higher extraction temperatures can lead to softening of plant tissue, destruction of phenolic compound connections with proteins and polysaccharides, and increased solubility of phenolic compounds, which can improve mass transfer. This also leads to an increase in the extraction rate of the above compounds at higher extraction temperatures [27]. Shaddel et al. (2013) in the

Therefore, according to the parameter with a significant effect, the fitted equation for this answer is as follows: $Y=71.55+10.44A$

Rajai et al. (2010) showed that the extraction of phenolic compounds from pistachio green skin was almost constant until 20 minutes with high speed and from 20 to 45 with low speed and from 45 to 60 minutes. Also, the extraction of phenolic compounds from pistachio green skin showed that the extraction process increases up to 65°C and is stable from 65 to 85°C and does not change significantly [33].

Zaman et al. (2012) showed the trend of increasing temperature on*jackfruit* They checked that it was found that first with the increase in temperature, we have an increase in phenolic compounds, but after a while, when the temperature increases a lot, due to decomposition, we will have a decrease in phenolic compounds[34]. Therefore, according to the parameter with a significant effect, the fitted equation for this answer is as follows: $Y=65.66+5.42A$

Sources Change	Sum of squares	Degrees οf	average of squares	F value	Prob > F
		freedom			
Model	184.28		92.14	31.58	< 0.0001
A	176.0		176.04	60.34	< 0.0001
B	8.2		8.24	2.82	0.1238
Residual	29.18	10	2.92		

extraction of phenolic compounds from the pulp using
 $Table 4.$ Analysis of variance of the lines Table 4- Analysis of variance of the linear model of the parameter of phenolic compounds

3-3- Investigation of antioxidant activity

The results of analysis of variance in table (5) show that the linear model for the amount of antioxidant activity was statistically significant $(0.05)p <$), but the goodness of fit test was not significant (<0.05) .*p*) which indicates the fit of the fitted model. Meaningful expression of the temperature model $(05/0 \text{ p/s})$ was Figure (1-c) shows the three-dimensional representation of the effect of temperature and extraction time. With increasing extraction temperature, the amount of antioxidant activity has increased significantly. Figure (1-c) shows that the increase in temperature in the extraction process had a greater effect on the increase in antioxidant activity. Many researchers have reported that the amount of phenolic compounds has a significant effect on antioxidant activity, which is due to the high reductive ability of these compounds and the ability to give hydrogen to active radicals such as*DPPH* is[35].

Phenolic compounds also increased with increasing temperature, so the effect of increasing temperature in increasing antioxidant activity can be easily justified. Shaddel et al. (2013) stated the effect of temperature on free radical inhibition*DPPH* It is very significant and the amount of free radical inhibition power*DPPH* It increases to a certain extent with increasing temperature [22]. Dong Rui et al. (2011) investigated the effect of 5 to 40 minutes on cherry seeds.*DPPH* And then that reduction showed that the best time was 30 minutes. They also investigated the effect of temperatures (30, 40, 50, 60, 70 degrees Celsius) on cherry seeds, which showed that up to 60 degrees, the free radical inhibition power*DPPH* increases, and then due to the decomposition of antioxidant compounds, the reduction in the ability to inhibit free radicals*DPPH* We will have that the best temperature was 60 degrees[36].

Kishek et al. (2010) stated the power of free radical inhibition*DPPH* In ginger, it increased up to 30 minutes and remained constant after that. Also, the power to inhibit free radicals*DPPH* Ginger increased from 20 to 54 degrees Celsius and then decreased due to the decomposition of antioxidant compounds [37]. Therefore, according to the parameter with a significant effect, the fitted equation for this answer is as follows: Y=62.66+3.96A

Table 5- Variance analysis of the linear model of the antioxidant activity parameter

B one thousand

Figure 1- The effect of independent variables on the amount of anthocyanin extraction (A and B), phenolic compounds (P and T), antioxidant activity (C and C).

3-4- Optimizing the extraction process

In the process of extracting sour tea extract, achieving the highest amount of phenolic compounds, anthocyanin and also the maximum antioxidant activityIt was considered as the desired goals of the experiments in the statistical analyses. Optimum operation conditions were done using numerical optimization technique. For this purposeAt first, optimization objectives, response

levels and independent variables were set. Using utility function $technique⁴$ The best answers were obtained. The results of two optimal points are shown in table (7).

Table 6- Results of optimization of sour tea extract extraction process

Nu mb er	tempe rature	ti m e	anthocyani n(mg/ml)	DPP $H\left(\%\right)$	TPC(mgG AL/100ml)	Desi rabil ity
1	71.41	1 8. 8 1	72.493	62.4 062	65.2564	0.82 3
2	77.13	1 0. θ 0	68.5267	62.3 116	65.2566	0.80 9

5-3- Investigating the physical and chemical characteristics of produced soft drinks during 3 months of storage

3-5-1- Examining changes in the antioxidant activity of soft drinks during the storage period

The analysis of variance table (7) and also Figure (2- A) showed that there was no statistically significant difference between the 4 formulations of sour tea drink in terms of antioxidant activity (<0.05*P*According to Figure (7), the highest amount of antioxidant activity related to the formulation $A(0.1)$ gram of stevia $+30$ ml of sour tea extract + 50 ml liter of carbonated water). which had the highest amount of stevia compared to other formulas. Figure (3-a) shows A decreasing trend in the amount of antioxidant activity، It has existed during the maintenance period. The highest amount of antioxidant activity was observed in the month of zero, and after that, no significant difference was observed in terms of the amount of antioxidant activity in the first, second, and third months.05/0<*P*The lowest amount of antioxidant activity was related to the third month.

Figure (4-a) shows the mutual effect of storage time and different formulations of soft drink on the antioxidant activity of sour tea soft drink. According to figure (4-a), the interaction effect of time and different formulations of sour tea drink has decreased the amount of antioxidant activity, and all the formulations of sour tea drink have decreased their antioxidant activity with the passage of time only in

 \overline{a}

the formula*C* (5 grams of sugar $+30$ ml of sour tea extract + 50 ml of carbonated water) which had the highest amount of sugar, an increase in the amount of antioxidant activity was seen from the second month onwards. It was also found that the lowest amount of antioxidant activity related to the formula*C* In the first month, the highest amount of antioxidant activity was related to the same formulation, but in the third month. According to the figure, there is a significant difference in the amount of antioxidant activity between the formulations in the month of Safar.*A* 0.1 gram of stevia $+30$ ml of sour tea extract $+50$ ml liter of carbonated water) and $B(0.02 \text{ grams of} \text{stevia} + 1.5)$ grams of sugar $+30$ ml of sour tea extract $+50$ ml of carbonated water) and D (0.02 grams of stevia $+3$ grams of sugar $+30$ ml of sour tea extract $+50$ ml of carbonated water) but the formulation*C* With the lowest amount of antioxidant activity, it had a significant difference with the other three formulations. In the first month between formulations*B and D* There was no significant difference in terms of the amount of antioxidants, but other formulas had significant differences with each other. In the second month, between the formulations*A and C* Statistically significant difference was not observed in terms of the amount of antioxidant, also the formulations $B \n_gD$ Also, there was no statistically significant difference with each other. In the third month between formulations*A and B* No statistically significant difference was observed in terms of the amount of antioxidants, but other formulations had a statistically significant difference in this regard (<0.05).*P*Finally, according to Figure (4-A), it is clear that the lowest amount of antioxidant related to the formulation*C* In the first month, the highest amount of antioxidant activity was related to the same formulation, but in the third month.

In this regard, Carino⁵ and colleagues in (2006) conducted a research that proved that the methanolic extracts of different species of Stevia because of the presence of flavonoids⁶Alkaloids⁷ Xanthophylls⁸and hydroxycinnamic $acids^9$ They show significant antioxidant properties [38]. Also, other research

⁴- Desirability function method

 $\frac{5}{5}$ - Cute

⁶ - Flavonoids

⁷ -Alchaloeids

⁸ - Xanthophils

⁹
- Hydroxy Cinnamic Acids

showed that the presence of flavonoid compounds in stevia extracts makes these extracts have unique antioxidant power [38,39]. Also, Wheeler et al. in (2008) showed Stevioside, as one of the three dominant compounds in Stevia rebadiana extracts, has the highest antioxidant power [40]. Arena¹⁰ and colleagues in(1999) showed The antioxidant activity of orange juice prepared from orange concentrateFrom 2 months Maintenance increases. $piga¹¹$ and colleagues in (2002) as well Mandarin orange juice¹² particle for direct objectat 4° C for 15 days maintained and showed that its antioxidant activity increased, but in contrast to Del Caro¹³et al. in (2004) show gave antioxidant capacity (*TEAC¹⁴*) obtained from the method*DPPH* For orange juice stored in the same conditions, there was a slight decrease.Now, if a decrease in antioxidant activity is observed, it may be related to a decrease in phenolic compounds and vitamins*C* compared to fresh juice, and if an increase in antioxidant activity is observed, it is usually attributed to Maillard reaction products. Panel¹⁵et al. in (2004) showed that several food compounds such as carotenoids, vitamins*C*, Vitamin*AND*, phenolic compounds and their mutual effects lead to the total antioxidant activity of foods, and it is difficult to measure the total antioxidant based on specific active compounds [41,42,43]. Jackback¹⁶ and his colleagues in (2007) conducted a study on antioxidant

- 10 Arena
- 11 Call
- 12 mandarin
- 13 -Del Caro

- 15 Pinelo
- 16 -Jacob

activity and anthocyanin from red fruit extracts, the general results showed that there is a direct relationship between anthocyanin content and their antioxidant activity, and red fruit extracts can be used as a source good antioxidant compounds in the human diet[44]

¹⁴ -trolox equivalent antioxidant capacity

Phenolic	Antioxidant activity	Anthocyani	pH	of Degrees	Source of change	
compounds		n		freedom		
ns 47/65	ns 33/58	$***$ 613/0	$***$ 077/0		Levels of repetitions	
ns 14/85	** $76/1485$	** 090/0	** 235/0		levels of treatments	
08/40 82/46	06/142 97/21	278/0 89/5	002/0 85/1	41	error of coefficient variation (CV	

Table 7- Variance analysis of the changes of investigated traits during the storage period of soft drinks

n.s: no statistically significant difference

*: Meaningful

3-5-2- Examining the changes of total anthocyanin

TableAnalysis of variance (7), show gave in terms of anthocyanin content between the formula C_2D Significant differenceThere was no statistics, but there was a statistically significant difference between the other formulas at the level of (0.05).*P*<) existed. Also, according to figure (2-b), it was determined that the highest and lowest amount of anthocyanin was related to the formulations, respectively*B*و*C* have been. Figure (3-b) shows that the highest and lowest amount of anthocyanin was related to the zero and third months, respectively, and it was found that the amount of anthocyanins decreased with the passage of time. According to table (7), a completely significant difference was observed between all months of storage in terms of the amount of anthocyanin. Figure (4-b) shows the interaction effect of storage time and different formulations of sour tea drink on the amount of anthocyanin. which indicated that the amount of anthocyanin in sour tea drink formulas had decreased over time. In this figure, it is clear that on the zero day between the three formulas*B*And*C*And*D* There was no significant difference in the amount of anthocyanin, but between these three formulas*A* which had the highest amount of anthocyanin in the month of Safar, there was a statistically significant difference. In the first month also between the three formulas*A*And*B and C* There was no significant difference (<0.05).*P*). But between these three formulas and formulas*D* which had the lowest amount of anthocyanin in the first month, there was a statistically significant difference in terms of the amount of anthocyanin $(P<0.05)$. In the second and third month, there was a statistically significant difference between all sour tea drink formulas in terms of anthocyanin content. The highest amount of anthocyanins related to the formula*A* In the zero month and the lowest amount is related to the formula*C* It was in the third month.

In this regard, Darvingas¹⁷AndColleagues in (1968) during research showed that all tested sugars (such as sucrose, fructose, glucoxylose) all increased

**: the completely completely the completely the completely the meaningful meaningful anthocyanin degradation in the same way [45]. During the research done by Tinsley and Bukian (1960) about the anthocyanin Pelargonidin 3-monoglucoside in strawberry was done, it was found that the decomposition of anthocyanin increases in the presence of browning reactions and compounds such as glucuronic acid, fructose, hydroxymethylfurfural and amino acids. The materials resulting from decomposition are insoluble red-brown compounds that gradually precipitate. The rate of destruction of anthocyanins reaches the maximum in conditions where the oxidation of vitamin C increases. Rousseau¹⁸ and togetherRan (2007) showed that the addition of sugars and salts has a negative effect on stability

18 - red

 17 - Daraving

It has anthocyanins. Therefore, the amount of anthocyanin in the formula can be higher*B* due to its lower amount of sucrose compared to the formula*C and D* attributed [46,47].

Boshaishi in the year (2004) showed that with the increase*pH* The degradation of anthocyanins of all the studied apple varieties increased and in*pH* Acids of anthocyanins show more resistance. Therefore, the amount of anthocyanins in the formula is less*A* relative to the formula*B* Considering the absence of sucrose in this formula, it can be justified according to the results obtained from this research.*A* compared to other formulas*pH*is more^[48]. See¹⁹ and his colleagues inyear (1943) andAlso Pederson et alIn the year (1974) decrease Simultaneously confirm anthocyanin and ascorbic acid during storage of fruit extracts. On their behalf, it was suggested to carry out the reaction between these two substances [6]. *Martio* Colleagues in 2002 showed that anthocyanins in pomegranate juice (which also includes cyanidin 3-glucoside) have little stability during storage and their amount decreases with time [49]. Hemti Kakhki et al. in 2016, in the study of the shelf life of the concentrate during the storage of barberry concentrate at 4 degrees Celsius, observed that the intensity of the color decreased during 6 months of storage.[10]

3-5-3- Examining changes in phenolic compounds

Variance analysis table(7), sign gave in terms of the amount of phenolic compounds between the formula*A and C* Statistically significant difference was not observed, but other formulas were differentStatistically significant at the level $(p<0.05)$ with had each other

Figure (2-c) shows the highest amount of phenolic compounds related to the formulation*D* and the lowest amount related to the formulation*B* Figure (3-c) shows that the highest amount of phenolic compounds in soda was observed in the first month, and only between the second and third months, no significant difference was observed in terms of the amount of phenolic compounds between soda formulations. Figure (4-c) shows the effect of storage time and different formulations of soft drink on the amount of phenolic compounds of sour tea soft drink. According to the form can be received in the zero month between formulations*A, B, and C*There is no statistically significant difference in the amount of phenolic compounds between these three formulas*D* There was a statistically significant difference in the first month only between the formulas*A and C* There was no significant difference in the amount of phenolic compounds. In the second month, between formulas*B and C and D* There was no significant difference in

terms of the amount of phenolic compounds, but between these three formulas*A* which had the highest amount of phenolic compounds in the second month, a statistically significant difference was observed. In the third month only between formulas*A and C* No significant difference was observed in terms of the amount of phenolic compounds $(P<0.05)$. According to Figure (4-c) most of the phenolic compounds in the formula*D*It was observed in the first month. As it is clear in Figure (3-c), in the first month, a significant increase in phenolic compounds was observed compared to zero month, and after that, the amount of phenolic compounds decreased.

hard work²⁰ and colleagues in the year (2004) showedAntioxidant capacity (*TEAC*²¹) Obtained from the method*DPPH* For orange juice stored in the same conditions, there was a slight decrease. Now, if a decrease in antioxidant activity is observed, it may be related to a decrease in phenolic compounds and vitamin C compared to fresh juice, and if an increase in antioxidant activity is observed, it is usually related to reactive products.Maillard is attributed. Klimza²² and colleagues in (2006) showed that after 4 months of storage of orange drink under the experimental conditions used, the amount of phenolic compounds decreased and at the end of storage, the amount of phenolic compounds increased. The reason for this could be the formation of materials during storage that reacted with silicic folin reagent and increased the total phenolic compounds [42,48].

3-5-4- Review of changes*pH*

The variance analysis table (7) showed In terms of amount*pH* between the three formulas*B and C and D* There was no statistically significant difference, but the formula*A*having the most*pH* Statistically significant differenceat the level $(P<0.05)$ with three formulashad another Figure (3-d), shows that A downward trend in the rate*pH* From the first month of maintenance there has been over time. It is also found that the highest amount*pH* It was observed in the month of zero and between the zero and the first month in terms of amount*pH* There was no significant difference($05/0 < P$) But other months of storage have statistically significant differences in terms of amount*pH* have been $(05/0)$. Figure $(4-d)$ the mutual effect of storage time and different formulations of soft drinks on the amount*pH* Soda shows sour tea. In this figure, it is clear that in the

 19 -Riding

 20 -Del Caro

²¹ -trolox equivalent antioxidant capacity

²² -Klimcza

month of Safar، Two formulas*C*And*D* In terms of amount*pH* There was no significant difference between them, but other formulas had significant differences in this respect (P<0.05). It was also found that in the first, second and third months of all four formulas of sour tea drink in terms of amount*pH* They had statistically significant differences with each other. Most of the measure*pH* Also related to the formula*A* It was in the first month and as it can be seen from the figure (3-D), the amount*pH* From the first month onwards, it has decreased with the passage of time*pH* And acidity in most cases is caused by chemical and biological reactions. Therefore, the reduction*pH* It can be justified by the growth of yeasts, such as by the consumption of sugar in soft drinks and the production of acid ،cause a decrease*pH* Examples are.([4,49,50].

Research has shown that although all kinds of microorganisms can be found in soft drinks, only a few of them that are acid-loving have an effective presence. Factors such as*pH*Acidity, sugar content and the presence of preservatives usually prevent the growth of yeasts, but yeasts that produce spoilage*lipolytica S. cerevisiae Candida and Zygosaccharomyces bailii*Sometimes they can overcome these conditions and therefore they are the most important group of microorganisms in soft drinks that can tolerate acidic conditions [51]. The light²³ and colleagues in (2002) also achieved similar results and $Edgek^{24}$ and colleagues in (1995) also showed that in fermented beverages after 48 hours of storage*pH* It has decreased from 1/5 to 3/4 [52,53]. Also, in

2008, Mirzaei et al., during their investigations on the stability of carbonated soft drinks containing sucrose and corn syrup rich in fructose during the storage period, concluded that during the storage period and due to two factors temperature and time,*pH*The samples of soft drinks containing corn syrup rich in fructose and the control samples have significantly decreased, which can be explained by the growth of yeasts in these conditions.[49]. Elhami et al. in (1384), Hosni in (1384), Hemti Kakhki and his colleagues in (1366) during various researches identified significant changes in the amount of*pH*Samples were not created. [4,6,10].

 23 -Battey

²⁴- Adegoke

Figure 2- The effect of different soda formulations on antioxidant activity (a), total anthocyanin content (b), phenolic compound content (c), pH (d)

FormulaA: $(0.1 \text{ gram of} \text{stevia} + 30 \text{ ml of} \text{extract} + 50)$ ml of carbonated water)

Formula*B*: (0.02 grams of stevia + 1.5 grams of sugar + 30 ml of sour tea extract + 50 ml of carbonated water)

Formula*C*: (5 grams of sugar + 30 ml of sour tea extract + 50 ml of carbonated water)

Formula*D*:(0.02 grams of stevia + 3 grams of sugar + 30 ml of sour tea extract $+50$ ml of carbonated water)

Figure 3- Changes in antioxidant activity (a), total anthocyanin (b), amount of phenolic compounds (c), pH (d) during three months of storage Soft drinks

Figure 4- Interaction effect of storage time and type of soft drink formulation on antioxidant activity (a), anthocyanin content (b), phenolic compound content (c), pH (d).

4- Conclusion

By conducting physicochemical tests on sour tea extracts with different conditions in terms of temperature and extraction time, it was determined that the optimal conditions for extracting from sour tea is 71.41 degrees Celsius in 18.81 minutes, which is optimal under these conditions. The amount of anthocyanin was 493.72 mg/liter, the amount of antioxidant activity was 62.4062%, and the amount of total phenolic compounds was 65.2564 mg/100 ml. Also, by performing physicochemical tests on four formulations of sour tea drink, it was stored for three months. At 4 degrees Celsius, it was determined that the use of natural sweetener stevia increased the antioxidant activity of sour tea drink compared to sour tea drink without stevia due to its active antioxidant compounds. It was also found that only in the first month of storage, a significant decrease in the amount of antioxidant activity was seen, and after three months of storage, the sour tea drink with a high antioxidant activity (around 50%) is still in a good condition in terms of the amount of antioxidant activity. has enjoyed It was also found that reducing the use of sugar reduced the degradation of anthocyanin compounds in the sour tea drink, but with the passage of time, there was a significant decrease in the amount of anthocyanin compounds in the sour tea drink. However, after three months of storage, the sour tea drink was still in good condition. In terms of the amount of anthocyanin, it was higher than other industrial carbonated soft drinks. The passage of time causes a significant decrease in the amount*pH* Soda was from the first month of storage. The phenolic compounds of sour tea soda also showed a slight decrease in the third month compared to fresh soda. In general, it can be concluded that the production of carbonated soft drinks from medicinal plants can be considered as a suitable alternative for industrial soft

drinks. . because they have good physicochemical stability and even with a slight decrease in the quality of chemical properties during the storage period, they still have a better quality than carbonated industrial soft drinks. Also, preserving the anthocyanins of sour tea drink is a good substitute for sugar in the formulation of this drink.

6- Resources

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بهینه سازی فرایند استخراج عصاره چای ترش به روش سطح پاسخ و ارزیابی فرموالسیون نوشیدنی حاصل از آن

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