



Scientific Research

Effect of concentration process on bioactive compounds of sugar beet thin juice: investigation of its physicochemical characteristics

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ABSTRACT

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In this research, the effect of concentration process (evaporation) on bioactive compounds and qualitative characteristics of thin juice and thick syrup during beet harvest was investigated. Phenolic and anthocyanin compounds of thin juice and quality attributes such as brix, degree of purity and soluble color were interest. For this purpose, from the beginning of October to the end of January 2016, samples were taken for thin juice from the last stage of the purification process and for thick syrup from the last stage of the concentration process, and all the qualitative chemical tests were carried out according to the ICUMSA reference book. In thin juice, anthocyanin amount was not constant during the time of beet harvest, but the total phenol amount was significantly different in the first and fourth months. There was no significant difference in the amount of protein in the first, third and fourth months. The highest brix of thin juice was 13.14% in the first and second months, and its degree of purity increased from the beginning to the end of the harvest season. The highest soluble color of thin juice was observed in the third and fourth months of harvesting. Thick syrup had the highest brix (53% on average) in the first and second months, and this syrup had a high soluble color at the time of harvesting, except for the second month. The results of investigation indicated the accuracy of the purification and evaporation processes to remove as much impurities as possible in the raw juice extracted from sugar beet cossettes.

1. Introduction

Sugar beet with scientific name *Beta vulgaris* It is a plant that is very important in terms of food and industrial value and is affordable. The presence of the sweet substances of sucrose stored in the root part of the beet plant caused sugar to be extracted from sugar beet for the first time in 1786 by Margraf's student named Achard.[1]. In today's society, sugar refers to substances that have a sweet taste and are presented in the form of white crystals [2]. Sugar is obtained from sugar beet or sugar cane and in some countries from palm sap. Sugar is widely used as a raw material in the preparation of many foods. Despite the production of natural and unnatural sweeteners, sugar consumption still has a special place in the food industry [3]. In order to produce white sugar from sugar beet, the following steps must be taken: 1- Washing the beet, 2- Grinding, 3- Diffusion (extraction), 4- Refining, 5- Evaporation (concentration), 6- Crystallization, 7- Separation, 8- Drying and 9- packaging. In the diffusion stage, if fresh water (condensate water + steam released from burning sulfur) at a temperature of 70 °C comes into contact with sugar beet slices, the extraction process is performed and the primary syrup is obtained, which has a black to gray color and is called raw syrup. can [4]. Raw syrup contains fine slag particles and various non-sugar substances (such as color compounds, amino acids, pectin, minerals, etc.), so it must go through the purification process. Raw syrup is purified in the purification section. The clean syrup that is obtained after a purification stage is called diluted syrup. The amount of dry matter of diluted syrup is between 12-15%, which should be enough to form sugar crystals. Therefore, concentration should be done in the evaporation part. The most important function of evaporation is to separate water from the syrup, which results in the thickening of the thin syrup and turning it into a thick syrup. Finally, the amount of dry matter of the thick syrup reached 60-70% to be sent to the crystallization part. The researchers found that the phenolic and color compounds that have high molecular weight, in addition to being absorbed on the surface of the crystal, enter into it. Sugar changes color over time under certain conditions, and this color

change is due to the presence of residual compounds on the surface and inside the crystal. These compounds mainly include: phenolic, melanoidin, melanin, caramel and alkaloid compounds resulting from pH changes. Among the above compounds, those with phenolic structure are of special importance. These compounds are present in the sugar beet plant and enter the raw syrup during the diffusion stage during extraction and go through all the production stages and finally are placed on the surface and inside the sugar crystals [5]. In the sugar industry, the main and fundamental goal is to extract the maximum amount of sucrose. Each of the bioactive compounds (phenols, proteins, anthocyanins) reduce production efficiency as impurities. According to the proven principle, for each unit of impurity, 1.5 unit of sucrose sugar is wasted [6].

During the vegetative growth period, sugar beet lacks stem and can be seen as sets of large horizontal to vertical leaves. Length of growth period for production sugar Loaf It is 6 to 9 months. It usually has good growth and quality in the mountainous climate, and in Iran, areas such as Euclid, Shahrekord, and Torbat Heydarieh have the highest cultivation level.[7]. Sugar beet has a wide adaptability to various environmental conditions and is relatively resistant to cold and heat. It is resistant to drought and soil salinity. Environmental factors such as heat, light, day length and soil moisture largely determine the growth and storage of sugar in the roots. Fertile soils with good drainage, medium texture and neutral to slightly alkaline acidity are ideal for sugar beet.[8]. Beet preparation in sugar factories is done in two parts. First, beet unloading and storage in silos, second, beet washing and turning into slices. In the slice mill, the beets are grated by the device and poured into thin slices on the conveyor belt to enter the diffusion tower. In the diffusion tower, if fresh water with a temperature of 70 °C comes into contact with it, raw syrup is obtained. Usually, fresh water is a mixture of condensed water with steam released from burning sulfur to adjust its pH in the range of 5.5-8.5. The color of the obtained raw syrup is black to gray, which is the result of melanin production due to the entry of air oxygen and tyrosinase enzyme, which remains as a colloid in

the syrup. If the color of the raw syrup is milky, it indicates the presence of microbial contamination of the syrup, the temperature of this type of syrup is usually low [9]. Raw sherbet is a sherbet in which the impurities are in the form of fine slag particles and in a floating state along with many different non-sugar substances. These compounds are extracted from sugar beet slices due to the use of high temperature water in diffusion. So, in order to convert sucrose into crystals from this syrup, it must be purified [10, 11].

In the process of sugar production, the most important and main step is the purification of raw syrup. At this stage, the more non-sugar compounds are removed, according to the formula for determining the degree of purity, the ratio of sucrose to dry ingredients in the syrup will reach the highest possible level and a syrup will be obtained that will make it possible to produce high quality white sugar [12]. In the purification process, lime is added to raw syrup in two stages, the purpose of which is to precipitate colloids and insoluble calcium salts. The next step is to add CO₂ gas. What is called carbonation, the excess of lime over pH 8.10-11 with CO₂ synthesizes and produces calcium carbonate. After the first and second carbonation, the syrup is filtered and passes through mechanical, industrial and press filters, and finally a diluted syrup is obtained, which has a dry substance or Brix between 13 and 15%. This syrup should reach the point that in the next stage (crystallization) sugar crystals can easily be obtained from it. The process of syrup concentration is done in the evaporator until the thick syrup reaches 60-70% Brix [12, 13]. As functional compounds, polyphenols are the main component of bioactive compounds and have a unique position among natural bioactive products [14, 15]. Polyphenols include flavonoids, tannins, anthocyanins, etc., which are abundantly found in tuberos plants such as sugar beet and in the roots of other plants [16, 17]. Types of polyphenols include phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanones, flavanols, isoflavones, proanthocyanins), anthocyanins and lignans [18, 19]. Phenolic compounds act effectively as hydrogen donors, so they act as effective

antioxidants [20]. The antioxidant property of plants depends on the amount of each of the polyphenolic compounds [21]. Phenolic compounds have many biological properties such as antioxidant properties, scavenging free radicals and anti-inflammatory properties [22-27]. These compounds prevent or delay oxidative damage in fats and other important molecules and prevent cancer and heart diseases, Alzheimer's, Parkinson's and diabetes [28-31]. Many evidences and studies show that polyphenols, in addition to preventing the oxidation of LDL, also prevent the clotting of platelets in the vessels and the destruction of red blood cells [20].

Anthocyanins are a subgroup of flavonoids and are responsible for creating red, purple and blue colors in many flowers, fruits and vegetables [16, 32, 33]. Anthocyanins have a C₆C₃C₆ flavonoid skeleton and are polyhydroxy and polymethoxy glycosylated derivatives of 2-phenylbenzopyrylium cation, i.e. flavylium cation [34]. Anthocyanins, glycosidic anthocyanidins and acylglycosides are soluble in water [16].

The aim of this study was to measure bioactive phenolic and anthocyanin compounds and qualitative characteristics such as brix, solution color and degree of purity in dilute and thick syrup resulting from the concentration process of Miandoab sugar factory during the sugar beet harvest season.

2- Materials and methods

All tests and samplings were done in Miandoab sugar factory. All chemical compounds used in the analysis were from Merck.

2-1- Measurement of dissolved solids (brix)

Before the bioactive compounds of diluted and concentrated syrups were tested, the samples were filtered by Whatman No. 1 filter paper and Brix was read by refractometer after bringing the sample temperature to 20°C [35].

2-2- Measuring the color of the solution

For color solution, syrups were first filtered through Whatman No. 1 filter paper until filtered samples were poured into glass cells. The

number read from the spectrophotometer was multiplied by 100,000. Distilled water was used to calibrate the device.[35].

(ICU) color solution= read $\times 100000$ / density \times Brix example

It should be noted that the density number was determined from the ICUMSA reference book.

2-3- Determining the degree of purity

For the degree of purity of thin and thick syrup, first 26 g of syrup was weighed and some lead acetate was added to them and the volume was brought to 100 mL with distilled water. Normal filter paper was used to filter the solution. The filtered solution was poured into a polarimeter tube and placed in the special place of the saccharomytic device and showed the sugar percentage of the syrups based on the z° degree [35].

Degree of syrup purity= Balaritration of thin and thick syrup/Brix thin and thick syrup

2-4- Measurement of total phenol

Total phenol was measured by the Folin-Ciocalto method using a 765 nm spectrophotometer. First, 1 g of thin and concentrated syrup was filtered in a 100 mL flask and 70 mL of distilled water was added to it, then 5 mL of Folin reagent was added and stirred for 8 min at laboratory temperature. Then 15 mL of sodium carbonate solution was added and made up to volume with distilled water. It was kept at laboratory temperature for 2 h. The desired solution was poured into a glass cell and the absorbance value of the solution was read against the blank (control sample). Finally, the amount of total phenol in terms of gallic acid equivalent was calculated by the following formula:

The amount of total phenol was expressed from the absorption rate of the sample and its comparison with the calibration curve in terms of the equivalent of milligrams of gallic acid per 100 g of the sample [36].

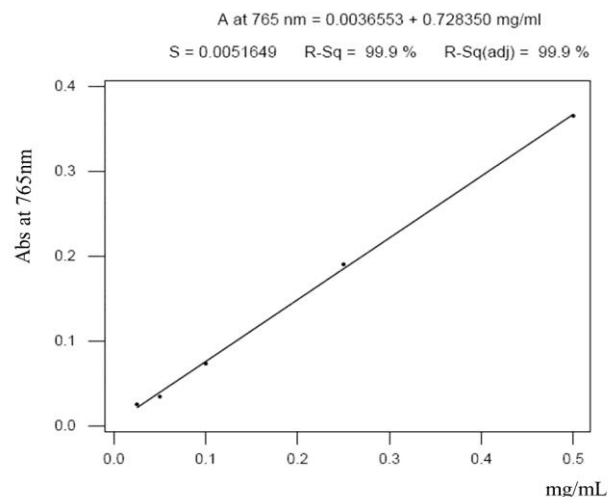


Fig 1 The standard curve of phenol using gallic acid

2-5- Protein measurement by Bradford method

For total protein, 1 mL of diluted and concentrated syrup was mixed with 10 mL of Bradford solution and mixed well for 20 min. The absorption value was read by a spectrophotometer at 595 nm. A mixture of 10 mL of Bradford solution with 1 mL of distilled water was used to calibrate the spectrophotometer [38].

Finally, the amount of protein was obtained from the following relationship in terms of mg/ml:

$$y = -0.0082X^2 + 0.3458X - 0.0148$$

6-2- Measurement of total anthocyanin amount

The pH difference method was used to measure anthocyanin in different treatments. First, 5 g of diluted and concentrated syrup was mixed with 25 mL of methanol-water solvent (50:50) in a well-capped 50 mL falcon. Then, two dilutions (1 mL of the prepared sample) were prepared from the sample using buffer solutions of potassium chloride with pH 1 and sodium acetate with pH 4.5 and placed in the dark for 15 minutes at laboratory temperature to reach equilibrium. The spectrophotometer was set with distilled water as a control sample at wavelengths of 510 and 700 nm. Then, the absorbance of both dilutions prepared at these wavelengths was measured.

The absorption values of the samples (A) were calculated as follows:

$$A = (A_{\lambda 520} - A_{\lambda 700})_{pH 1.0} - (A_{\lambda 520} - A_{\lambda 700})_{pH 4.5}$$

Finally, the concentration of total anthocyanin (TA) (mg/L) was calculated with the following formula:

$$TA = \frac{A \times MW \times DF \times 100}{\epsilon \times l}$$

MW=449 and $\epsilon=26900$ are respectively equal to molecular absorption and molar absorption of anthocyanin indicator of pomegranate juice, cyanidin 3-glycoside (Gill et al, 200), dilution factor DF, 1 spectrophotometer cell length (cm).

2-7- Statistical analysis of data

Investigating the effect of condensation process (evaporation) on dilute syrup during the sugar beet harvesting season was used in a completely randomized design. Experiments were performed in three repetitions, and analysis of variance and comparison of means were performed with the least mean square method at the 95% probability level. SAS software version 1/9 was used for statistical analysis and Excel

software was used to draw the curves.

3. Results and Discussion

3-1-Changes of dissolved solids in thin and thick syrup during the beet harvesting season

In the diluted syrup, the dissolved solids showed a somewhat constant value during the beet harvest season (Figure 2a). As it is clear in Figure (2b), the solids dissolved in diluted syrup during 4 months of the beet harvest season showed a statistically significant difference between the first, second, third and fourth months at the 5% probability level. Statistically, there was a significant difference between the first month and the third month, as well as the second month and the third and fourth months, but no significant difference was observed between the rest of the months ($P \leq 0.05$).

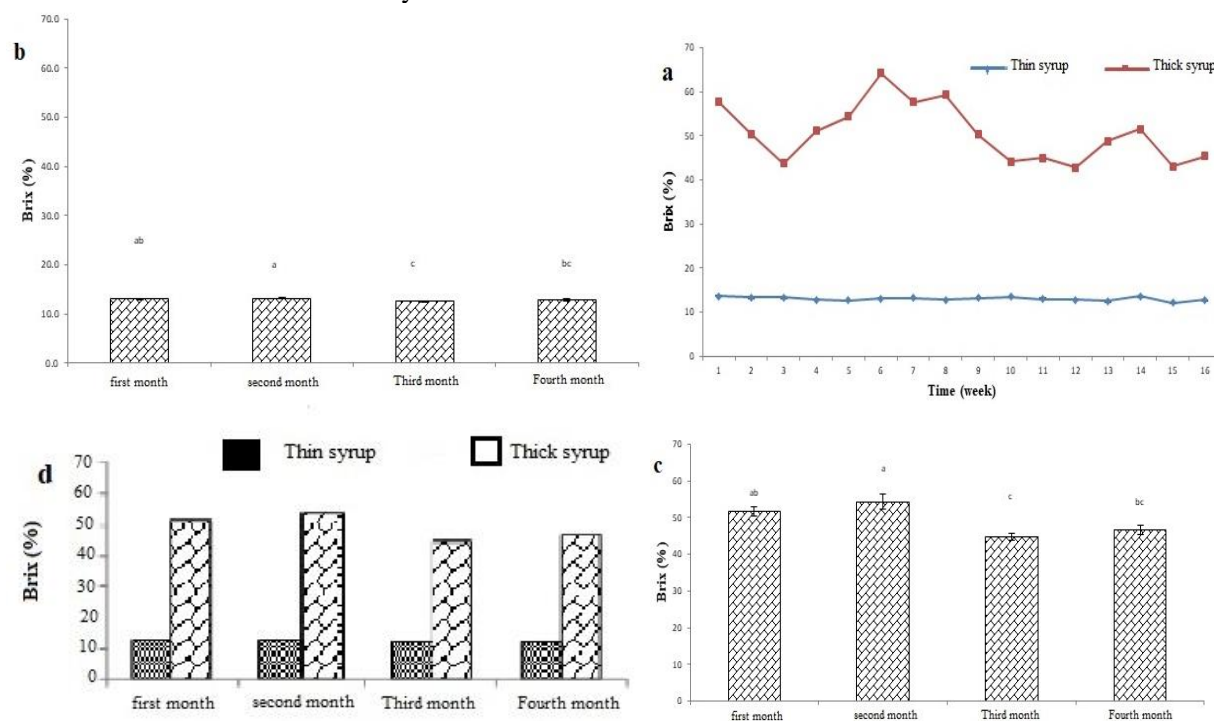


Fig 2 Effect of time on dissolved solids in the thin juice and thick syrup before (a), after (b) and before and after (c) evaporation during the beet harvest season. Lowercase Latin letters indicate significant differences ($P \leq 0.05$) in each month.

The dissolved solids of thick syrup fluctuated during the beet harvesting season. As shown in Figure (2c), the dissolved solids of thick syrup

increased to 69% in the sixth week and then decreased to 42% in the twelfth week. According to Figure (2d), the solids dissolved in

the thick syrup showed a statistically significant difference between the first, second, third and fourth months at the 5% probability level. Statistically, there was a significant difference between the first month and the third month, as well as the second month and the third and fourth months, but no significant difference was observed between the rest of the months ($P \leq 0.05$).

2-3-Changes in degree of purity of thin and thick syrup during the beet harvest season

In thin syrup, the amount of purity during the beet harvest season was not the same. As shown in Figure (3a), the lowest value of the purity of diluted syrup belonged to the first week and the highest value to the fifteenth week. According to

figure (3b), the degree of purity of diluted syrup showed a statistically significant difference at the 5% probability level between the first month and the second, third and fourth months, but there was no significant difference in the rest of the months ($P \leq 0.05$).

The purity level of thick syrup fluctuated during the beet harvest season, as shown in Figure (3c), the lowest and the highest purity level of thick syrup were related to the first and fifteenth week, respectively. According to figure (3d), the amount of purity of concentrated syrup showed a statistically significant difference at the 5% probability level between the first month and the second, third and fourth months, but no significant difference was observed between the rest of the months ($P \leq 0.05$).

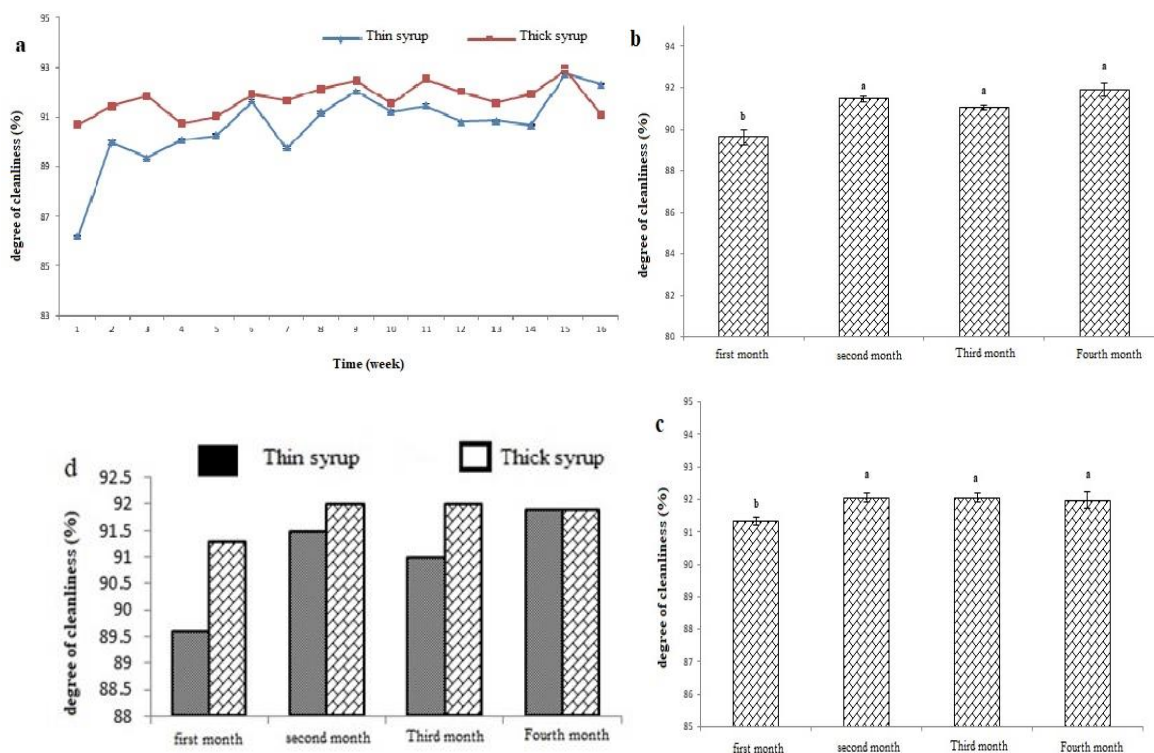


Fig 3 Trend of changes in degree of purity thin juice and thick syrup during different weeks (a), the effect of time on the amount of Purity degree in the thin juice before (b), after (c) and before and after (d) evaporation during the beet harvest season. Non-identical lowercase Latin letters indicate significant differences ($P \leq 0.05$) in each month.

3-3-Changes in the amount of color of thin and thick syrup solution during the beet harvest season

In dilute syrup, the amount of soluble color fluctuated during the beet harvest season. As

shown in Figure (4a), the lowest and highest color values of dilute syrup solution were related to the 9th and 13th weeks, respectively. According to Figure (4b), the color value of the dilute syrup solution showed a statistically significant difference at the 5% probability level

between the first month and the third month and between the second month and the third and fourth months, but there was no significant difference between the rest of the months. ($P \leq 0.05$).

The amount of color in the thick syrup solution was not constant during the beet harvesting season. As can be seen in Figure (4c), the lowest amount of color of thick syrup solution was observed in the twelfth week, and in the

thirteenth week, there was a sharp increase that reached the highest value. According to Figure (4d), the amount of color of the thick syrup solution showed a statistically significant difference at the 5% probability level between the second month and the first, third and fourth months, but no significant difference was observed between the rest of the months ($P \leq 0.05$).

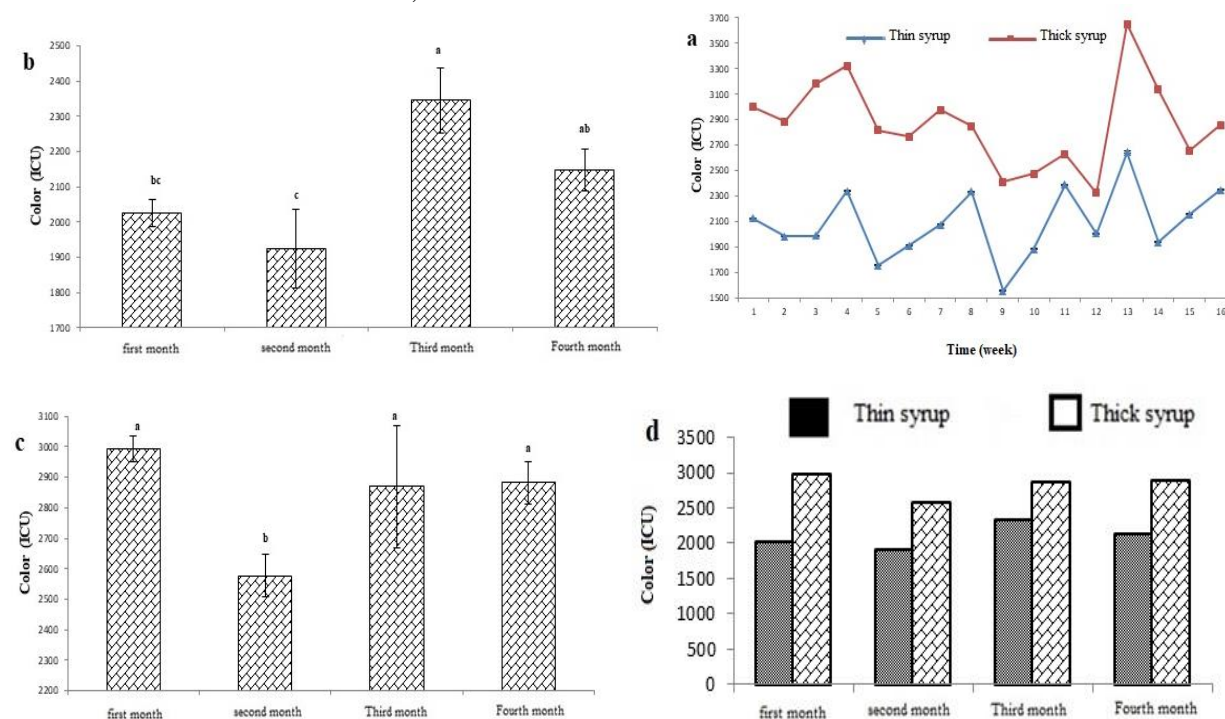


Fig 4 Trend of changes in soluble color of thin juice and thick syrup during different weeks (a), the effect of time on color in the thin juice before (b), after (c) and before and after (d) evaporation during the beet harvest season. Lowercase Latin letters indicate significant differences ($P \leq 0.05$) in each month.

4-3-Changes of total phenol of thin and thick syrup during the beet harvest season

The amount of total phenol in diluted syrup had significant changes during the beet harvest season. As shown in Figure (5a), in the diluted syrup, the lowest amount of total phenol was 0.1 mg GAE/100g, which corresponded to the first week, and the highest amount was 0.2 mg GAE/100g to the third week. According to Figure (5b), the amount of total phenol of diluted syrup showed a statistically significant difference between the first month and the fourth month at the 5% probability level, but there was no significant difference between the rest of the months.

($P \leq 0.05$). The amount of total phenol in thick syrup was not constant during the beet harvesting season. As can be seen in Figure (5c), the highest increase of phenol in thick syrup belonged to the sixth week, whose value is 0.6 mg GAE/100g, and the lowest decrease belonged to the sixteenth week, whose value is 0.4 mg GAE/100g. Was. In Figure (5d), the results of the statistical analysis at the 5% probability level show that there was a significant difference in the amount of total phenol of the thick syrup between the first month and the third and fourth months, but there was no significant difference between the rest of the months ($P \leq 0.05$).

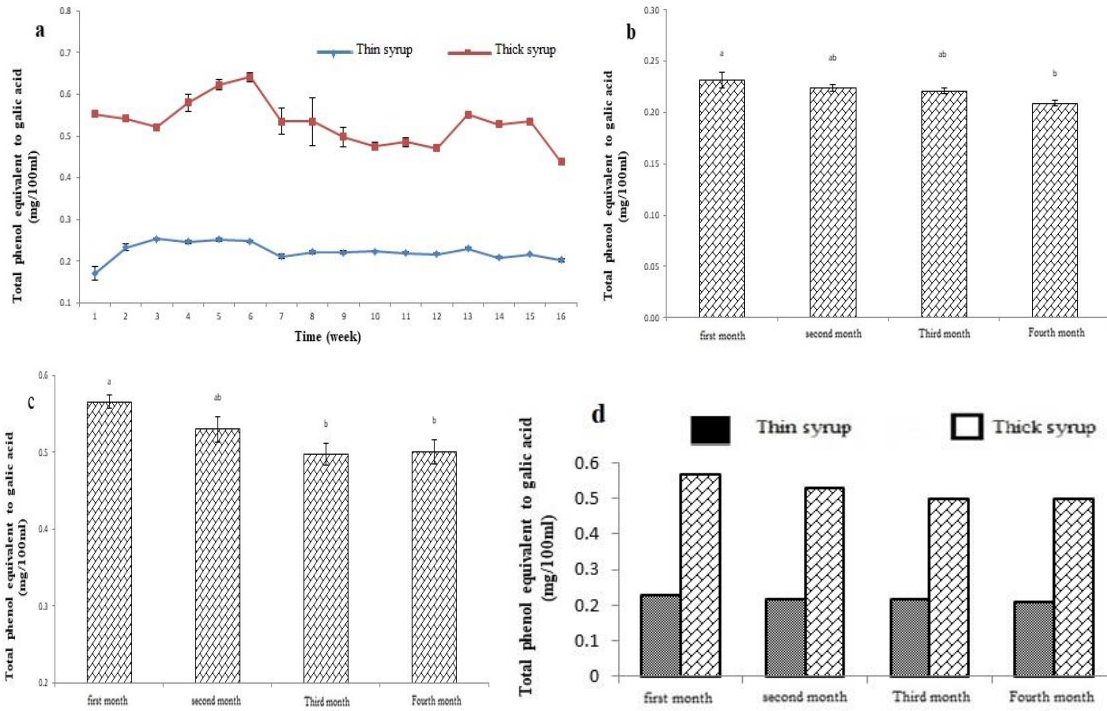


Fig 5 Trend of changes in total phenol of thin juice and thick syrup during different weeks (a), the effect of time on total phenol in the thin juice before (b), after (c) and before and after (d) evaporation during the beet harvest season. Lowercase Latin letters indicate significant differences ($P \leq 0.05$) in each month.

5-3-Changes of the total protein of thin and thick syrup during the beet harvest season

The total protein content of diluted syrup was not constant during the beet harvesting season. As shown in Figure (6a).

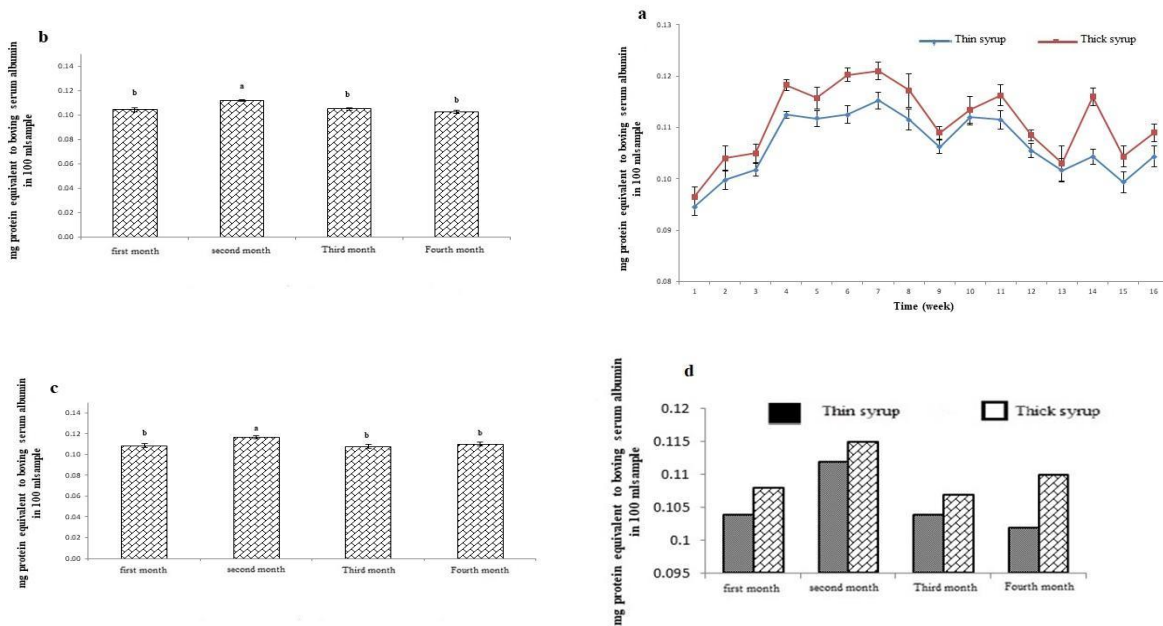


Fig 6 Trend of changes in protein of thin juice and thick syrup during different weeks (a), the effect of time on protein in the thin juice before (b), after (c) and before and after (d) evaporation during the beet harvest season. Lowercase Latin letters indicate significant differences ($P \leq 0.05$) in each month.

The lowest amount of total protein in diluted syrup was 0.091 mg/ml, which was related to the first week, and the highest amount was 0.117 mg/ml to the seventh week. According to Figure (6b), the amount of total protein of diluted syrup showed a statistically significant difference at the 5% probability level between the second month and the first, third and fourth months, but there was no significant difference between the rest of the months ($P \leq 0.05$).

In thick syrup, the amount of total protein during the beet harvesting season was not constant. As can be seen in Figure (6c), the total protein of the concentrated syrup in the first week was at the lowest value of 0.095 mg/ml and then increased to 0.126 mg/ml (seventh week). According to figure (6d), the statistical analysis of the total protein content of the thick syrup at the 5% probability level shows that there is a significant difference between the second month and the first, third and fourth months, but there was no significant difference between the rest of the months ($P \leq 0.05$).

6-3-Changes of total anthocyanins

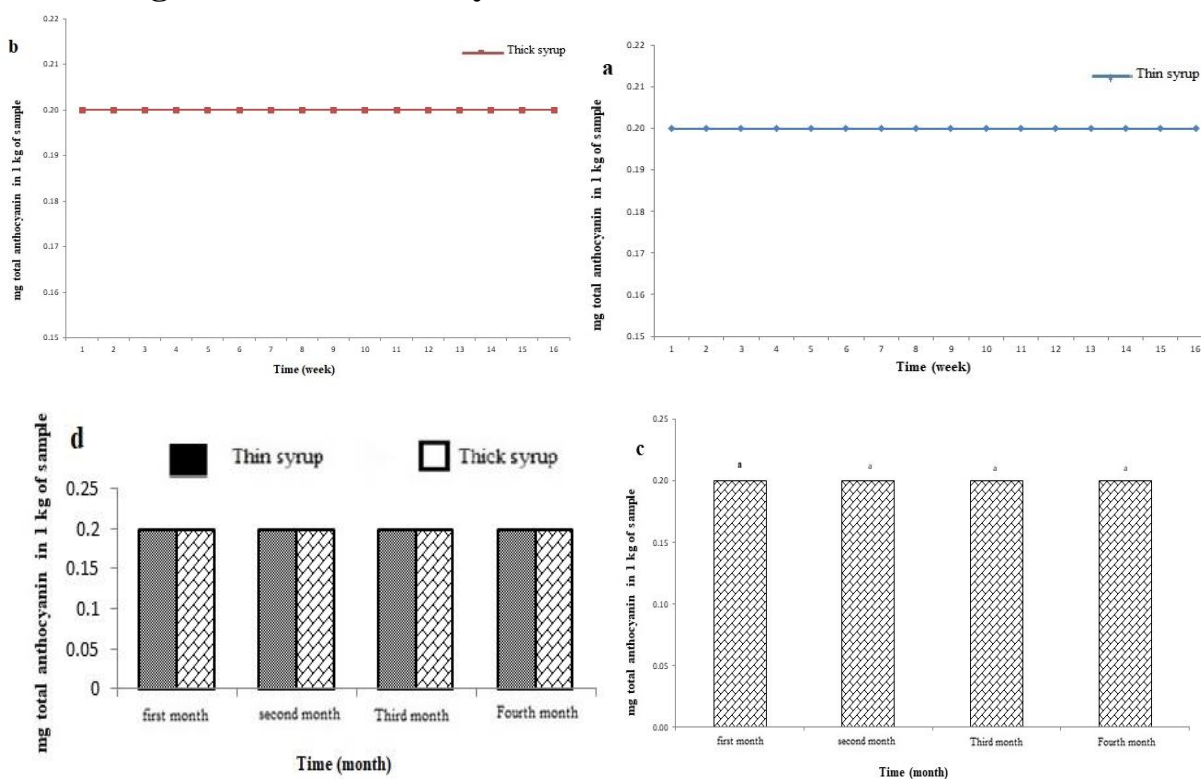


Fig 7 Trend of changes of anthocyanin in thin juice (a), in thick syrup (b), effect of time on total anthocyanin of thin juice and thick syrup (before and after evaporation) (c) and the effect of time on total anthocyanin of thin and thick

syrup (before and after evaporation) (d) during the beet harvest season. Lowercase Latin letters indicate significant differences (P≤0.05) in each month.

4 - Conclusion

In general, among the quality characteristics of diluted syrup that were investigated in this study, the Brix of diluted syrup was almost constant during the 4 months of the beet harvest season, but other quality parameters such as the color of the solution and the degree of purity of the diluted syrup varied during the 4 months of harvesting. Also, bioactive components of total phenol, anthocyanin and protein were investigated in diluted syrup, and only anthocyanin was stable during 4 months of beet harvesting season. They were. Also, the bioactive compounds of concentrated syrup, total phenol, anthocyanin and protein were also investigated. During 4 months, the beet harvest season was stable. The results confirm the accuracy of the purification and evaporation processes in order to remove as many impurities as possible in the raw syrup of sugar production from sugar beet in a factory. In the sugar industry, the main and fundamental goal is to extract the maximum amount of sucrose.

5- Resources

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تاثیر فرآیند تغلیظ بر ترکیبات زیست فعال شربت رقیق چغندر قند: بررسی ویژگی‌های فیزیکوشیمیایی آن

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چکیده

اطلاعات مقاله

در این تحقیق اثر فرآیند تغلیظ (اوپراسیون) بر ترکیبات زیست فعال و ویژگی‌های کیفی شربت رقیق و شربت غلیظ طی زمان برداشت چغندر بررسی شد. ترکیبات فنولی و آنتوسیانینی شربت رقیق و ویژگی‌های کیفی مانند بریکس، درجه‌ی خلوص و رنگ محلول مورد توجه بودند. برای این منظور از اوایل مهرماه تا اواخر دی‌ماه سال ۹۴ برای شربت رقیق از مرحله‌ی آخر فرآیند تصفیه و برای شربت غلیظ از مرحله‌ی آخر فرآیند تغلیظ نمونه‌برداری شد و کلیه آزمایشات شیمیایی کیفی بر اساس کتاب مرجع ایکومزا انجام شد. در شربت رقیق مقدار آنتوسیانین طی زمان برداشت چغندر ثابت بود، اما مقدار فنول کل در ماه‌های اول و چهارم تفاوت معنی‌داری داشت. مقدار پروتئین در ماه‌های اول، سوم و چهارم تفاوت معنی‌داری نداشت. بیشترین بریکس شربت رقیق در ماه‌های اول و دوم ۱۳/۱۴٪ بود و درجه‌ی خلوص آن از ابتدا تا انتهای فصل برداشت روند افزایشی داشت. بیشترین رنگ محلول شربت رقیق در ماه‌های سوم و چهارم برداشت مشاهده شد. شربت غلیظ در ماه‌های اول و دوم از بیشترین بریکس (به‌طور متوسط ۵۳٪) برخوردار بود و این شربت در زمان برداشت به جزء ماه دوم، رنگ محلول بالایی داشت. نتایج حاصل از این تحقیق صحت عملکرد فرآیندهای تصفیه و اوپراسیون برای حذف هر چه بیشتر ناخالصی‌های موجود در شربت خام استخراج شده از خلال‌های چغندر قند را نشان داد.

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