Ensuring transparency of pomegranate juice during its storage

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Abstract. Due to the constantly growing trend of using turbidity and stability indicators as indicators of the quality of fruit juices, the main goal of our research is to identify the role of components of pomegranate juices in processes that are accompanied by the formation of turbid suspensions in them and precipitation during their storage as a finished product. The experiments involved the fruits of 22 varieties of pomegranate of Azerbaijani reproduction. The fruits were manually peeled and the juice was obtained from the grains using a citrus juicer-press type Kenwood JE290. Freshly squeezed juices were filtered through a two-layer cloth-and-paper filter. Then samples were taken from them for chemical analysis. After that, they were poured into glass bottles, processed in a pasteurizer for 30 minutes at a temperature of 75° C and transferred to storage in a darkened room. After 9 months of storage at a temperature of 18 ... 22° C, the percentage of raw precipitation in each juice sample was determined using a method that included separating it using a centrifuge and weighing it. The data was processed using the correlation analysis method. It was found that there is a correlation between the data on the mass fractions of catechins, ascorbic acid and protein in freshly squeezed juices and the mass fractions of precipitation in them. It is concluded that increasing the stability of juices can be achieved due to their pre-membrane treatment aimed at removing colloids.

1. Introduction

From the physicochemical point of view, the turbidity of juices is a decrease in the transparency of the liquid caused by the presence of coarse particles of plant tissue with a particle size of more than 0.5 mm, having a predominantly heterogeneous composition, as well as colloidal high-molecular substances dissolved in the juice, causing the effect of light scattering (Tyndall effect) [1].

Transparency is one of the main organoleptic indicators, which is taken into account when assessing the quality of clarified and/or stabilized juices. There are differences between the processes of clarification and stabilization of juices (and respectively between clarified and stabilized juices). Clarification—the process of mechanical separation of suspended solids from the liquid phase of the juice by sedimentation, filtration or centrifugation, used alone or in combination with each other. Stabilization is a complex process, which is a combination of mechanical separation of turbid suspension particles (clarification) from the juice and its processing by biochemical (enzyme) and physico-chemical methods in order to remove high-molecular compounds that are in the juice in a dissolved state and can cause the formation of turbidity and / or sediment at further stages of product
manufacture or storage. The differences between the technologies of clarification and stabilization determine the differences in the quality of the obtained products. Clarified juices are unstable to the formation of secondary turbidity and / or precipitation, since they do not have the properties of stabilized juices [2].

The level of transparency (or stability) depends on the number and nature of the particles of turbid suspension, as well as the concentration of a number of substances that are in the juice in the colloidal state.

2. The purpose of the study
Turbidity and stability indicators do not belong to the normalized indicators of quality or safety of juices. In industrial and commercial practice, these indicators are mainly used only for the evaluation of Apple juice. Due to the ever-growing trend of using turbidity and stability indicators as quality indicators of a number of other fruit juices, the main purpose of this article is to increase awareness of the processes that lead to a violation of the stability (transparency) of pomegranate juice in order to learn how to manage these processes.

3. The object of the study
The objects of the study were juices of direct pressing of fruit grains of 22 varieties of pomegranate from the Reference point of the research Institute of fruit and tea growing, which is located in the Shirvan natural and economic zone of Azerbaijan.

4. Materials and methods

4.1 Organization of work and venue
The fruits were collected in early November from all sides of three to five adult trees of the same variety, selecting as an average sample 20 pieces typical in shape, color and degree of maturity of the fruits, which were then placed in a single layer in wooden trays. In the same trays, they were delivered by covered vehicles to the laboratory of processing and storage Technologies of the research Institute of fruit and tea growing, located in the Guba district about 500 km from the Geokchay stronghold. Obtaining juices was carried out on the next day after the fruit harvest.

4.2 Chemical analyses.
The content of soluble solids was determined in juices by refractometric method according to GOST P 51433-99, simple sugars - by Bertrand method according to GOST 8756.134-87, organic acids - by titration in the presence of a color indicator according to Interstate standard ISO 750-2013, ascorbic acid - by iodometric method according to GOST 28556-89, pH - by potentiometric method according to GOST 26188-84.

The amount of protein in juices was determined by the Kjeldahl method in the Department of biotechnology and bioecology of the Belarusian State Technological University [3]. The method is based on the separation of protein from other nitrogen-containing substances by precipitation of copper sulfate in an alkaline medium. The protein content in grams was determined by multiplying the calculated amount of nitrogen by a conversion factor of 6.25.

Determination of the content of the total amount of water-soluble polyphenols provided for their complete extraction into the extract, separation of the extract, filtration and titration with 0.1 n. potassium permanganate solution by Leventhal. First, all substances oxidized by this reagent are titrated, then that part of them that remained in the extract after processing it with activated carbon capable of adsorbing polyphenols. According to the difference in the amount of potassium permanganate, which went to oxidation for the first and second time, the content of phenols is
determined using 0.004157 as the conversion factor of milliliters of 0.1 N potassium permanganate solution into grams of phenols. This principle is embedded in the basis of the method for determining tannins in medicinal raw materials according to GOST 24027.2-80 and the corresponding article of the State Pharmacopoeia of the Russian Federation, which is also devoted to the definition of these substances in medicinal raw materials. The total content of anthocyanins was determined by pH-differential spectrophotometry according to GOST P 53773-2010. The total mass concentration (mass fraction) of anthocyanins was determined on the basis of changes in the absorption of light with a wavelength of 510 nm with a change in the acidity of juice solutions with a pH from 1.5 to 4.5.

The total content of catechins and leucoanthocyanins in the juices was determined by spectrophotometric method [4-5].

In aged pasteurized juices, the precipitate was determined according to GOST 8756.9-78. This standard method is based on separating the precipitate from the juice or extract by centrifugation with preheating the juice or extract in a water bath and determining the mass of the precipitate released. The analyses were performed in three biological repetitions, the data obtained were processed statistically using the Microsoft Excel spreadsheet package and presented as arithmetic averages.

4.3 Obtaining juices and storage conditions before further tests.
Washed under the tap pomegranate fruits of each variety were cut manually with a sharp knife into 4-6 approximately equal shares, then manually released the juicy seeds (grains) that were in them. From pomegranate seeds of each variety, juice was obtained using a citrus juicer-press Kenwood JE290. Fresh juice was subjected to filtration through installation of prefabricated laboratory equipment, arranged according to a simple scheme: a flask, a Bunsen funnel is inserted daneva with a double-layer paper filter, to process the attached bulb pump to generate thrust. The filtered juices were poured into glass bottles capable of holding 0.33 liters of juice, and pasteurized for 30 minutes at a temperature of 75 ° C. The Pasteurized juices were subjected to exposure in a darkened room at a temperature of 18 ... 22 ° C. Juices were removed from storage after 9 months, when the turbid suspension completely settled to the bottom of the bottles, and the juice on top of it became perfectly transparent.

4.4 Study of the correlation between individual indicators of the chemical composition of juices and the mass fractions of precipitation in them.
The aged pasteurized juices were heated, then subjected to centrifugation treatment aimed at separating the precipitates that fell in them, which were then weighed.

A comparative analysis of the influence of each related group of substances found in juices at the early stages of their production on the processes leading to the formation of turbid suspension in them and precipitation during long-term storage in pasteurized form was conducted using standard methods of statistical and correlation analysis.

5. Discussion of the results
Table 1 provides data on the concentration (mass fraction) of some biologically active and nutrients in freshly squeezed filtered fruit juices of 22 varieties of pomegranate of Azerbaijani reproduction and precipitation in them for 9 months of storage in a darkened room at a temperature of 18-22° C.

Table 2 presents some statistical indicators characterizing the presence or absence of a reliable relationship in each pair of data on individual indicators of the chemical composition of freshly squeezed filtered juices of fruits of 22 varieties of pomegranates and the percentage of precipitation in them (for 9 months, storage in pasteurized form at a temperature of 18-22°C).
Table 1. The chemical composition of freshly squeezed filtered pomegranate juices in varietal section and the percentage of precipitation in them for 9 months of storage in pasteurized form at a temperature of 18 ... 22°C:

| Variety of pomegranate | Indicators of the chemical composition of juices |
|------------------------|-------------------------------------------------|
|                        | 1  | 2   | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |
| Guleysha pink          | 16.8| 1.8 | 0.169| 12.47| 0.99| 6.16| 0.21| 28.4| 19.2| 0.094|
| İridanaly              | 15.4| 2.2 | 0.231| 12.04| 1.76| 2.82| 0.83| 14.7| 14.4| 0.116|
| Wild pomegranate       | 17.7| 1.2 | 0.169| 12.16| 3.20| 8.98| 0.42| 24.1| 20.7| 0.185|
| Kyrymyz Kabukh         | 17.3| 1.8 | 0.315| 12.88| 1.06| 5.63| 0.62| 39.8| 18.0| 0.128|
| Bala Mursal            | 17.6| 2.0 | 0.213| 13.26| 1.34| 5.28| 0.42| 38.6| 13.1| 0.076|
| Clone Guleysha pink    | 16.1| 4.0 | 0.252| 12.92| 0.38| 3.17| 0.62| 35.9| 5.6 | 0.131|
| İrada                  | 16.3| 1.8 | 0.189| 12.00| 0.74| 6.51| 0.62| 36.3| 16.9| 0.263|
| Siyah Dane (Iranian 9-4)| 16.3| 1.8 | 0.231| 12.14| 1.22| 4.58| 0.42| 20.1| 14.6| 0.055|
| Kazake improved        | 15.5| 2.0 | 0.252| 11.59| 0.64| 4.93| 0.21| 17.0| 24.1| 0.129|
| Geokhchay Meles        | 16.6| 1.8 | 0.169| 12.12| 1.22| 6.86| 0.62| 22.8| 9.2 | 0.109|
| Spring                 | 15.7| 4.0 | 0.294| 12.55| 1.98| 2.82| 0.21| 11.2| 17.0| 0.112|
| Mardakan-20            | 15.3| 2.0 | 0.231| 11.55| 0.54| 4.58| 0.42| 40.4| 12.3| 0.140|
| Shirin Nar             | 16.5| 4.5 | 0.231| 13.42| 0.40| 2.46| 0.62| 2.73| 8.9 | 0.097|
| VIR - 1                | 16.8| 3.5 | 0.252| 13.26| 1.38| 2.99| 0.42| 13.1| 15.1| 0.102|
| Azerbaijan (3-10-12)   | 16.4| 1.5 | 0.351| 12.42| 0.77| 6.34| 0.62| 41.6| 18.2| 0.099|
| Clone Veles            | 17.4| 2.6 | 0.252| 13.84| 0.53| 3.34| 0.42| 29.6| 15.8| 0.182|
| Malta                  | 16.4| 4.0 | 0.189| 12.38| 0.42| 2.64| 0.21| 8.0 | 21.3| 0.138|
| Nasimi (P1-8-6)        | 16.2| 1.6 | 0.275| 12.34| 0.64| 3.87| 0.42| 27.6| 26.6| 0.153|
| California - 630       | 16.7| 1.8 | 0.315| 12.30| 0.41| 3.70| 0.42| 29.5| 14.3| 0.043|
| Iran 3-1 (Iran-1)      | 16.2| 1.8 | 0.252| 12.68| 0.50| 5.22| 0.42| 28.0| 18.2| 0.118|
| Yeni Guleysha          | 15.2| 2.0 | 0.213| 11.41| 1.06| 4.75| 0.21| 23.1| 16.5| 0.105|
| Nukha red (3-8-4)      | 16.6| 1.6 | 0.231| 12.47| 0.85| 4.58| 0.21| 38.5| 11.5| 0.098|

1-Soluble dry matter, g / 100 g; 2-pH; 3-Protein (N x 6.25), g / 100 g; 4-Total sugar, g / 100 g; 5-Organic acids, g / 100 g; 6-Ascorbic acid, mg / 100 g; 7-Water-soluble polyphenols, g / 100 g; 8-Anthocyanins, mg / 100 g; 9-Catechins, mg / 100 g; 10-Crude precipitate, g/100 g.

Table 2. Values of correlation coefficients (R), student's t-test and probability of reliability (P) for each pair of data on the quantitative content of individual biologically active and nutrients in filtered juices of direct pressing of fruits of different varieties of pomegranates (n = 22) and precipitation in them for 9 months storage in pasteurized form at a temperature of 18-22°C C

| Pairs      | Data                                                                 | R     | T     | P    |
|------------|----------------------------------------------------------------------|-------|-------|------|
| 1          | On the mass fraction of solids and the mass fraction of sediment     | 0.06  | 0.26  | 0.197|
| 2          | On protein mass fraction (N x 6.25) and mass fraction of sediment    | -0.73 | -6.12 | <0.001|
| 3          | On concentration of hydrogen ions (pH) and mass fraction of sediment | -0.28 | -1.44 | 0.852|
| 4          | On the mass fraction of total sugar and the mass fraction of sediment | -0.29 | -1.50 | 0.866|
| 5          | On the mass fraction of organic acids the mass fraction of sediment  | 0.29  | 1.50  | 0.866|
| 6          | On the mass fraction of water-soluble polyphenols and the mass fraction of sediment | 0.15  | 0.71  | 0.517|
| 7          | On the mass fraction of anthocyanins and the mass fraction of sediment | 0.06  | 0.26  | 0.197|
| 8          | On the mass fraction of catechins and the mass fraction of sediment  | 0.71  | 5.75  | <0.001|
| 9          | On the mass fraction of ascorbic acid and the mass fraction of sediment | 0.60  | 4.13  | <0.001|

Table 2 shows that the correlation coefficients calculated for protein mass fraction (N x 6.25) and sediment mass fraction (R = - 0.73), catechin mass fraction and sediment mass fraction (R=0.71), and ascorbic acid mass fraction and sediment mass fraction (R=0.60) satisfy any probability and are therefore reliable. Hence, there is a direct causal relationship between the mass fraction of catechins
and ascorbic acid in freshly squeezed pomegranate juices and the masses of precipitation falling in them. In other words, the higher the mass fraction of catechins and ascorbic acid in freshly squeezed juices, the more the stability of the same juices is disturbed during their long-term storage in pasteurized form and the more sediment will fall out in them. We in this case can not say anything about the role that leucoanthocyanins play in these processes, since the content of this group of phenolic substances in freshly squeezed pomegranate juices varied from traces to 1.0 wt. %. Their content in pomegranate juices increases later, as a result of various factors of its processing. Such factors include heat treatment, which intensifies the transformation of juice polyphenols, especially anthocyanins, which then continue until they degrade completely.

But it does not mean that the value of the correlation coefficient for the data on the mass fraction of protein (N x 6.25) in freshly squeezed juices and the mass fraction of precipitation in them, equal to-0.73, can serve as a strong basis for the recognition of the inhibitory role of nitrogenous substances in these processes. This can not be that the higher the mass fraction of nitrogenous substances in freshly squeezed juices, the higher the stability of the juice and the less precipitate falls in it when stored in pasteurized form.

In order to clarify this issue, it was also necessary to conduct statistical processing of data on pairs of data concerning the quantitative content in fresh juices of certain groups of related substances, which showed that there is a weak conjugation in the variation of data on the mass fraction of nitrogenous substances (N x 6.25) and the mass fraction of catechins (R = -0.48, P> 0.95).

The conjugacy in the data pair "mass fraction of catechins and mass fraction of ascorbic acid" was higher than in the data pair mass fraction of nitrogenous substances (N x 6.25) and mass fraction of catechins. And in the data pair "mass fractions of nitrogenous substances and mass fractions of ascorbic acid" no reliable conjugation was revealed.

This suggests that juices with a high initial protein content contain, as a rule, less catechins and ascorbic acid than juices with a relatively low content. If juices with a high initial content of nitrogenous substances were also characterized by a high content of catechins, this would lead to the formation of "catastrophically" large amounts of precipitation. When polyphenols interact with protein substances, strong complexes are formed, with which the turbidity of juices, the enlargement of Muti particles and their precipitation are associated.

The role of ascorbic acid in these processes is that polyphenolic substances protect it from oxidation, catalyzed by metal ions. The mechanism of action of polyphenolic substances is to block the catalytic action of metals by binding them into very stable complexes. The result is the appearance of dark-colored oxidation products of phenolic compounds, which gradually condense into large insoluble particles and precipitate.

Correlation coefficients calculated for data on other indicators of the chemical composition of freshly squeezed juices and the percentage of precipitation in them after 9 months of storage in pasteurized form, had very low levels of significance from 0.13 to 0.80. The absence of a reliable pair correlation between some indicators of the chemical composition of freshly squeezed juices and the percentage of sediment can not serve as a complete proof of the absence of causal relationship between them. Sometimes the existence of a causal relationship between the content of reactive components and the stability of the juice during storage is difficult to detect due to other factors hiding this relationship.

At first glance, the correlation coefficients for data on titrated acidity and sediment percentage (R = 0.29) and pH and sediment percentage (R = - 0.28) were completely unexpected. If we conditionally accept their levels of significance reliable, it turns out that with an increase in total acidity and a decrease in pH (which is natural), the percentage of sediment in juices increases contrary to the known position of a positive effect on the stability of low pH anthocyanins. But this is understandable given the influence of a "strong" factor-ascorbic acid. After all, as a rule, high acidity and low pH are
characterized by juices with a relatively high concentration of ascorbic acid (table 1). Therefore, in the juices of sweet pomegranates (Spring, Shirin Nar, California 630, Malta, etc.), with a reduced content of ascorbic acid, sediment, as a rule, falls little.

From the discussion of the obtained data, it can be concluded that the violation of the stability of pomegranate juice during storage is due to the so-called "polyphenolic turbidity" - a kind of colloidal turbidity of juices. In this case, it is not only a purely phenolic opacity. At all stages of production and storage of pomegranate juice, products of polymerization and condensation of phenolic compounds are formed; the resulting products, as a rule, enter into numerous reactions of interaction with proteins, metals, amino acids.

Polyphenolic opacities may include not only catechins, but also anthocyanins, which protect ascorbic acid from oxidation; thus, anthocyanin derivatives are found in sediments that fell during storage of pomegranate juices [6-7].

The stability of anthocyanins is significantly affected by the chemical composition of the raw material. There was a decrease in anthocyanins with an increase in ascorbic acid and hydrogen peroxide [8].

Catechins are a fairly extensive group of plant organic compounds: the number of known flavan-3-ols exceeds 1000. One of the most important functions of catechins is participation in redox processes as effective antioxidants.

From the results of our studies, which we have already mentioned, it can be concluded that to improve the stability of pomegranate juice can be useful treatment of fresh juice, aimed at removing part of the catechins contained therein, belonging to a large group of phenolic compounds that are able to protect in aqueous solutions of ascorbic acid from oxidation.

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Catechins can become the ancestors of dark-colored insoluble substances, although they are colorless compounds before oxidation. This is due to the fact that under the influence of oxygen, catechins condense to form polymerized dark-colored products that precipitate.

Along with measures aimed at reducing the concentration of catechins, it is also necessary to prevent the possible interaction of other phenolic compounds with proteins, which is accompanied by enlargement of the particles and turbidity of the juice. It should be borne in mind that strong bonds with proteins form only oligomeric forms of phenolic compounds containing a sufficient number of hydroxyl groups. The relatively large size makes it easy to separate them from other forms of polyphenolic substances with smaller sizes.

Both can be achieved by ultrafiltration, the purpose of which is precisely the removal of not only suspended solids, but also colloids, including polyphenolic nature. The latter tend to interact with proteins and their presence in the juice makes it impossible to obtain a transparent juice during storage [9, 10, 11].

However, the ultrafiltration method has one significant drawback, which is the relatively low performance of membrane plants. Therefore, we will first have to implement the stage of pre-clarification with the use of bentonite and other means, so that it would be possible to increase the performance of the subsequent ultrafiltration.

Modern pomegranate juice production lines make it easy to do this. After all, they are usually composed of equipment for pre-purification of juice with the use of all kinds of vats, vacuum rotating or plate filters, and membrane installation.

Anthocyanins undergo changes not only under the influence of the chemical composition of the environment, but also as a result of many other factors, such as microbes and enzymes.
With this in mind, juices and extracts are passed through the membranes to remove part of the nutrients that support the growth and development of yeast, as well as the yeast itself, in the process of life which appear catalyzing oxidation components. For this purpose, the juice is first subjected to pre-cleaning from inclusions with a size of about 1 micron. In industry, this can be done using filter presses, rotating filters, etc., or cartridge filters with pore sizes ranging from 0.45 to 1.25 microns [12].

The filtrate can be passed through microfiltration membranes with pores of 0.1-10.0 microns to clean it from yeast cells and bacteria.

More complete purification is achieved by using ultrafiltration membranes with pore sizes of 0.005-0.05 microns, which provide a delay of colloidal particles, oil emulsions, pigments and complete delay of pigments with oligomeric and polymer structure (with a molecular weight of 20,000 to 50,000). When applied to pomegranate juice, this treatment requires careful selection of membranes so that it does not lead to the loss of proper anthocyanins and a decrease in the color intensity of the product, which is one of the main indicators of its quality.

6. Conclusion
The role of some components of the chemical composition of pomegranate juices in the processes that are accompanied by the formation of turbid suspensions and precipitation is studied. It is concluded that the violation of the stability of pomegranate juice during storage is due to the so-called "polyphenolic turbidity" - a kind of colloidal turbidity of juices. It is assumed that the most effective method in improving the stability of pasteurized pomegranate juice during storage can serve as ultrafiltration.

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