Pectin-containing antioxidant drink based on extracts of grape pomace and *Chamerion*

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Abstract. The cause of many pathological processes in the human body that entail various serious diseases is damage to cellular structures by free oxygen radicals. This has led to the urgency of developing drinks with high antioxidant and immunomodulatory properties. Various types of *Chamerion, C. angustifolium* L. and *C. caucasicum* and pomace of the Ancellotta grape variety growing in the South of Russia were selected as the objects of research. The results of determining the amino acid composition, the content of total polyphenols, flavonoids, anthocyanins and tannins confirmed the high antioxidant properties of *C. angustifolium* L. and *C. caucasicum*. It was found that the amino acid composition does not differ significantly by the type of *Chamerion*. The content of amino acids is not high, except for a relatively high number of four essential amino acids, such as leucine, valine, phenylalanine and isoleucine. Research results have shown that the type of *Chamerion* affects the content of antioxidant compounds. It was found that a small quantitative predominance of common phenolic compounds, flavonoids, anthocyanins and tannins is observed in *C. angustifolium* L. At the same time, the highest content of antioxidant compounds is observed in leaves and flowers. Quantitative evaluation of the anti-radical and anti-oxidative activity of the resulting beverage showed its high ability to bind free radicals, which is further confirmed by the indicators of restoring power (4.91 mmol Fe²⁺/1 kg of raw material).

1. Introduction

Recently, scientists of different specialties have come to the conclusion that the causes of many pathological processes in the body, leading to various diseases and eventually to aging, are the same processes associated with damage to cell membranes and other structures inside the cell by free oxygen radicals [1]. It is established that depending on the type of structural damage, either to inherited substance (DNA) or to the outer membrane, various changes occur in the human body: cancer develops or other disorders are observed. At the same time, as the body ages, the activity of free radicals increases, which, in turn, leads to an increase in the risk of various age-related diseases. The identified causes of these negative changes determine the urgency of the problem of developing food products with high antioxidant and immunological properties.

It is known that the body gets most of its antioxidants from food. At the same time, it was found that the antioxidant activity of the human diet is 68% determined by the consumption of beverages [2]. It follows that the most effective way to correct the antioxidant and immunological status of modern humans is to consider nutritional compositions in a liquid form.
The most common type of food products with an antioxidant effect are tea drinks. Among juices, nectars and non-carbonated beverages, tea drinks take the second place both in volume and in production growth, causing increased interest of consumers around the world.

Tea and health is a new global trend in the food consumer market. At the same time, in recent years, consumers have shown more and more interest in cold drinks based on tea, which makes it possible to combine the tea base with various food additives that enrich its taste.

The most physiologically active additive is pectin substances [3]. It is established that pectins entering the human body as part of food products perform a regulatory function. They bind biologically active molecules contained in gastric juice, such as superoxide radical, estrogens, endotoxin, leptin, tumor necrosis factor, pancreatic amylase, xanthine oxidase, and food protein antigens. It is assumed that by binding low-molecular bioregulators in the lumen of the upper gastrointestinal tract, pectins inhibit their reabsorption and influence the processes of digestion, carcinogenesis, immune response, free-radical damage and inflammation [4]. On the example of immunomodulatory activity, a unique property of pectins was revealed - polypotency, that is, the potential for pectins to have a multidirectional physiological effect [5].

Grape pomace, being a secondary raw material resource of winemaking, contains not only pectin, but also phenolic compounds [6], which defines them in this study as a physiologically valuable raw material component.

It is advisable, in our opinion, to consider the Chamerion as another raw material. Thus, from the literature data, it is known about the content of a wide range of biologically active substances (BAS) in different parts of the C. angustifolium L. Thus, the flowers contain traces of alkaloids, vitamin C (from 90 to 588 mg% per 100 g of raw grass) [7–9], tannins; petals contain anthocyanins; leaves contain pectin, lycopene, lignin (up to 21.67% weight), chlorophyll a and β-carotene (up to 4.16% weight), sugars, organic acids, coumarins, alkaloids (0.1 – 1%), flavonol and anthocyanin compounds (up to 30.11% weight), organic acids (2.9%), tannins (up to 5.65–20% weight), triterpenoids (1.3–1.9%), essential oils, phenol–carboxylic acids, tannin (up to 10 mg%) [10–12]. The content of these compounds determines the pronounced immunomodulatory effect of Chamerion [13–15].

In connection with the above, the relevance of developing a pectin-containing beverage with antioxidant and immunomodulatory effects based on extracts of grape pomace and Chamerion is quite high.

2. Materials and methods

As the basis for the development of a tea drink, we selected Chamerion, which has a historical tradition of consumption in Russia since the XII century. Since the XIII century, this traditionally Russian drink has often been called koporsky tea. Chamerion received this name because of the large areas of plantations cultivated by the monks of the settlement of Koporye in the Petersburg province. It should be noted that great Britain, which owned huge tea plantations in India and Ceylon, until the end of the XIX century preferred “Russian tea” made of Chamerion to the famous Indian tea with its taste qualities. From the 17th to the 19th centuries, Chamerion took an honorable place in Russian exports, significantly surpassing such traditional Russian products as gold and honey. The First World War and the revolution of 1917 contributed to the gradual oblivion of Chamerion, both on the Russian and international markets. In this paper, we have selected C. angustifolium L. and C. caucasicum grown in the Krasnodar and Stavropol territories as the objects of research (Fig. 1, 2).

The objects of research were also pomace of Syrah, Ancellotta and Pinot Franc grape varieties and their extracts, as varieties with a high content of phenolic compounds [16].

Studies of the chemical composition of Chamerion were carried out according to generally accepted methods. The quantitative content of ascorbic acid from the acidified water extract was determined titration by the Thielmans reaction. Anthocyanins were detected by spectrophotometric method by measuring the optical density of acid extraction. The quantitative content of free amino acids was carried out by capillary electrophoresis.
This type of Chamerion is a perennial plant, the height of which is usually from 50 to 150 cm, but can reach up to two meters. It has erect stems. Leaves on the stems are many, they are shiny, and their edges can be either small-toothed or whole. They are dark green above, and bluish below, but may be red-pink. The plant has large pink-purple flowers, which can be also white. It grows in the foothill and forest-steppe zone of the Krasnodar territory.

This type of Chamerion grows in the Stavropol territory, in the Western Caucasus, in Adygea in the river valleys and in the Alpine belt. This is a low type of Chamerion, growing up to 50 cm. It is distinguished by creeping stems and linear-lanceolate leaves about 3 cm long and up to 1 cm wide. Brushes of inflorescences of such Chamerion are short, and petals are rounded.

The total content of flavonoids was measured by the photocolorimetric method based on the intensity of the reaction with solutions of sodium nitrite and aluminum chloride at a wavelength of 510 nm. The total content of tannins was also determined by the photocolorimetric method based on the reaction of the extract with the vanillin reagent. Antiradical activity was determined using the DPPH method based on the ability of feedstock antioxidants to bind a stable Chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) at a wavelength of 517 nm [17, 18]. Antiradical activity was expressed as a concentration of the initial extract in mg / ml, at which 50% of the radicals were bound.

The restoring power of extracts was determined by the FRAP method [19]. The method is based on the ability of the active substances of the original extract to restore trivalent iron. Antioxidant activity was determined using the TEAC method (trolox equivalent to antioxidant activity) [20]. The method is based on measuring the discoloration of the color of a long-lived blue cation radical when exposed to an antioxidant. The transmission coefficient was determined at 734 nm. The results were expressed relative to trolox in mol/kg.

The antioxidant activity of the samples was determined in the linoleic acid system [21], based on the ability of the antioxidants of the studied raw materials to inhibit the oxidation of linoleic acid under conditions close to the state of a living cell – at a temperature of 40°C and pH 7.0 for 120 hours.

The method of quantitative determination of pectin substances in plant raw materials is based on the extraction of pectin from plant raw materials and its transfer to the dissolved state. The basis of the study of the extracts of hydropectin and protopectin is the calcium-pectate method and precipitation with ethanol.

3. Results and discussion
To assess the nutritional value of the selected types of Chamerion, we conducted studies in the comparative assessment of the amino acid composition, the results of which are shown in Table 1.
It was found that the amino acid composition does not differ significantly by the type of *Chamerion*. The content of amino acids is not high, except for a relatively high number of four essential amino acids, such as leucine, valine, phenylalanine and isoleucine.

**Table 1. Amino acid composition of biomass proteins of *Chamerion***

| Amino Acid          | *Chamerion angustifolium* L. | *Chamerion caucasicum* L. |
|---------------------|-----------------------------|---------------------------|
| Lysine              | 0.42±0.01                   | 0.45±0.01                 |
| Histidine           | 0.24±0.01                   | 0.30±0.01                 |
| Arginine            | 0.56±0.01                   | 0.54±0.01                 |
| Aspartic acid       | 1.27±0.03                   | 1.31±0.04                 |
| Threonine           | 0.47±0.01                   | 0.48±0.01                 |
| Serine              | 0.52±0.01                   | 0.54±0.01                 |
| Glutamic acid       | 1.77±0.03                   | 1.83±0.03                 |
| Proline             | 0.62±0.02                   | 0.64±0.02                 |
| Glycine             | 0.55±0.01                   | 0.53±0.01                 |
| Alanine             | 0.60±0.02                   | 0.61±0.02                 |
| Valine              | 0.61±0.01                   | 0.65±0.01                 |
| Methionine + cysteine| 0.12±0.01                  | 0.14±0.01                 |
| Isoleucine          | 0.52±0.01                   | 0.57±0.01                 |
| Leucine             | 0.85±0.02                   | 0.90±0.02                 |
| Tyrosine            | 0.32±0.01                   | 0.37±0.01                 |
| Phenylalanine       | 0.58±0.01                   | 0.54±0.01                 |

The results of determining the content of antioxidants in the studied species of *Chamerion*, taking into account its morphological groups, are shown in table 2.

The table shows that the type of *Chamerion* affects the content of antioxidant compounds. It was found that a small quantitative predominance of almost all compounds is observed for *C. angustifolium* L., so in further studies we selected this species. It should also be noted that the highest content of antioxidant compounds is observed in leaves and flowers than in stems.

**Table 2. The content of antioxidant compounds in various types of *Chamerion***

| Morphological group | Phenolic substances in terms of gallic acid, mg/g | Flavonoids in terms of miquelianin, mg/g | Tannins, mg/g in terms of oenethin B | Anthocyanins, mcg/g in terms of cyanidin-3-O-glucoside |
|--------------------|-----------------------------------------------|----------------------------------------|-------------------------------------|-----------------------------------------------------|
|                    | *C. angustifolium* L. | *C. caucasicum* L. | *C. angustifolium* L. | *C. caucasicum* L. | *C. angustifolium* L. | *C. caucasicum* L. |
| leaves             | 252.46             | 251.02                | 43.52                | 39.42                | 203.86                | 195.77                | 229.84                | 221.78                |
| flowers            | 237.86             | 220.07                | 82.58                | 78.54                | 135.97                | 128.87                | 207.30                | 202.41                |
| stems              | 129.54             | 110.34                | 10.74                | 10.32                | 64.02                 | 60.05                 | 147.73                | 139.46                |

One of the main valuable components of grape pomace is pectin substances. We conducted studies to determine the fractional composition and total content of pectin substances (figure 3).
The results of experimental studies showed that the lowest content of hydratopectin (1.93%) was observed in the pomace of Syrah grapes, the highest was in the Ancellotta variety (3.95%). A high content of protopectin was found in Pinot Franc pomace (2.01%), and a low content was found in Syrah. The total content of pectin substances in the pomace of the studied grape varieties ranges from 2.83 – 5.66%.

The results of the study of the content of phenolic compounds and anthocyanins in the pomace of the Ancellotta grape variety, conducted earlier, showed that the total content of phenolic compounds in them is 640 mg/dm3, anthocyanins are from 2.4 mg/dm3 [16].

The results obtained made it advisable to conduct further studies with pomace of the Ancellotta grape variety as a variety with a high content of pectin substances and phenolic compounds.

Extraction was used to obtain extracts from the study objects. As it is known, this process is a multi-factor one, depending on a number of technological factors [22]. In order to ensure food safety, water was selected as an extractant. Extraction of extractive substances was performed at pH=3.0, temperature of 60°C and duration of 120 minutes and mass flow ratio of 1:10.

The biochemical and technological parameters of the obtained extracts are shown in Table 3. From the table data, it follows that the obtained extracts equally have high technological and antioxidant properties, which makes it advisable to use them in a blend mixture as a basis for creating a drink with the introduction of various flavor components.

**Table 3. Biochemical and technological parameters of extracts**

| Name of the indicator                              | Pomace of Ancellotta grape variety | *Chamerion angustifolium* L. |
|----------------------------------------------------|------------------------------------|-----------------------------|
| Pectin substances, %                               | 0.82±0.02                         | 0.32±0.02                   |
| Titrated acidity, % tartaric acid                  | 7.5±0.07                          | 3.2±0.05                    |
| Content of phenolic substances in terms of gallic acid, mg / ml | 2.3±0.03                          | 2.7±0.03                    |
| Content of coloring substances, g / dm³            | 0.43±0.04                         | 0.37±0.02                   |
| Vitamin C content, mg / g                          | 5.0±0.11                          | 7.2±0.09                    |
| Antioxidant activity, micromolTE / dm³             | 2419.58±70.5                      | 2620.74±69.7                |

The obtained extracts from grape pomace and *C. angustifolium* L. were mixed in the ratio of 70% and 30%, respectively, according to the results of the tasting evaluation of the food composition. Glucose
and stevioside were used as flavoring additives. The characteristics of the resulting pectin-containing beverage are shown in table 4.

Table 4. Quality indicators of the developed pectin-containing beverage

| Indicator                                                                 | Value  |
|--------------------------------------------------------------------------|--------|
| Phenolic substances in terms of gallic acid mg/g                          | 374.2  |
| Flavonoids in terms of miquelianin, mg/g                                 | 47.3   |
| Anthocyanins, mcg/g of feedstock, in terms of cyanidin-3-O-glucoside     | 199.4  |
| Tannins, mg/g in terms of oenethein B                                    | 47.4   |
| Antiradical activity, E<sub>50</sub>, mg/ml                              | 9.1    |
| Antioxidant activity, micromolTE/g of feedstock                          | 28.8   |
| Restoring power, mmol Fe<sup>2+</sup>/1 kg of feedstock                  | 4.91   |
| Antioxidant activity, % inh.                                            | 77.8   |

The experimental data obtained confirm the high antioxidant properties.

4. Conclusion

The selected types of Chamerion and pomace of the Ancellotta grape variety have shown that they can be considered as raw components being a source of pectin and antioxidant compounds. In the studied grape pomace, the lowest content of hydratopectin (1.93%) was observed in the pomace of the Syrah grape variety, the highest was in the Ancellotta variety (3.95%). A high content of protopectin was found in Pinot-franc pomace (2.01%), and a low content was found in Syrah. The total content of pectin substances in the pomace of the studied grape varieties ranges from 2.83 to 5.66%. Taking into account not only the total content of pectin, but also phenolic substances, the practical interest for the production of a drink with an immunomodulatory effect is the pomace of the Ancellotta grape variety.

The results of determining the amino acid composition, the content of total polyphenols, flavonoids, anthocyanins, and tannins confirm the high antioxidant properties of *C. angustifolium* L. and *C. caucasicum*. Quantitative evaluation of the antiradical and antioxidative activity of the resulting beverage showed its high ability to bind free radicals, which is further confirmed by the indicators of restoring power (4.91 mmol Fe<sup>2+</sup>/1 kg of raw material).

The studied raw materials are a promising source of antioxidants, and the use of extracts from them in food products with a directed antioxidant effect will help to effectively combat oxidative stress and increase the immune status of a person.

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