

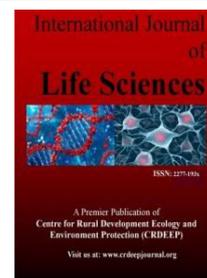
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Antioxidant Activity of *Prunus cerasifera* products

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ABSTRACT

Prune (lat. Prunus cerasifera) is a perennial fruit plant of the family Rosaceae, which has recently become of great interest in many countries of the world. Studies have been carried out on the chemical composition and physiological activity of a prune. In fact, there are no such studies in this area in Georgia. The aim of our work was to study the biologically active compounds, as well as the antioxidant activity of prune (which is widespread in Georgia, and in particular in Adjara) and products obtained from it, using modern instrumental research methods: quantitative determination of total phenols with the Folin-Chocalteu reagent and antioxidant activity - with the DPPH method. The prune fruit and products obtained from it, are characterized by high antioxidant activity, which is associated with a high content of biologically active compounds, including phenolic compounds, and a particularly high content of antioxidant active catechins.

Introduction

Antioxidant activity of prune and products obtained from it.

The problem of preserving human health and extending his life has been serious and urgent. Substantial changes in human lifestyle have led to the specificity and growth of diseases. Deficiencies in the structure and quality of nutrition are accompanied by the inability of the body's corresponding defense systems to adequately respond to the environment, what sharply increases the risk of developing many diseases. Functional and therapeutic-and-prophylactic food products that enhance the protective functions of the body and maintain and improve the condition of the body, reduce the risk of developing diseases, and all this due to ingredients that have a positive effect on one or more physiological functions. These ingredients include vitamins, organic acids, phenolic compounds (antioxidants), etc.

Our country is considered to be the place, where apples, pears, plums, prunes, nuts, mespilus, and other crops originated. Fruit cultures are the best raw material for the production of functional products due to their high nutritional, medicinal and dietary properties [1]. Prune (lat. *Prunus cerasifera*) is a perennial fruit plant of the family of Rosaceae. The tree reaches a height of 3-10 m, sometimes it is a woody shrub, highly branched, with or without thorns. It grows well in uncultivated areas, forms a dense thorny mass that can be found along roads, canals on the windward side, less demanding on the soil, resistant to diseases, drought and frost. Productivity from an adult tree is up to 300 kg [1,2]. Natural hybrids of the Prune species have a wide range of distribution - Europe, North-West Africa, the Caucasus, Iran and Turkey. They have a large area of distribution, as well as a wide range of ecological adaptations. Both wild and cultivated prunes are highly polymorphic. Depending on the shape and size of the fruit, the color, shape of the seed and the nature of its surface, prunes differ in varieties. The fruit is round, flat-round or ovoid. The color of fruits can be yellow, red and purple, sometimes almost black [3]. At the present time, when there is a great demand for the purity of biologically active substances and the quality of the raw materials produced, it is relevant to develop new, more accurate, objective and simple methods of analysis that provide a quick control of substances in plant raw materials and products obtained from it. Fruits contain a lot of phenolic compounds, their concentration depends on the degree of ripeness, as well as geographical and seasonal factors [4]. The interest in phenolic compounds has recently increased due to its beneficial health potential [5,6]. Some beneficial properties include anticarcinogenic action and reduce risks of heart disease [6-8]. One of the most interesting functions is its antioxidant property. They can play an important role in reducing the risk of certain pathological conditions. Antioxidants neutralize free radicals and other reactive substances that cause degenerative reactions in the body with the above mentioned diseases [9,10]. Prune and "Georgian" products, obtained from it, have recently become especially popular. The best quality juice, compote, jam, "Tkłapi" and well-known sauce "Satsebeli" are the unique attributes of the Georgian table.

The prune fruits can be consumed raw as well. Interest in prunes has recently grown significantly, both according to Georgian and foreign data. Literary sources found by us show that prune (*Prunus spinosa*) is an excellent resource in Georgia, but the fruits and products, obtained from it, have not been actually chemically studied. The development of the agricultural sector and tourism infrastructure is important both for the region and the country as a whole. It is very important to study natural resources and determine biochemical diversity. In today's ecological environment, the focus is on functional products, and scientists are looking for new raw materials to manufacture these products. The protective function of such products is partly due to the presence of phenolic compounds [11]. Phenolic compounds are indispensable components of fruits and vegetables, they affect the character of food (sensory and nutritious), its color and taste [12]. These compounds have high antioxidant activity, which is associated with several potential health benefits [13].

Materials and methods

Plum fruit and production her processing juice, jam, Preserve, Korao, Tklapi, Sauce. Chemicals- Folin–Ciocalteu reagent, 2,2-Diphenyl-1-picrylhydrazil, the ABTS method (2,2-Azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid, Gallic acid (Sigma-Aldrich), Na₂CO₃. Spectrophotometer (Mettler Toledo).

Total phenolics assay - The Folin–Ciocalteu method [20] was used for the determination of the total phenolics. In brief, an aliquot (1 mL) of the appropriate diluted extracts was added to a 25 mL volumetric flask, containing 5 mL of distilled water. Then, 1.0 mL of Folin–Ciocalteu reagent was added and the contents mixed. After 3 min, 10 mL Na₂CO₃ solution of concentration 7% was added and made up to a total volume of 25 mL distilled water. Their absorbances were read at 765 nm against distilled water as the blank. A calibration curve was constructed using gallic acid standard solutions (0–100 mg/L). The concentration of total phenolics is expressed as the gallic acid equivalent (GAE) per 1 g of fresh sample. All samples were prepared in triplicate.

The formula used to determine phenols is provided

$$X = (D \cdot K \cdot V \cdot F) \times 1000 / m$$

X – amount of phenols mg/kg; *D* – optical density; *K* – coefficient; *F* – factor of dilution; *V* – volume of extract in ml; *m* – mass of the raw material used for extraction in grams.

DPPH method for determining antioxidant activity- methods based on different principles for determining the total antioxidant activity are mainly used for reactions occurring during radical mechanisms between specific colored radicals and extracts with antioxidant activity, where the variability of the optical density of a solution is determined spectrophotometrically; both the specific substance and the general antioxidant activity of compounds are evaluated [22; 23; 25]. There are: the DPPH method of binding organic radicals ((2,2-Diphenyl-1-picrylhydrazil), the ABTS method (2,2-Azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) and others [19; 21]. One of the most common methods is free radical colorimetry DPPH with 50% radical inhibition. The method was first described by Blois in 1958 and subsequently modified several times [26]. The DPPH method for determining antioxidant activity is a fast, simple and accurate test method. It is used to determine the ability of various compounds to bind free radicals, as well as to measure the antioxidant activity in food and juices [19; 20; 24].

Antioxidant activity :The DPPH assay was done by using 1 mL of samples extract that was mixed with 1,5 mL of DPPH solution in methanol (0,02 mg mL⁻¹), The mixture was homogenized for 30 min at room temperature and then the absorbance was determined at 517 nm. Antioxidant activity DPPH - 50% inhibition mg of samples was calculated using the following formula 1 and 2: AA % inhibition = [A(DPPH) – A(sample)*100]/A(DPPH) (1),

Where; A (DPPH) - Absorbance of 0.01 mM DPPH at 517 nm

A(sample) - Absorbance of sample at 517 nm

AA of mg sample = $m \cdot 50 \cdot 1000 / V \cdot AA\%$ (2),

Where *m* – weight of sample,

V – volume of sample,

50 - % of inhibition,

1000 – unit conversion from gram to mg

Sampling methodology Plum and product phenols and antioxidant activity

Sample name	AA -0.1 mM DPPH-0.5% inhibition mg by fresh mass sample	Total phenols (Gallic acid) mg/100g fresh mass	Anthocyanin pigments (C-3-g) mg/100g fresh mass	Catechine mg/100g fresh mass
Whole fruit	9,56	1807,44	109,77	1628,48
Pulp	8,22	1875,92	84,86	1539,52
Skin	4,71	3320,86	374,35	2751,67
Juice	20,56	1094,51	68,79	971,21
Heated juice	35,77	1045,42	47,82	960,01
Jam	21,99	1378,06	76,77	1112,63
Preserve	38,68	748,66	13,39	352,09
Korao	5,12	2676,74	180,35	1792,58
Tklapi	4,90	4748,01	321,05	4403,20
Sauce	6,56	2425,46	154,58	2078,41
Spices	2,16	3498,38	0,00	0,00

Results

The prune fruit is distinguished by high antioxidant activity (9.56 mg), which is associated with a high content of biologically active compounds, including phenolic compounds (1.8 g / 100 g), of which a significant proportion is accounted for by antioxidant-active catechins (1.6 g / 100 g). These compounds are unevenly distributed in the pulp and skin of the fruit after juicing. Juice contains almost twice as many phenolic compounds (1.09 g / 100 g) and, therefore, catechins (8.8 mg / 100 g) than pulp (1.8 g / 100 g) and rind (3.3 g / 100 g). Some of these compounds are insoluble in water and therefore do not pass into juice. When heated, the juice loses some of the phenolic compounds (1.04 g / 100 g), and the content of anthocyanins (47.82 mg / 100 g) and catechins (0.96 mg g / 100 g) in the juice also decreases. There is a correlation between antioxidant activity and the content of biologically active compounds. The peel of the fruit is distinguished by high activity (4.71 mg AA), which is almost twice as high as in the fruit (9.56 mg AA) and pulp (8.22 mg AA), while in juice (20.56 mg AA) it is almost 5 times more active. When the juice is heated, the antioxidant activity decreases by almost 2 times (35.77 mg AA). Processing fruits into preserve and jam significantly reduces the content of biologically active substances (1.37 and 0.7 g / 100 g, respectively). The content of catechins (76.77 and 13.39 mg / 100 g, respectively) and anthocyanins (1.17 and 0.35 mg / 100 g, respectively) is also significantly reduced. Such Georgian traditional products as Korao (juice concentrate) and Tklapi (crushed mass without juice) are obtained from prunes as well. In the case of Korao, the amount of dry matter increases significantly, practically the juice is concentrated 5 times, which in turn leads to an increase in the amount of phenolic compounds (2.67 g / 100g) 2.5 times (instead of the expected 5 times), which is caused by thermal impact. The content of catechins (180.35 mg / 100 g) increases slightly, while the amount of anthocyanins doubles (1.79 g / 100 g). The concentration of phenolic compounds (4.7 g / 100 g) is higher in Tklapi, and the content of catechins (321.05 mg / 100 g) and anthocyanins (4.4 g / 100 g) also increases. Korao (5.12 mg) and Tklapi (4.90 mg) have high antioxidant activity, which probably leads to their use in traditional medicine against inflammatory processes. The content of phenolic compounds in the sauce Satsebeli (2.4 g / 100g) increases with antioxidant activity (2.16 mg) and, consequently, the content of phenolic compounds (3.49 g / 100g) at the expense of spices. The antioxidant activity of the sauce is also increased (6.56 mg).

Conclusions

There has been determined and studied a quantitative content of anthocyanins and catechins of common and main flavonoids of polyphenols in prune fruits, widespread in Adjara, as well as their variability during the processing of fruits. It has been established that the content of anthocyanins in products, obtained by heating (preserve, jam, sauce) is significantly reduced, while in products obtained by heating at a lower temperature (korao, tklapi, juice), their content is more preserved. Prune fruits and products, obtained from it, are distinguished by high antioxidant activity.

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