Determination of bioactive compounds of pomegranate peel to incorporate as a functional food ingredient in food production

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Abstract. Each component of pomegranate peel is examined in this research, with particular attention paid to the tannin content, chemical cosmetics, amino acids, carbohydrates, and polysaccharides. Utilizing atomic adsorption spectrometry, elemental analysis identified potassium and calcium as the key elements. The Kjeldahl method's amino acid composition showed that all of the required amino acids were present, indicating the protein derived from pomegranate waste's nutritional significance. Polysaccharides were found and described, including pectin-containing compounds and water-soluble polysaccharides. Applying spectrophotometry and high-performance liquid chromatography, the tannin content was found to be about 5.5%. Because of its antioxidant and antibacterial qualities, tannins have potential benefits in the culinary, pharmaceutical, and veterinary industries. The study sheds light on the nutritional value and bioactive components of pomegranate peel waste, underlining its multiple values. The information provided by this research is important in using pomegranate peel as a sustainable resource for several applications.

1 Introduction

In recent years, in the group of food additives that regulate product consistency, much attention has been paid to stabilization systems, including several components: an emulsifier, a stabilizer, and a thickener [1]. The qualitative composition and ratio of components can be very diverse, depending on the nature of the food product, its consistency, production technology, storage conditions, and method of sale [2].

Today, some manufacturers are increasingly including plant origins in the composition of food additives, since their effect on humans has been proven to be effective for centuries. They are used not only in nutrition but also in treatment since any food supplement contains a whole complex of biologically active substances [3]. It is important to understand that most plant supplements are not one substance but a unique combination of biologically active components. It is for this reason that in the description of the beneficial

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properties of biological additives, one can always find a wide range of effects on a living organism [4].

Tannins belong to a group of phenolic compounds of plant origin with a molecular weight of 500 to 3000, containing a large number of hydroxyl groups. They are found in the bark, wood, leaves, and fruits of many plants: chestnut, oak, spruce, larch, eucalyptus, tea, cocoa, pomegranate, etc. In water, ethyl alcohol, glycerin, and tannins, depending on their molecular weight, form true or colloidal solutions that have a slightly acidic reaction.

There are hydrolysable and condensed (non-hydrolysable) tannins [5]. Esters of gallic acid or the related digallic and trigallic acids with a polyhydric alcohol (such as glucose) are known as hydrolyzable tannins. Consequently, hydrolyzable tannins are characterized by the presence of aromatic structures with carboxyl and phenolic groups. Tannins of this structure are easily susceptible to acid and alkaline hydrolysis with the formation of carbohydrates and phenolcarboxylic acids [6].

Many properties of tannins have a high biological value for the human body and animals. In this regard, we studied the maximum tannin yield from waste pomegranate peels of the Kyzyl-anor variety. The peel of pomegranate fruits is distinguished by a high content (18-20%) of tannins; in wild pomegranate, they reach 28-35%; they also contain pectin substances (4-6%), approximately 16% cellulose, 7-8% hemicellulose, up to 20-25 mg% ascorbic acid, and up to 1.80% of various alkaloids (peltierine, isopelletierine, and methylpelletierine) [7,8].

Punica granatum L. (Punicaceae), also known as pomegranate, is one of the world's most popular fruits due to its delicious flavor, high nutritional value, and therapeutic characteristics. Therefore, this study was focused on the extraction of bioactive substances and uses it in food production.

2 Material and methodology

Atomic absorption spectrometers: Nov AA 350, Preekem Topex+ oxidizer, Mettler Toledo UV/VIS UV5 Bio spectrophotometer.

Test conditions: temperature 20–22 °C; humidity 45.0-55.0%; pressure 730–745 mmHg. In the next stage, the method of burning the sample with an oxidizer of the Preekem Topex+ brand (GOST 31671-2012 EN 13805:2002). Nov AA 350 atomic absorption spectrometers are made in compliance with approved and standard operating procedures to measure the mass concentration of elements in aqueous solutions of food goods, soils, biological objects, etc. Spectral range: 185–900 nm. Photometric range: 0–3 abs [9-10].

2.1 Determination of protein and amino acid content

To study the properties of pomegranate peel proteins, the quantitative amino acid composition was determined on a T-339 analyzer [11-13].

2.2 Tannin Analysis using HPLC

Tannin analysis was carried out by HPLC using gradient elution mode and a diode array detector (DAD). Acetonitrile and a buffer solution were used as the mobile phase. Spectral data were studied in the spectral range from 200 to 400 nm [14].

Table 1. Chromatography conditions.

Component of the HPLC	Standard
Mobile phase (isocratic mode)	Acetonitrile – buffer solution pH=2.92 (30: 70)
Injection volume	5 µl.
The speed of the mobile phase	0.75 ml/min.
Column	Eclipse XDB – C18. 5.0 microns, 4.6x250mm.
Detector	diode matrix detector, wavelength 254.

Chromatographic analysis was utilized according to the above table 1 standards. Mobile phase applicable Acetonitrile – buffer solution and pH=2.92 maintain and injection volume should be 5 µl. The speed of the mobile phase is adjusting 0.75 ml/min. The column Eclipse XDB – C18. 5.0 microns, 4.6x250mm. Diode matrix detector used with wavelength 254.

2.3 Determination of minerals

A sample of crushed peel, weighing 0.5 g, was placed in a flask. 5 ml of chemically pure nitric acid was poured into each flask. The flasks were sealed with a tight lid that featured a hole guarded by a plastic gasket to allow surplus brine to escape the flask. At the next stage, the amount of elements Mg, K, Ca, and Zn was studied using atomic adsorption spectrometry using a Nov AA 350 device (Germany, according to GOST 32343-2013) [15-16].

The assembled samples under study were placed in a microwave system. In this case, a sensor for measuring temperature and pressure was placed in housing with a zero flask. The first heating was carried out to a temperature of 140 °C and held for 2 minutes; the second was carried out to a temperature of 1800C and held for 15 minutes. The third to a temperature of 190 °C and held for 15 minutes.

The permissible pressure is 45 atmospheres; if it is exceeded during the operation of the microwave system, the system will automatically turn off. Once the sample is burned, the system cools down [15-16].

The concentrations of the obtained samples were corrected. To determine Mg, we diluted 1 ml of a liquid with 70 ml of distilled water; to determine calcium Ca, we diluted 1 ml of a liquid with 10 ml of distilled water; to determine Zn, we diluted 1 ml of a liquid with 2 ml of distilled water; and to determine potassium K, we diluted 1 ml of a liquid with 140 ml of distilled water. The contents of the glasses were stirred with a glass rod until a homogeneous mass was obtained [17-18].

2.4 Determination of the amino acid composition

To determine the amino acid composition, protein was obtained from pomegranate peel using the Kjeldahl method. The total amount of protein in the raw material is 3.0%. 100 g of dry raw material is extracted in a medium of 0.2 N. NaOH in a ratio of 1:15 with stirring with a magnetic stirrer for 1 hour at room temperature. Then 80% of the amino acids were precipitated in a solution of dry ammonium sulfate [$(NH_4)_2SO4$] with continuous stirring with a magnetic stirrer. The solution was left to stand for one night in the refrigerator to form a precipitate. The product was centrifuged at 6000 min-1 for 30 min [19].

The resulting precipitate was dialyzed against running water for 24 hours and then against running water overnight in the refrigerator. After dialysis, the protein solution was dried in a freeze-drying unit at a temperature of – 350 C and high vacuum. Then the yield of total protein was determined by the Kjeldahl method [20].

Next, the amino acid composition was determined. An exact sample (50 mg) was hydrolyzed in 5 and 7 N media. hydrochloric acid (in a 200-fold ratio with protein) for 24 hours at 1100 C in a vacuum. The hydrolyzate was evaporated on a rotary evaporator and transferred to a T-339 amino acid analyzer to determine the amino acid composition. It is important to remove hydrochloric acid quickly enough. With its slow removal, partial destruction of amino acids is possible.

2.5 Determination of carbohydrate composition

To determine the carbohydrate composition of pomegranate peel, the peel was crushed and sifted through a sieve with a diameter of 0.5 mm. After this, the raw materials are processed with 80% ethyl alcohol to remove alcohol-soluble sugars, low molecular weight compounds and other related impurities. Polysaccharides were separated from one sample of raw materials in the usual sequence. Water-soluble neutral polysaccharides (WSNPs) were extracted at room temperature with water, and the yield was 2.0% [11-13].

2.6 Determination of tannin content of pomegranate peel

To obtain tannin, gravimetric methods or quantitative precipitation of tannin with gelatin are used. To do this, 2.5±0.01 g of gelatin is dissolved in 100 ml of distilled water and left for 30 minutes in a dark place, and 500 ml of a 10% NaCl solution is added. After 30 minutes, the gelatin solution is removed, excess water is drained and filled with 10% NaCl solution [21,22]. A solution of gelatin and salt in a 1:1 ratio is added to a crushed sieve with a diameter of 0.05 mm (in the LZM-1 device) pomegranate seed extract, resulting in the formation of a white cloudy precipitate. The deposited sediment is a precipitate of tannin and gelatin. The precipitate was isolated in the filtrate and dried in a water bath at 40-450C. 5.35 g of polyphenolic compound (flavor) was extracted from 450 ml of extract.

3 Results and Discussion

Experiments were carried out to determine the quantitative composition of microelements. Mg along the resonance line 285.2 nm, photometric range 0.4 abs, Ca 422.6 nm, photometric range 0.6 abs, Zn 213.8 nm photometric range 0.2 abs and K 766.5 nm photometric range 0.5 abs. If the photometric range is from 0.2 to 0.8 abs, then using Beer-Lambert law. Let's determine the quantity. From the results, it is clear that the photometric range of the determined metals is in the range of 0.2-0.8 abs.

Table 2. The mineral content of pomegranate peel.

Mineral	**Ingredient content (mg/kg)**
	***Variety* Red pomegranate (Uzbekistan)**
Mg (magnesium)	50
K (potassium)	200
Ca (calcium)	370
Zn (zinc)	4

The table shows that pomegranate peel contains more potassium and calcium than other elements.

The table presents the results of studies on the amino acid composition of nitrogenous substances in pomegranate peel. The data is shown in Table 3.

Table 3. Amino acid composition of pomegranate peel proteins.

Amino acid name	Content, %	Amino acid name	Content, %
Asparagine	0.87	Valin	0.59
Threonine	0.47	Methionine	0.24
Serin	0.54	Isoleucine	0.34
Glutamine	1.96	Leucine	0.98
Proline	0.57	Tyrosine	0.37
Glycine	0.62	Phenylalanine	0.51
Alanine	0.67	Histidine	0.29
Cysteine	0.54	Lysine	0.44
Arginine	0.76		
Total: 10.76			

It was revealed that nitrogenous substances contain all essential amino acids - threonine, valine, methionine, isoleucine, arginine, lysine, phenylalanine, and histidine. This speaks volumes about the nutritional value of the pomegranate waste protein. The protein is well balanced in non-essential amino acids.

3.1 Carbohydrates content in pomegranate peel

The resulting polysaccharide is an amorphous powder of light cream colour, soluble in water. The relative viscosity of a 1% solution is μrel – 1.5; a qualitative reaction to the detection of starch gives a negative reaction.

Pectin substances (PS) are extracted from the remainder of the raw material after removing water-soluble polysaccharides; their yield was 4.0%. Pectin substances are an amorphous powder of a dark cream colour, sour taste, and slimy to the touch, which does not dissolve in organic solvents, but dissolves well in water (pH 4.8), and has a relative viscosity of a 1% solution of μrel-3.2. The molecular weight of pectin, according to the viscometric method, is 3000.

To determine the monosaccharide composition, the BPPS and PV samples were subjected to complete acid hydrolysis. Monosaccharides were found in the hydrolysis products of VPPS: arabinose, xylose, galactose, and traces of rhamnose, and galacturonic acid, arabinose, galactose, traces of glucose and rhamnose were found in pectin substances.

Determination of polysaccharides in pomegranate peel showed that it contains many water-soluble polysaccharides and pectin substances. Diet is very important for the physical and mental fitness and it will be having advantages for the stress [23]. Also, the proper fruit selection, types of cultivars and the method of irrigation for the plant will have impact on the final outcome of the fruits [24].

3.2 The tannin content of pomegranate peel

Determination of purity and amount of tannin by spectrophotometry and high-performance liquid chromatography. Polyphenolic tannin compounds are determined by spectrometry. The method absorbance at a wavelength of 278±2 nm was compared as a standard. The tannin yield in pomegranate peel is about 5.5%. These results are shown in Figure 1

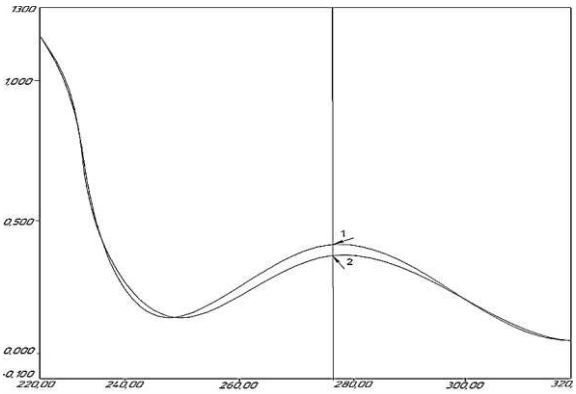

Fig. 1. Tannin absorption from pomegranate peel. (1-tannin standard; 2- 2-tannin from pomegranate peel).

The spectrophotometric method for determining tannins in pomegranate peel is the most accurate compared to other methods and has been successfully used to determine tannins in other herbal preparations. Determination of tannin purity using high-performance liquid chromatography. Data reduction to Figure 2 and 3.

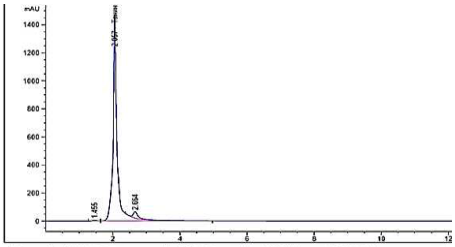

Fig 2. Standard tannin.

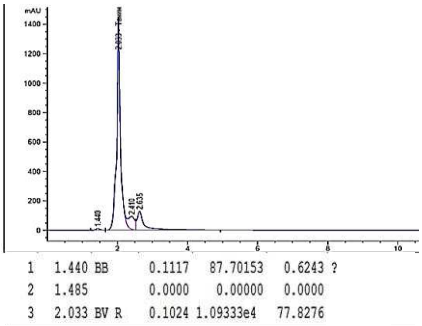

Fig 3. Tannin sample.

The organoleptic characteristics of the resulting substance were compared with standard tannin. Tannins have antibacterial and antioxidant activity. Due to these properties, they are widely used in medicine and food production. Application in the veterinary and feed industries today has great potential. Many properties of tannins have high biological value for the human body and animals [25].

4 Conclusion

Thus, the tannin from the waste pomegranate peel is about 5.5% and the main elements are potassium and calcium. The water-dissolved polysaccharide of pomegranate peel contains monosaccharides: arabinose, xylose, galactose, and traces of rhamnose, and galacturonic acid, arabinose, galactose, traces of glucose and rhamnose are found in pectin substances. Nitrogenous substances contain all the essential amino acids - threonine, valine, methionine, isoleucine, arginine, lysine, phenylalanine, and histidine. This speaks volumes about the nutritional value of the pomegranate waste protein.

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