Industrially applied methods for the production of pomegranate polyphenols

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Abstract. Utilization of by-products of the production of pomegranate juice is an urgent task. However, there are no ready-made solutions for complex disposal of solid parts of the grenade in the markets of technological equipment. The time has come to analyze the accumulated experience of some companies that are closer to the production of dietary supplements in the processing of small batches of this raw material and to highlight from it the main thing that can be guided by the organization of a larger-scale processing of peels and seeds, which are formed in juice shops whole mountains and they are simply taken to the landfill. This review so far concerns only their experience, given that the analysis of all innovative ideas would take up a lot of space and would not fit into the framework of a single scientific article.

Key words: Pomegranate peel, processing, industrially applied technologies.

1. Introduction

The pomegranate (Punica granatum L.) belongs to the family Punicaceae and has been grown since ancient times to produce delicious fruits and as an ornamental garden plant. The global acreage allocated for this plant is about 300 thousand hectares. The world production of pomegranate fruits reached 3.0 million tons per year.

Pomegranate is a typical succulent plant.

The skin (a.k.a. rind, husk, or pericarp) of the pomegranate fruit is tough and leathery. Membranous walls, known as carpels, compartmentalize the interior of the fruit, along with white spongy pith. The resulting compartments (a.k.a. locules) contain 600–800 sacs called arils. One seed and juicy pulp is contained in each aril. In total, the arils consist of approximately 80% juice pulp and 20% seeds [1].

The ratio between the individual parts of the pomegranate fruit may vary depending on the variety of pomegranate, as well as the place and year of its cultivation, but in general they can be attributed to fruits with a relatively low juice content of 50 % or less.

Large piles of peels and seeds on the territory of large canning shops during the pomegranate processing season suggest that you will again have to take them to the landfill with additional labor and equipment, whereas processing them directly on the spot would help improve economic performance and production cleanliness.

These by-products of the production of pomegranate juice are perishable products, which in their raw form consist of 70 % (peel) and 55 % (pressed seeds) of water. The peel at a temperature of 0° C can maintain its quality for 21 days, at 2° C-up to 18 days, at 5° C-up to 14 days [2]. Drying allows you to convert the peel and seeds into a concentrated secondary raw material that is stored for a long time and is a more convenient object for further transportation and processing in any place and at any appropriate time.

While the peel is an extremely rich source of polyphenols, monosaccharides, organic acids, and minerals, the seeds are exceptionally rich in protein, starch, and storage lipids.

Comprehensive utilization of by-products of pomegranate juice can generate the development of this well-established production through diversification, which involves the production of products with added value, the cost of which does not include the cost of raw materials and development of new sales markets.

It remains only to choose a technology suitable for processing solid residues, which is not as easy as it may seem at first glance.

And the point here is not in the absence of bright ideas, but, strange as it may seem, in their abundance and diversity [3-5].

True, these ideas can be grouped based on their technological component and the functionality of the future technology, but even so, the number of such groups will be large and will not be limited to two or three groups.

Some of them are based on a simple grinding and drying of pomegranates with or without their peel in two stages (first-to a residual humidity of 30-35 % at a temperature of up to 90° C; then - to a residual humidity of 6-8 % at a temperature not exceeding 40° C), with simultaneous disintegration of the processed materials [6-7]. The developers of most other ideas consider such a simplified approach impractical, given the diverse nature of the biological action of the components of the pomegranate fruit. They follow an approach based on the separation of the raw material into separate parts and the selective extraction of related components of the raw material. At the same time, each researcher sees the extraction process itself and the further processing of the separated extracts in his own way [8-11].

Some companies that are closer to the production of dietary supplements, cosmetic and pharmacological ingredients have already mastered and continue to master modern extraction technologies of two types, some of which are designed to grind the solid parts remaining after

the juice is obtained, and others provide for the extraction of fruits directly without first dividing them into separate parts.

The time has come to analyze the accumulated experience of these firms in the processing of small batches of this raw material and to highlight from it the main thing that can guide the organization of a larger-scale processing of peels and seeds, which are formed in the juice shops of the whole mountains and they are simply taken to the landfill.

This review concerns only scientific and practical experience in the field of extraction of polyphenols of individual pomegranate peel. It does not address such issues as the extraction of oil from individual pomegranate seeds, the extraction of a mixture of peel and seeds (pulp), since the information in these areas is very extensive and requires separate analysis and discussion.

2. Industrially applied patents and solutions

Recently, pomegranate polyphenols have been widely recognized by scientists, companies, and public figures because of their unique pharmaceutical and conservative properties in the physiological system. In this regard, the number of studies concerning the health benefits of pomegranate polyphenols is also growing, and most of them are conducted on products of POM Wonderful (USA), which enter the market with ingredients with 100% pomegranate juice concentrate and a polyphenol extract with the brand name POMx.

POM x extract is produced according to a patented technology [12], which includes:

- creating a mixture containing the solid parts of the pomegranate (this is the pericarp, internal partitions and seeds, which are obtained and collected after the primary juice is removed from the pomegranate fruit by pressing, grinding or other methods used to extract the pomegranate juice) in an aqueous solution, with water accounting for 20-50 or 80 % of the total mass of the solid parts of the pomegranate;

- adding to the mixture a decomposing agent designed to help the phytochemicals to be released from the tissues and / or cells of the solid parts of the mixture, and this decomposing agent is one or more enzymes from the list consisting of cellulase, hemicellulase and pectinase;

- heating the mixture to temperatures of 60, 85, 120, 160, 185 or 210^{o} F (preferably to 110 - 160^{o} F), at which the released phytochemicals begin to enter into chemical reactions and / or polymerization reactions and, thereby, new phytochemical compounds or reaction products are converted, and holding at the specified temperature for 195-45 minutes;

- removal of insoluble solid residues from the mixture to obtain a clarified liquid part of the mixture, which contains much higher molecular weight polyphenols, including punicalagins, than in pomegranate juice.

The last stage can be carried out with the use of a clarifying agent such as bentonite, or it is performed by microfiltration with a cut - off by molecular weight of 1000-5500 Da.

Further processing of the clarified extract is also possible: passing it through an adsorption resin that can bind to at least one polyphenol; using a solvent to elute the specified polyphenol from this resin; partial evaporation of the used solvent and concentration of the mixture to 50-90 °Brix.

One of the options for implementing this method is as follows.

The solid parts of the garnet are transferred to the Reitz mills with sieves in inches. The material is ground to a fine puree, 50% pure water is added to it and heated to approximately 125 ° F [(125° F − 32) × 5/9 = 51.67° C)] with the addition of the enzymes pectinase (Rohapect ® DA6L), cellulase / pectinase (Rohapect ® CL) and hemicellulase / pectinase (Rohapect ® BIL), which contribute to the destruction of the colloidal structure of the tissue and, as a result, the release and transition to the resulting extract of sugars, minerals, anthocyanins and other polyphenols, and is kept for 2 hours at this temperature. The mixture is then pumped from the extraction plant to the primary processing plant, where it is kept in tanks for 1 hour, after which 50-100 pounds of bentonite is added to it as part of its 125-gallon water suspension (about 0.47 m^3) per 8000 gallons of the mixture (about 30.28 m^3). The treated mixture is then passed through a Westphalia 755 decanter to remove the resulting sediment, and the remaining liquid portion is treated in a Schmidt evaporator, rectified and pasteurized at 205° F for 45 seconds, after which it is discharged from the evaporator and filtered through Koch Micro-Filtration membranes at a threshold molecular weight of soluble solids of the liquid extract of 4500 Da. The filtered liquid extract is fed back to the evaporator for final concentration (starting its treatment from a temperature of approximately 185-195° F) to a soluble dry matter content of 70 °Brix. The resulting concentrate is cooled to a temperature below 45° F and pumped into a dosing chamber, where it is mixed with other batches of the extract and standardized.

Comparison of this extract with natural pomegranate juice showed that the concentration of punicalagin α and β isomers in the extract of solid parts of pomegranate is 26 times higher (21.80 and 4.79 mg / ml) than in pomegranate juice (0.15 and 0.02 mg/ml). This also applies to ellagic acid glycosides (19.65 mg/ml in the extract versus 0.33 mg/ml in the juice), punicalin (with an extract content of 3.62 mg/ml), and total polyphenols.

POM Wonderful's POMx Polyphenol Extract is available in capsules: 1 capsule (1000 mg) contains 753 mg of polyphenols.

This company also offers various compositions containing POM x extract, which can be health food products, beverages, medicinal products, dietary supplements, vitamin supplements, botanical extracts.

In its ads, the company claims that its products have "great health-enhancing abilities" provide "heart-healthy benefits, "and are"40 percent more effective than Viagra" in this regard.

The company believes that pomegranate polyphenols are intended to support prostate health and reduce the risk of cardiovascular disease, and to fight prostate cancer and erectile dysfunction.

The US Federal Trade Commission (FTC) does not agree with such "bold statements" of this company [The source online, 1].

However, the company believes that these claims are supported by medical research, for which the aforementioned company has allocated 25 million $ US.

These claims are also supported by independent sources.

When testing the effect of POM x extract on serum and atherogenicity coefficient in patients with hypocholesterolemia, in whose life there were factors that increase the risk of developing cardiovascular diseases and who received the hypolipidemic drug simvastatin for this reason, it was found that this extract causes anti - atherogenic effects that can reduce the risk of developing atherosclerosis. The addition of POM x in simvastatin therapy improved oxidative stress in patients with hypercholesterolemia and promoted the transition of serum lipids to a state more consistent with their original natural status [13].

Critics of this technology rightly believe that its main drawback is several thermal treatments, which can result in the degradation of some punicalagins, reducing the health benefits of pomegranate extract.

The method is also attributed to such a disadvantage as obtaining a target product that does not contain some phytochemicals (smaller ellagitanins, anthocyanins, etc.), characteristic only of the juice fraction, and generates not only polyphenols, but also several other substances, in particular sugars, organic acids, the presence of which during treatment with this extract can cause side effects, and, therefore, is undesirable.

It is claimed that the first in vivo study on the benefits of pomegranate for heart health was conducted in 2013 by the Catalan Institute of Cardiovascular Sciences (ICCC) using an extract with the brand name Pomanox. This study showed that diets enriched with pomegranate polyphenols can prevent or slow down endothelial dysfunction – the first signs of atherosclerosis and cardiovascular disease. The significant health benefits of this extract are also indicated by recent studies up to 2018 [Source online, 2].

Pomanox polyphenols are obtained using a technology patented by the Spanish company Probeltebio [14], which differs from its analogues mainly in that it is designed to process not the peel, but the whole fruit of the pomegranate.

The advantages of this technology can be attributed to the fact that it is based on a pure water process and the target product here is not a mixture of polyphenols, but punicalagins - the key antioxidant compounds of pomegranate.

The preferred version of this invention provides for heat treatment of the primary extract (before or after clarification, but in any case-before passing it through the resin columns) for 5-30 seconds at a temperature of 80-90° C. In this case, the pH of the extract, which is subjected to heat treatment, can vary in the range from 1.0 to 5.0 (its optimal value is 3.5 ...5.0).

The prepared extract is passed through columns with a sorbent designed to delay polyphenols. Next, the polyphenols are extracted from the sorbent, for which a weak solution of alkali is passed through the sorbent. The resulting eluate is concentrated by nanofiltration and / or reverse osmosis to remove water, salts, and other impurities as completely as possible, and is converted to a solid form by freeze-drying, rotary vacuum evaporation, or spray drying with or without carriers such as maltodextrins or soluble fibers.

The extract is prepared at room temperature in order to make it difficult to transfer products such as free ellagic acid into it, and to obtain a completely water-soluble pomegranate extract. At the very beginning of the process, the enzyme tannase is inactivated, which means that the hydrolysis of ellagitannins to ellagic acid is prevented due to the fact that acid is added to the water used as a solvent, bringing the pH of the solvent used to 0.5 -1.5. Tannase is produced by filamentous fungi that can infect pomegranate fruits during the growing season, during harvest, during processing, storage, transportation, and marketing.

In addition, after separation, the extract is subjected to heat treatment, which also contributes to the inactivation of enzymes and / or their spores that can form and secrete tannase and other undesirable substances. The final pomegranate extract is completely devoid of tannase-forming microorganisms and / or an enzyme that can hydrolyze punicalagins at the time when the extract is introduced into a food or drink.

To extract the polyphenols adsorbed on the resin, a slightly alkaline aqueous solution containing no organic solvents and demineralized water is used. This means that no organic solvents come into contact with the aqueous extract, which contributes to the production of the target product, which is particularly suitable for use in the food, cosmetics and pharmaceutical industries.

The stages of obtaining the adsorption and elution of polyphenols from the sorbent, as well as the subsequent stage of concentrating the eluate by membrane methods, allow separating the sugar fraction from the fraction of ellagitannins and polyphenols and obtaining a solid final product without mixing the concentrate with maltodextrins and other additives.

The method is carried out as follows.

The raw material is washed and fed into a blender-mixer, in which the pomegranate fruits are crushed and mixed with demineralized acidified water and enzymes for 15-150 minutes in the temperature range from 4 to 30° C (pH from 3.5 to 5.0). After this, the resulting extract is

separated, cleaned from large suspensions using a centrifuge and heated for 5-30 seconds at a temperature of 80-90° C.

In this invention, polymer resins made of styrene and divinylbenzene are used as adsorbing resins, such as Amberlite series resins, for example, Amberlite XAD-16 (manufactured by Rohm&Haas, USA). Other suitable resins are commercially available from The Dow Chemical Company (USA) XFS-4257, XFS-4022, XUS-40323 and XUS-40322 series resins.

Styrene-divinylbenzene (SDVB) resin Amberlite XAD-16 consists of particles ranging in size from 100 to 200 microns and has a macroreticular structure.

The prepared extract is injected into a TFE vacuum aspiration column, where it passes through the resin. The purpose of loading the extract into the column is to quantitatively retain the polyphenols in the TFE cartridge while the matrix impurities are removed.

After the target substance is retained by the sorbent (as judged by the color of the extract: at first it is dark brown, after the retention of polyphenols by the resin becomes pale yellow), the sorbent is washed from foreign impurities. For this purpose, distilled water is passed through it until the sweet pale yellow eluate becomes transparent. The remaining water is removed from the resin by vacuum aspiration.

At the final stage, a quantitative elution of the target component occurs, and the choice of one or another adsorption resin for this purpose may be associated with the difference in the size of certain groups of polyphenols.

In this method, Amberlite XAD-16 is used as a polyphenol sorbent with the following sorption characteristics: ~800 m^2/g; average pore diameter ~150 Å (~15 nm; ~0.015 microns). To extract the polyphenols bound to the resin, 50 M of sodium bicarbonate at room temperature is used.

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The resulting eluate has a purity of 55-70 % in punicalagins. The degree of reduction of punicalagins from resin is 85-90 %.

The eluate is concentrated by evaporation or ultrafiltration in a tangential flow (TFF), but preferably by nanofiltration or reverse osmosis, until the content of punicalagins in it reaches 10 %, after which it is dried in a lyophilic drying unit, a vacuum rotary evaporator or a spray dryer (which is preferable) to obtain a solid final product.

Let's look at this in a concrete example.

1000 g of pomegranate fruit is ground into a puree and mixed with 1000 ml of acidified demineralized water. They are kept for several minutes with the agitator turned on, after which the water phase is separated from the solid residue on the filter. The solid residue is washed with 100 ml of demineralized water, and water, washing water is added to the previously isolated water phase. The water phase with a volume of approximately 1700 ml is then purified in a centrifuge to remove the solid particles that have passed through the filter. After removing the solid, 1495 ml of the crude water extract remains.

A sample of crude aqueous extract (1495 ml) containing 9.62 g of punicalagin α and β is loaded into a column containing XAD 1180 series adsorption resin. The liquid phase extracted at the end of the column no longer contains punicalagins.

The resin is washed with the same volume of demineralized water to remove the remaining ballast substances on its surface.

After that, the punicalagins are eluted from the resin using a constant pH shift strategy, which is facilitated by the passage of 50 M of sodium bicarbonate through the resin in an amount that is 2 times the amount of resin. Then the elution is completed by passing demineralized water through the resin until at least 90% of the punicalagins from the amount originally bound to the resin are extracted. The eluate comes out with a content of approximately 9.42 g of punicalagins with a purity of approximately 73.4 %.

In accordance with this invention, extracts are obtained in solid form with a content of less than 5 wt. % ellagic acid and not less than 30 wt. % punicalagins, 0.1 wt. % of anthocyanins (delphinidin 3,5-diglucoside, cyanidin 3,5-diglucoside, delphinidin 3-glucoside, cyanidin 3-glucoside, and pelargonidin 3-glucoside).

Pomanox is placed in capsules covered with an intestinal-soluble soft shell; one capsule of Pomanox (200 mg) contains 64-100 mg of polyphenols.

Table 1. Pharmaceutical form and composition of Pomanox powder pomegranate extract. (Source online, 3).

Components	Pomanox formulation		
	P15	P20	P30
Punicalagins	> 15 %	> 20 %	> 30 %
The amount of polyphenols	> 32 %	> 37 %	> 50 %
Elagic acid	< 8 %	< 8 %	< 8 %

The solubility of Pomanox in water is up to 100 g/l, which makes it ideal for use in a mixture with beverages, at the same time it has heat resistance and a neutral astringent taste, which can also be attributed to its advantages; antioxidant activity on the ORAC scale -5.700 micromol eq. Trolox/g or more; the optimal dose is from 265 to 750 mg / day, depending on the application.

As part of its ongoing commitment to offering the best pomegranate juices and ingredients, Stiebs (Madera, California), an American firm founded by George Stieb in 1963 as Stiebs Pomegranate Products, operates. These are pomegranate juice, juice concentrates, extracts, pomegranate seeds(arils), powders, mixtures, and formulations certified by the US Department of Agriculture as organic products. Pomegranate oil is produced here by drying the seeds and cold pressing them. It is recommended to use it in the preparation of certain products, such as chewing gums, cosmetic moisturizers and products to increase the elasticity of the skin of the face. It is particularly important as an ingredient for promoting the health of women in the pre-and post-menopausal periods. Punica granatum L. Peel Extract This company is a water-soluble antioxidant powder for use as an additive in cosmetic products, beverages, and other food products. The product has a beneficial effect on the heart, is standardized for 50 % content of total polyphenols, 5 % - punicalagins and 2 % - ellagic acid. The recommended oral dosage is 1000 mg per day [Source online, 4].

Conclusion

As can be seen, the technologies considered have significant differences depending on whether they are aimed at producing liquid concentrates containing not only polyphenols, but also other hydrophilic substances, or seek to isolate the polyphenols from the rest of the extract.

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