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Samir Hafizov and Gharib Hafizov *

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Article

Obtaining Clots of Various Polyphenols in A Co-Production with an Extract with the Texture of Dry Red Wine from the Peel of Pomegranate Fruits: From Abstract Ideas to Their Technical Embodiment

A Method for Obtaining Clots of Various Polyphenols and Dry Red Wine from Pomegranate Peel

Samir Hafizov and Gharib Hafizov *

Research Institute of Fruit and Tea Growing of the Ministry of Agriculture of the Republic of Azerbaijan

* Correspondence: hafizov-54 @mail.ru

THEORY/CALCULATIONS

In the process of understanding what should not be done and what will work, the basic idea of extracting polyphenols from the initial substrate during fermentation, triggered by the ethanol-forming yeast S. Cerevisiae, was put forward.

It was expected that this would be accompanied by many positive effects: Microorganisms would convert the substrate (monosaccharides released from the peel into solution along with polyphenols) into alcohol, which, together with polyphenols, would become an important component of the primary extract. After purifying the extract from polyphenols in a clean way, the extract will become more transparent and its taste will improve, which, with an abundance of coloring substances and a certain percentage of alcohol, will make it look like dry wine.

Another idea, directly related to the purification of the extract, was based on the following expectations: Due to the difference in polarity (solubility) and molecular weight, the ability of individual forms of polyphenols to remain in aqueous alcohol media for a long time will also be different. Some of them may precipitate first (ellagic acid), protein precipitators can be used to extract the second group (punicalagin, proanthocyanidins), and the precipitation of the third (a-glycosides - rutin, quercetin, and others) will have to wait until complete clarification occurs.

Such a clean and low-cost cleaning would be a real godsend.

Abstract

Since pomegranate peel polyphenols have several biological activities, their demand and competition for sales markets are growing. Therefore, manufacturers of polyphenols of this type should be able to force the buyer to choose their product rather than a competitor, and for this it is necessary to achieve cost reduction and offer them at a lower price. This study was conducted precisely with the aim of developing a new way to obtain them, commensurate with such difficult tasks as cost and cost reduction. It has been tested for this: 1- extraction of polyphenols from the raw coarse peel from manual or machine peeling of pomegranates into an extract as a result of its fermentation using ethanol-forming yeast Saccharomyces cerevisiae; 2- separation of the resulting extract as a result of natural association and provoked (using gelatin). The technical result is a new method for producing three clots of different polyphenols in co-production with red dry wine "Aztanna". Although in the proposed method, the degree of presence of target substances in these clots (39-54% of their dry matter) is lower than in some commercial polyphenolic preparations (40-90% of dry matter and higher), it does not require, like them, the use of drying, grinding, heating, stirring and adsorption chromatography systems. It is also important that the best known methods are patented and protected by law, while the proposed solution can be freely implemented in the existing workshop of any winery, and without investing additional funds for the purchase of new equipment.

Keywords: pomegranate peel; fermentation without air access; wine extract; self-association of polyphenols; ellagic acid; gelatin–tannate; a - glycosides

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1. Introduction

The global fruit production of the Punica granatum plant has exceeded 3 million tons, since the peel accounts for about 50-60% of the total weight of this specific fruit, and about 1.62 million tons of waste are generated [1].

Pomegranate peel has long attracted the attention of researchers as a valuable resource for the food industry and a source of polyphenols and tannins [2–7].

The difference between polyphenols and tannins is that polyphenols are a wide class of plant compounds (antioxidants, flavonoids, tannins), while tannins are a specific subgroup of polyphenols known for their ability to "tan" skin (bind proteins), giving it strength, but also having astringent properties and antioxidant activity. activity. All tannins are polyphenols, but not all polyphenols are tannins.

There are 4 groups of tannins found in plants: complex tannins, condensed tannins, gallotanins and ellagitannins.

Gallotanins and ellagitannins are hydrolyzable tannins. The most notable group among pomegranate tannins are the ellagitannins, which are formed from gallotanins. In pomegranate, they are mainly found in the form of punicalagin (isomers of punicalagin α and β), which accounts for 65.75% to 85% of the total amount of tannins. Punicalin can be a derivative of punicalagin, both punicalagin and punicalin are converted to ellagic acid during hydrolysis. [8,9]. The concentration of punicalagin ranged from 181 to 255 mg/g in the peel of six Spanish pomegranate varieties [10]. Other tannins found in the skin of pomegranate fruits include punicalin, pedunculagin, granatin A, granatin B, corylagin, tellimagrandin, gallagylhexoside, and others [11].

In addition to tannins, pomegranate peel is rich in other polyphenols, including phenolic acids (gallic, ellagic, vanillic, caffeic, ferulic, cinnamic, chlorogenic, p-coumaric), flavonoids (anthocyanins, kaempferol, luteolin, rutin, catechins, quercetin, rutin, hesperidin, procyanidins), triterpenes (oleanic acid and ursolic acid), derivatives of phenylethyl alcohol (hydroxytyrosol). [12].

In the skins of six Chinese pomegranate varieties, the average content of gallic and ellagic acids was 0.57 mg/g and 1.34 mg/g, respectively [13]. According to other authors [11], 100 g of pomegranate peel contains 123.79, 35.89, 20.56 and 4.48 mg of gallic, ellagic, caffeic and p-coumaric acids. The average rutin content in the peel of six Italian pomegranate varieties was 4.5 mg/g [14]. About 30% of the total amount of anthocyanins in pomegranate fruits was accounted for by their peel [15]. Eight different anthocyanins were identified in the peel of the Nana variety from Tunisia: pelargonidin-3-glycoside, pelargonidin-3,5-diglycoside, delphinidin-3-glycoside, delphinidin-3,5-diglycoside, cyanidin-3-diglycoside, cyanidin-3,5-diglycoside, cyanidin-3-pentoside and cyanidin-3-rutinoside [16].

Pomegranate peel polyphenols are strong antioxidants, fight inflammation, help lower cholesterol, control blood sugar levels, have antibacterial and anthelmintic effects, promote wound healing and improve digestion [17–20]. They showed good absorption by the body [21,22]. Pomegranate extract significantly reduced the formation of circulating trimethylamine N-oxide from choline and L-carnitine in vitro, high levels of which are associated with metabolic diseases, adverse outcomes of heart failure and atherogenic effects in animals and humans [23], and also showed the ability to stimulate cell regeneration [24].

Industrially applied extraction technologies of commercial pomegranate polyphenols are the prerogative of several patent-holding companies specializing in liquid and dry extracts for the food industry and biomedicine. First, the peel is dried and crushed, then a multicomponent aqueous or water-alcohol peel extract is obtained from it using thermal maceration, which is then concentrated, and finally, the resulting solutions are separated using column chromatography. At the same time, the efficiency of the peel and the yield of the target substances are very low, and some of the procedures included in this scheme are technologically vulnerable and expensive.

The purpose of this study was to simplify the production of polyphenols, as well as to make more complete use of the peel and increase the economic justification of the technology.

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The relevance of the work is due to the need to increase the production of biologically active substances and compounds that increase the body's resistance to the effects of adverse environmental factors that support and correct health. The need for research is justified by the important role of antioxidants in human life. The basis of the work is the high demand for dry and liquid pomegranate extracts.

2. Materials and Methods

2.1. The Transformation Object

The peel from the manual cleaning of three samples of intensely colored pomegranates, which we purchased at the end of October at the central market in Guba (Azerbaijan), was used as the initial objects of technological processing.

2.2. Auxiliary Substances:

- Granulated sugar according to gost (State standard OF Russia) 33222-2015;
- Gevrin GV1 dry wine yeast (England) for white, pink and red wines with an alcohol content of up to 18% (known in other countries under the Fermivin brand). This is a French strain of wine yeast S. cerevisia Bordeaux strain 7013 h, native to the Bordeaux region (France);
- Food grade gelatin conforming to GOST 11293-2017 standard. This standard specifies requirements for the quality and properties of gelatin intended for the food industry, including its safety and suitability for various applications such as beverage clarification.
- Clean drinking water passed through a Thomas Compact filter.

2.3. Extraction

The intensification of the extraction of target substances from the substrate into the solution requires grinding, heating and mixing - the finer the grinding of the starting material and the higher the process temperature, the faster the extraction of target components into the solution, and mixing only enhances this effect.

However, when extracting an object with a low cellulose content and a high content of soluble fibers and simple sugars, such as pomegranate peel, this can also lead to undesirable consequences.: the pieces of the peel will boil, and the extraction mixture will turn into a sticky suspension, which will greatly complicate the further separation and separation of the extract [25,26]. In addition, drying the peel, grinding it and thermal maceration with constant stirring can lead to degradation of some of its biologically active substances and require a lot of energy, which makes the use of such extraction beyond the experimental and laboratory scale problematic.

Therefore, three main tasks had to be solved in the extraction stage: 1- reducing the cost of the stage (cost reduction) due to its simplification and reduction of energy consumption; 2- maximum preservation of the original independence (texture) peel, facilitating the separation of the liquid phase from the solid and their further separate processing; 3- maximum preservation of coloring and other substances of the natural composition of the peel.

In the course of thinking about what you can not do and what will really work, the idea came up of using the conversion of fresh, unpolished coarse peel as a starting substrate and initiating the conversion of sugar, which goes into solution along with polyphenols, into alcohol using ethanol-forming yeast, which is able to do this at room temperature.

So sugar coming out of the peel into the solution will always be consumed, and alcohol will appear instead, and all this will happen at a lower temperature, this should contribute not only to an increase in the degree of extraction of polyphenols, but also to a significant decrease in the viscosity of the extraction mixture and the preservation of active target substances.

Fermentation of raw coarse-grained peel using S. Cerevisiae (stem Gevrin GV1) under anaerobic conditions (in a glass vessel with a water seal) was tested. The fermentation of the peel was carried

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out at room temperature. A different ratio of peel and solvent was used for all experiments from 1:1 to 1:10 (g/ml) with incubation periods of one and two weeks. Six primary extract samples were obtained for each peel sample. The fermentation process was visually monitored by the intensity of foaming and gas formation.

2.4. Separation of the Extract

In this study, the precipitation and fractionation of polyphenols from the obtained extracts was tested based on their polarity (solubility).

The primary extracts were aged for three months, during which time three precipitates with different polyphenols were isolated on the filter.

2.5. The Nomenclature of the Studied Chemical Parameters:

- Dry substances. It is determined by dehydrating a sample of plant material to a constant mass in a drying cabinet according to [GOST (State Standard of Russia) 33977-2016];
- Soluble dry substances. Their determination is based on the refractometric method (GOST 28562-90);
- Simple sugars - according to the Bertrand method (GOST 8756.134-87), which is based on the ability of the aldehyde group of sugars to reduce CuO to Cu2O (precipitating red) when interacting with Fehling® reagent;
- Total acidity - titration with 0.1 N alkali solution in the presence of a color indicator, 0.0064 - conversion coefficient of 0.1 N NaOH solution to citric acid (Interstate standard ISO 750 – 2013);
- •Ascorbic acid - by the iodometric method (GOST 24556-89).
- Hydrogen index - by the potentiometric method in pH units (GOST 26188-2016).
- Total polyphenols - according to GOST 24027.2-80 by titration of the indigocarmine residue, not consumed for the oxidation of phenolic substances, 0.1 N solution of potassium permanganate.

Since other compounds are involved in the reaction with permanganate, first all substances oxidized by this reagent are titrated, and then that part of the extract that remains after treatment with activated carbon, capable of adsorbing only polyphenols.

The amount of phenols is determined by the difference between the amount of permanganate consumed for oxidation in the first and second cases, using a coefficient of 0.004157 to convert milliliters of 0.1 N potassium permanganate solution into grams of phenols.

This principle is the basis of Article 1.5.3.0008.18 of the Russian Pharmacopoeia (XIV edition) concerning the methodology for determining tannins in medicinal raw materials.

All chemicals used must be kept analytically clean.

- Crude protein - by the Keldal method (6.25 is the conversion factor used to calculate the protein content).
- Fibers are produced by the enzymatic gravimetric method according to GOST 34844-2002, this standard applies to special-purpose food products, biologically active and food additives.

2.6. Significance of Experimental Data

All experiments were conducted randomly to avoid a systematic error. Linear, quadratic, and interactive effects of independent variables on response variables were considered at a confidence level of 95% (Statgraphics Centurion XV, version 15.1.02, StatPoint, Inc., Warrenton, Virginia, USA).

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3. Results and Dıscussıon

3.1. Preparation of the Wine Peel Extract and İts Separation

3.1.1. Chemical Analysis of the İnitial Peel

The main components of full-fledged alcoholic fermentation are carbon sources (mainly simple sugars - glucose and fructose) and nitrogen, as well as minerals (magnesium, calcium, potassium, phosphates, zinc).

Table 1 shows the yield and component composition of the tested peel samples 1, 2, and 3 from manually peeled fruits of three local pomegranate varieties.

It shows that in the total weight of the fruit, depending on the variety, the peel was: 1- 32.00±0.64 %; 2-38.97± 0.80% and 3-29.70±0.40 %.

In peel samples 1, 2, and 3, the content of water, monosaccharides, sucrose, titrated acids, and amounts of polyphenols, ash, protein, and fat varied within the limits of 64.00±1.62-69.70±1.65; 10.08±0.08-20.89±0.10; 0.00-0.66±0.01; 2.68±0.03-5.69±0.04; 2.60±0.02-3.60±0.04; 0.50±0.01-0.75±0.01; 1.66±0.02-2.50±0.02 and 0.50±0.01-1.00±0.01 G/100 g of their crude weight, respectively. In samples of peel 1-3, the content of water, monosaccharides, sucrose, titrated acids, total polyphenols, ash, protein and fat averaged 65.90±.1.65; 14.25±0.12; 0.22±0.01; 4.25±0.03; 3.14±0.03; 0.61±0.01; 2.05±0.02 and 0.77±0.01 G/100 g of crude weight.

Table 1. Yield and component composition of the peel of three local intensively colored pomegranate varieties (G/100 g of raw weight).

Indicators	Peel samples:			
	1	2	3	1- 3
Peel yield (% of the total weight of the fruit)	32.00±0.64	38.97±0.80	29.70±0.40	33.56±0.80
Water	64.00±1.62	69.70±1.65	64.02±1.70	65.90±1.65
Total polyphenols Monosaccharides	2.60±0.02	3.22±0.03	3.60±0.04	3.14±0.03
Sucrose	20.89±0.10	11.79±0.09	10.08±0.08	14.25±0.12
Titrated acids (by citric acid)	0.00	0.00	0.66±0.01	0.22±0.01
Ash	5.69±0.04	2.68±0.03	4.39±0.04	4.25±0.03
Protein (N x 6.25)	0.50±0.01	0.57±0.01	0.75±0.01	0.61±0.01
Fat	2.00±0.02	1.66±0.02	2.50±0.02	2.05±0.02
	0.50±0.01	0.80±0.01	1.00±0.01	0.77±0.01
p-value	0.05	0.05	0.05	0.05

A simple recalculation showed that the potentially soluble part (monosaccharides, sucrose, titratable acids, polyphenols) in the dry matter of the tested peel samples is equal to: (1) ≈ 81.05 %; (2) ≈58.38 %; (3)≈52.03%; (1)-(3)≈64.10 %. In experiments and production practice, the yield of soluble solids from pomegranate peel is usually 5-10% lower due to incomplete extraction.

3.1.2. Directed Fermentation of Pomegranate Peel Without Air Access Using *S. Cerevisiae*

The most common biotechnological method used to transform a plant substrate is bioconversion (biotransformation), the transformation of organic compounds (biological raw materials) into other useful substances under the action of enzyme systems.

In this study, this problem was solved by alcoholic fermentation, a particular and very common type of fermentation (the process of splitting organic substances by microorganisms with the release of energy, for example, yeast), where sugars are converted into alcohol and carbon dioxide.

At the same time, our task was not to conduct a special study on optimizing the technological parameters of fermentation, it was decided to proceed from the well-known rules, given that using

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time-tested approaches is not "stomping on the spot", but a guarantee of stability and efficiency in the production of products.

From the manufacturer's description, we learned that the Gervin GV1 strain provides pure fermentation at temperatures from 15° C to 25° C, has an alcohol tolerance of up to 12-14% and they can simply be scattered over the surface of the prepared wort (they do not require mixing).

Winemakers also know that increasing the fermentation temperature to 26-33° C stimulates the rapid growth of yeast, but with specialized fermentation (wine/beer) it is unacceptable, as it can degrade the taste and aroma. Therefore, when making wine, fermentation is usually carried out at a temperature of 18-22° C.

Earlier, we came to the conclusion that when the peel is crushed and its aqueous extraction is heated, it becomes difficult to separate the liquid phase in the presence of boiled tiny pieces of peel and colloidal turbidity and a significant increase in its viscosity [27]. In addition, it enhances the co-extraction of sugars and other related substances, and the content of targeted antioxidants in the liquid phase, on the contrary, decreases [28].

Therefore, in this study, we used coarse-grained peel from manually cleaned pomegranates, and the extraction of target substances from it into solution was carried out at a temperature of 18-22 ° C, that is, we replaced thermal maceration with “cold”.

Visual observation of the turbidity of the extract and the formation of gas and foam showed that with such a large size of the peel pieces and such a relatively low temperature, viscous biocoloids remained in the peel, and the period of maximum active conversion of sugar into ethanol in the medium occurred on the last days of the first week, the first days of the second. By the end of the second week of fermentation, turbidity appeared at the bottom of the glass vessel with a water seal, where this process took place, since during this time ellagic acid had already begun to precipitate. But in general, the condition of the extract and the remainder of the peel made it easy to separate the main part of the resulting extract by gravity. The pieces of the peel remained independent, so it was easy to continue working with the extract, and the solid residue was easily pressed out when the part of the extract absorbed into it was extracted.

The reasons for such positive effects:

- the process was carried out at room temperature, and not when heated, as in known methods, because the peel did not boil and remained in its original form.;
- with such a low extraction temperature and the use of non-ground material, the transition to a solution of sticky substances, such as soluble pectin, slows down.;
- during alcoholic fermentation, there was no oversaturation of the liquid phase with dry substances, since sugar was constantly converted to ethanol, and the volume of the solvent was constantly increasing due to the formed ethanol.

Putrefactive, acetic acid and other pathogenic bacteria in wine begin to actively develop when the pH level rises above 3.5-3.6. High pH (low acidity) It reduces biological stability, contributing to bacterial growth, turbidity and souring.

Therefore, one of the important conditions for successful fermentation is the pH balance.

However, since the content of soluble acids in the tested peel samples was initially high, fermentation mixtures with a fairly low pH of 2.8-3.2 were obtained, which consistently ensured their microbiological purity. S. cerevisiae prefers just such a slightly acidic environment.

If there is a lack of nutrients, especially nitrogen, fermentation can stop, and the yeast begins to produce hydrogen sulfide. Therefore, in such cases, special preparations and additives (stimulants, mineral salts) are used, thereby regulating the biotechnological functions of yeast and allowing them to increase their vitality, fermentation rate and improve the quality of the final products.

However, studying the effects of special drugs requires special studies that we have not planned.

Our experiments with the peel have shown that it contains enough nitrogen and ash elements for the growth and nutrition of S. Cerevisiae yeast, but not enough sugar to form an extract with the required ethanol content, as in dry wines.

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Theoretically, 1 kg of sugar (sucrose) yields 0.511 kg (or about 640 ml) of pure alcohol and the same amount of carbon dioxide, which in volume percentages gives about 0.6% alcohol per 1% fermented sugar.

Fermented sugar is carbohydrates (glucose, fructose, sucrose) processed by yeast during fermentation into alcohol, carbon dioxide, and heat. Simple sugars (monosaccharides) are easily absorbed by yeast, turning into a wine material until the sugar completely disappears or fermentation stops.

In winemaking and brewing, this process converts the sugars of the wort into alcohol, reducing the overall sweetness of the drink.

In the peel samples 1-3 used by us, the mass fraction of simple sugars (monosaccharides and sucrose) varied in the range of 10.74-20.89% (Table. 1), for each 400 g peel (exactly the amount included in the experiments), 42.96 g of natural monosaccharides accounted for at worst, and 83.56 g at best.

Since the average volume of the extract in our experiments was ≈ 675 ml/ 400 g of the average raw peel, and the theoretical yield of ethyl alcohol during glucose fermentation is 0.511 kg or about 0.647 liters of pure 100% alcohol/1 kg of glucose, the strength of the wine extract within the limits of our experiments, could not be higher than 4.12-8.01% vol., while in most European wines it is in the range of 9-14% vol.

Another fundamental factor of fermentation is also the ratio between the peel and the solvent (hydromodule).

To see how the change in the hydromodule affected the quantitative extraction of dry matter and the amount of polyphenols during two weeks of fermentation of peel samples (1) and (3) at 18-22° C with the addition of 0.13% S. Cerevisiae and 18% granulated sugar from the total mass of the fermentation mixture, it is necessary to refer to the data in Table 2.

Table 2. The effect of the hydromodule on the quantitative extraction of dry matter and the amount of polyphenols during two weeks of fermentation of the peel sample (1) using S. Cerevisiae.

The ratio between raw peel and water (G/ml)	**The degree of extraction of the dry substance, % of its total weight in the peel samples:**		**The degree of extraction of the sum of polyphenols, % of their total weight in the peel samples:**	
	(1)	(3)	(1)	(3)
1:1	63.16 ±1.23	42.50±0.80	63.29±0.59	69.00±0.65
1:1.5	67.22± 1.33	45.14±0.88	66.63±0.62	72.22±0.70
1:4	70.58±1.36	47.19± 0.91	67.97±0.63	73.10±0.71
1:8	72.60±1.40	48.95±0.94	70.63±0.66	74.00±0.72
p-value	0.05	0.05	0.05	0.05

Since the bulk of the dry matter of the peel is made up of easily soluble simple sugars (the proportion of sugars in the dry matter of the peel sample (1) is 58.03%, and in the dry matter of the peel sample (3) is 40.19%), the degree of extraction of dry matter from the peel sample (1) with any hydraulic module was higher than from peel sample (3).

With a 1:1 hydromodule, 63.16±1.23% of solids and 63.29 ±0.59% of polyphenols from their total weight in the peel sample (1) were extracted; with a 1:8 hydromodule, the yield of solids increased to 72.60 ±1.40%, and of polyphenols – 70.63±0.66%.

With the 1:1 hydromodule, 42.50±0.80% of solids and 69.00±0.65% of polyphenols were extracted from their total weight in the peel sample (3); with the 1:8 hydromodule, the yield of solids increased to 48.95±0.94%, polyphenols -74.00±0.72%.

As you can see, the larger the volume of water used, the easier it is to hydrate and dissolve dry substances and polyphenols.

Table 3 shows how, depending on the hydraulic module, the degree of use of total sugar (the sugar of the peel itself and added) and protein changed during two weeks of fermentation of the peel

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sample (1) at 18-22 ° C with the addition of 0.13% S. Cerevisiae and 18% granulated sugar from the total weight.

Table 3. Effect of the hydromodule on the degree of protein and sugar utilization during two weeks of fermentation of the peel sample (1) using S. Cerevisiae.

The ratio between raw peel and water (G/ml)	The degree of protein utilization, % of the total weight of protein in the sample peel (1)	The degree of sugar utilization, % of the total weight of sugar in the sample peel (1) and added
1:1	70.00±1.40	85.26±0.80
1:1.5	74.00±1.50	86.06±0.82
1:4	75.07±1.45	88.19±0.86
1:8	74.00±1.48	87.00±0.83
p-value	0.05	0.05

This table shows that with an increase in the volume of water used, the degree of S. Cerevisiae protein and sugar utilization increases, reaching a maximum with a 1:4 hydromodule (75.07±1.45% and 88.19±0.86%, respectively). But when there is too much water (as with hydro module 1:8), the concentration of sugars and protein falls below the optimal level, which slows down the growth of S. cerevisiae and slightly reduces the degree of protein and sugar utilization.

It follows that too much water can dilute the nutrients to a suboptimal level, affecting the efficiency of fermentation.

The potentially negative effect of excess water on yeast activity may also be associated with an increase in pH.

If the plant material contains little protein but a lot of soluble sugars, then yeast in a more aqueous environment will consume available amino acids more efficiently before sugars become a limiting factor, or vice versa.

Therefore, it is necessary to find a balance for maximum efficiency.

With hydromodule 1/1.5-1.6, 162.5-175 ml (on average 168.8 ml) of extract was obtained from 100 g of peel samples 1-3, and with hydromodule 1:4 - 412.5-425 ml (average 418.8 ml).

Considering that the water-alcohol extract in the framework of our idea should have the most intense color and strength, like dry wine, we chose the ratio between the samples of peel 1-3 and water 1:1.5-1.6 (G/ml). The 1:1.5-1.6 hydraulic module is a hydraulic module with a minimum volume of 600-640 ml of water, which was enough to cover the entire volume occupied by 400 g of raw coarse-grained peel in a flat-bottomed narrow-necked vessel with a total capacity of 1400 cm^3, which we used to conduct experiments on fermentation of the peel without air access.

Since there is not enough sugar in the peel to obtain a wine extract with the strength of European dry wines (9-14% vol.), we had to add granulated sugar at the rate of 72 g of granulated sugar / 400 g of each tested sample of peel 1-3.

Due to the natural sugar of the peel and added, it was possible to obtain wine extracts (due to their richness in polyphenols, they can also be called polyphenolic), with the same ethanol content as in commercial dry wines (9-14% vol.).

When using this amount of added sugar at one time, there was no high osmotic load or rapid accumulation of alcohol as a result of excess sugar.

3.1.3. Separation and Separation of the Liquid Fermentation Mixture

Aging is necessary for the maturation of any wine - clarification and acquisition of the best taste qualities.

Therefore, we could just wait until the wine extract was completely transparent, then filter the wine extract and discard the precipitate in it, as wine makers usually do.

But given the challenges of this study, this was unacceptable to us, and we needed to apply a different approach that considers polyphenols as the main target product. Therefore, we had to

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think about how to separate them from the extract, and without causing any damage to the naturalness of the extract itself.

Pomegranate peel is known to contain a rich complex of soluble and insoluble (fiber-related) polyphenols. Soluble polyphenols (of medium polarity and especially high) include flavonoids (anthocyanins, quercetin, rutin, etc. in the form of glycosides), oxycoric acids (chlorogenic, n-coumaric, etc.), punicalagin and ellagitanins, which have a high antioxidant capacity and are easily extracted by water, especially when infused. Insoluble polyphenols (low polar and nonpolar) are bound to the cell walls (polysaccharides) and make up a significant part of the polyphenolic complex of pomegranate peel, being partially released during heat treatment or hydrolysis.

A study was conducted that showed that in pomegranate peel the ratio between free (water-soluble), acid hydrolyzable (extracted from the remainder of the peel from its aqueous treatment with 1.25% H2SO4 solution) and alkaline hydrolyzable (extracted from the remainder of the peel from two previous treatments with 1.25% NaOH solution) fractions of polyphenols in favor of the free fraction. It was exactly as follows: free phenols 8.11 G/100 g; acid hydrolyzable - 1.56 G/100 g; alkaline hydrolyzable - 4.05 G/ 100 g of air-dry peel [29].

In the course of experiments to extract free phenols from pomegranate peel using a 50:50 mixture of ethanol and water, then the remaining bound forms of phenols by alkaline hydrolysis (32 min, 1:48 rpm). 1.5 mol/l NaOH) using ultrasound, it was found that the concentration of the sum of free polyphenols and separately punicalagin (the sum of α- and β-anomeric forms) and ellagic acid in this by-product is 10159 ± 395 mg, 1197 ± 79 mg and 515 ± 19 mg/100 g of dry peel, respectively, and the concentration of bound phenols in the insoluble solid residue is 4230 ± 190 mg/ 100 g of its total dry weight [30].

Our experiments concerned the extraction of only a fraction of free phenols from the peel.

Their extraction into solution and natural coagulation in solutions during their subsequent settling were based on extensive procedures, which, due to their specificity, could not be carried out quickly. Hence the extension of the overall production cycle to three months, as in the case of commercial wines.

Although the first target substances (mainly ellagic acid) fell out of the extract already in the first three days of its exposure, further phenolic precipitation occurred monotonously without fluctuations, and it took 3 months to fully clarify the extract.

Primary wine extract and freshly squeezed solid residue from fermentation of the peel sample (1) with hydromodule 1:1.5 and a temperature of 18-22° C with the addition of 0.13% S. cerevisiae and 18% granulated sugar from its total mass were with the same component composition as in Table 4.

Table 4. Chemical composition of wine extract and freshly squeezed solid residue from fermentation of peel samples (1) using S. Cerevisiae.

Parameters	Unit of measurement	Wine extract	Freshly squeezed solid residue
Dry substances	Г/100 г	-	11.87±0.13
Soluble dry substances	° Brix	10.70±0.10	-
Total polyphenols	Г/100 мл	0.99±0.01	1.34±0.01
Monosaccharides	-	1.18±0.02	1.57±0.02
Sucrose	-	0.36±0.01	0.52±0.01
Titrated acidity (by citric acid)		2.81±0.02	2.81±0.03
Ascorbic acid	Мг/100 г	3.17±0.03	3.34±0.04
Protein (N x 5.25)	Г/100 г	-	0.80±0.01
Ash	Г/100 г		0.37±0.01
Ethanol	Мл/100 мл	14.00±0.12	-
p-value		0.05	0.05

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Note. *The yield of wine extract with a total polyphenol content of 0.99 G/100 ml: 700 ml /400 g of the peel sample (1); the yield of insoluble solid residue: freshly squeezed 257.6 g, after drying 47.6 g/400 g of the* peel sample (1).

To calculate which part of the polyphenols was extracted from the peel sample (1) into solution, and which part remained in the insoluble solid residue, it is necessary to return to the data in Table 1. It shows that in 100 g of the peel sample (1), the polyphenols are 2.60 g, while in 400 g of the peel sample (1) they should be: 2.60 x 4 = 10.40 g (100%).

Now let's go back to the data in Table 4.

Based on them, it can be calculated that the amount of total polyphenols in 700 ml of the extract is: 0.99 x 7= 6.93 g or 66.63% of their total weight in 400 g of the peel sample (1). In the freshly squeezed solid residue with a dry matter content of 11.87 G/100 g (257.6 g) total polyphenols remained: 1.34 x 2.58 = 3.46 g or 33.37% of their total weight in 400 g of the peel sample (1).

Already at the beginning of the exposure of the aqueous alcohol solution of the extract at room temperature, it became clear that it takes only 3 days to separate the first batch of polyphenols (mainly ellagic acid) from it. At the same time, the natural aggregation of polyphenols of the remaining part of the extract took place slowly over 3 months.

Table 5 shows how the ratio between soluble and insoluble polyphenols changed during different time periods of settling the wine extract from fermentation of the peel sample (3) at a 1:1.6 hydromodule and a temperature of 18-22 ° C with the addition of 0.13 wt. % of dry S. cerevisiae and 18 wt. % granulated sugar.

Table 5. The change in the balance between soluble and insoluble polyphenols in the wine extract from fermentation of the peel sample (3) during its three month settling at 18-22° C.

Duration of settling, day	**% from the total weight of the amount of polyphenols in the initial extract.**	
	Insoluble polyphenols	Soluble polyphenols
3	12.22±0.24	87.78±1.70
30	18.33±0.36	81.67±1.63
90	26.00±0.49	74.00±1.38
p-value	0.05	0.05

Note. *Yield of wine extract: 650 ml/400 g of peel sample (3) with an initial total polyphenol content of 3.60 G/100 g of crude weight; total polyphenol content in wine extract: 1.70 G/100 ml.*

Based on the data in this table, the total potential of 650 ml of wine extract for absolutely pure polyphenols is: 1.70 x 6.5 = 11.05 grams or 76.74% of their total weight in 400 grams of peel sample (3), which corresponds to ≈ 80 mg/gram of peel dry matter.

After 3 months of settling, the ratio between insoluble and soluble fractions of polyphenols in the extract from fermentation of the peel sample (3) was 26.00/74.00 (%/%).

This means that the potential of 650 ml of this extract for the insoluble fraction of polyphenols is 2.87 grams or 19.93% of their total weight in 400 g of the peel sample (3).

Is this a lot or a little?

For comparison, one of the published papers tested the release of ellagic acid from its bound forms by alkaline hydrolysis using ultrasound. Using optimal hydrolysis conditions (32 min, 1/48 agitator rotation, revolutions/sec, alkali concentration 1.5 mol/l NaOH), 59 ± 3 mg of punicalagin and 1457 ± 71 mg of ellagic acid were obtained from 100 g of dry peel [30].

The Chinese authors performed a mechanochemical complementary extraction of pomegranate peel at a liquid-solid ratio of 50 ml/g and a temperature of 78 ° C for 67 minutes. Under these conditions, a total of 12 phytochemicals were identified, and the total phenolic content reached 258.09 ± 1.35 mg GAE/g dry weight of the extract. [31].

In another published paper, the authors focused on the extraction of biologically active compounds from pomegranate peel using a combination of water and ethanol under pressure. It

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was found that the optimal conditions are a process temperature of 200 ° C and an ethanol content of 77%. The yield of the total amount of phenolic compounds and punicalagin under these conditions was 164.3 ± 10.7 mg and 17 ± 3.6 mg/g of peel dry matter, respectively. [32]. These results confirm a higher degree of extraction of the total amount of phenolic compounds using a combination of water and ethanol under pressure than in our proposed method of extracting polyphenols by alcoholic fermentation (≈ 80 mg/g of dry matter), but not with respect to the isolation of punicalagin.

In another published study [33], which was based on the extraction of the peel using a 50% methanol solution in combination with ultrasound treatment, the yield of total polyphenols was 72.21 mg/g of the initial pomegranate peel powder, which is comparable to the yield of total polyphenols in our proposed method.

This suggests that in these well-known approaches that require the use of pressure, ultrasonic treatment and chemical reagents, the yield of target substances from the peel into the solution may be slightly higher than in our method, while our proposed method is much simpler technologically and environmentally friendly.

3.2. Commercial Analogues of the Target Products

1. *Spanish Tannat* is a dry, tart red wine made from red grapes grown in the Basque Country. Synonyms of this variety are: Madiran, Arriaga, Mustru, Bordelaise Belt. French Tannat is a very strong, rather tart wine with rich color and good aging potential, it is also used for blending with other strong Cabernet varieties. In Uruguay, they prefer to combine Tannat with something more subtle and diametrically opposed.

In Russia, prices for dry tart (tannic) wines start from 160-300 rubles / 0.75 liters. Popular varieties with pronounced astringency - Cabernet Sauvignon, Saperavi, Merlot, Matrasa are in the price range of 500-2000 rubles (as of March 11, 1926: 1 US $/79.22 Russian rubles).

2. *Ellagic acid,* chemical formula C14H6O8, a powerful antioxidant found in pomegranates, berries (strawberries, raspberries) and nuts. Protects cells from free radical damage, has anti-inflammatory, cardioprotective and potential anti-cancer properties. In cosmetology, it is used as a whitening and protective agent for the skin, and in medicine it is used to maintain the cardiovascular system. However, due to its poor solubility in water, its water-soluble derivatives are used.

Soluble salts of ellagic acid are obtained as a result of its reaction with alkalis such as sodium hydroxide (NaOH) and potassium hydroxide (KOH), as well as with amino acids or other bases. These reactions lead to the formation of water-soluble derivatives (ellagates) suitable for use in medicine and cosmetology. Such salts can be sodium, potassium salts of ellagic acid or its complexes with amino acids. Soluble salts, such as sodium salts, are used in dietary supplements and cosmetics due to their antioxidant and anti-inflammatory properties. Ellagic acid is treated with a base (e.g., sodium hydroxide) in a suitable solvent. Deprotonation of one or more of its -OH groups occurs, which leads to the formation of the corresponding salt.

Elagic acid is a part of the following food additives and berry extracts: Xantho Plus, Smokerade, URX, ZenThonic. Although elagic acid is not a vital substance for humans, it has the potential to be one of the most effective anti-cancer substances. Shaanxi Hongkang Biological Technology Co., Ltd. offers a cosmetic extract containing up to 95% elagic acid, produced by HPLC, at a price from 186.58 to 201.48 US dollars per 1 kg. In Russia, elagic acid preparations (CAS No. 476-66-4, chemical formula C14H6O8, molecular weight 302.19) of 40-95% purity are offered at a price of 5 grams/ 17-34.5 US $.

3. *Gelatin-tannate (Adiarin).* It is available as a powder for the preparation of a suspension for oral administration in case of diarrhea, in a dosage of 250 mg. There is also another form of release – capsules (the composition of one capsule: gelatin-tannate – 500 mg, corn starch – 73.3 mg, magnesium stearate – 11.7 mg). Drugs from manufacturers Novintethical Pharma (Switzerland), Noventure (Spain), Labomar (Italy) in Russian pharmacies, they cost US$3.5-6.2/ pack of 8-10 sachets of 250 mg each.

4. *Protein - carbohydrate flour and dietary fiber.* Products of this type have gained popularity because they help reduce the risk of heart and other chronic diseases.

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The powder from direct drying and grinding of pomegranate peel contains alkaloids, so tea is made from it against salmonellosis, dysentery and other dangerous intestinal infections. This powder is offered in the NaturoMama bio- and organic products store (Moscow) at a price of 490 rubles (more than US $ 5) per 100 g.

However, if the maximum amount of monosaccharides and polyphenols is removed from the pomegranate peel, then its remainder will consist mainly of dietary fiber, which helps to get rid of excess weight and obesity.

In the study [34], the content of water, protein, fat, pectin, hemicellulose, cellulose, lignin, neutral soluble fiber (NRK), acid-soluble fiber (CRC), titratable acidity and pH were determined both in the peel of pomegranate fruits and in the remaining alcohol-insoluble solid residue. Their functional properties, such as bulk density, swelling capacity, water retention capacity (EUB), oil retention capacity (EUM) and color, were also studied. This study found that the dried peel and the alcohol-insoluble residue of the peel contain, respectively, 12.87 and 15.88% water, 5.20 and 7.80% protein, 1.06 and 3.11% fat, 28.90 and 37.30% neutral soluble fiber (NRF), as well as 18.02 and 23.50% acid-soluble fiber (CRC). The content of pectin, hemicellulose, cellulose and lignin in the above-mentioned two preparations was 27.90 and 29.54%, 10.88 and 13.80%, 26.22 and 30.86% and 5.67 and 7.47%, respectively. The essential amino acids in both drugs are threonine, valine, isoleucine, leucine, phenylalanine and lysine. The predominant essential amino acid in both preparations is lysine, which is very important in the preparation of poultry feed. The minimum content is accounted for by isoleucine (peel) and theronin (residue). The alcohol-insoluble residue of the peel, reduced to a fine powder, was deficient in essential amino acids such as methionine and tryptophan. Attempts to replace some of the flour intended for making shaped bread with it have led to a decrease in the ash content in bread and an increase in the mass fraction of water, protein and fiber.

It is noted that the peel of pomegranates has a higher energy value than, for example, alfalfa, apple pomace, citrus peel, corn silage and tomato cake. In terms of crude protein content (36 g/kg of dry matter), the peel is inferior to pomegranate seeds (154 g), but surpasses seeds in terms of ash elements (54 g versus 24 g) and soluble carbohydrates (695.9 g versus 135 g/kg of dry matter) [35].

If their content in energy products is too high, polyphenols have a bad effect on protein digestibility, which prevents the direct use of the peel as feed for farm animals.

If you adjust the dose of polyphenols and transfer their content to an acceptably low level for dietary supplements, they will not only do no harm, but will even become necessary as antioxidants to help get rid of excess weight and obesity.

Clinical trials were conducted on bread, in its manufacture 2.5, 5 and 7.5% of flour was replaced by pomegranate peel in powder; they showed that due to the enrichment of fiber and antioxidant components of pomegranate peel, varieties of bread for weight loss can be obtained. [36].

Nutritionists know that a very important indicator regarding dietary fiber and its use as functional ingredients in food is the ratio between insoluble dietary fiber (NPS) and soluble dietary fiber (RPA); beneficial to human health is the ratio of NPS/RPA, which ranges from 1 to 2.3. In pomegranate peel this ratio varies in the range from 1.6 to 1.7, and such a balance can be considered successful in terms of meeting the above-mentioned evaluation criteria [37].

3.3. Main Aspects of the Technical Result

In this study, it was possible to develop an alternative solution, the main elements of which are:

1- Mixing coarse pomegranate peel (it should be the peel of freshly harvested or aged pomegranates of intensely colored varieties) with clean drinking water and granulated sugar in a ratio of 1:1.5-1.6:0.18 (weight/weight/weight), respectively;

2- Fermentation without air access for two weeks at 18-22° C using S. Cerevisiae;

3. Pumping the formed wine extract (up to 74-75% of the total volume of the extract) through a gauze filter, unloading and pressing the post-fermentation residue of the peel to separate the part of the extract absorbed into it (up to 25-26% of the total volume of the extract), and mixing both fractions of the extract.

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2- Settling the separated extract for 2.5-3 months and purifying it from insoluble polyphenols as a result of their natural and induced precipitation.

Target products: individual clots of ellagic acid and a-glycosides, gelatin-tannate, clarified extract with the texture of dry red wine.

A graphical method of transmitting information that clearly shows the steps of performing actions is often clearer than a text description.

Figures 1–4 shows the photos (visual algorithms) that sequentially display the stages of the new technology.

Figure 1. Fermentation using S. Cerevisiae: **a**- pomegranate fruits; **b**- peel from their manual cleaning; **c** - a mixture of peel and granulated sugar in a vessel with a water seal; **d** -alcoholic fermentation.

Figure 1 shows, that the proven method for extracting polyphenols from the peel into a solution has the advantages of simplicity, ease of use, and high practicality, and is based on alcoholic fermentation.

Alcoholic fermentation is a particular and very common type of fermentation (the process of splitting organic substances by microorganisms with the release of energy, for example, yeast), where sugars are converted into alcohol and carbon dioxide [38].

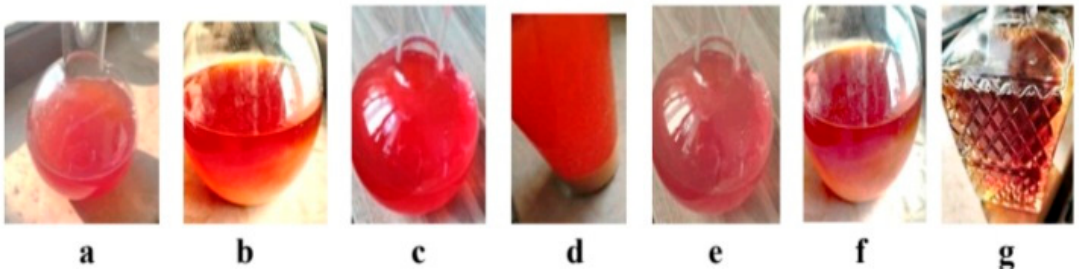

Figure 2. Processing of wine extract in 3 stages: stage 1 - initial wine extract (a), after 3 days of exposure (b) and after removal of sediment on the filter (c); Stage 2 - extract residue from stage 1 after treatment with 5% gelatin solution (d), and after removing the sediment on the filter (e); stage 3 - the remainder of the extract from the 2nd stage after exposure to complete clarification (f) and after removing the sediment on the filter (g).

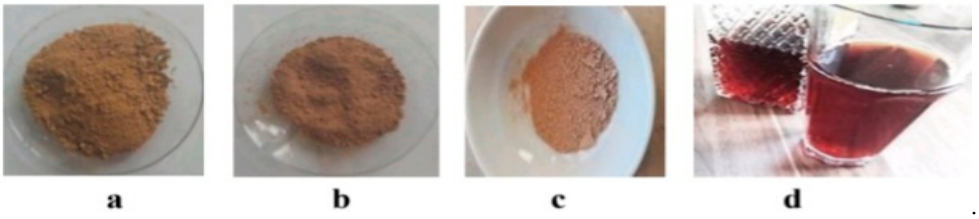

Figure 3. Final products from the association of polyphenols from wine extract: a- ellagic acid; b-gelatin-tannate; c- a-glycosides; d- Aztanna dry red wine.

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Figure 4. Intermediate (b - after drying) and final (c-after drying and grinding) conversion products of the solid insoluble residue of the peel (a).

The deposition of pure antioxidants (punicalagin, ellagic acid) from pomegranate peel extracts for the pharmaceutical and food industries has been studied and continues to be studied [39,40].

Solid-phase extraction, despite the many advantages of separation, has significant disadvantages. Although solid-phase extraction has been used as an extract separation method for decades, it is usually only on a relatively small scale to produce a particularly high purity product or to perform exceptionally complex separations. After each use of the sorbent, it is necessary to process it to restore its adsorption capacity. It is also not easy to choose a sorbent that would demonstrate not only good adsorption, but also desorption, that is, it would not only absorb polyphenols well, but also give them away. But even when selecting a sorbent that shows the most important advantages of advanced synthetic functional materials, it is often necessary to find a compromise between cost, environmental impact and high extraction efficiency. In addition, the adsorption capacity of many sorbents may face some practical problems when using highly concentrated raw materials. The capacity values usually range from <1 to 300 mg of separable substance per g of sorbent [41].

Figure 2 shows how we obtained and isolated three precipitates from the wine extract in three time stages.: 1- self-association of ellagic acid occurred due to hydrogen bonds and hydrophobic interactions; 2- precipitation of tannins remaining in the extract after removal of ellagic acid using gelatin was triggered; 3- self-association of a-glycosides remaining in the extract after removal of ellagic acid and astringent tannides occurred.

As it can be seen from it, this separation of the wine extract formed during the fermentation of pomegranate peel, combined with the achievement of its complete clarification, is a very illustrative example of how effective a process that previously seemed self-evident and win-win can be in a new application.

The peel also contains a lot of sugar, protein and fiber, which, like the polyphenols of its extract precipitate, have the potential to be used in obtaining sought-after products.

The reorientation from single-product to multi-product production often becomes a key factor in its rationalization, especially low-productivity [42].

Figures 3 and 4 are just a demonstration of the successful reorientation of the biotechnological process from the production of one end product to the joint production of several end products (coproduction), which we performed experimentally during this study.

3.4. Scientific Experience on Fermentation of Pomegranate Peel Using S. Cerevisiae

A known method for the production of ethanol from pomegranate peel, including drying, grinding, hydrolysis with 5% HNO_3 solution at 100 ° C for 30 minutes, detoxification of the hydrolysate using 2.5% activated carbon (which reduced the content of phenolic compounds by 62%) and fermentation for 5 days with ethanologenic yeast S. Cerevisiae; using the Metschnikowia strain sp. Y31 ethanol yield was increased to 0.42±0.08 g/g of reducing sugars [43]. Another method of ethanol production from pomegranate peel includes drying, grinding, hydrolysis with 3% HNO3 solution at 75 ° C for one hour and fermentation using S. Cerevisiae for 7 days; maximum ethanol yield (up to 0.43± 0.04 and 0.41±0.03 g/g of reducing sugars) It was obtained using the strains Metchnikowia sp. Y31 and M. cibodasensis Y34 [44].

The main disadvantage of these methods, which aim to convert the lignocellulose part of the peel first into sugar and then into ethanol, is the need to use nitric acid, which is associated with a potential danger to personnel and the environment. In addition, thermal hydrolysis using an

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aggressive solution of nitric acid is more an element of chemical technology than food technology, and requires the use of a chemically resistant reactor and significant energy expenditure in the production of commercial volumes of ethanol.

Among the well-known developments in the field of obtaining this type of bioethanol is one [45], which includes the stages of hydrothermal peel treatment, saccharification and fermentation using S. Cerevisiae, supported by external carbon sources. It is carried out in three main stages:

- Hydrothermal treatment: Pomegranate peel is harvested, washed, dried, crushed and subjected to pre-hydrothermal treatment (for example, at 115 ° C for 40 minutes) to remove pectin and phenolic substances that interfere with the absorption of cellulose, making cellulose more accessible;
- Saccharification: The remainder of the peel from hydrothermal treatment is processed using cellulase enzymes capable of breaking down the fiber of the pre-processed peel into fermentable sugars (glucose);
- Yeast (S. cerevisiae) is added to the sugared mixture from the previous stage, which simultaneously converts glucose into ethanol under optimal conditions (pH ~5.65, temperature ~40.3 °C, dry matter content ~12.8% by weight). The activity of S. cerevisiae is maintained by external carbon/nitrogen sources (e.g. glucose, sucrose, meat peptone). At a consumption of S. cerevisiae of 30 g/l, 90.4% of the initial sugar was consumed; the maximum ethanol yield was 12.9 g/l, which corresponded to 95.09% of its theoretical yield.

Although this method does not use HNO_3, that is, it is cleaner than the previous two methods, but it also has significant drawbacks. Its first disadvantage is that hydrothermal treatment is accompanied by the irretrievable loss of not only a significant part of the pectin and phenolic substances, but also the natural sugar of the peel, as well as soluble fractions of nitrogenous and mineral substances that feed on ethanol-forming yeast. If we assume that the peel is very rich in simple sugars, and in its ligninocellulose complex, the main place is occupied not by cellulose (the product of complete cleavage of which is glucose), but by lignin, the answer to this question suggests itself.

Another method has been proposed, which sets the task of changing the initial structure of natural oligo- and polysaccharides of the peel [46], which includes two main stages. First, the peel is dried, crushed, and treated for 2 minutes with hot (100 ° C) steam so that the endogenous yeast is inactivated (stage 1). Then water (10 l / 1 kg of peel) and S. cerevisiae (25 g / 10 l) are added and fermentation is carried out without air access for 72 hours at 25 ° C and stirring (380 rpm).

The main result of such harsh hydrothermal treatment was a decrease in the molecular weight of oligo- and polysaccharides from a maximum value of 705 kDa to a value of 26 kDa. In vitro experiments showed that polysaccharides were then used more easily by Bifidobacterium breve and Lactobacillus plantarum bacteria than polysaccharides from control samples. Also, this treatment also led to an increase in the quantitative extraction of tannin, its content in extracts increased to 70 g / 100 g of dry extract (in experiments with spontaneous fermentation of the peel under the same conditions without the participation of S. cerevisiae - 30-35 g of tannin/ 100 g of dry extract). The yield of the dry extract obtained by lyophilization of the filler liquid ranged from 28% to 50% relative to the dry peel. Its yield was significantly lower in the samples fermented with S. cerevisiae compared to the corresponding control samples, probably due to the consumption of yeast components of the peel, such as free sugars, mineral salts, proteins. On the other hand, extracts from S. cerevisiae were processed more easily than control samples, which contained more free sugars (their hygroscopicity was much higher).

From the above data, it turns out that fermentation with S. cerevisiae at a ratio between the peel and water of 1/10 (kg/l) allowed to obtain an extract containing 70 g of polyphenols / 100 g of dry extract. With a dry extract yield of 28% of the total weight of the dry peel, the potential of this method for polyphenols is 196 g / 1 kg of dry peel.

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If we assume that the extraction rate of polyphenols in this method was 100%, then there were 19.6 g of polyphenols in 100 g of the initial dry peel. If there were 13.72 g of polyphenols in 100 g of the initial dry peel, then in this case the degree of their extraction would be high, amounting to 70%.

At the same time, hydrothermal treatment, if we included it as an element of our technology, would significantly complicate the separation of the extract, would have a bad effect on the naturalness of the extract (could change its color and smell), and it could potentially contribute to the enrichment of the extract not only with polyphenols, but also with viscous biocoloids.

3.5. Advantages and Disadvantages of Known Methods of Separation of Pomegranate Peel Extracts

The precipitation of polyphenols from pomegranate peel extracts is an urgent topic at the intersection of food chemistry, pharmacy and ecology. The main purpose of such studies is usually either to purify the extract (remove excess tannins) [47], or to concentrate the active substances by evaporation under vacuum or using membranes [48].

This issue is being actively studied, as it is a key step in obtaining pure antioxidants (punicalagin, ellagic acid) for the pharmaceutical and food industries.

The research is carried out in three main directions: natural aging (sedimentation), directed (provoked) deposition and adsorption on macroporous resins:

3.5.1. Natural (Spontaneous) Deposition

This phenomenon is often referred to as "turbidity" or "aging" of the extract.

- Self-association: When cooled or stored for a long time, ellagitannin molecules can aggregate due to hydrogen bonds and hydrophobic interactions. This phenomenon is often observed during the storage of aqueous extracts.

Pomegranate polyphenols, especially punicalagins, are prone to spontaneous precipitation for several reasons:

- Hydrolysis: Under the influence of enzymes or acidity, punicalagin can hydrolyze, releasing ellagic acid, which has an extremely low solubility in water and precipitates as a crystalline precipitate.
- Interaction with proteins: If residual proteins are present in the extract, they form complexes with tannins, which expand and settle over time.
- Temperature factor: When the saturated hot extract is cooled, the solubility of polyphenols decreases, which causes "turbidity" and natural precipitation.

Another important aspect concerns the use of environmentally friendly solvents (DES). In particular, a glycerin-urea mixture was tested as such a solvent [49]. The article is interesting because it describes how changing the composition of a solvent and adding water causes the loss of polyphenolic complexes.

Precipitation of polyphenols with gelatin is a classic method based on the formation of insoluble complexes between proteins and tannins (tannins). In the case of pomegranate peel, which is extremely rich in ellagitannins (for example, punicalagin), this process has proven to be particularly effective [50].

When gelatin is added to an aqueous extract, hydrogen bonds bind between the hydroxyl groups of polyphenols and the peptide groups of the protein. This leads to the formation of large aggregates that precipitate.

The main factors influencing the output of polyphenols:

- Gelatin preparation: The gelatin must be soaked in cold water (1:5) and then dissolved at 50-60 °C before being introduced into the extract.
- pH of the medium: Optimal precipitation usually occurs near the isoelectric point of gelatin (pH 4.7–5.2).
- Temperature: An increase in temperature can increase the solubility of the complex, so the process is often carried out at room temperature or cooled to a temperature of 10-15 °C.

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- Gelatin concentration: Excess protein can cause the opposite effect (stabilization of the colloid).

3.5.2. Induced (Targeted) Precipitation by Chemical Agents

This is the most studied method based on the ability of polyphenols (especially ellagitannins, which are rich in pomegranate peel) to bind to certain macromolecules.

Scientists and technologists use various methods of directional "precipitation" to separate target substances from sugars and organic acids (Table 6).

Table 6. Methods of directional precipitation of polyphenols.

Method	Mechanism of action
pH change	Regulation of acidity (usually by alkalinization followed by severe acidification) allows certain fractions of phenolic compounds to precipitate.
Protein deposition	The use of gelatin/casein/albumin; tannins bind to proteins and precipitate (the classic "pasting" method); the "gelatin index" is often investigated in the literature to assess the concentration of tannins..
Alcohol precipitation	Adding ethanol in certain concentrations helps to separate polysaccharides (pectins) from polyphenols, or vice versa - to plant complex polyphenolic complexes.
Use of salts (salting out)	The addition of ammonium sulfate or sodium chloride reduces the solubility of organic compounds, causing them to precipitate.
Metal ions	The addition of iron (Fe3+), aluminum, or calcium salts leads to the formation of insoluble chelate complexes (metal phenolates).
Synthetic polymers	The use of polyvinyl polypyrrolidone (PVPP) is a standard in plant oenology and chemistry for the selective removal of polyphenols.

The most effective method of induced precipitation of ellagic acid from an aqueous extract and its further crystallization is the hydrolysis of ellagitannins [51]. The study describes the process of obtaining high purity ellagic acid (90%) by acid hydrolysis. The method is based on the fact that when heated with acid, soluble tannins decompose to ellagic acid, which has an extremely low solubility in water and precipitates. It is also claimed that the use of distilled water at 60 °C followed by acidification with acetic acid makes it possible to effectively convert polyphenols into the insoluble form of ellagic acid. [52].

The main essence of the ultrafiltration separation method is that the processed extract is passed through membranes with a certain pore size to concentrate high-molecular compounds (punicalagin).

The published work investigates the use of ultrafiltration (10 kDa membrane) as a method of "physical deposition" (retention) of polyphenols. An increase in the concentration of ellagic acid by 118% in the retentate (membrane sediment) was achieved. [53].

3.5.3. Modern Deposition Alternatives

Although classical deposition is used, modern laboratories are more likely to use adsorption on macroporous resins and ultrafiltration.

In the scientific literature of recent years (2021-2026), the focus has shifted from simple precipitation to targeted isolation (recovery) and purification (purification) of specific substances such as punicalagin and ellagic acid. [54,55].

In modern industry, the term "precipitation" is often replaced by "adsorption on macroporous resins" [56]. A recent review details the purification of punicalagine using Amberlite XAD-16 resins and MPLC (medium pressure chromatography) technologies [55]. A combination of ultrasound and

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flash chromatography for the fractionation of polyphenols was also tested [57]. The method makes it possible to selectively "plant" target fractions on the sorbent.

In recent years, in the research and production of pure pomegranate polyphenols, preference has been given to macroporous adsorption resins HPD100, HPD400, HPD-417, HPD450 and HPD600 from the Chinese company Cangzhou Bon Adsorber Technology Co., Ltd. LLC (Hebei). The macroporous HPD adsorption resin, which was developed by this company in collaboration with the Nation Engineering Scientific Research Center for Natural Medicines, is produced here on a par with traditional adsorption resin, which has disadvantages such as rapid destructibility, poor absorption selectivity, and low capacity [58].

The production of commercial polyphenols from pomegranate peel is an industrial process involving the extraction of active compounds such as ellagitanins (punicalagin, ellagic acid, etc.) from dried peel using water, ethanol, or a mixture thereof, concentrating the extract and separating it to produce a standardized powder, which is then used in nutraceuticals, cosmetics, and the food industry as an antioxidant [59,60].

The essence of the extract separation method using macroporous resin (Amberlite, XAD) is that the prepared extract is passed through a column, the polyphenols are retained by the resin, and then washed out (desorbed) with ethanol. The key points are as follows:

- Preparation of raw materials: The pomegranate peel is peeled, dried and powdered.
- Extraction: The peel powder is extracted using suitable solvents (hot water or water-alcohol mixtures) for the extraction of polyphenols.
- Fractionation (adsorption chromatography): The extract is filtered, concentrated (for example, by dilution evaporation) and passed through a column with silica gel, a resin capable of adsorbing one or more polyphenols.
- Polyphenols "stick" to the resin (they are adsorbed).
- Removal of impurities from the resin surface and desorption of polyphenols: First, the resin is washed with deionized water until a light flow is obtained and the remaining water is removed by vacuum aspiration, after which it is treated with an organic solvent, for example, 5 times the volume relative to the volume of the column 80 wt. % ethanol, an eluate containing ethanol, is collected and concentrated at reduced pressure and ethanol is simultaneously regenerated from it.
- The desorbant is dehydrated in a spray dryer or vacuum dryer to obtain a powder.

Main target components:

- Punicalagins: Highly active tannins that break down in the body to ellagic acid.
- Ellagic acid: A powerful antioxidant with anti-inflammatory properties.

The isolation and purification of polyphenols from pomegranate peel extracts using macroporous resin forms the basis of all industrially applied technologies in this field. They also provide for the preparation and preparation of extracts according to a scheme that includes drying the peel, grinding, water extraction in special extractors during heating and stirring, separation of the resulting extract and concentration during dilution until approximately 1/3 of the initial volume is reached.

This scheme was generally developed in accordance with the objectives of the business in the production of pomegranate extracts for biomedicine, taking into account the growing demand for pomegranate peel polyphenols [61].

However, it is not without significant drawbacks. As we already pointed out in one of the previous parts of this article, when heated and stirred, the smallest particles of the feedstock quickly boil and stick together, and a lot of sugar and colloids pass into the solution. In addition, such a scheme may not always be justified due to its multi-stage nature and high energy costs for drying and extracting the peel, as well as concentrating the primary extract before separating it.

Since the polyphenols in the peel are several times less than, for example, simple sugars and fibers, the ratio between the pure target substance and the initial dry peel can be 1:100

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(weight/weight) or more (especially when trying to obtain individual polyphenols rather than total ones). At the same time, hundreds of kg of resin may be required to isolate 1 kg of pure polyphenols from 100 kg of dried resin.

Hence, the high prices for pure polyphenols (for 500 mg of encapsulated pomegranate extract of European production with 40% punicalagin content, you have to pay 47.31 euros).

In this scenario, any attempt to increase the profitability of the production of pure pomegranate polyphenols only by optimizing technological parameters may be unsuccessful. To do this, it is necessary at least to simplify and reduce the cost of the process at the root and redirect it to the use of other substances of the component composition of the peel, that is, to give it a co-product character, which was the main objective of this study.

3.6. Statement of the Appropriateness of the Proposed Approaches

The expediency of our proposed approaches, based on their technical simplicity and proximity to the conditions of food production, is obvious:

- *Direct fermentation (extraction of polyphenols) of coarse-grained raw peel from machine or manual peeling of pomegranates for 2 weeks at a temperature of 18-22 ° C with the addition of water and sugar in such a way that the added sugar and peel sugar are sufficient to obtain a wine extract with the same residual sugar content and the same strength (2-4 G/100 ml and 9-13 vol.%, respectively) as well as European dry wines.*

Justification: This is important, since in the known methods, the extraction of polyphenols from pomegranate peel is more technologically difficult. To do this, it is dried, crushed and subjected to harsh acid-water hydrothermal treatment or hydrothermal treatment with hot steam, and only after that it is fermented. This is much more difficult technically and leads to severe physical consequences.

In our method, these disadvantages can be avoided by using whole pieces of peel and using “cold” (at a temperature of 18-22 ° C) enzyme treatment.

Monitoring of the conversion of monosaccharides of the peel and added sugar to ethanol showed that fermentation under these conditions was almost completed in 1.5-2 weeks.

- *Separation of the resulting extract as a result of natural and gelatin-induced precipitation of polyphenols.*

Separation of the extract by column chromatography requires high competence and strict specialization in the production of dietary supplements. Although it is necessary for the complete purification of polyphenols from a large number of related substances (simple sugars, acids, biocolloids and minerals) and obtaining them in their pure form, at the same time it is very expensive.

Our goal was to simplify the process of obtaining polyphenols as much as possible. We had a lot of time for this, just as long as it took to fully clarify the extract and get the finished wine from it.

Therefore, it was possible to simply wait until insoluble polyphenols fell out of the extract and separate the cumulative precipitate, or it was possible to carry out a certain intervention aimed at its separation in several time periods in order to obtain several polyphenol clots of different polarities (solubility).

We chose the second option, which consists precisely in the fact that the primary extract is maintained for 2-3 months (this is the minimum time required for any wine to mature). During this time, three acts are performed to separate the precipitate (the first at the very beginning, the second immediately after the first with the help of added gelatin, the third at the very end of the entire time allotted for settling). The precipitates containing polyphenols are filtered and washed on a filter with water until it is completely discolored, then they are dried at a temperature not exceeding 60 ° C and crushed to obtain three polyphenolic dry clots of 32-49% purity. And after that, the remaining part of the extract is stored in tanks as a clarified dry red wine ready for bottling, which, by analogy with commercial Spanish tart wines, was given the name "AzTanna".

- *Acceptability of the proposed process to the technical conditions of food production*

The equipment for the proposed technology is the same as for fermenting wort with pulp, and the same requirements apply to it.

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Figure 5 shows the main devices needed to implement the technology we propose:

A vinifier (for example, from BeerMachines) for fermenting wort together with pulp (skin, bones) with automatic discharge of solid residue (Figure 5, a) is a professional container equipped with mechanisms for stirring ("caps") and temperature control, which accelerates the extraction of color and flavor. Unlike a conventional fermentation tank with a water seal, which only isolates the wine from oxygen, the vinifier actively controls the fermentation process. It is designed for the production of red wines (fermentation on a pulp), ensuring constant contact of the wort with the skin, which is impossible in a conventional container without manual stirring. They have hatches for unloading cake, taps for sampling and level control. The vessel is 75% full.

Screw (screw) press (Figure 5b), can be used for transportation, dewatering and compaction of solid household waste. The mechanism, consisting of a rotating screw in a perforated housing, significantly reduces the volume and humidity of waste. The waste enters the loading funnel, is moved by an auger, and is pressed, in which moisture is removed through a mesh/perforation. Often, the diameter of the auger increases and the gap decreases towards the outlet for maximum pressure. The body and screw are made of stainless steel (AISI 304, 316) to protect against corrosion. The closed design prevents the spread of odors. Efficiency: automatic operation, low power consumption and high spin rate.

An intermediate tank for collecting gravity wort and for pressing solid residue (Figure 5c) provides tightness, protection from oxidation and ease of pumping, preventing contact with air and preserving the quality of the primary wine. It is made of food grade stainless steel (AISI 304/316) to ensure hygiene and durability. It includes maintenance hatches, drain taps, pump connections, measuring rulers, and sometimes cooling jackets for temperature control. They are installed next to the vinifier so that the liquid phase can be collected in it before sending it to the wine sump.

A conical-bottomed wine container with an automatic wall washer (Figure 5d) is a specialized stainless steel food-grade tank. It is equipped with washing heads (sprinklers/CIP systems) to remove sediment stuck to the tank walls and clean it without disassembly. The conical bottom ensures the natural collection of sediment, simplifying its removal without overflow.

Nutch filter dryer (Figure 5e) with a hinged bottom for unloading sludge. Its design is based on a vertical cylindrical tank into which the initial product is loaded. The vacuum pump is connected through a receiving tank and is necessary to create a vacuum. Inside, in the lower part, there is a lattice partition - the so-called false bottom or filter base. A filter material is placed on it, most often a mesh or a special filter cloth. In some models, the housing is equipped with a thermoregulation jacket, which allows you to control the temperature of the process or dry the cake. The solution is fed into the upper part of the tank, where it is distributed over the filter surface. A vacuum pump pumps air out of the area under the partition, creating a vacuum. This accelerates the filtration process under the influence of pressure drop and gravity, the liquid passes through the filter layer, being cleaned of solid impurities. After the process is completed, the precipitate can be rinsed to remove any remaining solution, and then dried if required. In case of insufficient filtrate quality, repeated purification is possible. In industrial models, automated side valve discharge systems are sometimes used.

There are open and closed Nutch filters. An open nut filter is a container without an airtight lid into which the solution is supplied. Filtration takes place under the influence of vacuum and gravity. After separation of the liquid phase, the precipitate is easily removed manually. Such filters are convenient for laboratories or small industries where simplicity of design and ease of maintenance are important. Visual control of the process allows you to quickly respond to changes, and access to the filter layer simplifies its replacement and cleaning.

Nutch filter dryers allow filtration, rinsing and drying in one unit. Drying is carried out after filtration and washing of the sludge by purging with hot gas (convective method) or heating through a jacket (thermal conductivity), which ensures the production of a dry product. This makes it possible to obtain high-quality dry sediment without intermediate extraction from the device. The use of a

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nutch filter significantly optimizes the technological process by combining several stages in one device.

Figure 5. The main devices for the implementation of the proposed technology: a- vinifier; b - screw press; c - intermediate tank for collecting the liquid phase of the fermented liquid phase; d - sump (tank) with automatic wall washing; e- Nutch filter. .

You will also need an container for storing / bottling finished wine in the form of special stainless steel food containers (tanks) with a floating lid (such as containers from the Chinese manufacturer Cassman with a hole for injecting a small amount of nitrogen to create an environment with low positive pressure inside the tank and completely isolate the wine from oxygen).

The application of well-known processes and devices in our new method is based on the proof of achievement of a new technical result due to their original combination and a new field of use. This was not obvious to a specialist, even if the components were known to him.

In our case, well-known processes are used for a completely new purpose, which has not been practiced before, solving a specific problem. The use of well-known processes and the use of well-known devices in combination, which makes it possible to simplify the technological scheme, eliminating unnecessary stages, is considered an inventive step.

- *The proposed method in a concise formulation*

Item 1. *A method of bio-processing pomegranate peel*, including the stages of extracting target substances into a solution and separating the resulting extract, is based on the fact that the peel from manual or machine cleaning of fresh or aged pomegranates (large pieces of irregular shape) is used as the initial object. First, the peel is weighed and passed under the shower. Then the peel, water and granulated sugar are fed into the vinifier in a weight ratio of 1:1.5-1.6:0.18, respectively, and Gervin GV1 dry wine yeast (at the rate of 0.25 g / liter of the fermentation mixture). Fermentation is carried out for two weeks at a temperature of 18-22° C, then the main part of the liquid phase (74-75% of the total volume of the extract) is decanted, the insoluble remainder of the peel is also discharged from the vinifier, and it is pressed to obtain the second part of the extract (25-26% of the total volume of the extract). Both liquids are cleaned of large inclusions using a gauze filter and collected together in a primary extract receiver. The mixed extract is sent to a container with a conical bottom for settling in three time stages, each of which ends with separation of the precipitate using a nut filter. The first precipitate is separated after three days. A 5% gelatin solution is added to the remaining part of the extract, left for 3 days and the second precipitate is separated. The third precipitate is separated at the very end of the entire time (3 months) allotted for clarifying the extract. The precipitates separated by a nutch filter are dried in it, then crushed separately in a disintegrator to a fine powder to produce clots of ellagic acid, gelatin tannate and a-glycosides with a polyphenol purity of at least 50%, 38% and 47%, respectively. The clarified extract (95% of its original volume) is stored as a dry red wine called Aztanna (depending on the batch of wine, its strength is 9-14 vol%). Individual batches of wine are mixed to obtain a standard sample with a strength of 11 vol%. The insoluble residue of the peel is dried (in a roller dryer) and crushed (in an integrator) to obtain a powdered additive for fortifying food with dietary fiber, which contains protein 3.8-4.00 G / 100 g of dry weight, crude fiber - 63-67% G / 100 g of dry weight. Its yield is 11.86-16.25 g / 100 g of the original raw peel.

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Item 2. *The method of bio-processing pomegranate peel according to item 1*, characterized in that only two precipitates are filtered out - after three days and at the very end of settling the extract to obtain two polyphenol clots: 1 - ellagic acid; 2- a mixture of ellagitanins and a-glycosides.

- *Prospects for commercialization*

The ratio between the conversion substance and auxiliary materials and the target yield are important indicators for attesting to changes in the cost of production as a result of technical and organizational innovations within the framework of the proposed technical solution.

Let's consider this using the example of a sample of peel 3 with an initial content of the sum of polyphnols 3.60 G/100 g of crude mass and total sugar 10.74 G/100 g of crude mass.

100 kg of coarse peel of this sample is weighed on a scale, loaded onto a conveyor, washed in the shower, and sent to the vinifier receiving device. 160 liters of clean drinking water, 18 kg of pre-sifted granulated sugar are added there, and 62 g of Gervin GV1 dry wine yeast are added to them. After two weeks of fermentation at a temperature of 18-22 ° C, the liquid phase (120.0 liters) is decanted and part of the liquid absorbed into the solid post-fermentation residue (42.5 liters) is also separated - this time in a pressing device, both fractions are cleaned of large inclusions using a gauze filter and collected together in a receiver for primary extraction. The mixed extract with a dry matter content of 10.4% is sent to a container with a conical bottom for settling in three time stages, each of which ends with separation of the precipitate using a nut filter. The first precipitate is separated after three days. To the remaining part of the extract, add 2 liters of a 5% gelatin solution for precipitation of tannins, prepared from 100 g of dry gelatin gelatin, leave for 3 days and separate the second precipitate. Only gelatin obtained by acid hydrolysis can be used, as it has a high positive charge. Experimentally, it was found that Erbigel acidic dry gelatin produced by Erbigel Geisenheim AG, with a Blum number gelling capacity of 90-100, is the most optimal for use. Erbigel gelatin is rapidly and completely dissolved by filling it with cold water and further dissolving it in hot water (95 ° C). The third precipitate is separated at the very end of the entire time (3 months) allotted for clarifying the extract. The precipitates separated by a nutch filter are dried in it, then crushed separately in a disintegrator to a fine powder to obtain dry clots of ellagic acid (670 g), gelatin tannate (150 g) and a-glycosides (425 g) with a polyphenol purity of at least 54.30%, 39.49% and 49.36%, respectively. The remainder of the extract (154.4 liters) is collected in a storage container as dry red wine Aztanna (alcohol strength 9 vol%). The insoluble residue of the peel of the peel is dried (in a roller dryer) and crushed (in an integrator) to obtain 16.25 kg of a powdered additive for fortifying food with dietary fibers with a protein content of 3.50 G / 100 g of dry weight, crude fiber 16.25 g / 100 g of the original raw peel.

Taking into account the market prices for these products, the manufacturer's prices for the above-mentioned products (which will be twice as low as market prices) may be: dry wine Aztanna 3.00 $ US/0.75 l; ellagic acid 8.00 $ US/5 g; gelatin-tannate 2.00 $ US/2.5 g; a-glycosides 7.00 $ US/ 5 g.

Then the price of the total volume of wine from processing 100 kg of a 3 peel sample (154.4 liters) will be ≈617 $ US; polyphenolic clots (1 kg 245 g) - 1787 $ US; wine and polyphenolic clots - 2404 $ US. If 10 tons of raw peel are processed per season, the total price sold from the warehouse will be about 240,000 US dollars.

Calculating the profitability of their production requires a production check, but with such low production and non-production costs (as can be seen from the previous paragraph), it should be high.

4. Conclusıon

Accordingly, this will simplify the extraction of polyphenols into the extract and its separation, expand the range and ensure the purity of products, use the pomegranate peel as fully as possible without damaging the environment, create the maximum amount of valuable results with minimal resource expenditure and increase the economic attractiveness of the new technology as a whole, which exactly corresponds to the objectives of this study.

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