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A Method for the Bioprocessing of Pomegranate Peel

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Abstract

As global demand for pomegranate peel polyphenols grows, optimizing production costs is essential to increase market competitiveness. This study proposes a comprehensive bioextraction and purification system designed to minimize capital costs (CAPEX) and operational complexity. The technique includes two main stages: (1) extraction of polyphenols from the raw pomegranate peel during active fermentation mediated by Saccharomyces cerevisiae, and (2) sequential isolation of polyphenolic fractions by spontaneous and directed precipitation with gelatin. As a result, three different polyphenolic precipitates were successfully obtained from the pomegranate peel in co-production with the red dry wine "Aztanna". Although the purity of the polyphenol clots (39–54% of the dry matter) was lower than that of commercial polyphenolic preparations (40–90%) isolated from peel extracts using adsorption resins, their production in the developed and proposed version can be carried out without the use of drying, grinding, thermal maceration of the peel with stirring and adsorption chromatography. The proposed technology does not require the cost of purchasing new equipment, as it can be easily implemented in any workshop for the production of recycled wine from the pomace of colored fruits and berries. In fact, it is an easily scalable, successful alternative to expensive and not always economically feasible industrially applied methods for producing pomegranate polyphenols.

1 INTRODUCTION

Global fruit production of *Punica granatum* has exceeded 3 million tons. Since the exocarp (peel) accounts for approximately 50–60% of the total fruit mass, roughly 1.62 million tons of bio-waste are generated annually [1]. Pomegranate peel has long been recognized as a valuable resource for the food industry, serving as a concentrated source of polyphenols and tannins [2–7]. Structurally, polyphenols comprise a broad class of plant-derived antioxidants and flavonoids, while tannins represent a specific functional subgroup known for their ability to bind and precipitate proteins—a process that imparts astringency and significant antioxidant potential. While all tannins are polyphenolic, the converse is not true.

In the plant kingdom, tannins are classified into four primary groups: complex tannins, condensed tannins (proanthocyanidins), gallotannins, and ellagitannins. The latter two constitute the hydrolyzable tannin class. In pomegranate, ellagitannins are the most prominent fraction, primarily occurring as punicalagin (α and β isomers), which represents 65.75% to 85% of the total tannin content. Both punicalagin and its derivative, punicalin, undergo hydrolysis to form ellagic acid [8–9]. The quantitative profile of these metabolites varies significantly by cultivar; for example, the peel of six Spanish pomegranate varieties exhibited punicalagin concentrations ranging from 181 to 255 mg/g [10]. Other identified tannins in the exocarp include pedunculagin, granatin A, granatin B, corylagin, tellimagrandin, and gallagylhexoside [11].

Beyond tannins, pomegranate peel is rich in various phenolic acids-including gallic, ellagic, vanillic, caffeic, ferulic, cinnamic, chlorogenic, and p-coumaric acids-as well as flavonoids such as kaempferol,

luteolin, rutin, quercetin, and hesperidin. It also contains triterpenes (oleanic and ursolic acids) and phenylethyl alcohol derivatives like hydroxytyrosol [12]. In Chinese pomegranate varieties, average gallic and ellagic acid contents were recorded at 0.57 mg/g and 1.34 mg/g [13], while other studies report values of 123.79, 35.89, 20.56, and 4.48 mg per 100 g for gallic, ellagic, caffeic, and p-coumaric acids, respectively [11]. Furthermore, the peel contains approximately 30% of the fruit's total anthocyanins, with eight distinct glycosides identified in Tunisian varieties, including delphinidin and cyanidin derivatives [15, 16].

These polyphenols exhibit potent biological activities, including anti-inflammatory, hypocholesterolemic, and antimicrobial effects [17–20]. They demonstrate high systemic bioavailability [21–22] and have been shown to reduce the formation of pro-atherogenic metabolites like trimethylamine N-oxide (TMAO) in vitro [23]. Furthermore, they stimulate cellular regeneration and provide antibacterial and anthelmintic protection [24].

The peculiarity of pomegranate fruits as a raw material for juice production is that the peel and seeds account for more than half of their total weight. Tons of these wastes are generated annually, which, due to their composition and taking into account the costs already incurred by the time of their formation, it would be more correct to call bioactive by-products.

Despite this, many factories are forced to throw them away due to the high logistical and economic costs and complexity of processing. Large financial investments are required for the purchase of efficient drying plants and the construction of workshops for the extraction of oil and polyphenols. At the same time, the benefits of selling seed oils or polyphenols from the peel for the cosmetic and pharmaceutical industries may not always justify the creation of such non-core industries for a juice factory.

Despite this, interest in the waste-free processing of pomegranate, which is based on the process of so-called "green extraction" (extraction of hydrophilic and lipophilic compounds), is growing. And this is easily explained: an integrated approach eliminates the need to bury large volumes of raw materials, accounting for up to 70% of the weight of processed fruits, and prevents environmental pollution.\

Currently, industrial extraction technologies of pomegranate polyphenols are largely restricted by proprietary patents. Conventional workflows involve energy-intensive desiccation, mechanical comminution, and thermal maceration, followed by complex adsorption chromatography. These methods are characterized by low efficiency and high operational costs. This study aims to simplify the production of pomegranate polyphenols while maximizing substrate utilization and economic viability, addressing the global demand for bioactive compounds that support human health and environmental resistance.

2 MATERIALS AND METHODS

2.1 The Experimental Substrate

The exocarp (peel) obtained from the manual peeling of three samples of intensely pigmented pomegranates (*Punica granatum* L.) served as the primary substrate for technological processing. These fruit samples were procured in late October from the central market in Guba, Azerbaijan

2.2 Auxiliary substances:

The experimental process utilized several auxiliary agents to facilitate fermentation and subsequent extract stabilization. White crystalline sugar (sucrose) was employed as the supplemental carbon source, conforming to the GOST 33222 – 2015 standard (State Standard of Russia). The primary microbial agent was Gervin GV1 (commercially associated with the Fermivin 7013 brand), an active dry wine yeast. This *Saccharomyces cerevisiae* strain, characterized by its high ethanol tolerance (up to 18% v/v), is technically recognized as the Bordeaux 7013 strain, though it was originally isolated by INRAe in the Corbières region of France.

For the selective purification and clarification of the polyphenol-rich extract, food-grade gelatin was used in compliance with the GOST 11293 – 2017 standard. This agent serves as a protein-based fining medium designed to facilitate the sedimentation of specific polyphenolic fractions through hydrogen bonding and hydrophobic interactions. All aqueous phases and media were prepared using potable water purified via a Thomas Compact reverse osmosis system, ensuring a substrate free of suspended solids and chlorine, which could otherwise interfere with microbial kinetics or polyphenolic stability.

2.3 Extraction

The intensification of target substance extraction from a substrate into solution traditionally necessitates mechanical comminution, thermal treatment, and agitation. Reducing particle size increases the surface area available for mass transfer, while elevated temperatures enhance the diffusion coefficients of target components; mechanical mixing further accelerates these kinetics. However, when processing substrates with low cellulose content and high concentrations of soluble fibers and monosaccharides-such as pomegranate peel-intensive thermal and mechanical treatment can induce undesirable rheological changes. Specifically, excessive heat can lead to the hydrolytic breakdown of the pectin-rich peel matrix, converting the extraction mixture into a high-viscosity colloidal suspension (mucilage) that severely hinders subsequent phase separation and purification [25–26]. Additionally, the desiccation, grinding, and thermal maceration of the peel can result in the oxidative or thermal degradation of bioactive compounds, while the high energy requirements of these unit operations compromise the economic viability of the process beyond a laboratory scale.

Consequently, three primary objectives were defined for the extraction stage: (1) minimizing operational costs through process simplification and reduced energy consumption; (2) maintaining the structural integrity (texture) of the exocarp to facilitate efficient solid-liquid separation and secondary processing of the solid residue; and (3) maximizing the retention of pigments and other sensitive phytochemical constituents. To achieve these goals, a methodology was proposed utilizing fresh, coarse-grained exocarp as the primary substrate. This process leverages *Saccharomyces cerevisiae* to initiate the

bioconversion of sugars-released into solution alongside polyphenols-into ethanol at ambient temperatures.

By continuously consuming the monosaccharides as they diffuse from the peel, the yeast effectively modulates the osmotic pressure of the medium while generating ethanol, which acts as an *in-situ* co-solvent. This biochemical environment facilitates a higher degree of polyphenol recovery while significantly reducing the viscosity of the extraction medium and ensuring the stability of thermolabile target substances. The fermentation of the raw, coarse exocarp was executed using *S. cerevisiae* (strain Gervin GV1) under anaerobic conditions in glass vessels fitted with hydraulic air locks. All experimental runs were conducted at room temperature. Substrate-to-solvent ratios ranged from 1:1 to 1:10 (g/mL), with incubation durations of one and two weeks. For each sample of exocarp, six distinct primary extract samples were produced. The progression of the fermentation was monitored qualitatively through the intensity of CO_2 gas evolution and foam formation.

2.4 Separation and Fractionation of the Extract

The fractionation and precipitation of polyphenols from the resulting fermented extracts were evaluated based on their differential solubility and relative polarities within the hydro-ethanolic matrix. To facilitate phase separation and stabilization, the primary extracts were subjected to a maturation period of three months. This duration allowed for the sequential sedimentation of three distinct polyphenolic precipitates, which were recovered via filtration. This tiered approach leverages the varying physicochemical properties of the target molecules-such as molecular weight and hydrophobicity-to isolate specific compound groups from the supernatant at different stages of the clarification process.

2.5 Analytical Parameters and Methodologies

The following chemical parameters were evaluated using standardized analytical procedures:

- **Dry Matter Content**: Determined by desiccation of the plant material to a constant mass in a drying oven according to GOST 33977 – 2016.
- **Total Soluble Solids (TSS)**: Quantified via the refractometric method in accordance with GOST 28562-90.
- **Reducing Sugars**: Determined using the Bertrand method (GOST 8756.13-87). This technique relies on the capacity of the carbohydrate aldehyde groups to reduce cupric oxide (CuO) to cuprous oxide (Cu_2O), resulting in a red precipitate upon reaction with Fehling's reagent.
- **Titratable Acidity**: Measured through titration with a $0.1\ N$ alkaline solution in the presence of a colorimetric indicator. Results were converted to citric acid equivalents using a coefficient of 0.0064 (ISO 750:2013).
- **Ascorbic Acid**: Quantified by iodometric titration according to GOST 24556-89.
- **pH Value**: Determined using the potentiometric method (GOST 26188 – 2016).
- **Total Polyphenols**: Analyzed via permanganatometric titration according to GOST 24027.2–80. This method involves titrating the residual indigo carmine-used as a redox indicator—with a $0.1\ N$

potassium permanganate ($KMnO_4$) solution. To isolate the phenolic fraction from other oxidizable compounds, the extract is titrated twice: initially to determine total oxidizable substances, and subsequently after treatment with activated charcoal to adsorb the polyphenolic fraction. The total phenolic content is calculated from the difference in $KMnO_4$ consumption using a conversion factor of 0.004157 g of phenols per milliliter of $0.1\ N\ KMnO_4$. This protocol is consistent with Article 1.5.3.0008.18 of the State Pharmacopoeia of the Russian Federation (XIV Edition) for the determination of tannins in medicinal plant substrates. All reagents employed were of analytical grade.

- **Crude Protein**: Determined by the Kjeldahl method, utilizing a nitrogen-to-protein conversion factor of 6.25
- **Dietary Fiber**: Determined via the enzymatic-gravimetric method according to GOST 34844 – 2002, applicable to bioactive additives and specialized food products.

2.6 Statistical Analysis

To mitigate systematic bias, all experimental trials were performed using a randomized design. The linear, quadratic, and interaction effects of the independent variables on the response variables were evaluated at a 95% confidence level $(p < 0.05)$. All statistical modeling and data processing were conducted using Statgraphics Centurion XV software (version 15.1.02, StatPoint Technologies, Inc., Warrenton, VA, USA).

3 THEORY/CALCULATIONS

To optimize the extraction process, we proposed the recovery of polyphenols from the initial substrate during fermentation mediated by Saccharomyces cerevisiae.

This approach was expected to yield several advantages: the yeast would ferment the monosaccharides-released from the peel alongside the polyphenols-into ethanol. This ethanol, in conjunction with the polyphenols, would constitute the primary extract.

Subsequent selective purification of these polyphenols would enhance the extract's clarity and organoleptic profile. Given the high concentration of pigments and the specific ethanol content, the final product would resemble a dry wine in both appearance and character.

Furthermore, a refinement strategy was developed based on the differential solubility, polarity, and molecular weights of various polyphenol fractions within hydro-ethanolic media. Specifically, compounds such as ellagic acid are expected to precipitate preferentially, while a second group-including punicalagins and proanthocyanidins-can be isolated using protein-based fining agents. The third group, comprising α-glycosides (such as rutin and quercetin), would remain in solution until complete clarification occurs.

This sequential, low-cost purification method represents a highly efficient and sustainable processing route.

4 RESULTS AND DISCUSSION

4.1 Preparation of the wine peel extract and its separation

4.1.1 Chemical analysis of the initial peel

The efficiency of complete alcoholic fermentation depends on the availability of carbon sources-primarily fermentable monosaccharides such as glucose and fructose-alongside nitrogen and essential minerals, including magnesium, calcium, potassium, phosphates, and zinc. Table 1 presents the yield and biochemical composition of the tested exocarp (peel) samples (1, 2, and 3) obtained from the manual separation of three local pomegranate varieties.

The data indicate that the peel constituted a significant portion of the total fruit mass, varying by variety: 32.00 ± 0.64% for sample 1, 38.97 ± 0.80% for sample 2, and 29.70 ± 0.40% for sample 3. In peel samples 1, 2, and 3, the concentrations of water, monosaccharides, sucrose, titratable acids, total polyphenols, ash, protein, and fat varied within the ranges 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 fresh weight, respectively.

Across the three samples, the average values for water, monosaccharides, sucrose, titratable acids, total polyphenols, ash, protein, and fat were 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 fresh weight. These results confirm that the pomegranate exocarp is a nutrient-dense substrate suitable for fermentation-assisted extraction.

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

Calculations regarding the theoretical soluble fraction of the exocarp-comprising monosaccharides, sucrose, titratable acids, and polyphenols—as a percentage of total dry matter yielded the following values: (**1**) ≈ 81.05%; (**2**) ≈ 58.38%; (**3**) ≈ 52.03%; and a mean for samples (**1**)-(**3**) of ≈ 64.10%. In both experimental and industrial contexts, the actual recovery of soluble solids from pomegranate peel is typically 5–10% lower than these theoretical values due to inherent extraction inefficiencies.

4.1.2 Controlled fermentation of pomegranate exocarp under anaerobic conditions using S. cerevisiae

Biotransformation is the primary biotechnological method employed for the enzymatic conversion of organic substrates into target metabolites. In this study, bioconversion was achieved via alcoholic fermentation-a specific metabolic pathway in which microorganisms catabolize organic compounds to release energy, converting sugars into ethanol and CO_{2}. Rather than conducting a specific parameter optimization study, established oenological protocols were adopted; utilizing validated methodologies ensures procedural stability and industrial reproducibility, avoiding unnecessary procedural stagnation.

According to the manufacturer's technical data, the *Saccharomyces cerevisiae* strain Gervin GV1 facilitates efficient fermentation at temperatures between 15° C and 25° C, exhibits an ethanol tolerance of up to 12–14%, and allows for direct surface inoculation (dry pitching) without the requirement for

mechanical agitation. While elevated temperatures (26–33° C) accelerate yeast proliferation, such kinetics are generally avoided in specialized fermentation (e.g., wine or beer production) as they can negatively impact the organoleptic profile. Consequently, fermentation in this study was maintained at 18–22° C.

Previous research suggests that mechanical comminution and thermal aqueous extraction of the exocarp impede solid-liquid separation due to the formation of colloidal turbidity, increased viscosity, and the presence of hydrolyzed particulate matter [27]. Furthermore, thermal treatment facilitates the co-extraction of non-target saccharides, which may inversely correlate with the concentration of desired antioxidants in the liquid phase [28]. To mitigate these issues, this study employed coarse-grained exocarp from manually separated pomegranates, substituting thermal maceration with ambient-temperature ("cold") extraction at 18–22 ° C.

Qualitative observations of extract turbidity and gas evolution indicated that the use of large exocarp fragments at these temperatures prevented the release of viscous biocolloids into the medium. The peak kinetic phase of sugar-to-ethanol conversion occurred between the final days of the first week and the initial days of the second. By the conclusion of the second week, sedimentation was observed at the base of the anaerobic vessel (equipped with a hydraulic airlock), signaling the spontaneous precipitation of ellagic acid. The structural integrity of the exocarp fragments allowed for efficient gravitational separation (decantation). The remaining solid residue was easily pressed to recover the occluded extract, facilitating high-efficiency phase separation.

Several factors contributed to the positive outcomes observed during the fermentation-assisted extraction. First, by maintaining the process at room temperature rather than utilizing thermal maceration, the structural integrity of the exocarp was preserved. In conventional high-temperature methods, the tissue undergoes thermal degradation; in this study, the "cold" extraction prevented such breakdown, allowing the peel to remain structurally intact. Second, the lower temperature and the use of unground material significantly attenuated the solubilization kinetics of mucilaginous substances, such as soluble pectins. This restricted the transition of these polysaccharides into the liquid phase, thereby maintaining low viscosity and facilitating easier filtration. During the alcoholic fermentation, the liquid phase avoided oversaturation with dissolved solids due to the continuous bioconversion of monosaccharides into ethanol and CO_2. The biosynthetic production of ethanol also provided a volumetric contribution to the solvent phase, acting as a co-solvent that further facilitated the stabilization of the extracted polyphenols. Microbiological stability is another critical factor. Spoilage microorganisms, including acetic acid bacteria and putrefactive pathogens, typically proliferate when the pH rises above 3.5–3.6. A high pH (low acidity) reduces the biological stability of the medium, increasing the risk of bacterial growth, turbidity, and acidification. In this study, the high initial concentration of organic acids in the pomegranate exocarp resulted in fermentation mixtures with a pH range of 2.8–3.2. This naturally acidic environment consistently ensured microbiological purity, as S. cerevisiae is well-adapted to such conditions while most competing spoilage bacteria are inhibited. While a lack of essential nutrients-specifically nitrogen-can lead to "stuck" fermentation and the undesirable production

of hydrogen sulfide, our analysis indicates that the pomegranate exocarp contains sufficient nitrogen and mineral constituents (ash) to support the metabolic requirements of the S. cerevisiae strain. However, the endogenous sugar content was insufficient to achieve the ethanol concentrations characteristic of dry wines.Stoichiometrically, the fermentation of 1 kg of sucrose theoretically yields 0.511 kg (approximately 640 ml) of pure ethanol and an equivalent mass of CO_2. This corresponds to an approximate yield of 0.6% vol. alcohol per 1% fermented sugar. In the exocarp samples (**1**–**3**) used here, the mass fraction of fermentable sugars ranged from 10.74–20.89% (Table 1). For the 400 g of peel used in each experimental trial, this equated to a range of 42.96 g to 83.56 g of natural sugars. Given that the average extract volume was $\approx$ 675 ml per 400 g of raw peel, the theoretical alcohol strength would be limited to 4.12–8.01% vol., which is lower than the 9–14% vol. range typical of European dry wines. Another fundamental factor is the liquid-to-solid ratio, or hydromodule. To evaluate how changes in the hydromodule affected the quantitative recovery of dry matter and polyphenols, experiments were conducted over a two-week fermentation period for samples (**1**) and (**3**) at 18–22° C with the addition of 0.13% S. cerevisiae and 18% granulated sugar. The resulting data are detailed 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 a significant portion of the total dry matter (DM) of the exocarp is composed of highly soluble monosaccharides-representing **58.03%** of the DM in sample (**1**) and **40.19%** in sample (**3**) - the extraction efficiency for dry matter was consistently higher for sample (**1**) across all tested hydromodules. At a **1:1** hydromodule, **63.16 ± 1.23%** of the total solids and **63.29 ± 0.59%** of the total polyphenols were recovered from sample (**1**); increasing the hydromodule to **1:8** resulted in a dry matter yield of **72.60 ± 1.40%** and a polyphenolic yield of **70.63 ± 0.66%**. In contrast, sample (**3**) yielded **42.50 ± 0.80%** of solids and **69.00 ± 0.65%** of polyphenols at a **1:1** hydromodule, which increased to **48.95 ± 0.94%** and **74.00 ± 0.72%**, respectively, at a **1:8** ratio.

Consistent with mass transfer and diffusion principles, increasing the solvent volume facilitates the hydration and solubilization of both total solids and polyphenolic constituents. Table 3 delineates the

metabolic utilization of total carbohydrates (including both endogenous sugars and exogenous additions) and proteins during a two-week fermentation of sample (**1**) at **18–22°C**, utilizing an inoculation of **0.13%** *S. cerevisiae* and the addition of **18%** sucrose relative to the total mass of the fermentation mixture.

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

Data analysis indicates that as the solvent volume increases, the metabolic assimilation of proteins and carbohydrates by *S. cerevisiae* also rises, peaking at a **1:4** hydromodule (75.07 ± 1.45% and 88.19 ± 0.86%, respectively). However, at higher dilutions-such as a **1:8** hydromodule-the concentrations of nitrogenous compounds and fermentable sugars fall below the optimal threshold for microbial proliferation. This nutrient dilution slows the growth kinetics of *S. cerevisiae* and results in a slight decrease in the efficiency of protein and sugar utilization. Consequently, excessive water volumes may compromise fermentation efficiency by reducing nutrient density to suboptimal levels. This inhibitory effect may also be compounded by an increase in pH resulting from the dilution of endogenous organic acids, which shifts the medium away from the yeast's preferred acidic range. In substrates with limited nitrogen but high soluble sugars, a more aqueous medium may cause the yeast to deplete available amino acids more rapidly, potentially leading to metabolic imbalances before carbohydrates become the limiting factor.

To optimize the process for maximum efficiency, a balanced hydromodule is required. At a ratio of **1:1.5–1.6**, an average of 168.8 ml (range: **162.5–175 ml**) of extract was recovered per **100 g** of exocarp samples **1–3**, whereas a **1:4** ratio yielded an average of 418.8 ml (range: **412.5–425 ml**). To ensure the resulting hydro-ethanolic extract exhibited the intense pigmentation and alcoholic strength characteristic of dry wines, the **1:1.5–1.6** (g/ml) ratio was selected. This hydromodule, requiring a minimum of **600–640 ml** of water, provided sufficient volume to fully submerge **400 g** of coarse exocarp within the **1400 cm^3** flat-bottomed, narrow-necked vessels used for anaerobic fermentation. Given that the endogenous sugar content of the peel was insufficient to achieve the standard 9–14% vol. ethanol range found in European dry wines, exogenous sucrose was added at a rate of **72 g** per **400 g** of each tested sample **1–3**. The combined endogenous and exogenous sugar concentrations allowed for the

production of polyphenol-enriched wine extracts with an ethanol content consistent with commercial dry wines (9–14% vol.). The specific dosage of added sugar was calibrated to prevent excessive osmotic stress on the yeast membranes and to avoid the inhibitory effects of rapid ethanol accumulation.

4.1.3 Separation and separation of the liquid fermentation mixture

Maturation is an essential phase for the clarification and development of optimal sensory characteristics in any fermented product. While a standard oenological approach involves waiting for complete transparency before filtering and discarding the sediment, such a method was unsuitable for this study. Given that polyphenols were the primary target, it was necessary to employ a strategy focused on their recovery without compromising the natural composition of the extract. Pomegranate exocarp contains a complex profile of both soluble and insoluble (matrix-bound) polyphenols. Soluble fractions, ranging from medium to high polarity, include flavonoids—such as anthocyanins, quercetin, and rutin in their glycosidic forms—alongside hydroxycinnamic acids (e.g., chlorogenic and p-coumaric acids), punicalagins, and ellagitannins. These compounds exhibit high antioxidant capacity and are readily extracted into aqueous media, particularly during prolonged infusion. In contrast, insoluble polyphenols exhibit low to zero polarity and are bound to cell wall polysaccharides. These represent a significant portion of the pomegranate polyphenolic complex and are typically released only through thermal treatment or enzymatic hydrolysis.

A previous study established the distribution of polyphenolic fractions in pomegranate exocarp as free (water-soluble), acid-hydrolyzable (extracted with 1.25% H_2SO_4), and alkaline-hydrolyzable (extracted with 1.25% $NaOH$). Results favored the free fraction, with concentrations of **8.11 g** for free phenols, **1.56 g** for acid-hydrolyzable, and **4.05 g** for alkaline-hydrolyzable per **100 g** of air-dried exocarp [29]. Further experiments utilizing a **50:50** ethanol-water mixture to extract free phenols, followed by ultrasound-assisted alkaline hydrolysis (**32 min**, **48 rpm**, **1.5 mol/l** $NaOH$), determined that the cumulative concentration of free polyphenols, punicalagin anomers (α and β), and ellagic acid was **10159 ± 395 mg**, **1197 ± 79 mg**, and **515 ± 19 mg** per **100 g** of dry exocarp, respectively. The concentration of bound phenols in the insoluble residue was recorded at **4230 ± 190 mg** per **100 g** of total dry weight [30].

Our research focuses specifically on the extraction of the free phenolic fraction. The transition of these compounds into solution and their subsequent spontaneous sedimentation are exhaustive processes that cannot be accelerated without affecting quality. Consequently, the production cycle was extended to **3 months**, mirroring commercial wine aging. Although the initial target substances (predominantly ellagic acid) precipitated from the extract within the first **three days** of maturation, subsequent sedimentation occurred steadily, requiring the full **3 months** for complete clarification. The primary wine extract and the recovered solid residue from the fermentation of sample **1**-conducted with a **1:1.5** hydromodule at **18–22° C** with 0.13%. cerevisiae and 18% exogenous sucrose-maintained the chemical composition detailed in Table 4.

To determine the distribution of polyphenols between the liquid extract and the insoluble solid residue for sample (**1**), calculations were derived from the biochemical profiles in Table 1. Given that the total phenolic content in **100 g** of exocarp sample (**1**) is **2.60 g**, the total polyphenolic mass in the **400 g** substrate is **10.40 g** (100%). Based on the data in Table 4, the amount of total polyphenols in **700 ml** of the resulting extract is **6.93 g** (**0.99 x 7**), representing 66.63% of the initial mass. The freshly pressed solid residue, with a dry matter content of **11.87 g/100 g** (**257.6 g**), retained **3.46 g** of total polyphenols (**1.34 x 2.58**), or 33.37% of the total weight in the **400 g** sample.

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	º 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

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).

During the maturation of the hydro-ethanolic extract at room temperature, the initial fraction of polyphenols-predominantly ellagic acid-precipitates within **3 days**. In contrast, the spontaneous aggregation of the remaining polyphenolic complex occurred gradually over **3 months**. Table 5 illustrates the temporal shift in the ratio between soluble and insoluble polyphenols during the sedimentation of the wine extract derived from sample (**3**). These experimental conditions utilized a **1:1.6** hydromodule at **18–22°C**, with the addition of **0.13 wt. %** dry *S. cerevisiae* and **18 wt. %** sucrose.

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 analytical data, the total phenolic potential of **650 ml** of the wine extract is **11.05 grams** (**1.70 x 6.5**). This recovery represents 76.74% of the total polyphenolic mass found in **400 grams** of exocarp sample (**3**), which corresponds to $\approx$ **80 mg/gram** of dry matter. After a three-month maturation period, the ratio between the insoluble and soluble polyphenolic fractions in the extract from sample (**3**) was determined to be 26.00/74.00 (%/%). Consequently, the potential of **650 ml** of this extract for the insoluble polyphenolic fraction is **2.87 grams**, or 19.93% of the total mass in the **400 g** sample (**3**).

To evaluate the significance of these findings, comparisons with established extraction methodologies are instructive. For example, research into the release of ellagic acid from its bound forms via ultrasound-assisted alkaline hydrolysis (**32 min**, **1/48** agitation rate, **1.5 mol/l NaOH**) yielded $59 \pm 3 \, \varvec{m} \backslash \varvec{g}$ of punicalagin and $1457 \pm 71 \, \varvec{m} \backslash \varvec{g}$ of ellagic acid per **100 g** of dry peel [30]. Similarly, mechanochemically-assisted extraction at a liquid-to-solid ratio of **50 ml/g** and a temperature of **78° C** for **67 minutes** identified 12 phytochemicals, with a total phenolic content reaching $258.09 \pm 1.35 \, \varvec{m} \backslash \varvec{g}$ **GAE/g** dry weight of the extract [31].

Further studies investigating the recovery of bioactive compounds using pressurized water-ethanol mixtures determined that optimal conditions (200° C, 77% ethanol) resulted in a total phenolic yield of $164.3 \pm 10.7 \, \varvec{m} \backslash \varvec{g}$ and a punicalagin yield of $17 \pm 3.6 \, \varvec{m} \backslash \varvec{g} / \varvec{g}$ of dry matter [32]. While these results indicate a higher total phenolic extraction efficiency compared to the proposed fermentation-assisted method ($\approx$ **80 mg/g**), the proposed approach remains competitive in the isolation of punicalagins. Additionally, research utilizing ultrasound-assisted extraction with a 50% methanol solution yielded **72.21 mg/g** of total polyphenols [33], a value comparable to the results of the present method. This suggests that while methodologies requiring high pressure, ultrasonic treatment, and chemical reagents may achieve slightly higher yields, the proposed fermentation-assisted process offers a significantly streamlined, cost-effective, and environmentally sustainable alternative.

4.2 Commercial analogues of the target products

1. Spanish Tannat is a dry, tart red wine produced from red grapes grown in the Basque Country. Synonyms for this variety include Madiran, Arriaga, Mustru, and Bordelaise Belt. French Tannat is a robust, notably tart wine with deep color and significant aging potential; it is also frequently used for blending with strong Cabernet varieties. In Uruguay, producers typically combine Tannat with more subtle and diametrically opposed varieties.

In Russia, prices for dry, tart (tannic) wines start from 160–300 rubles per 0.75 liters. Popular varieties with pronounced astringency-such as Cabernet Sauvignon, Saperavi, Merlot, and Matrasa-fall within a price range of 500–2000 rubles (as of March 11, 1926: 1 USD/79.22 Russian rubles).

1. Ellagic acid, chemical formula $C_{14}H_6O_8$, is a powerful antioxidant found in pomegranates, berries (strawberries, raspberries), and nuts. It protects cells from free radical damage and possesses anti-inflammatory, cardioprotective, and potential anti-cancer properties. In cosmetology, it is utilized as a whitening and protective agent for the skin, while in medicine, it is used to support the cardiovascular system. However, due to its poor solubility in water, its water-soluble derivatives are employed instead.

Soluble salts of ellagic acid are obtained through its reaction with alkalis, such as sodium hydroxide ($NaOH$) and potassium hydroxide (KOH), or with amino acids and other bases. These reactions result in the formation of water-soluble derivatives (ellagates) suitable for medical and cosmetic use. These salts may include sodium or potassium salts of ellagic acid, or its complexes with amino acids. Soluble salts, such as sodium salts, are used in dietary supplements and cosmetics for their antioxidant and anti-inflammatory properties. Ellagic acid is treated with a base (e.g., sodium hydroxide) in a suitable solvent, causing the deprotonation of one or more of its $-OH$ groups, which leads to the formation of the corresponding salt.

Ellagic acid is a component of the following food additives and berry extracts: Xantho Plus, Smokerade, URX, and ZenThonic. Although ellagic acid is not a vital substance for humans, it has the potential to be one of the most effective anti-cancer compounds. Shaanxi Hongkang Biological Technology Co., Ltd. offers a cosmetic extract containing up to 95% ellagic acid, produced via HPLC, at a price ranging from 186.58 to 201.48 USD per 1 kg. In Russia, ellagic acid preparations (CAS No. 476-66-4, chemical formula $C_{14}H_6O_8$, molecular weight 302.19) with 40–95% purity are offered at a price of 17.00–34.50 USD per 5 grams.

1. Gelatin-tannate (Adiarin) is available as a powder for oral suspension to treat diarrhea in 250 mg dosages. It is also available in capsule form (composition per capsule: gelatin-tannate – 500 mg, corn starch – 73.3 mg, magnesium stearate – 11.7 mg). Medications from manufacturers such as Novintethical Pharma (Switzerland), Noventure (Spain), and Labomar (Italy) cost approximately 3.50–6.20 USD per pack of 8–10 sachets (250 mg each) in Russian pharmacies.
2. Protein-carbohydrate flour and dietary fiber products have gained popularity due to their role in reducing the risk of heart disease and other chronic conditions. Powder produced by direct drying and grinding of pomegranate peel contains alkaloids; consequently, it is used to make tea effective against salmonellosis, dysentery, and other dangerous intestinal infections. This powder is available at the NaturoMama bio- and organic products store (Moscow) for 490 rubles (over 5.00 USD) per 100 g.

However, if the maximum amount of monosaccharides and polyphenols is removed from the pomegranate peel, the remainder consists mainly of dietary fiber, which aids in managing excess weight and obesity. In study [34], the content of water, protein, fat, pectin, hemicellulose, cellulose, lignin, neutral soluble fiber (NRF), and acid-soluble fiber (CRC), along with titratable acidity and pH, were determined for both pomegranate peel and the remaining alcohol-insoluble solid residue. Functional properties—including bulk density, swelling capacity, water retention capacity (WRC), oil retention capacity (ORC), and color—were also investigated. This study found that the dried peel and the alcohol-insoluble residue 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), and 18.02% and 23.50% acid-soluble fiber (CRC). The pectin, hemicellulose, cellulose, and lignin content in these two preparations were 27.90% and 29.54%, 10.88% and 13.80%, 26.22% and 30.86%, and 5.67% and 7.47%, respectively.

Essential amino acids in both preparations include threonine, valine, isoleucine, leucine, phenylalanine, and lysine. The predominant essential amino acid is lysine, which is highly significant for poultry feed production. The lowest concentrations are isoleucine (in the peel) and threonine (in the residue). The alcohol-insoluble residue, reduced to a fine powder, was deficient in methionine and tryptophan. Attempts to partially replace flour in shaped bread with this residue led to decreased ash content and increased mass fractions of water, protein, and fiber.

It is noted that pomegranate peel has a higher energy value than alfalfa, apple pomace, citrus peel, corn silage, or tomato pomace. In terms of crude protein content (36 g/kg of dry matter), the peel is inferior to pomegranate seeds (154 g), but it surpasses seeds in ash elements (54 g vs 24 g) and soluble carbohydrates (695.9 g vs 135 g/kg of dry matter) [35]. If polyphenol levels are too high, they negatively impact protein digestibility, preventing the direct use of raw peel as livestock feed. However, if the polyphenol dose is adjusted to levels acceptable for dietary supplements, they serve as necessary antioxidants for weight management. Clinical trials on bread, where 2.5%, 5%, and 7.5% of flour was replaced by pomegranate peel powder, showed that enrichment with fiber and antioxidant components can produce bread varieties suitable for weight loss [36].

Nutritionists recognize that the ratio between insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) is a critical indicator for functional food ingredients. A health-beneficial IDF/SDF ratio ranges from 1 to 2.3. In pomegranate peel, this ratio varies from 1.6 to 1.7, a balance considered successful for meeting these evaluation criteria[37].

4.3 Main aspects of the technical result

In this study, an alternative solution was developed, the primary elements of which include:

1. Mixing coarse pomegranate peel (utilizing freshly harvested or aged fruits of intensely colored varieties) with purified drinking water and granulated sugar in a weight-to-weight ratio of **1:1.5–1.6:0.18**, respectively.
2. Fermentation under anaerobic conditions for a duration of **14 days** at a temperature of **18–22°C** using *Saccharomyces cerevisiae*.
3. Recovery of the resulting wine extract (comprising **74–75%** of the total volume) via gauze filtration, followed by the mechanical pressing of the post-fermentation peel residue to isolate the absorbed fraction (**25–26%** of the total volume). Both fractions of the extract are subsequently combined.
4. Sedimentation of the separated extract for a period of **2.5-3 months**, facilitating the removal of insoluble polyphenols through natural and induced precipitation.

The target products of this process are: discrete aggregates of ellagic acid and α —glycosides, gelatin-tannate, and a clarified extract exhibiting the texture and characteristics of a dry red wine.

A graphical representation of these procedural steps is often more effective for conveying technical results than a standard textual description.

Figure1. 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.

Figure2. 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 1 illustrates that the established method for extracting polyphenols from the peel into solution offers simplicity, ease of use, and high practicality, and is based on alcoholic fermentation.

Alcoholic fermentation is a specific and prevalent form of fermentation (the metabolic process by which microorganisms, such as yeast, decompose organic substances to release energy), wherein sugars are converted into ethanol and carbon dioxide [38].

The recovery of pure antioxidants (punicalagin, ellagic acid) from pomegranate peel extracts for the pharmaceutical and food industries has been and remains a subject of ongoing study [39–40].

Despite its advantages for separation, solid-phase extraction (SPE) presents significant drawbacks. Although SPE has been utilized as an extract separation method for decades, it is typically restricted to relatively small scales for producing high-purity products or performing exceptionally complex separations. The sorbent requires regeneration after each use to restore its adsorption capacity. Furthermore, selecting a sorbent that exhibits both high adsorption and efficient desorption-releasing the polyphenols-is challenging. Even when utilizing sorbents with the advantages of advanced synthetic functional materials, a compromise between cost, environmental impact, and extraction efficiency is often required. Additionally, the adsorption capacity of many sorbents faces practical limitations when processing highly concentrated raw materials. Capacity values typically range from to of analyte per gram of sorbent [41].

Figure 2 illustrates the isolation of three distinct precipitates from the wine extract through a three-stage process: (1) self-association of ellagic acid driven by hydrogen bonding and hydrophobic interactions; (2) gelatin-triggered precipitation of the remaining tannins following the removal of ellagic acid; and (3) self-association of the residual -glycosides after the removal of both ellagic acid and astringent tannins.

As shown, this fractional separation of the wine extract-derived from the fermentation of pomegranate peel-coupled with its complete clarification, serves as a compelling example of the efficacy achieved by repurposing an established process for a novel application.

The peel also contains significant quantities of sugars, proteins, and fibers which, similar to the precipitated polyphenols, have the potential to be utilized in the development of high-demand products.

The transition from single-product to multi-product manufacturing is often a key factor in process rationalization, particularly for low-productivity operations [42].

Figures 3 and 4 demonstrate the successful reorientation of the biotechnological process from the synthesis of a single end-product to the joint production of multiple end-products (coproduction), as experimentally validated in this study.

4.4 Scientific experience on fermentation of pomegranate peel using S. Cerevisiae

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

The main disadvantage of these methods, which target the conversion of the lignocellulosic fraction into sugar and then ethanol, is the required use of nitric acid, which poses a potential danger to personnel and the environment. Additionally, thermal hydrolysis using an aggressive nitric acid solution is more characteristic of industrial chemical technology than food technology, requiring chemically resistant reactors and significant energy expenditure for the production of commercial volumes of ethanol.

Among the well-known developments in the field of bioethanol production is a method [45] that includes 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 hydrothermal pretreatment (e.g., at 115°C for 40 minutes) to remove pectin and phenolic substances that interfere with cellulose accessibility, making the cellulose more available;
- Saccharification: The residue from the hydrothermal treatment is processed using cellulase enzymes capable of breaking down the fiber into fermentable sugars (glucose);
- Yeast (*S. cerevisiae*) is added to the saccharified 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, or meat peptone). At an *S. cerevisiae* concentration of 30 g/l, 90.4% of the initial sugar was consumed; the maximum ethanol yield was 12.9 g/l, corresponding to 95.09% of its theoretical yield.

Although this method does not use HNO3, making it cleaner than the previous two methods, it also has significant drawbacks. Its primary disadvantage is that hydrothermal treatment causes the irretrievable loss of not only a significant portion of the pectin and phenolic substances, but also the natural sugars of the peel and the soluble fractions of nitrogenous and mineral substances that nourish the yeast. If we assume the peel is rich in simple sugars and that its lignocellulosic complex is primarily composed of lignin rather than cellulose (the complete cleavage of which produces glucose), the inefficiency of this approach becomes evident.

Another proposed method focuses on modifying the initial structure of the natural oligo- and polysaccharides in the peel [46] and includes two main stages. First, the peel is dried, crushed, and treated for 2 minutes with hot (100°C) steam to inactivate endogenous yeast (stage 1). Then, water (10 l / 1 kg of peel) and *S. cerevisiae* (25 g / 10 l) are added, and fermentation is carried out under anaerobic conditions for 72 hours at 25°C with stirring (380 rpm). The main result of this rigorous hydrothermal treatment was a decrease in the molecular weight of oligo- and polysaccharides from a maximum of 705 kDa to 26 kDa. *In vitro* experiments showed that these polysaccharides were utilized more easily by *Bifidobacterium breve* and *Lactobacillus plantarum* bacteria than those from control samples. Furthermore, this treatment led to an increase in quantitative tannin extraction; the tannin content in extracts increased to 70 g/100 g of dry extract (compared to 30–35 g of tannin / 100 g of dry extract in spontaneous fermentation controls). The yield of dry extract obtained by lyophilization ranged from 28%

to 50% relative to the dry peel mass. The yield was significantly lower in samples fermented with *S. cerevisiae* compared to control samples, likely due to the yeast's consumption of peel components such as free sugars, mineral salts, and proteins. Conversely, the *S. cerevisiae* extracts were easier to process than control samples, which contained more free sugars and exhibited higher hygroscopicity.

From the above data, it appears that fermentation with *S. cerevisiae* at a peel-to-water ratio of 1/10 (kg/l) produced an extract containing 70 g of polyphenols / 100 g of dry extract. With a dry extract yield of 28% of the total dry peel weight, the potential of this method for polyphenol recovery is 196 g / 1 kg of dry peel.

If we assume an extraction rate of 100%, there were 19.6 g of polyphenols in 100 g of the initial dry peel. If the initial dry peel contained 13.72 g of polyphenols per 100 g, the degree of extraction would be high, amounting to 70%.

At the same time, incorporating hydrothermal treatment into our technology would significantly complicate extract separation and negatively affect the organoleptic properties (color and aroma). It could also potentially enrich the extract with undesirable viscous biocolloids.

4.5 Advantages and disadvantages of known methods of separation of pomegranate peel extracts

The precipitation of polyphenols from pomegranate peel extracts represents a critical area of research at the intersection of food chemistry, pharmaceutical science, and environmental ecology. The primary objective of such studies is typically either the purification of the extract through the removal of excess tannins [47] or the concentration of active substances via vacuum evaporation or membrane filtration [48].

This field is subject to active investigation, as it constitutes a pivotal step in isolating pure antioxidants, such as punicalagin and ellagic acid, for the pharmaceutical and food industries.

Current research is pursued in three primary directions: natural sedimentation (aging), induced precipitation, and adsorption onto macroporous resins.

4.5.1 Natural (spontaneous) deposition

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

- **Self-association**: Upon cooling or prolonged storage, ellagitannin molecules can aggregate due to hydrogen bonding and hydrophobic interactions. This phenomenon is frequently observed during the storage of aqueous extracts.

Pomegranate polyphenols, particularly punicalagins, are susceptible to spontaneous precipitation for several reasons:

- **Hydrolysis**: Under enzymatic or acidic influence, punicalagin can hydrolyze to release ellagic acid, which has extremely low solubility in water and settles as a crystalline precipitate.
- **Interaction with proteins**: If residual proteins are present in the extract, they form complexes with tannins that enlarge and sediment over time.
- **Temperature factor**: When a saturated hot extract cools, the solubility of polyphenols decreases, leading to turbidity and natural precipitation.

Another significant aspect concerns the use of environmentally friendly deep eutectic solvents (DES). Specifically, a glycerin-urea mixture has been evaluated as such a solvent [49]. The study is notable for describing how modifying the solvent composition and adding water induces the loss of polyphenolic complexes.

The precipitation of polyphenols with gelatin is a conventional method based on the formation of insoluble complexes between proteins and tannins. In the case of pomegranate peel, which is exceptionally rich in ellagitannins (such as punicalagin), this process has proven to be particularly effective [50].

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

The primary factors influencing the yield of polyphenols include:

- **Gelatin preparation**: Gelatin must be hydrated in cold water (1:5 ratio) and then dissolved at 50–60°C prior to being introduced into the extract.
- **pH of the medium**: Optimal precipitation typically occurs near the isoelectric point of gelatin (pH 4.7–5.2).
- **Temperature**: An increase in temperature can enhance the solubility of the complex; therefore, the process is often conducted at room temperature or cooled to 10–15°C.
- **Gelatin concentration**: An excess of protein can cause the opposite effect, leading to the stabilization of the colloid.

4.5.2 Induced (targeted) precipitation by chemical agents

This represents the most extensively researched method, leveraging the capacity of polyphenols-particularly ellagitannins, which are highly prevalent in pomegranate peel-to bind to specific macromolecules.

Researchers and technologists employ various methods of induced precipitation to isolate target compounds 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 for the induced precipitation and subsequent crystallization of ellagic acid from an aqueous extract is the hydrolysis of ellagitannins [51]. This study describes the production of high-purity ellagic acid (90%) through acid hydrolysis. The method relies on the thermal decomposition of soluble tannins in an acidic medium into ellagic acid, which, due to its extremely low aqueous solubility, forms a precipitate. It has also been reported that utilizing distilled water at 60°C, followed by acidification with acetic acid, enables the effective conversion of polyphenols into the insoluble form of ellagic acid [52].

The core principle of the ultrafiltration separation method involves passing the processed extract through membranes with specific pore sizes to concentrate high-molecular-weight compounds, such as punicalagin. Research has investigated the application of ultrafiltration (using a 10 kDa membrane) as a means of "physical deposition" (retention) of polyphenols. A 118% increase in ellagic acid concentration within the retentate was achieved [53].

4.5.3 Modern deposition alternatives

Although conventional precipitation is still utilized, modern laboratories increasingly favor adsorption on macroporous resins and ultrafiltration.

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

In modern industry, the term "precipitation" is frequently replaced by "adsorption on macroporous resins" [56]. A recent review details the purification of punicalagin using Amberlite XAD-16 resins and medium-

pressure liquid chromatography (MPLC) technologies [55]. A combination of ultrasound and flash chromatography for the fractionation of polyphenols has also been evaluated [57]. This approach enables the selective immobilization of target fractions on the sorbent.

In recent years, research and industrial production of high-purity pomegranate polyphenols have prioritized macroporous adsorption resins such as HPD100, HPD400, HPD-417, HPD450, and HPD600 from Cangzhou Bon Adsorber Technology Co., Ltd. (Hebei). Developed in collaboration with the National Engineering Scientific Research Center for Natural Medicines, these HPD resins offer significant improvements over traditional adsorption resins, which often suffer from poor mechanical stability, low adsorption selectivity, and limited capacity [58].

The production of commercial-grade polyphenols from pomegranate peel is an industrial process involving the extraction of active compounds-such as ellagitannins (punicalagin, ellagic acid, etc.)-from dried peel using water, ethanol, or aqueous-ethanol mixtures. The resulting extract is concentrated and separated to produce a standardized powder for use as an antioxidant in the nutraceutical, cosmetic, and food industries [59–60].

The core of the extract separation method using macroporous resins (e.g., Amberlite XAD) involves passing the prepared extract through a column where polyphenols are retained by the resin and subsequently eluted (desorbed) using ethanol. The key procedural steps are as follows:

- Preparation of raw materials: The pomegranate peel is separated, dried, and pulverized into a powder.
- Extraction: The peel powder is processed with suitable solvents (hot water or hydro-alcoholic mixtures) to extract the polyphenols.
- Fractionation (adsorption chromatography): The extract is filtered, concentrated (e.g., via vacuum evaporation), and passed through a column containing a resin or silica gel capable of adsorbing specific polyphenols.
- Adsorption: The target polyphenols are selectively adsorbed onto the resin matrix.
- Purification and desorption: The resin is first washed with deionized water to remove impurities until the effluent is clear. Residual water is removed by vacuum aspiration. The polyphenols are then eluted using an organic solvent (e.g., five column volumes of 80 wt% ethanol). The resulting ethanol-rich eluate is collected, concentrated under reduced pressure, and the ethanol is simultaneously recovered for reuse.
- Drying: The desorbate is dehydrated using a spray dryer or vacuum dryer to obtain a final powder.

Main target components:

- Punicalagins: Highly bioactive tannins that metabolize into ellagic acid within the body.
- Ellagic acid: A potent antioxidant with established anti-inflammatory properties.

The isolation and purification of polyphenols via macroporous resins form the technical foundation for most industrial applications in this field. These processes typically follow a scheme including peel drying, grinding, aqueous extraction with heating and agitation, extract separation, and concentration to approximately one-third of the initial volume.

This framework was developed primarily to meet commercial objectives in the production of pomegranate extracts for biomedicine, reflecting the increasing global demand for these polyphenols [61].

However, this approach has significant drawbacks. As previously noted, the fine particles of the feedstock tend to agglomerate and foul the system during heated agitation, while excessive sugars and colloids leach into the solution. Furthermore, the multi-stage nature of this scheme incurs high energy costs for drying, extraction, and the concentration of the primary extract prior to separation.

Since the concentration of polyphenols in the peel is significantly lower than that of simple sugars and fibers, the ratio of the pure target substance to the initial dry peel mass can reach 1:100 (w/w) or higher-particularly when isolating individual compounds rather than total polyphenols. Consequently, hundreds of kilograms of resin may be required to isolate just 1 kg of pure polyphenols from 100 kg of processed material.

This inefficiency is reflected in the high market price of high-purity polyphenols; for example, 500 mg of European-produced encapsulated pomegranate extract with 40% punicalagin content can cost 47.31 euros.

In this context, attempts to enhance profitability solely by optimizing existing technological parameters are likely to be insufficient. Instead, a fundamental simplification of the process is required to reduce costs, alongside a strategic shift toward utilizing the other components of the peel. Adopting a co-production framework is the central objective of the current study.

4.6 Statement of the appropriateness of the proposed approaches

The expediency of our proposed approaches, based on their technical simplicity and alignment with food production conditions, is evident:

- **Direct fermentation (polyphenol extraction)** of coarse-grained raw peel obtained from manual or mechanical peeling is conducted for 2 weeks at 18–22°C. Water and sugar are added such that the combined endogenous and exogenous sugars are sufficient to produce a wine extract with a residual sugar content (2–4 g/100 ml) and alcohol strength (9–13 vol.%) comparable to European dry wines. **Justification**: This is significant because established methods for extracting polyphenols from pomegranate peel are technologically more complex. They typically require drying, grinding, and aggressive acid-water hydrothermal treatment or high-temperature steam processing before fermentation. Such methods are technically demanding and lead to significant physical degradation

of the material. In our approach, these drawbacks are avoided by using whole peel pieces and a "cold" (18–22°C) enzymatic treatment. Monitoring the conversion of peel monosaccharides and added sugars to ethanol showed that fermentation under these conditions is nearly complete within 1.5-2 weeks.

- **Separation of the resulting extract** through natural and gelatin-induced precipitation of polyphenols. Separating the extract via column chromatography requires high technical competence and specialized facilities for dietary supplement production. While necessary for the complete purification of polyphenols from associated substances (simple sugars, acids, biocolloids, and minerals), it is also prohibitively expensive. Our objective was to simplify the recovery process as much as possible, utilizing the time required for the extract to fully clarify and mature into wine. Consequently, rather than a single collection, we implemented a phased intervention to obtain several polyphenol fractions of varying polarities and solubilities. We chose an approach where the primary extract is maintained for 2–3 months-the minimum duration for wine maturation. During this period, three stages of precipitate separation are performed: the first at the beginning, the second immediately following via gelatin induction, and the third at the conclusion of the settling period. The polyphenol-containing precipitates are filtered and washed with water until colorless, then dried at temperatures below 60°C and ground to produce three dry polyphenol fractions with 32–49% purity. The remaining clarified extract is stored in tanks as a dry red wine ready for bottling; by analogy with commercial Spanish tart wines, it has been designated "AzTanna."
- **Acceptability of the proposed process to the technical conditions of food production** The equipment required for the proposed technology is identical to that used for fermenting must with pomace, and follows the same operational requirements. Figure 5 illustrates the primary devices needed for implementation: **A vinifier** (e.g., from BeerMachines) for fermenting must together with solids (skins, seeds) with automatic residue discharge (Fig. 5, a) is a professional vessel equipped with temperature control and stirring mechanisms ("cap" management) to accelerate color and flavor extraction. Unlike conventional fermentation tanks with simple water seals that only isolate the wine from oxygen, a vinifier actively controls the fermentation process. It is designed for red wine production (fermentation on the skins), ensuring constant contact between the must and the solid phase, which is otherwise impossible without manual intervention. These units include discharge hatches for pomace and taps for sampling and level control. The vessel is typically filled to 75% capacity. **A screw (auger) press** (Fig. 5, b) is used for the transportation, dewatering, and compaction of solid residues. The mechanism, featuring a rotating screw within a perforated housing, significantly reduces waste volume and moisture content. Solid waste enters the loading hopper, is moved by the auger, and is pressed to remove moisture through a mesh screen. The auger diameter often increases while the gap decreases toward the outlet to maximize pressure. Both the body and screw are constructed from stainless steel (AISI 304 or 316) for corrosion resistance. The closed design prevents odor dispersal, ensuring high efficiency and low power consumption. **An intermediate tank** for collecting gravity-run must and pressed liquids (Fig. 5, c) ensures an airtight environment to prevent oxidation and maintain wine quality. Constructed from food-grade stainless steel (AISI 304/316), it includes maintenance hatches, drain taps, pump connections, and level

gauges. These are installed adjacent to the vinifier to collect the liquid phase before it is transferred to the settling tanks. **A conical-bottomed wine container** with an automatic CIP (Clean-in-Place) system (Fig. 5, d) is a specialized food-grade tank. It is equipped with rotating spray heads to remove sediment from the walls without disassembly. The conical bottom facilitates the natural collection and easy removal of sediment. **A Nutsche filter dryer** (Fig. 5, e) with a hinged bottom for sludge discharge consists of a vertical cylindrical tank. A vacuum pump, connected via a receiving tank, creates the necessary pressure drop. The lower section contains a perforated false bottom covered with a filter medium (mesh or cloth). Some models feature a thermoregulation jacket for temperature control or cake drying. The solution is fed into the upper section, and the vacuum accelerates filtration by drawing the liquid through the filter layer. After filtration, the precipitate can be rinsed and subsequently dried within the same unit using hot gas (convection) or jacket heating (conduction). This multi-functional design optimizes the process by combining filtration, washing, and drying into a single stage, yielding high-quality dry sediment without intermediate handling.

You will also require specialized food-grade stainless steel containers (tanks) with floating lids for the storage and bottling of the finished wine (such as those from the Chinese manufacturer Cassman, which feature a port for injecting a small amount of nitrogen to maintain a low positive pressure environment and completely isolate the wine from oxygen).

The application of established processes and devices in this novel method is justified by the achievement of a new technical result arising from their unique combination and application in a new field. This outcome would not be obvious to a specialist, even if the individual components were already known to them. In this case, conventional processes are utilized for a completely new purpose to address a specific challenge. The combined use of established processes and devices simplifies the technological workflow by eliminating redundant stages, which constitutes an inventive step.

• *The proposed method in a concise formulation*

Item 1. A method for the bioprocessing of pomegranate peel, including the stages of extracting target substances into a solution and separating the resulting extract, is characterized by using the peel from manual or mechanical cleaning of fresh or aged pomegranates (large pieces of irregular shape) as the starting material. First, the peel is weighed and rinsed via a water spray. The peel, water, and granulated sugar are then fed into a vinifier in a weight ratio of 1:1.5–1.6:0.18, respectively, along with Gervin GV1 dry wine yeast (at a concentration of 0.25 g per liter of the fermentation mixture). Fermentation is conducted for two weeks at a temperature of 18–22°C. Subsequently, the primary liquid phase (74–75% of the total extract volume) is decanted, and the insoluble peel residue is discharged from the vinifier and pressed to recover the secondary extract (25–26% of the total extract volume).

Both liquid fractions are filtered through gauze to remove macro-impurities and collected in a primary extract receiver. The combined extract is transferred to a conical-bottomed container for settling in three distinct stages, each concluding with precipitate separation using a Nutsche filter. The first precipitate is separated after three days. A 5% gelatin solution is then added to the remaining extract, which is left for

an additional 3 days before the second precipitate is separated. The third precipitate is isolated at the conclusion of the total clarification period (3 months). The precipitates collected by the Nutsche filter are dried in situ and then ground separately in a disintegrator into a fine powder, yielding fractions of ellagic acid, gelatin tannate, and alpha-glycosides with polyphenol purities of at least 50%, 38%, and 47%, respectively. The clarified extract (95% of its original volume) is stored as a dry red wine designated "Aztanna" (with an alcohol content of 9–14 vol%, depending on the batch). Individual batches are blended to achieve a standardized strength of 11 vol%. Finally, the insoluble peel residue is dried in a roller dryer and pulverized in an integrator to produce a powdered dietary fiber additive containing 3.8-4.00 g/100 g (dry weight) of protein and 63–67% crude fiber. The yield of this additive is 11.86–16.25 g per 100 g of the original raw peel.

Figure 6 shows a complete diagram of the method we have developed and proposed for producıng clots of varıous polyphenols and dry red wıne from pomegranate peel.

Item 2. The method of bioprocessing pomegranate peel according to Item 1, characterized by the filtration of only two precipitates-after three days and at the conclusion of the settling period-to obtain two polyphenol fractions: (1) ellagic acid and (2) a mixture of ellagitannins and alpha-glycosides.

• Prospects for commercialization

The ratio between the substrate and auxiliary materials, along with the target yield, are critical indicators for evaluating production cost fluctuations resulting from technical and organizational innovations within the proposed framework.

This is illustrated using a sample (Peel 3) with an initial total polyphenol content of 3.60 g/100 g (wet weight) and a total sugar content of 10.74 g/100 g (wet weight).

In this scenario, 100 kg of coarse peel is weighed, transferred via conveyor, rinsed, and fed into the vinifier. To this, 160 liters of clean drinking water, 18 kg of pre-sifted granulated sugar, and 62 g of Gervin GV1 dry wine yeast are added. Following two weeks of fermentation at 18–22°C, the decanted liquid phase (120.0 liters) and the liquid recovered from the solid residue via pressing (42.5 liters) are combined. Both fractions are filtered to remove large inclusions and transferred to a primary extraction receiver. The mixed extract, with a dry matter content of 10.4%, is sent to a conical-bottomed container for three-stage settling.

The first precipitate is isolated after three days. To the remaining extract, 2 liters of a 5% gelatin solution (prepared from 100 g of acidic dry gelatin) are added to induce tannin precipitation; after 3 days, the second precipitate is separated. Only gelatin derived from acid hydrolysis is utilized due to its high positive charge. Experimental results indicate that Erbigel acidic dry gelatin (Erbslöh Geisenheim AG, Bloom number 90–100) is optimal, as it dissolves rapidly when hydrated in cold water followed by dissolution in hot water (95°C). The third precipitate is separated at the end of the 3-month clarification period. The precipitates isolated by the Nutsche filter are dried and ground into fine powders, yielding dry

fractions of ellagic acid (670 g), gelatin tannate (150 g), and alpha-glycosides (425 g) with polyphenol purities of 54.30%, 39.49%, and 49.36%, respectively.

The residual extract (154.4 liters) is collected as Aztanna dry red wine (9 vol% alcohol). The insoluble peel residue is processed to yield 16.25 kg of a powdered dietary fiber additive with a protein content of 3.50 g/100 g and a crude fiber content representing 16.25 g per 100 g of the original raw peel.

Based on current market trends, estimated manufacturer prices (set at approximately 50% of retail rates) are as follows: Aztanna dry wine at $3.00 USD per 0.75 L; ellagic acid at $8.00 USD per 5 g; gelatin-tannate at $2.00 USD per 2.5 g; and alpha-glycosides at $7.00 USD per 5 g.

Under these parameters, the total value of wine produced from 100 kg of peel is approximately $617 USD, while the polyphenolic fractions (totaling 1.245 kg) are valued at $1,787 USD, bringing the total to $2,404 USD. If 10 tons of raw peel are processed per season, the projected wholesale revenue is approximately $240,000 USD. While a full profitability analysis requires a production-scale validation, the low operational and non-production costs suggest a high profit margin.

5 CONCLUSION

Consequently, this approach simplifies polyphenol extraction and separation, diversifies the product range, and ensures high product purity. It enables the comprehensive utilization of pomegranate peel biomass in an environmentally sustainable manner, maximizing value addition while minimizing resource expenditure. These improvements collectively enhance the overall economic viability of the proposed technology, aligning precisely with the core objectives of this study.

Declarations

Competing interests

The authors declare that they have no conflict of interest.

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Data Availability Statement

The data and materials of the study are published on Preprints.org under the DOI identifier: 10.20944/preprints202603.1555.v1. The source code and databases are available in the Zenodo repository under an open license.

The data set can be obtained from the corresponding author (hafizov-54@mail.ru) after agreeing on the purposes of use.

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Figures



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.

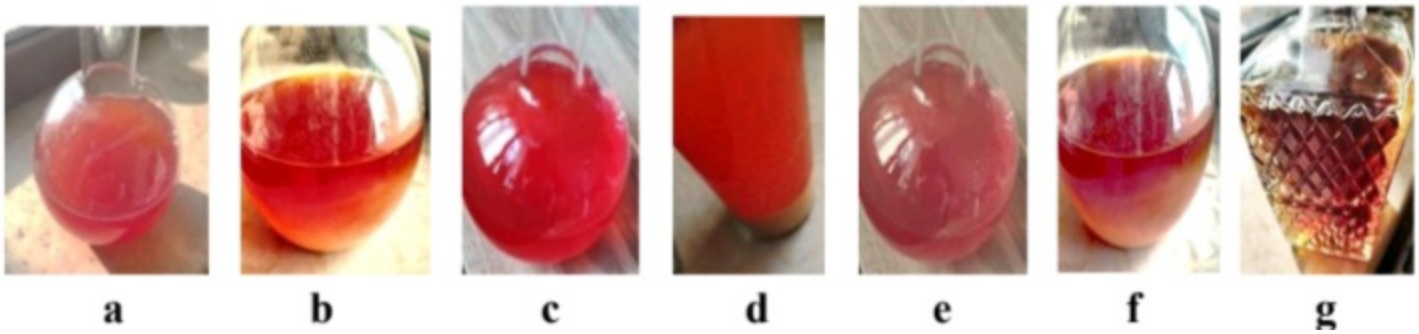


Figure 2

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).

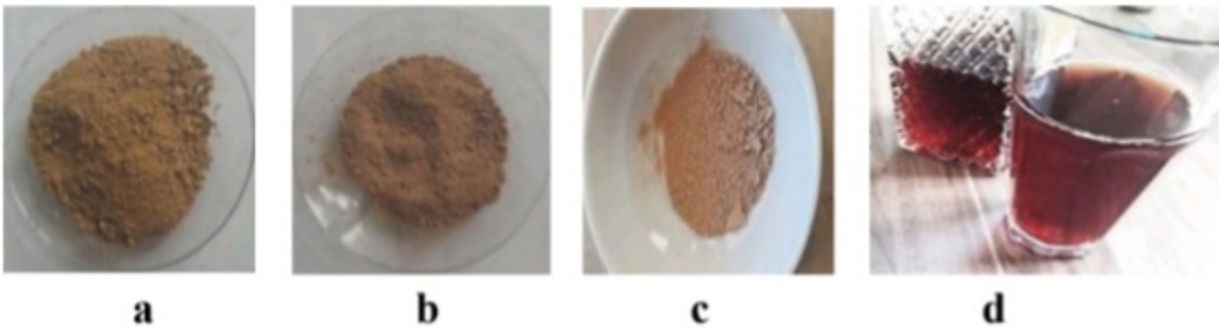


Figure 3

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

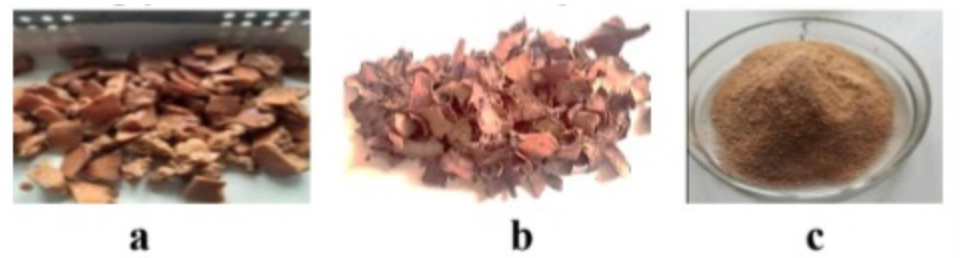


Figure 4

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



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.

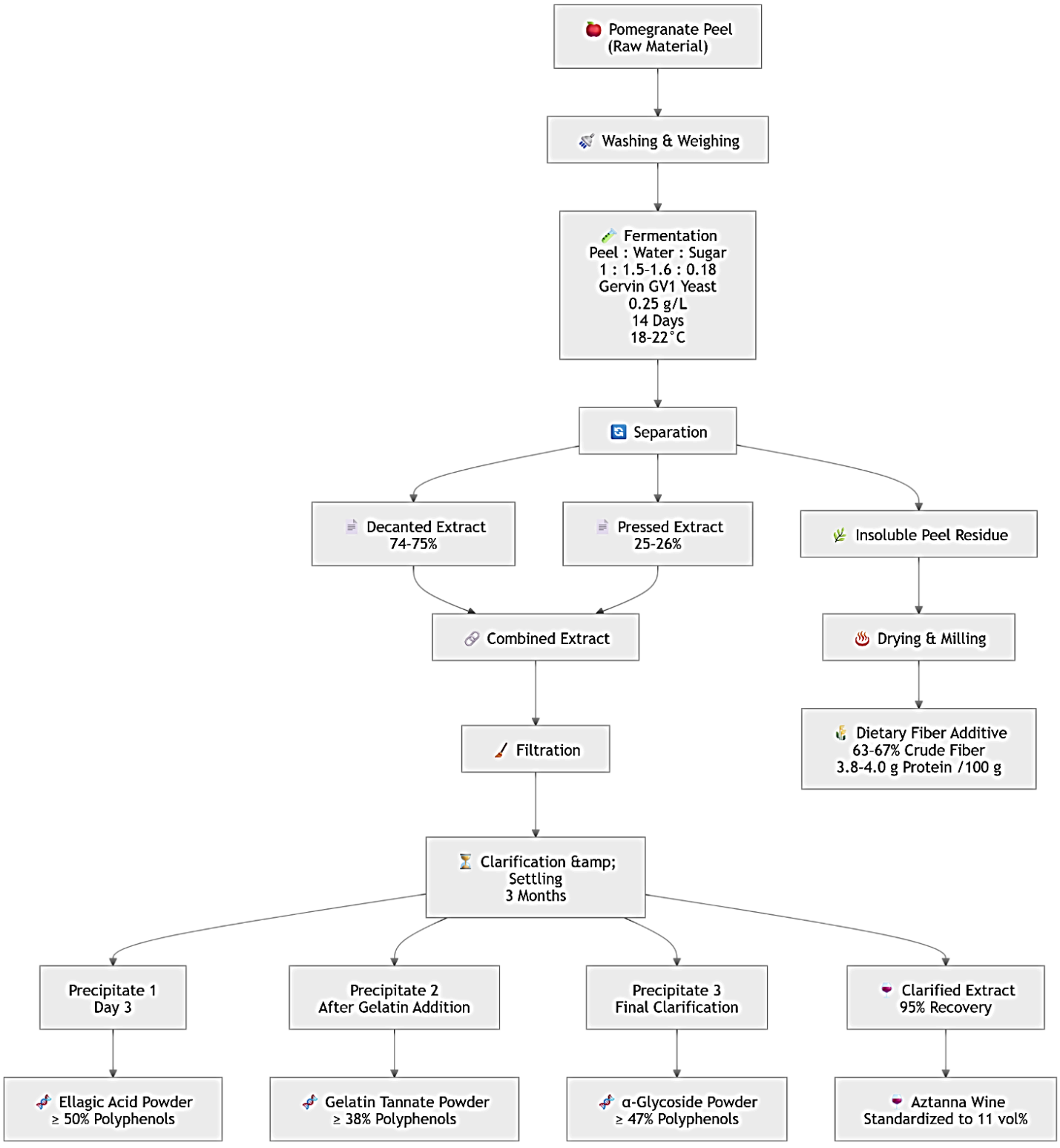


Figure 6

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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GA.png