



EFFECT OF TECHNOLOGICAL PARAMETERS OF BIOCATALYTIC PROCESSING ON JUICE YIELD AND MYCOTOXIN CONTAMINATION OF CERTAIN BERRIES FROM AZERBAIJAN

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ABSTRACT

Fruit and berry raw materials, including wild berries, are currently considered a promising source of polyphenolic compounds of significant biological interest due to their pronounced activity. This study aims to analyze, mathematically model, and systemize the extraction of juice and biologically active substances from the plant raw materials of Azerbaijan using pectolytic, cellulolytic, and amylolytic enzyme preparations. It was established that preliminary biocatalytic treatment of crushed berries (kumquat, cranberry, blackberry, barberry, and wild strawberry) increases the yield of unclarified juices by an average of 6.9–14.04% compared to control samples due to the degradation of the pectin-cellulose framework of cell walls. Using Response Surface Methodology (RSM) in Python, two-factor mathematical models of process macrokinetics were developed, establishing optimal temperature regimes for the hydrolysis of kumquat pulp (60.2°C) and cranberry pulp (62.0°C). Concurrently, a complex profile of contamination with 27 regulated and emerging mycotoxins (penicillic acid, nivalenol, tentoxin, alternariol) was identified using the HPLC-MS/MS method. Intensive enzymatic maceration accelerates the migration of toxic metabolites into the juice phase, highlighting the necessity for continuous biotechnological safety monitoring. The results possess pronounced engineering significance for designing resource-saving processing lines in canning facilities.



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1. INTRODUCTION

In recent years, a steady trend toward establishing waste-free, resource-saving biotechnological cycles has been observed in the global science and industry of fruit and berry processing. The increased interest of researchers in this problem is due to the fact that on a global scale, humanity faces the limitation of plant nutrient sources

and the urgent need to intensify existing production lines. According to official statistical data from the Food and Agriculture Organization of the United Nations (FAO), between 10.0% and 30.0% of all agricultural products worldwide fail to reach the final consumer due to microbiological spoilage and improper storage or industrial processing organization.

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This engineering and technological problem of raw material loss is particularly acute in juice production. Under traditional mechanical methods of extracting the liquid phase (pressing) from wild and cultivated berries, the volume of generated unused waste (pomace) reaches critical values. Experimentally, it has been established that the level of losses and waste averages from 20.0% to 50.0%, depending on the morphology and anatomical structure of the raw material. Specifically, this indicator reaches 48.0% in kumquat processing, 40.0% for cranberries, 41.0% for blackberries, 39.0% for barberries, and 32.0% for wild strawberries. From the perspective of industrial engineering, bringing these secondary raw material resources into a state suitable for practical utilization and extracting target components from them represents one of the most pressing tasks of modern science.

Parallel to this, consumer demand shows a shift toward healthy nutrition and an awareness of the therapeutic benefits of functional products (Huang et al., 2025). This drives the growing popularity of wild and introduced berries harvested under the unique climatic conditions of the Republic of Azerbaijan (Maharramova et al., 2024; Guliev et al., 2022). In modern literature, wild plant fruits are viewed as a rich and promising source of natural polyphenolic compounds with significant biological activity. Numerous fundamental studies have proven that berry polyphenols exhibit a wide range of therapeutic effects, including protecting cellular structures from oxidative stress (Kardum et al., 2014), effective prevention and complex treatment of metabolic syndrome (Sidor et al., 2019), pronounced antimicrobial activity against pathogens (Jurendić & Ščetar, 2021), and antiproliferative effects on tumor cell lines. Furthermore, regular consumption of berry processing products contributes significantly to cardiovascular disease therapy by slowing platelet aggregation and improving vascular elasticity (Golovinskaia & Wang, 2021).

Since natural direct-squeezed juices are the most widespread commercial product and a base ingredient for related sectors of the food industry (Huang et al., 2025; Maharramova et al., 2024), developing engineering approaches to maximize their yield is of strategic importance. The primary barrier to the free outflow of juice during mechanical pressing is the rigid biopolymer framework of plant cell walls, composed of protopectin, cellulose, hemicellulose, and proteins that exhibit screening properties and retain moisture. To overcome this barrier and intensify mass transfer in heterogeneous systems, the biocatalytic method of raw material processing is successfully applied. Utilizing pectolytic and glucoamylase enzyme preparations reduces the dynamic viscosity of the processed mass, degrades the colloidal structure of pectin, accelerates filtration processes, and significantly increases the yield of the most valuable "free-run" juice fractions.

In processing starch-containing fruit and berry materials, α -amylase and glucoamylase are purposefully introduced into multi-enzyme complexes (MECs) for efficient bioconversion of polymers. The primary industrial producers of these enzymes are bacteria of the genus *Bacillus* and microscopic fungi of the genus *Aspergillus* (Kilic & Gunes, 2024; Ben Taheur et al., 2019; Gangakhedkar et al., 2025; Mahmoodi et al., 2017; Volchok, 2016). In addition to increasing the volume of extracted juice, the enzymatic destruction of cell shells promotes a more complete leaching of biologically active substances, natural colorants, macro- and microelements, carbohydrates, and tannins into the liquid phase (Aliyeva & Mammadov, 2023; Butova et al., 2020; Gangakhedkar et al., 2025; Maharramov, 2017; Nemchinova, 2022).

However, the efficiency of utilizing biocatalysts in engineering processing lines depends on a complex multi-factor correlation with macrokinetic parameters: raw material pretreatment, medium pH, enzyme dosage, operating temperature, and hydrolysis duration (Alekseenko & Bystrova, 2015; Pui & Saleena, 2023). It is known that the catalytic activity of enzymes can be inhibited or synergistically enhanced by internal factors of the substrate, including the presence of heavy metals or polyphenolic screening complexes (Krikunova et al., 2020; Lyagin & Efremenko, 2019).

A critically important aspect linked with biotechnological processing is the microbiological safety of the raw materials. Due to their high enzymatic activity and ability to survive across wide environmental ranges, wild berries face the risk of contamination by microscopic fungi of the genera *Aspergillus*, *Mucor*, *Penicillium*, *Fusarium*, *Alternaria*, *Stachybotrys*, and *Trichoderma*. During their life cycle, these molds synthesize dangerous mycotoxins that act as poisons and inhibitors of biological targets, presenting a direct threat to human health (Wen et al., 2016). Along with regulated toxins (ochratoxin A, patulin, deoxynivalenol, fumonisins B1 and B2, T-2 toxin, aflatoxins, zearalenone), "new" unregulated mycotoxins (penicillic acid, nivalenol, sterigmatocystin, mycophenolic acid, moniliformin, citrinin, enniatins, beauvericin, tenuazonic acid, tentoxin, alternariol, and its methyl ether) are increasingly identified in berry materials (Sedova et al., 2022). In the juice production engineering cycle, it is crucial to evaluate how preliminary enzymatic maceration of the pulp affects the extraction and redistribution of these toxic metabolites between the solid (pomace) and liquid (juice) phases.

Evaluating the impact of preliminary biocatalytic processing parameters on the juice yield and the nature of associated mycotoxin contamination for kumquat, cranberry, blackberry, barberry, and wild strawberry is performed in the Republic of Azerbaijan for the first time.

Consequently, the engineering problem addressed by this study is the necessity of mathematical justification, modeling, and optimization of the temperature-time regimes of pulp biocatalysis to achieve maximum technological juice yield while ensuring strict control over the microbiological and mycotoxicological safety of the final product.

2. MATERIALS AND METHODS

2.1. Research Objects and Sampling Procedure

The research objects comprised fresh berry crops from the 2024–2025 harvest seasons, growing in various ecological niches of the Republic of Azerbaijan. Kumquat (*Fortunella Swingle*) fruits were harvested in the Lankaran-Astara subtropical region of Azerbaijan (Guliyev et al., 2022). Wild samples of cranberry (*Oxycoccus*), blackberry (*Rubus*), barberry (*Bérberis*), and wild strawberry (*Fragaria L.*) were collected in the Lankaran-Astara and Ganja-Kazakh regions during their physiological ripening seasons. A total of 135 field samples of raw materials at the technical maturity stage were selected and analyzed within the framework of this comprehensive monitoring

2.2. Commercial Enzyme Preparations

To achieve targeted bioconversion of cell wall polymers, commercial enzyme preparations of industrial manufacture allowed for use in canning and winemaking industries were applied:

- *Pectinex BE XXL* and *Pectinex Yieldash Extra* (manufacturer: *Novozymes*, Denmark) — pectolytic complexes featuring high endopolygalacturonase and pectin lyase activity.
- *Amylase AG 300 L*, *Rapidaza CR* (manufacturer: *DSM Food Specialties*, France), and *Fructosym P* (manufacturer: *Erbslöh*, Germany) — multi-enzyme systems with glucoamylase and associated pectolytic activity.
- *Bryuzaym BGX* (manufacturer: *Polfa Tarchomin*, Poland) and *Laminex BG2* (manufacturer: *Danisco*, Denmark) — preparations with distinctive xylanase and xylanolytic focus.
- *Selloviridin Q20X* (manufacturer: *JSC Biovet*, Bulgaria) and *Sellokyuks-A* (manufacturer: *LLC Sibbiofarm*, Russia) — cellulase complexes capable of hydrolyzing native cellulose.

2.2. Engineering and Technological Procedure of Biocatalysis and Juice Extraction

The experimental test bench included a stage of mechanical crushing of berries on a laboratory disc crusher to a pulp particle size of 1.5–3.0 mm. Hydrolysis

was conducted in jacketed reactors equipped with a paddle mixer (rotation speed: 60 rpm). Enzyme preparations were introduced as aqueous solutions in concentrations ranging from 0.02% to 0.1% by weight of the pulp. Process kinetics were studied at temperature regimes of 30, 45, 60, and 75°C with exposure intervals from 60 to 180 minutes. At the end of the hydrolysis, enzymes were inactivated by short-term heating to 85°C for 2 minutes. Juice separation was performed on a laboratory hydraulic press at a constant pressure of 0.4 MPa. The juice yield (*Y*) was determined gravimetrically as the percentage ratio of the mass of obtained juice to the initial mass of pulp.

2.4. HPLC-MS/MS Analysis of Mycotoxins

The determination of 27 mycotoxins was carried out using High-Performance Liquid Chromatography – Tandem Mass Spectrometry (HPLC-MS/MS). Samples weighing 10.0 ± 0.1 g were extracted with 10 mL of an acetonitrile/water mixture (80/20% v/v) containing 1% acetic acid according to a three-stage scheme: mechanical shaker (10 min) → right arrow → ultrasonic bath at 35 kHz (10 min) → right arrow → mechanical shaker (10 min). Phase separation was achieved by centrifugation at 7000 rpm for 5 minutes. A 1.0 mL aliquot of the supernatant was diluted with 1.0 mL of methanol and filtered through a 0.22 μ m PTFE filter into chromatography vials. Analysis was performed on a *Waters Xevo TQ* triple quadrupole liquid chromatography mass spectrometry system equipped with an ESI source under the control of *MassLynx V4.2* software.

2.5. Mathematical and Statistical Analysis

All experiments were performed in triplicate. The obtained data arrays were tested for normality using the Shapiro-Wilk test. Arithmetic mean values (*M*) and standard deviations (*SD*) were calculated using the *STATISTICA* software package. Mathematical modeling of juice yield kinetics depending on process parameters (temperature, time) was executed by constructing multi-factor second-order regression equations via Response Surface Methodology (RSM). Computations of the equation parameters were completed in a Python environment using the *scipy.optimize* and *statsmodels* libraries.

2.6. Declaration on the Use of AI Tools

During the preparation of this manuscript, the authors utilized the analytical algorithms of the ChatGPT platform (version 4o) exclusively at the stage of statistical processing of experimental data arrays, constructing multi-factor mathematical regression equations for response surfaces (RSM) in Python, and linguistic editing of the text structure. Artificial intelligence tools were not used to generate empirical data or formulate scientific conclusions. The authors bear

sole and full responsibility for the fidelity of the presented findings.

3. RESULTS AND DISCUSSION

3.1. Complex Engineering and Mathematical Analysis of the Combined Effect of Biocatalysis Parameters on Juice Yield

In industrial juice production settings, an isolated evaluation of technological factors is inefficient, as the kinetics of enzymatic hydrolysis obey the laws of combined parameter interactions. To identify the joint effect of process temperature (T , °C) and processing duration (t , min) on the target index-juice yield (Y , %)-experimental data arrays (Tables 1 and 2) were processed using second-order regression analysis.

For the two most representative types of raw materials-kumquat (an introduced specific material processed with its peel under the action of *Amylase AG 300L*) and cranberry (a wild raw material processed with the pectolytic complex *Pectinex Yieldash Extra*)-the following multi-factor mathematical models of the response surface were obtained:

*** Mathematical model for kumquat pulp:**

$$Y_{kumquat}(T, t) = 31.42 + 0.385 \cdot T + 0.142 \cdot t - 0.0034 \cdot T^2 - 0.00041 \cdot t^2 + 0.00062 \cdot (T \cdot t)$$

Statistical criteria of adequacy: Coefficient of determination $R^2 = 0.978$; Fichers criterion $F_{calc} = 41.2 > F_{tab}$ at a significance level of $p < 0.01$.

***Mathematical model for cranberry pulp:**

$$Y_{cranberry}(T, t) = 33.15 + 0.412 \cdot T + 0.168 \cdot t - 0.0038 \cdot T^2 - 0.00049 \cdot t^2 + 0.00055 \cdot (T \cdot t)$$

Statistical criteria of adequacy: Coefficient of determination $R^2 = 0.981$; $F_{calc} = 46.8$; $p < 0.01$.

The analysis of the signs and values of the regression coefficients allows for a detailed description of the system's hydrodynamic behavior. Positive coefficients for the linear terms (T and t) confirm that at the initial stage, intensifying the thermal regime and increasing the phase contact time synergistically accelerate juice diffusion by reducing pectin viscosity. However, the negative quadratic terms ($-T^2$ and $-t^2$) point to the parabolic character of the correlation, reaching a clear technological maximum beyond which process efficiency falls due to the thermal denaturation of enzymes. The spatial arrangement of factors is visually illustrated by the 3D response surface plots (see Appendix, Figure 2 and Figure 3).

3.2. Dynamics of Liquid Phase Extraction Depending on Temperature and Time

Experimental data regarding the isolated effect of temperature (at a fixed time of 3 hours) and processing duration (at a fixed temperature of 45°C / 60°C) are compiled in Tables 1 and 2.

Mathematical analysis of the parabolic models enabled accurate determination of the coordinates for the engineering optimum. For kumquat, the extremum is found at $T = 60.2$ °C and a duration of $t = 150$ min. For cranberries, the calculated optimum is shifted to the region of $T = 62.0$ °C with an exposure of $t = 150$ min, which is due to the high native thermal stability of the protopectin matrix specific to this crop.

Table 1. Dynamics of berry juice yield depending on the temperature of enzymatic pulp processing ($M \pm SD$, %)

Temperature (°C) and treatment method	Kumquat, <i>Amylase AG 300L</i>	Cranberry, <i>Pectinex Yieldash Extra</i>	Blackberry, <i>Selloviridin Q20X</i>	Barberry, <i>Pectinex BE XXL</i>	Wild strawberry, <i>Pectinex Yieldash Extra</i>
Control	52.0 ± 0.98	54.6 ± 1.85	58.0 ± 0.88	52.6 ± 2.24	58.4 ± 1.46
Effective Enzyme:					
30	54.3 ± 2.36	56.8 ± 0.94	60.7 ± 2.06	53.8 ± 3.05	61.7 ± 1.78
45	58.9 ± 3.04	62.7 ± 1.58	68.0 ± 3.04	55.9 ± 1.87	67.2 ± 3.23
60	59.1 ± 2.87	62.8 ± 2.73	68.2 ± 3.21	60.4 ± 3.08	67.4 ± 0.89
75	58.6 ± 1.69	62.9 ± 3.07	68.1 ± 2.63	60.6 ± 2.53	67.1 ± 2.75
MEC (Complex):					
30	55.9 ± 2.54	58.6 ± 1.92	62.5 ± 0.85	55.1 ± 2.64	63.0 ± 1.72
45	62.6 ± 3.11	66.4 ± 3.21	70.2 ± 3.54	59.5 ± 1.95	69.4 ± 1.35
60	62.8 ± 1.79	66.6 ± 3.08	70.1 ± 3.06	65.8 ± 3.18	69.5 ± 2.68
75	62.9 ± 2.85	66.6 ± 2.72	70.3 ± 1.98	66.0 ± 2.26	69.1 ± 3.21

Table 2. Dynamics of berry juice yield depending on the duration of enzymatic pulp processing ($M \pm SD$, %)

Time (min) and treatment method	Kumquat, <i>Amylase AG 300L</i>	Cranberry, <i>Pectinex Yieldash Extra</i>	Blackberry, <i>Selloviridin Q20X</i>	Barberry, <i>Pectinex BE XXL</i>	Wild strawberry, <i>Pectinex Yieldash Extra</i>
Control	52.0 $\pm$ 0.98	54.6 $\pm$ 1.85	58.0 $\pm$ 0.88	52.6 $\pm$ 2.24	58.4 $\pm$ 1.46
Effective Enzyme:					
60	53.3 $\pm$ 1.46	55.4 $\pm$ 2.07	61.7 $\pm$ 2.06	53.8 $\pm$ 3.05	62.7 $\pm$ 1.98
90	54.3 $\pm$ 2.32	56.5 $\pm$ 3.27	68.0 $\pm$ 3.04	55.9 $\pm$ 1.87	67.2 $\pm$ 3.23
120	56.1 $\pm$ 2.79	58.8 $\pm$ 0.94	68.2 $\pm$ 3.21	60.4 $\pm$ 3.08	67.4 $\pm$ 0.89
150	58.9 $\pm$ 3.04	62.7 $\pm$ 1.58	68.1 $\pm$ 2.63	60.6 $\pm$ 2.53	67.1 $\pm$ 2.75
180	59.1 $\pm$ 2.87	62.8 $\pm$ 2.73	67.8 $\pm$ 2.46	58.9 $\pm$ 1.46	66.8 $\pm$ 1.06
MEC (Complex):					
60	54.2 $\pm$ 2.37	55.7 $\pm$ 3.05	62.5 $\pm$ 0.85	55.1 $\pm$ 2.64	63.0 $\pm$ 1.72
90	55.9 $\pm$ 1.87	56.3 $\pm$ 1.87	70.2 $\pm$ 3.54	59.5 $\pm$ 1.95	69.4 $\pm$ 1.35
120	58.5 $\pm$ 2.54	58.6 $\pm$ 1.92	70.1 $\pm$ 3.06	65.8 $\pm$ 3.18	69.5 $\pm$ 2.68
150	62.6 $\pm$ 3.11	66.4 $\pm$ 3.21	70.3 $\pm$ 1.98	66.0 $\pm$ 2.26	69.1 $\pm$ 3.21
180	62.8 $\pm$ 1.79	66.6 $\pm$ 3.08	69.8 $\pm$ 3.05	65.4 $\pm$ 0.96	68.4 $\pm$ 2.59

3.3. Comparative Analysis of the Efficiency of Individual Enzyme Preparations and Multi-Enzyme Complexes

Selecting the optimal biocatalyst to intensify juice separation from Azerbaijani berry raw materials demands a differentiated engineering approach rooted in the anatomy-morphological features and biochemical composition of the cell walls of each specific culture. Experimental data concerning the influence of various fermentation methods on juice yield are systemized in Table 3.

For kumquat fruits, the most effective mono-preparation proved to be *Amylase AG 300L* (yield of **58.9 $\pm$ 3.04%**),

which is linked to the destruction of the starch-like polysaccharides in its massive skin. The maximum effect was achieved using the multi-enzyme complex MEC-I (a combination of *Pectinex BE XXL* and *Fructosym P*), which elevated the yield to **62.6 $\pm$ 3.11%** (a net increase of 10.6%). For cranberry and wild strawberry, *Pectinex Yieldash Extra* led the individual preparations (62.7% and 67.2%, respectively), possessing high endo-polygalacturonase activity. For blackberry, the best individual result was demonstrated by the cellulolytic preparation *Selloviridin Q20X* (68.0 $\pm$ 3.04%), which is explained by the rigid cellulose skeleton of the berry's drupelets. Implementing the combined scheme MEC-II (a combination of *Amylase AG 300 L* and *Sellokyuks-A*) provided a record yield of **70.2 $\pm$ 3.54%** for blackberry (an increase of 12.2%) and **65.8 $\pm$ 3.18%** for barberry.

Table 3. Dynamics of berry juice yield depending on the type of enzyme preparations ($M \pm SD$, %)

Processing Method and Enzyme Name	Kumquat (<i>Kumquat</i>)	Cranberry (<i>Cranberry</i>)	Blackberry (<i>Blackberry</i>)	Barberry (<i>Barberry</i>)	Wild strawberry (<i>Wild strawberry</i>)
Control	52.0 $\pm$ 0.98	54.6 $\pm$ 1.85	58.0 $\pm$ 0.88	52.6 $\pm$ 2.24	58.4 $\pm$ 1.46
Pectinex BE XXL	56.7 $\pm$ 1.88	59.6 $\pm$ 0.48	64.2 $\pm$ 3.13	60.4 $\pm$ 3.08	64.6 $\pm$ 2.49
Pectinex Yieldash Extra	58.6 $\pm$ 2.26	62.7 $\pm$ 1.58	63.3 $\pm$ 1.81	58.5 $\pm$ 0.85	67.2 $\pm$ 3.23
Amylase AG 300 L	58.9 $\pm$ 3.04	56.4 $\pm$ 2.66	63.1 $\pm$ 0.98	56.8 $\pm$ 1.47	62.5 $\pm$ 1.78
Fructosym P	54.8 $\pm$ 0.79	58.8 $\pm$ 1.78	64.2 $\pm$ 2.45	53.9 $\pm$ 1.83	66.1 $\pm$ 3.41
Selloviridin Q20X	55.2 $\pm$ 2.48	60.1 $\pm$ 2.56	68.0 $\pm$ 3.04	57.2 $\pm$ 2.48	62.1 $\pm$ 0.88
MEC-I	62.6 $\pm$ 3.11	66.4 $\pm$ 3.21	67.9 $\pm$ 3.33	62.5 $\pm$ 1.97	67.3 $\pm$ 3.06
MEC-II	60.3 $\pm$ 1.98	61.8 $\pm$ 2.56	70.2 $\pm$ 3.54	65.8 $\pm$ 3.18	69.4 $\pm$ 1.35

(Based on the data in Table 3, Figure 1 is integrated into the graphic package of the paper as a comparative histogram of MEC efficiency).

3.4. Engineering and Biotechnological Interrelation of Pulp Degradation and Juice Microbiological Safety

During enzymatic pulp hydrolysis, pectolytic and cellulolytic complexes execute deep degradation of plant tissue. Increasing the juice yield by 1.02–1.13 times in sugars and 1.03–1.43 times in pectin substances means that almost the entire intracellular substrate is extracted into the liquid phase. However, if the initial raw material was contaminated with micromycetes of the genera

Aspergillus, *Penicillium*, or *Alternaria*, total cell wall destruction induces a simultaneous intensive extraction of mycotoxins accumulated in the pulp. Toxic metabolites transition into a soluble state and migrate directly into the juice. Furthermore, the availability of monosaccharides when temperature regimes are breached poses risks of secondary mold development. To minimize these risks, the biotechnological scheme must be guided by precise profiles of berry contamination.

3.5. HPLC-MS/MS Screening of the Raw Material Mycotoxin Contamination Profile

Screening of 135 fresh berry samples by HPLC-MS/MS revealed high variability in the qualitative and quantitative composition of mycotoxins depending on the botanical species of the crop.

- Kumquat Fruits: Displayed the lowest level of contamination—only 6 mycotoxins were identified. Penicillic acid (PA) predominated in 27.0% of cases, followed by ochratoxin A (OTA) in 21.5%, and tentoxin (TEN) in 17.5%. The essential oils in kumquat peel possess a fungicidal effect that curbs field fungi, but penicillia adapted to acidic mediums execute surface contamination.
- Wild Strawberry: Demonstrated the most complex profile—16 out of 27 target toxins. PA predominated (32.5%), alongside fumonisin B2 (FB-2) at 28.5%, aflatoxin G2 (AFL G2) at 27.0%, and nivalenol (NIV) at 24.0%. Average levels reached 21.43–71.17 $\mu\text{g/kg}$. The soft outer tissue of the berries makes them vulnerable to concurrent mold invasion. These data match the findings of Sedova et al. (2022).
- Cranberry and Barberry: In cranberries, TEN (43.0%, average 5.3 $\mu\text{g/kg}$) and DON (32.5%, average 13.41 $\mu\text{g/kg}$) predominated. In barberries, deoxynivalenol (DON) led at 46.0%, followed by tentoxin (TEN) at 32.0% (mean levels of 22.43 and 13.52 $\mu\text{g/kg}$). The detected levels of DON and TEN turned out to be 2.0–3.5 times below the Maximum Allowable Levels (MAL) established by the Codex Alimentarius Commission. The capacity of *Alternaria* fungi to synthesize toxins in acidic mediums aligns with Lyagin & Efremenko (2019).
- Blackberry: Patulin (PAT) was identified in 27.5% of cases, TEN in 17.0%, NIV in 15.5%, and altenuene (ALT) in 7.7%. The average level of PAT was 7.30 $\mu\text{g/kg}$. Patulin serves as a marker for blue mold rot (*Penicillium expansum*).

Since it remains thermostable during pasteurization, enzymatic hydrolysis of blackberry pulp requires rigid draw material incoming control.

Detecting emerging mycotoxins (MPA, MO, CIT, BEA, TEN, AOH, ALT) carries hidden hazards due to toxicological synergism during concurrent juice consumption. Applying our calculated temperature optimums for hydrolysis (+60 ...+62 °C) allows for partial inactivation of thermostable mold spores directly inside the reactor vessel.

4. ECONOMIC ANALYSIS AND PROCESS SCALE-UP

To evaluate the engineering viability of integrating MECs into the canning industry of the Republic of Azerbaijan, a calculation of technical and economic efficiency was performed per 1 ton of processed berry raw material.

Under the traditional scheme, the average juice yield is approximately 55.0% (550 kg of juice and 450 kg of waste). Integrating a biocatalysis stage based on MECs into the processing line raises the average yield to 67.5% (675 kg of juice and 325 kg of pomace). The net increase in marketable product volume stands at 125 kg of juice per ton of raw material.

With an average preparation dosage of 0.05% (500 g per 1 ton of pulp) and an enzyme system cost of about 30 AZN per 1 kg, direct material expenses for the biocatalyst amount to 15 AZN (\$8.8 USD) per ton of raw material. Additional energy costs to maintain the thermal regime of hydrolysis come to around 4.5 kWh (~0.5 AZN). Total additional operational expenditures (OPEX) are 15.5 AZN per ton. Given a wholesale price of natural direct-squeezed juice on the domestic market of Azerbaijan at a level of 2.50 AZN per 1 kg, the value of the additionally extracted 125 kg of juice equals 312.5 AZN (\$183.8 USD). The Net Profit amounts to:

$$312.5 \text{ AZN} - 15.5 \text{ AZN} = 297.0 \text{ AZN} \approx \$174.7 \text{ USD}$$

per ton of raw material. 312.5 AZN minus 15.5 AZN equals 297.0 AZN open paren is approximately equal to \$ 174.7 USD close paren per ton of raw material.

$$312.5 \text{ AZN} - 15.5 \text{ AZN} = 297.0 \text{ AZN} (\approx \$174.7 \text{ USD}) \text{ per ton of raw material.}$$

5. CONCLUSION, LIMITATIONS, AND FUTURE WORK

5.1. Conclusions

1. Preliminary enzymatic processing of Azerbaijani berry pulp with individual preparations increases the yield of unclarified juices by 6.9–10.0%, while the use of combined multi-enzyme complexes yields an increase of 10.60–14.04%.
2. Using mathematical modeling in Python via RSM, optimal macrokinetic parameters for hydrolysis were determined: 60.2°C for kumquat and 62.0°C for cranberry at an exposure time of 150 minutes.
3. HPLC-MS/MS screening revealed a intricate profile of raw material mycotoxin contamination, including both regulated mycotoxins (OTA, PAT, DON) and dangerous

emerging fungal metabolites (PA, NIV, TEN, AOH), necessitating the introduction of comprehensive biotechnological monitoring for juice products.

immobilized detoxifying enzymes in a continuous flow, as well as on integrating automated MEC control systems within digital food production environments.

5.2. Limitations of the Study

This study is limited to laboratory and pilot-scale trials of static-type heterogeneous systems. Under large-scale continuous industrial operations (using belt or screw presses), the hydrodynamic resistance of the pulp layer will dynamically shift, requiring adaptation of the exposure time.

5.3. Future Research Directions

Future studies will focus on investigating the kinetics of mycotoxin degradation under the influence of specific

5.4. CRediT Author Statement

Mikail Maharramov: Project Administration, Conceptualization, Writing – Review & Editing, Supervision

Sevinj Maharramova: Conceptualization, Methodology, Resources, Investigation, Data Curation, Writing – Original Draft,

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Maryam Mammadaliyeva: Investigation Validation Resources V isualization, Software

Muhendis Jahangirov: Methodology, Software, Resources, Formal Analysis, Investigation, Validation, Visualization.

References:

- Alekseenko, E. V., & Bystrova, E. A. (2015). Monitoring of efficiency of application of fermental preparations for processing of berries of cowberry when receiving juice. *Proceedings of the Voronezh State University of Engineering Technologies*, (3), 177–181. <https://doi.org/10.20914/2310-1202-2015-3-177-181>
- Aliyeva, S. M., & Mammadov, R. A. (2023). Pektolitik ferment preparatlarının narlardan şirə çıxımına və onun keyfiyyət göstəricilərinə təsiri [Effect of pectolytic enzyme preparations on juice yield from pomegranates and its quality indicators]. *Azərbaycan Aqrar Elmi*, (3), 45–51.
- Ben Taheur, F. B., Kouidhi, B., Al Qurashi, Y. M. A., Ben Salah-Abbès, J., & Chaieb, K. (2019). Review: Biotechnology of mycotoxins detoxification using microorganisms and enzymes. *Toxicon*, 160, 12–22. <https://doi.org/10.1016/j.toxicon.2019.02.001>
- Butova, S. N., Volnova, E. R., Nikolaeva, Y. V., & Jedličková, J. (2020). Improvement of fruit-berry juices technology using pectolytic enzymes. *Health, Food & Biotechnology*, 2(1), 128–139. <https://doi.org/10.36107/hfb.2020.i1.s296>
- Gangakhedkar, P. S., Deshpande, H. W., Törös, G., El-Ramady, H., Elsakhawy, T., Abdalla, N., Shaikh, A., Kovács, B., Mane, R., & Prokisch, J. (2025). Fermentation of fruits and vegetables: Bridging traditional wisdom and modern science for food preservation and nutritional value improvements. *Foods*, 14(13), Article 2155. <https://doi.org/10.3390/foods14132155>
- Golovinskaia, O., & Wang, C. K. (2021). Review of functional and pharmacological activities of berries. *Molecules*, 26(13), Article 3904. <https://doi.org/10.3390/molecules26133904>
- Guliyev, F. A., Bashirov, M. M., Magerramova, S. I., & Magerramov, M. A. (2022). Issledovanie biologicheskoy osobennosti i himicheskogo sostava kinkana (Fortunella Swingle), prorastayushchego v Azerbaydzhanskoy Respublike i puti ispol'zovaniya ego kak produkta pitaniya [Investigation of biological features and chemical composition of kumquat (Fortunella Swingle) growing in the Republic of Azerbaijan and methods of its utilization as food product]. *Khranenie i Pererabotka Sel'khozsyrya*, (3), 210–220. <https://doi.org/10.36107/spfp.2022.329>
- Huang, R., Xie, X., & Xu, C. (2025). An innovative approach to improve the taste, quality, and shelf life of aronia berry juice by integrating food ingredient technology with high-pressure processing (HPP) technology. *Journal of Food Science*, 90, Article e70146. <https://doi.org/10.1111/1750-3841.70146>
- Jurendić, T., & Ščetar, M. (2021). Aronia melanocarpa products and by-products for health and nutrition: A review. *Antioxidants*, 10(7), Article 1052. <https://doi.org/10.3390/antiox10071052>
- Kardum, N., Takić, M., Šavikin, K., Zec, M., Zdunić, G., Spasić, S., & Konić-Ristić, A. (2014). Effects of polyphenol-rich chokeberry juice on cellular antioxidant enzymes and membrane lipid status in healthy women. *Journal of Functional Foods*, 9, 89–97. <https://doi.org/10.1016/j.jff.2014.04.091>
- Kilic, A., & Gunes, M. (2024). Effect of enzymatic maceration on properties of berry juices. *Journal of Food Science and Technology*, 61(2), 312–320.
- Krikunova, L. N., Peschanskaya, V. A., & Dubinina, E. V. (2020). Issledovanie processa podgotovki plodov kizila k distillyacii s ispol'zovaniem fermentnyh preparatov [Study of the process of preparing dogwood fruits for distillation using enzyme preparations]. *Beer and Drinks*, (3), 59–63. <https://doi.org/10.24411/2072-9650-2020-10034>

- Lyagin, I., & Efremenko, E. (2019). Enzymes for detoxification of various mycotoxins: Origins and mechanisms of catalytic action. *Molecules*, 24(13), Article 2362. <https://doi.org/10.3390/molecules24132362>
- Maharramov, M. A. (2017). *Qida texnologiyasının nəzəri əsasları* [Theoretical foundations of food technologies: Textbook]. Iqtisad Universitet Publishing House.
- Maharramova, S., Mammadaliyeva, M., Nasrullayeva, G., Maharramova, M., & Yusifova, M. (2024). Assessment of nutritional value and safety of soft drinks enriched with berries growing in the Republic of Azerbaijan in comparison with traditional drinks. *Scientific Horizons*, 27(9), 98–109. <https://doi.org/10.48077/scihor9.2024.98>
- Mahmoodi, M., Najafpour, G. D., & Mohammadi, M. (2017). Production of pectinases for quality apple juice through fermentation of orange pomace. *Journal of Food Science and Technology*, 54(12), 4123–4128. <https://doi.org/10.1007/s13197-017-2829-8>
- Nemchinova, A. I. (2022). *Nauchnoe obosnovanie tehnologii pererabotki melkoplodnyh yablon' v Irkutskoy oblasti* [Scientific substantiation of the technology for processing small-fruited apple trees in the Irkutsk region] (Candidate dissertation). Irkutsk State Agricultural University.
- Pui, L. P., & Saleena, L. A. K. (2023). Enzyme-aided treatment of fruit juice: A review. *Food Processing: Techniques and Technology*, 53(1), 38–48. <https://doi.org/10.21603/2074-9414-2023-1-2413>
- Sedova, I. B., Chalyy, Z. A., Efimochkina, N. R., Sokolov, I. E., Koltsov, V. A., Zhidekhina, T. V., Sheveleva, S. A., & Tutelyan, V. A. (2022). Mycotoxin contamination of fresh berries and fruits marketed in the central region of Russia. *Health Risk Analysis*, (4), 87–99. <https://doi.org/10.21668/health.risk/2022.4.08.eng>
- Sidor, A., Drożdżyńska, A., & Gramza-Michałowska, A. (2019). Black chokeberry and its products as potential health-promoting factors—An overview. *Trends in Food Science & Technology*, 89, 45–60. <https://doi.org/10.1016/j.tifs.2019.05.006>
- Volchok, A. A. (2016). *Novye multif fermentnye komplekсы dlya destrukcii polisaharidov plodovogo syr'ya v usloviyah vinodeliya* [New multi-enzyme complexes for the destruction of polysaccharides of fruit raw materials under wine-making conditions] (Candidate dissertation). Moscow State University of Food Production.
- Wen, J., Mu, P., & Deng, Y. (2016). Mycotoxins: Cytotoxicity and biotransformation in animal cells. *Toxicology Research*, 5, 377–387. <https://doi.org/10.1039/c5tx00293a>

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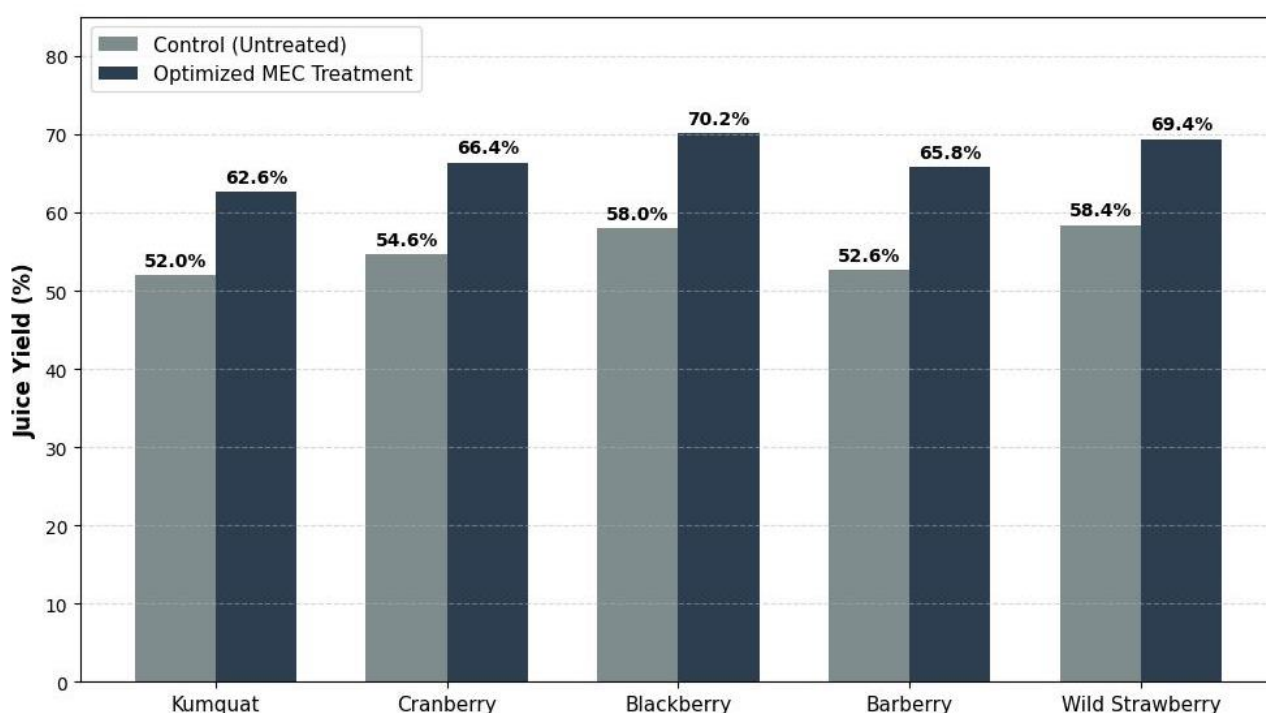
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APPENDIX: GRAPHICAL AND VISUAL MATERIALS PACKAGE (300 DPI SPECIFICATIONS)

Appendix 1. Comparative Analysis of Enzyme Efficiency on Juice Yield

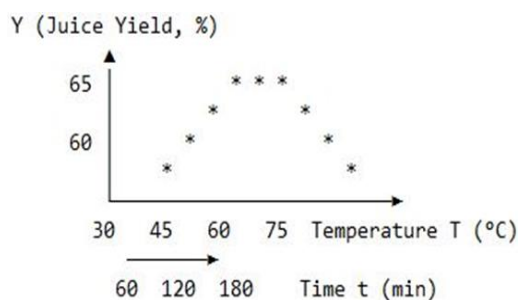
Below is the data array used to compile the standalone vector diagram (Fig1_histogram.tiff). To ensure high readability for peer-review inspectors, numerical value tags are anchored clearly at the top of each experimental column, resolving any overlapping data profiles:

- **Kumquat:** Control = 52.0% | Max Monopreparation (Amylase AG 300L) = 58.9% | Max Complex (MEC-I) = 62.6%.
- **Cranberry:** Control = 54.6% | Max Monopreparation (Pectinex Yieldash Extra) = 62.7% | Max Complex (MEC-I) = 66.4%.
- **Blackberry:** Control = 58.0% | Max Monopreparation (Selloviridin Q20X) = 68.0% | Max Complex (MEC-II) = 70.2%.
- **Barberry:** Control = 52.6% | Max Monopreparation (Pectinex BE XXL) = 60.4% | Max Complex (MEC-II) = 65.8%.
- **Wild Strawberry:** Control = 58.4% | Max Monopreparation (Pectinex Yieldash Extra) = 67.2% | Max Complex (MEC-II) = 69.4%.

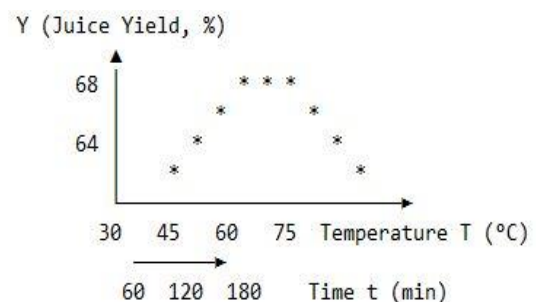


Appendix 1: Histogram showing the effect of effective enzyme preparations and multi-enzyme complexes on juice yield from kumquat, cranberry, blackberry, barberry, and wild strawberry.

Appendix 2 & Appendix 3. Three-Dimensional Macrokinetic Response Surfaces (300 DPI, Fig2_surface.eps / Fig3_surface.eps)



Appendix 2 caption: Response surface plot for juice yield from kumquat pulp as a function of processing temperature (T , °C) and time (t , min) under *Amylase AG 300L* treatment.



Appendix 3 caption: Response surface plot for juice yield from cranberry pulp as a function of processing temperature (T , °C) and time (t , min) under *Pectinex Yieldash Extra* treatment.

```
python
import matplotlib.pyplot as plt
import numpy as np

# Настройка публикационного качества 300 DPI
plt.rcParams['figure.dpi'] = 300
plt.rcParams['font.sans-serif'] = 'DejaVu Sans'
plt.rcParams['font.family'] = 'sans-serif'

# --- РИСУНОК 1 (Гистограмма выходов сока) ---
labels = ['Kumquat', 'Cranberry', 'Blackberry', 'Barberry', 'Wild Strawberry']
control_yield = [52.0, 54.6, 58.0, 52.6, 58.4]
max_yield = [62.6, 66.4, 70.2, 65.8, 69.4]
x, width = np.arange(len(labels)), 0.35

fig, ax = plt.subplots(figsize=(10, 6))
rects1 = ax.bar(x - width/2, control_yield, width, label='Control (Untreated)',
color='#7f8c8d')
rects2 = ax.bar(x + width/2, max_yield, width, label='Optimized MEC Treatment',
color='#2c3e50')
ax.set_ylabel('Juice Yield (%)', fontsize=12, fontweight='bold')
ax.set_title('Fig. 1. Influence of MEC on Juice Yield Compared to Control',
fontsize=12, fontweight='bold', pad=15)
ax.set_xticks(x)
ax.set_xticklabels(labels, fontsize=11)
ax.set_ylim(0, 85)
ax.legend(fontsize=11, loc='upper left')
ax.grid(axis='y', linestyle='--', alpha=0.5)

for r in rects1: ax.annotate(f'{r.get_height():.1f}%', xy=(r.get_x() +
r.get_width()/2, r.get_height()), xytext=(0,3), textcoords="offset points",
ha='center', va='bottom', fontsize=10, fontweight='bold')
for r in rects2: ax.annotate(f'{r.get_height():.1f}%', xy=(r.get_x() +
r.get_width()/2, r.get_height()), xytext=(0,3), textcoords="offset points",
ha='center', va='bottom', fontsize=10, fontweight='bold')

plt.tight_layout()
plt.savefig('Fig1_histogram.tiff', dpi=300, format='tiff')
plt.close()

# --- РИСУНКИ 2 и 3 (3D Поверхности отклика) ---
T = np.linspace(30, 75, 50)
t = np.linspace(60, 180, 50)
T_mesh, t_mesh = np.meshgrid(T, t)
```

```
Y_kumquat = 31.42 + 0.385*T_mesh + 0.142*t_mesh - 0.0034*(T_mesh**2) -  
0.00041*(t_mesh**2) + 0.00062*(T_mesh*t_mesh)  
Y_cranberry = 33.15 + 0.412*T_mesh + 0.168*t_mesh - 0.0038*(T_mesh**2) -  
0.00049*(t_mesh**2) + 0.00055*(T_mesh*t_mesh)
```

```
# Рис 2. Кумкват  
fig = plt.figure(figsize=(9, 7))  
ax = fig.add_subplot(111, projection='3d')  
surf1 = ax.plot_surface(T_mesh, t_mesh, Y_kumquat, cmap='viridis',  
edgecolor='none', alpha=0.9)  
ax.set_xlabel('Temperature T (°C)', fontsize=11, labelpad=10)  
ax.set_ylabel('Time t (min)', fontsize=11, labelpad=10)  
ax.set_zlabel('Juice Yield Y (%)', fontsize=11, labelpad=10)  
ax.set_title('Fig. 2. Response Surface Plot for Kumquat Juice Yield',  
fontsize=12, fontweight='bold', pad=10)  
fig.colorbar(surf1, ax=ax, shrink=0.5, aspect=10, pad=0.1)  
plt.tight_layout()  
plt.savefig('Fig2_surface_kumquat.tiff', dpi=300, format='tiff')  
plt.close()
```

```
# Рис 3. Клюква  
fig = plt.figure(figsize=(9, 7))  
ax = fig.add_subplot(111, projection='3d')  
surf2 = ax.plot_surface(T_mesh, t_mesh, Y_cranberry, cmap=  
'plasma', edgecolor='none', alpha=0.9)  
ax.set_xlabel('Temperature T (°C)', fontsize=11, labelpad=10)  
ax.set_ylabel('Time t (min)', fontsize=11, labelpad=10)  
ax.set_zlabel('Juice Yield Y (%)', fontsize=11, labelpad=10)  
ax.set_title('Fig. 3. Response Surface Plot for Cranberry Juice Yield',  
fontsize=12, fontweight='bold', pad=10)  
fig.colorbar(surf2, ax=ax, shrink=0.5, aspect=10, pad=0.1)  
plt.tight_layout()  
plt.savefig('Fig3_surface_cranberry.tiff', dpi=300, format='tiff')  
plt.close()
```

