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Assessment of Quality Indices in Japanese Quince (*Chaenomeles japonica* L.) Juice and Concentrate: Evaluating the Impact of Hydrolytic Enzymes and Clarifiers

Running title: Japanese quince juice and concentrate

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SUMMARY

Research background. Japanese quince (*Chaenomeles japonica* L.) is known for its relatively high levels of bioactive compounds, including phenolics, vitamin C, organic acids, dietary fibers, and pectins. Its acidic nature makes Japanese quince juice (JQJ) a potential alternative to lemon juice, offering preservative and acidifying properties in various products.

Experimental approach. This study aimed to evaluate the effects of different hydrolytic enzymes (EnartisZym 1000 S, EnartisZym RS, EnartisZym EZ Filter) and the clarifying agent bentonite from "Neoclar AF" (bentonite/activated carbon) on the quality indicators of JQJ and its concentrate (JQJC). Juice was extracted from frozen fruits, followed by clarification, filtration, and concentration through open water evaporation at 60 °C to achieve approximately 50 Brix %.

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Results and conclusions. The findings demonstrated that hydrolytic enzymes and clarifiers effectively reduced pectin content in JQJ and JQJC by 68.5 % and 57.0 %, respectively, significantly improving their clarity by 85.9 % and 64.9 %. The highest clarity was observed in samples treated with EnartisZym 1000 S and bentonite (0.028) compared to the control. Enzymatic treatment had a minimal impact on phenolic content, with enzyme-treated JQJ containing an average of 318.3 mg/100 mL and JQJC 3.12 g/100 g, compared to 326.2 mg/100 mL and 3.34 g/100 g in untreated samples, respectively. Vitamin C retention was high, with enzyme-treated JQJ containing 68.3–69.4 mg/100 mL and JQJC 231.4–236.9 mg/100 g, compared to 72.8 mg/100 mL and 244.9 mg/100 g in untreated samples, indicating that enzymatic treatment and mild processing effectively preserved ascorbic acid content. The $DPPH^{\bullet}$ radical scavenging activity was significantly higher in enzyme-treated JQJ, though it decreased during juice concentration. In both JQJ and JQJC, FRAP values were lower in enzyme-treated samples than in controls. Based on generalized scores acquired from AHP analysis, the enzyme EnartisZym EZ Filter, possessed both cellulolytic and pectolytic activities, was found to be the most efficient, ensuring the quality characteristics of JQJC nearly similar to those commercially available on the market such as lemon juice concentrate

Novelty and scientific contribution. This study provides valuable insights into the potential of JQJC as a natural acidifying alternative to lemon juice, addressing a gap in existing research. The obtained data demonstrate that JQJC not only offers preservative and acidifying properties but also retains significant nutritional benefits, making it a promising ingredient for food applications. As an innovative niche product, JQJC has the potential to enhance food preservation and quality naturally. Future research should further explore its applications in various food formulations to maximize its functional and commercial potential.

Keywords: enzymatic treatment; cloudy juice; titratable acidity; pectin; chemical compounds; antiradical activity

INTRODUCTION

The *Chaenomeles* genus is globally recognized as an ornamental plant; however, its cultivation as a fruit crop for processing has a more recent history. The cultivation of *Chaenomeles japonica* L. (Japanese quince, JQ) for fruit production began in Latvia in the last century, with the first large plantations established in the 1970s (*1*). Over the past two decades, JQ cultivation has gained popularity in northern European countries, particularly in the Baltic Sea region, with Latvia's cultivation area currently reaching 752 hectares (*2*).

JQ fruit has a distinct sour taste and a low pH, making it unsuitable for fresh consumption. However, it is widely processed into various products, including juice, puree, syrup, candies, jams, and marmalades (*3*). Additionally, less common products such as alcoholic beverages (wine, liqueurs), fruit chips, freeze-dried fruit pieces, and powder have also been developed (*3*). JQ fruit is rich in bioactive compounds such as polyphenols, vitamin C, organic acids, dietary fiber, and pectins (*4,5*). It is particularly high in polysaccharides, including cellulose, hemicellulose, and pectin – key components of dietary fiber. The pulp contains a significant amount of pectin, with soluble pectins predominantly present in the juice (*6*).

Similar to lemon juice, JQ juice (JQJ) can serve as a natural acidifier (*7*) while also providing additional antioxidant properties to food products (*8*). JQJ is either comparable to or richer in bioactive compounds than lemon juice. For instance, the ascorbic acid concentration in JQJ ranges from 45–78 mg/100 mL, compared to 23–40 mg/100 mL in lemon juice (*8,9*). Although the total acid content is higher in lemon juice (5.2–6.9 %), JQJ still contains a substantial amount of organic acids (2.6–5.6 %) (*10*). Furthermore, the concentration of polyphenolic compounds in JQJ is approximately twice that of lemon juice, ranging from 229.2–459.0 mg/100 mL compared to 84.8–196.8 mg/100 mL (*8,11*).

Juice concentration is a common industrial practice that facilitates storage and transportation (*12*). Widely used juice concentrates include apple, lemon, orange, grapefruit, tangerine, lime, pomegranate, pineapple, apricot, and mango. The quality of juice concentrates is influenced by several factors, including the physiological maturity of the fruit, raw material quality, processing technology, and storage conditions (*11*). One key quality indicator is the content of 5-hydroxymethylfurfural (HMF), an organic compound formed due to the dehydration of reducing sugars, mainly hexoses, under acidic conditions (*13*).

Pectin, a high-molecular-mass polysaccharide present in plant cell walls, plays a crucial role in juice quality. It consists of negatively charged functional groups distributed along a backbone of D-galacturonic acid monomers linked via α-1→4-glycosidic bonds. Pectins can hinder juice clarification by interacting with other compounds in the matrix (*14*). To address this, enzymatic treatments using hydrolytic enzymes such as pectinases and amylases are commonly employed to break down pectin and polysaccharides (*14*). Pectinases hydrolyze pectin, disrupting pectin-protein complexes and reducing viscosity, thereby improving filtration efficiency and lowering energy costs.

Enzymes, as efficient biocatalysts, are widely used at various stages of juice production. Specifically, pectinases play a vital role in improving clarity and stability by reducing viscosity (*15*). Industrial depectinization processes typically use commercial enzyme mixtures containing pectinases, pectinesterases, polygalacturonases, cellulases, and pectin lyases (*16*).

Despite the increasing interest in JQJ, only one preliminary study has investigated the preparation and quality evaluation of JQJ concentrate (*17*). The study found that treating JQJ with the enzyme preparation Ultrazym 100 G, which primarily exhibits polygalacturonase activity, resulted in relatively higher quality indices. Given the high juice yield, dominance of organic acids, and antioxidant properties, JQJ and its concentrate could serve as viable alternatives to lemon juice, potentially enhancing food preservation and extending shelf life.

With the rising demand for innovative and health-promoting food products, JQJ has the potential to become a niche product with global market appeal (*18*). The limited availability of research articles focusing on the analysis of JQJ as a potential acidifying alternative to lemon juice prompted the design of this study. This research aims to determine the effects of different hydrolytic enzymes and a clarifying agent on the quality indicators of JQJ. Specifically, the study seeks to assess how these treatments influence parameters such as clarity, pectin content, and overall quality of the JQJ, thereby identifying optimal methods for processing JQJ into a product (concentrate JQJC) that could serve as an effective acidifying alternative to lemon juice. It is hypothesized that the application of hydrolytic enzymes and a clarifying agent will significantly improve the quality of JQJ by enhancing its clarity, reducing pectin content, and preserving or even boosting its bioactive properties. Furthermore, it is proposed that processing JQJ into a concentrate using these treatments will yield a product that can serve as an effective, acidifying alternative to lemon juice, offering comparable or superior bioactive benefits while maintaining high product quality.

MATERIALS AND METHODS

Ethanol 96.3 % (Kalsnava Distillery, Latvia), Folin–Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), reagent potassium chloride, potassium ferrocyanide, sodium carbonate anhydrous, iron trichloride hexahydrate (Sigma-Aldrich, Germany), 2,4,6-tripyridyl-s-triazine, tannic acid, ascorbic acid (Fluka, England); aluminium chloride hexahydrate (Alfa Aesar, Thermo Fisher Scientific, Germany); sodium nitrite (VWR, France); sodium acetate trihydrate (AppliChem Chemicals, Germany); (+)-catechin, gallic acid monohydrate, sodium hydroxide pellets, hydrochloric acid (Merck, USA); activated carbon (Eiro Plus, Ukraine). Enzymes: EnartisZym 1000 S, EnartisZym RS, EnartisZym EZ Filter, and clarifying agent Neoclar AF (Enartis Italy). Analytical standard L-ascorbic acid was purchased from Merck KGaA (Darmstadt, Germany). Acetonitrile (MeCN), formic acid (HCOOH) (puriss r.a.) of liquid chromatography-mass spectrometry (LC-MS) grade were purchased from the same source. Ultrapure water (UPW) was produced using the reverse osmosis PureLab Flex Elga water purification system (Veolia Water Technologies, Paris, France).

The research used whole, fully ripe Japanese quince (*Chaenomeles japonica* L., JQ) fruits, which were harvested in late August at the Institute of Horticulture in Dobele (Latvia, location: 56°36'39.9"N 23°17'48.8"E). The ripeness of JQ fruit was determined using a developed protocol (ME 05 CHE 2017) at LatHort, based on the evaluation of fruit surface and seed color. This assessment was conducted organoleptically, using the following scale: 1 represents unripe fruit, characterized by green skin and white seeds, 2–4 represents intermediate ripeness stages, and 5 indicates fully ripe fruit, with yellow skin and dark brown seeds, where the color corresponds to botanical color standards. After harvesting, fruits (50 kg) were washed, dried, packed in airtight and moisture-proof polypropylene bags, frozen and stored at (−18±1) °C for 3 months until processing. The fruits were thawed at room temperature for 24 h, the juice was obtained with a Basket press 60K (Voran Maschinen GmbH, Austria). Immediately after squeezing, the Japanese quince juice (JQJ) was divided into five parts of 3 L each for two replications for enzymatic treatment. Raw juice was used as a control sample.

Japanese quince juice clarification with enzyme preparations

A total of three clarification enzymes and one clarifying agent “Neoclar AF” were used in the study. The characteristic parameters and amounts of enzymes and clarifying agent added to 1 L of JQJ are given in Table 1.

The conditions used for the enzymatic treatment of JQJ were selected based on “Enartis” company guidelines and recommendations and considering the observations made by other researchers (*19*). Enzymatic treatment of JQJ samples was performed using a water bath (WB-4MS, Biosan, Latvia). The samples were subjected to thermal treatment at 90 °C for 3 min to terminate the catalytic activity of enzymes. Then the processed juice samples were cooled and filtered through Whatman filter No. 1. Instead of enzymatic treatment, one JQJ sample was subjected to clarifying using the clarifying agent “Neoclar AF”. Clarification of JQJ with “Neoclar AF” has been done in refrigeration conditions at 4 °C for 48 h, then filtered through Whatman filter No. 1. All the filtrates were collected for further analysis and preparation of the concentrate.

Preparation of juice concentrate

Filtered JQJ was poured into wide, low glass beakers for evaporation. For concentration, the JQJ was evaporated in wide, low glass beakers using an open-form evaporation method in a laboratory water bath (JP Selecta™ Precisdig, Barcelona, Spain). The evaporation process was controlled by estimating the soluble solids content, which was done periodically with a refractometer (Atago PAL-

1, Japan) until the concentrate reached (50±1) Brix %. The obtained JQJ concentrate (JQJC) was cooled, filled in closed containers and stored at 4 °C until further analysis.

Physical and chemical analyses

The following chemical indices were determined for JQJ and its concentrate (JQJC): soluble solids, titratable acidy, pH, clarity, the content of total vitamin C, proanthocyanidins, phenolics, pectins and antioxidant activity done by free radical scavenging activity DPPH• and ferric reducing antioxidant power (FRAP) assays.

Soluble solids content

Soluble solids content (SSC) Brix % in juice and concentrate was determined using a digital refractometer type Pal-1 (Tokyo, Japan)**.**

Clarity

Clarity (TR) was determined by measuring the absorbance at 660 nm using a spectrophotometer UV 1800 (Shimadzu, Japan). Deionized water was used as a reference according to (*20*).

Titratable acidity

Titratable acidity (TA) of samples was assessed for 10 mL of water extracts, which were titrated with 0.1 mol/L NaOH to a pH of 8.1 according to general guidelines on objective tests (*21*).TA was expressed as % citric acid equivalent in fresh mass (FM).

Total phenolic content

The total phenolic (TP) content in JQJ and its concentrate was determined by a photometric method using Folin-Ciocalteau reagent described by Singleton *et al.* (*22*) Sample preparation: 1mL juice or (0.5±0.01) g of the concentrate sample transferred to a 50 mL flask; 30 mL of 80 % ethanol mixture was added, then vortexed and extracted in an ultrasonic bath for 30 min, centrifuged and filtered. TP content in the sample expressed as mg or g gallic acid equivalent (GAE) in 100 g FM. For the evaluation of antioxidant activity (DPPH•, FRAP) the same extracts were used.

Total proanthocyanidin content

The total proanthocyanidin (TT) content was estimated by the method of Price and Butler (*23*). Sample preparation and procedure for measuring: 1 g juice or 0.5 g of concentrate was transferred to

100 mL flask; 50 mL water was added, and boiled for 30 min. After filtration through a Whatman filter the solution was transferred to a 100 mL flask and water was added to reach 100 mL mark. Water extract 0.5 mL aliquots was finally transferred to vials, 1 mL 1 % $K_3Fe(CN)_6$ and 1 mL 1 % $FeCl_3$ were added, and final volume was adjusted to 10 mL with water. After five min, the solutions were measured spectrophotometrically at 720 nm. The results expressed as mg or g catechin equivalent (CE) per 100 g FM.

Pectin content

Pectin (PE) was determined by the carbazole method (*24*). Pectin was isolated from the JQJ and its concentrate by leaching with 96.0 % ethanol, and from the residues – by extracting with diluted 1 M NaOH solution. By adding 0.1 % carbazole solution and concentrated sulfuric acid to the extract, the sample was heated at 85 °C for l5 min. The determination was based on the reaction of pectin with carbazole, in an excess of concentrated sulfuric acid, for the development of a pink coloration. The intensity of the color was measured with a spectrophotometer UV 1800 (Shimadzu, Japan) at 525 nm. The pectin amount expressed as galacturonic acid equivalent (GalAE) mg/100 mL juice or g/100 g concentrate FM.

Vitamin C content

Vitamin C was determined by high performance liquid chromatography (HPLC) according to standard EN 14130:2003 (*25*) and expressed as mg/100 g FM.

DPPH• free radical scavenging activity

The DPPH• free radical scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl assay according to Floegel *et al*. (*26*), with minor modifications. Briefly, the test sample (0, 100 mL) was reacted with 2.9 mL of DPPH• solution (0.0039 g DPPH• in 100 mL 96.0 % ethanol). Absorbance of the research sample was measured at 515 nm using a spectrophotometer. The radical scavenging activity of the sample was expressed in mmol Trolox equivalents (TE) per 100 g FM.

Ferric reducing antioxidant power (FRAP)

The FRAP reducing antioxidant power was determined according to the Benzie and Strain (*27*) some modifications. The FRAP reagent solutions was prepared from 300 mmol acetate buffer pH 3.6, 10 mol TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mmol HCl, and 20 mmol $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution. Sample extracts (0.150 mL) reacted with 2.850 mL of the

FRAP solution for 10 min in dark. The changes in absorbance from red to blue were determined at 593 nm. The absorbance results were converted using a calibration curve of the standard and expressed as mmol Trolox equivalent (TE) per 100 g of juice and mol (TE) per 100 g of concentrate FM.

5-Hydroxymethylfurfural (HMF) content

The chromatography analysis for HMF determination was conducted following the method reported by Gökmen and Acar (*28*) using a Shimadzu LC-40 Nexera system (Shimadzu Corporation, Kyoto, Japan). The system comprised a Column Oven CTO-40C, Auto Sampler SIL-40C x3, Solvent Delivery Module LC-40D x3, Degassing Unit DGU-405, and System Controller SCL-40. Separation was performed on an Aqueous PerkinElmer C18 column (250 mm × 4.6 mm, 5 µm, USA) under isocratic elution mode at a column temperature of 35 °C. The mobile phase consisted of acetonitrile (CH_3CN) and water (H_2O) in a 10:90 ratio, with a sample injection volume of 10 µL. The total analysis time was up to 8 min, with a flow rate of 1.0 mL/min, and detection was carried out at a wavelength of 280 nm using a Photo Diode Array SPD-M40 detector. The concentration of 5-HMF was expressed as µg/100 g of the sample.

Statistical analysis

Experimental data processing was done by One-way analysis of variance (ANOVA) procedure using IBM® SPSS® Statistics v. 23 (*29*). The mean arithmetic value and standard deviation were calculated for the obtained results using MS Office program Excel 2019 version 18008 (build 10416.20073) (Microsoft Corporation, Redmond, Washington, USA). Tukey's multiple range test was used to specify significant differences ($p<0.05$) among the studied samples. Analytical Hierarchy Process (AHP) was used to evaluate the clarification efficiency of enzyme treatment. The key quality characteristics of JQJC samples were carefully selected for evaluation, with clarity identified as the primary parameter. Given the study's focus on developing JQJC as an alternative to lemon-based acidifiers, acidity was established as the second critical quality attribute. An index in the range of 1–9 was assigned to the evaluation indicators of the quince concentrate samples according to the intensity of relative importance: clarity (TR)-9, titratable acidity (TA)-7, pectin-5, antioxidant activity ($DPPH^{\bullet}$)-3 vitamin C-1. The priority coordinate vectors were calculated following the AHP recommended in the literature (*30*).

RESULTS AND DISCUSSION

Japanese quince juice

The effect of enzymatic treatment on the quality indicators of JQJ was observed in this study, and the results are summarized in Table 2**.** The obtained results showed that treatment with enzymes significantly ($p<0.05$) positively affected the clarity of the juice, reaching almost 90 % transparency compared with the control (non-treated) sample. The TR values of clarified JQJ fluctuated in the range from 0.021 to 0.053. As seen, the most pronounced influence of enzymes on the clarity of juice is ranked in the following order: 1000 S (91.2 %)>bentonite (88.3 %)>EZ (86.2 %)>RS (77.9 %). Hmid *et al*. (*31*) found that pomegranate juice clarity increases as the concentration of the used enzymes (in particular, pectinase and protease) increases. The authors indicated that applying enzymes at concentrations from 0.05 to 0.5 % (juice to enzyme ratio, *v/v*) substantially improved the clarity of juice. The potential applicability of polygalacturonase in the food industry has been demonstrated by Amobonye *et al*. (*32*), who noted that enzymatic treatment of pear juice with polygalacturonase substantially improved its clarity, positively affecting the browning index reduction and turbidity decrease.

The content of total acids (TA) and soluble solids (SSC) in the control JQJ sample, which before analysis was frozen and thawed, corresponded to 2.35 % and 7.80 Brix %, while the pH was 2.83, respectively. Similar results were reported by Nowak *et al*. (*33*), indicating an almost equal composition of fresh (unprocessed) juices, where the pH of quince juice was 2.8, and the soluble solids content was 7.5 Brix %. Enzymatic treatment of JQJ with pectolytic enzymes and bentonite affected TA ($p=0.002$) and pH ($p=0.001$). In turn, the content of SSC was not significantly changed ($p>0.05$) compared with the control sample. A more significant difference was determined by the pectinase treatment of pomegranate juice, the initial pH (4.13) of which was reduced from 3.69 (pectinase concentration 0.5 %) to 3.82 (pectinase concentration 0.05 %) during the clarification process (*31*). A similar pH reduction was observed by Amobonye *et al*. (*32*) after clarification of pear juice with polygalacturonase, where the difference between the control and treated juice was 3.6 %. The reduction in the pH during juice clarification is believed to result from galacturonic acid release, which is a pectin degradation product.

Treatment of JQJ with hydrolytic enzymes and bentonite significantly reduced the amount of pectin (PE) in the samples ($p<0.05$) compared to the control sample, which was an important objective of the study (Fig. 1). The most pronounced depectinization efficiency was observed after enzymatic treatment of JQJ with enzyme EZ, as nearly 88.9 % of the PE observed in the control sample was removed after enzyme exposure and filtration. A significantly lower but still relevant reduction in the PE content was observed in JQJ exposed to enzymatic treatment with RS and 1000 S, indicating a decrease of 81.9 % and 74.0 % PE compared with the initial value, respectively. Treatment with bentonite showed a significantly lower depectinization efficiency, causing a percentage decrease of

PE by 29.2 % compared with the initial amount. This can be explained by the composition of bentonite and the mechanism that removes pectin from the liquids. According to the datasheet provided along the "Neoclar AF" preparation, the bentonite does not contain pectin-degrading enzymes, the primary mechanism of pectin removal is associated with the interaction of negatively charged bentonite and activated charcoal with positively charged high-molecular-mass compounds, in particular, proteins, while precipitating it. Since the formation of protein-pectin complex, which proceeds more intensively under the conditions of low pH, greatly affects the clarity of juice (*34*), partial clarification can be achieved by adding bentonite to JQJ. A similar observation was made by Valdhuber and Pulko (*35*) , highlighting the ability of bentonite with a small addition of sodium to clarify apple juice effectively.

The concentration of total phenolics (TP) content in JQJ was found in the range from 316.55 to 326.25 GAE mg/100 g FM, with the control JQJ sample containing the highest content and the bentonite and enzyme-treated EZ sample the lowest. However, no significant influence of JQJ clarification on TP content was observed since the reduction amounted to 1.8–3.1 % compared to the control sample. The results of this study are not coinciding with the finding reported by Martino *et al.* (*36*) and Candrawinata *et al.* (*37*), indicating either the increase or decrease of TP content after juice clarification.

The amount of vitamin C in JQJ samples fluctuated in the range from 68.35 to 72.8 mg/100g FM, with the control JQJ sample containing the highest and enzyme-treated samples, *i.e*. 1000 S and RS, the lowest amount. It was observed that the addition of enzymes to the JQJ for clarification negatively affected vitamin C content. After clarification, the amount of vitamin C in JQJ decreased by 4.6–6.1 % compared to the control sample. The decrease in vitamin C content is conditioned perhaps by the sensitivity of this vitamin to heat, light, and oxygen the samples were exposed to during juice clarification. However, the research of Radziejewska-Kubzdela (*38*) focused on the analysis of bioactive compounds in strawberry mass depending on the type of treatment, i.e., ultrasonic, thermal and enzymatic, did not reveal the direct influence of enzymatic treatment with pectin-degrading enzymes on vitamin C content.

The concentration of proanthocyanidins (TT) in JQJ ranged from 242.4 to 314.3 expressed in CE mg/100 g FM, with the control JQJ sample having the highest and enzyme-treated EZ sample and bentonite-treated the lowest. Similar to vitamin C, the clarification process negatively affected the content of this type of biomolecule since the percentage reduction amounted from 8.5 to 22.9 %, the 1000 S enzyme having the most negligible effect, while bentonite had the most. A strong interaction between various substrates and tannins, among which the substrates considered included activated carbon, clays, bentonites, and so forth, was revealed in studies on tannin adsorption in water treatment (*39*). This observation is reinforced by Youn *et al.* (*40*) finding, indicating considerable

effectiveness of activated carbon or bentonite in apple juice, achieving 26.9 % and 23.1 % tannin mitigation efficiency, respectively. A study on the treatment of apple juice with various clarifiers performed by (*41,42*) indicated that bentonite and gelatin (even with the addition of chitosan) adsorb and reduce the content of phenolic compounds, including TT.

Analyzing the antiradical activity of JQJ, it was observed that the control JQJ sample showed a value of 2.6 TE mmol/100 g FM, while samples subjected to clarifying demonstrated slightly higher radical scavenging activity by 3.8 to 5.8 %, with RS and bentonite-treated samples having the highest values and 1000 S and EZ the lowest. The results of the FRAP assay showed significantly higher values ranging from 87.00 to 92.95 TE mmol/100 g FM, with the control JQJ sample demonstrating the highest value, while 1000 S and EZ were the lowest. A percentage reduction of antioxidant activity according to FRAP analysis revealed from 1.3 to 6.4 % loss, with 1000 S, EZ, and bentonite having the most substantial negative impact, while RS had negligible effects. A similar observation was made by Mazrou *et al.* (*41*), indicating that after treatment with bentonite, the turbidity of grape juice decreased, and it became more transparent; however, the content of TP (including TT) was substantially decreased. This observation is supported by data provided by Dey and Banerjee (*43*), which reveal that the clarification of apple juice using enzymes and activated charcoal resulted in a 19.8 % reduction in TP compared to cloudy juice samples. Similarly, activated carbon has been shown to modify the volatile composition of fruits, including grape juice (*44*), potentially influencing both the flavor and color of juice and wine. In a study by Liu *et al*. (*45*), activated carbon treatment was found to reduce ester concentrations in stored apples, which play a key role in fruit aroma. The action of hydrolytic enzymes, in turn, is aimed at breaking the ester bonds between macromolecules and active substances, such as phenolic compounds (*46*) and volatile organic compounds (*47*). This process facilitates the release of bound fractions, which are typically involved in various biochemical reactions or contribute to the bioactivity of the substances. Essentially, hydrolysis by these enzymes cleaves the ester linkages, resulting in the liberation of components that were previously bound within larger structures (*48*). However, Amobonye *et al.* (*32*) indicated that treatment of pear juice with the pectin-degrading enzyme polygalacturonase preserved antioxidant potential and TP content, as no significant changes were detected after treatment. Chemical compounds in different juice samples may react differently when treated with complex enzymes or activated carbon, which generally affects antioxidant activity and volatile compound composition.

Japanese quince juice concentrate

The concentration of fruit juices requires the partial removal of water without changes in solid composition, leaving all the original solid components, such as fruit carbohydrates, minerals and

vitamins, in the concentrated solution, which is essential for the food industry (*12*). The quality indicators of JQJ concentrate (JQJC) as a final product will determine its suitability for the needs of the food industry, which, according to the purpose of the research, would be included in the category of acidifiers, similar to lemon juice concentrate. Based on the quality indicators of lemon juice concentrate available on the market, JQJ samples were steamed to a soluble dry matter content of (50.5±0.5) Brix % after enzymatic treatment and filtration. The chemical composition, antioxidant activity and physical properties of JQJC are summarized and shown in Table 3.

TA and pH did not differ significantly between JQJC samples ($p>0.05$), showing values from 29.78 to 30.17 % and 2.53 to 2.56, respectively. According to Tamer *et al.* (*49*), lemon juice concentrate (Limkon Fruit Juice Concentrate Facilities, Adana, Turkey) with 45.4 % soluble solids content had a TA content of 15.66 g/100 g, which is almost 2 times lower than that observed in JQJC samples.

The obtained results showed that treatment with enzymes considerably ($p<0.05$) positively affected the clarity of the JQJC, on average reaching 64.9 % transparency compared with the control (non-treated) sample. The clarity values (TR) of clarified and concentrated JQJC fluctuated in the range from 0.33 to 0.48, compared to 1.16 in the control JQJ sample. The most pronounced influence of pretreatments on JQJC clarity can be assorted in the following order: 1000 S (71.6 %)>bentonite (65.5 %)>RS (63.8 %)>ES (58.6 %). The application of enzymatic treatment with enzyme 1000 S, containing polygalacturonase as a sole pectin-degrading enzyme, delivered a satisfactory JQJC clarity result and can be considered by the industry for potential utilization in fruit juice clarification.

The PE content in the control JQJC sample was 1.50 GalAE g/100 g, while from 0.49 to 0.73 GalAE g/100 g FM in the enzyme-treated samples, and 0.81 GalAE g/100 g FM in the bentonite-treated sample (Fig. 2). The most significant reduction in PE from JQJC was achieved when enzymes with cellulolytic and pectinolytic activities in RS and EZ preparations were utilized. A percentage reduction in PE content in these samples corresponded to 63.3 and 67.3 %, respectively. While a significantly ($p<0.05$) lower but still relevant decrease in the PE content was reached using 1000 S and bentonite preparations, the values corresponded to 51.3 and 46.0 % PE loss. According to these data, one can conclude that a combination of two or three enzyme preparations possessing both cellulolytic and pectinolytic activity, *i.e.* RS or EZ and 1000 S, can deliver sufficient quality of fruit juice in terms of clarity.

The amount of vitamin C in JQJC samples fluctuated in the range from 231.45 to 244.95 mg/100 g FM, with the control JQJ sample containing the highest and enzyme-treated samples, *i.e.* RS and 1000 S, the lowest amount. Burdurlu *et al.* (*50*) reported a very close value of vitamin C in lemon juice concentrate, corresponding to 225.0 mg/100 g. It was observed that adding enzymes to the JQJ for

clarification negatively affected vitamin C content. After clarification, the amount of vitamin C in JQJC, similar to JQJ, decreased by 3.3–5.5 % compared to the control sample. As seen, the concentration of vitamin C in JQJC compared to JQJ increased on average 3.4 times, indicating that evaporation conditions for removing excess water from JQJ may affect the content of bioactives. In a study performed by Hellín *et al.* (*17*), the content of vitamin C in JQJC with soluble solids content 70 Brix % achieved by a laboratory scale evaporator at 40 °C and 40 mbar vacuum was increased from 96 to 1048 mg/100 mL, corresponding to an increase of more than 10 times.

The concentration of TP in JQJC was found in the range from 3.07 to 3.34 GAE g/100 g FM, with the control JQJC sample having the highest content and the enzyme-treated sample 1000 S the lowest. The observed values were substantially higher than those reported by Tamer *et al.* (*49*) for lemon juice concentrate, corresponding to 116 GAE g/100 g. As a result of concentration, the content of TP in JQJC increased almost 10 times. The most substantial decrease in the content of TP was observed in JQJC treated with 1000 S, followed by EZ addition.

The concentration of TT in JQJC ranged from 1.75 to 2.93 CE g/100 g FM, with the control JQJ sample showing the highest and bentonite-treated and enzyme-treated EZ samples the lowest. Overall, the manipulations the JQJ samples were exposed to during the clarification process, i.e., enzymatic/bentonite treatment, filtration, and evaporation led to from 20.8 to 40.4 % TT content loss. Similar to JQJ, the most favorable effect of processing on the content of TT was observed in JQJC obtained after JQJ exposure to enzymatic treatment with enzyme preparations 1000 s and RS.

The use of cellulolytic/pectolytic enzymes and bentonite did not significantly ($p>0.05$) affect the antioxidant activity of JQJC according to DPPH$^{\bullet}$ and FRAP assays. DPPH$^{\bullet}$ radical scavenging activity values varied from 5.37 to 6.11 TE mmol/100 g FM, while the FRAP values from 0.51 to 0.55 TE mol/100 g FM. The control sample demonstrated slightly higher values in both antioxidant activity assays than JQJ exposed to the clarification process. The observed DPPH$^{\bullet}$ values disagree with those reported by Tamer *et al*. (*49*) for lemon juice concentrate, corresponding to 12.1 TE mmol/100 g, and are, on average 5.5, times smaller than observed in JQJC. Substantial differences were also observed between the FRAP values obtained in this study and those reported by Tamer *et al.* (*49*) for lemon juice concentrate, revealing that JQJC had a much stronger reducing power than lemon concentrate. Among the four fruit juice concentrates evaluated, *i.e.* tangerine, grape, lemon, and lime, Oikeh *et al.* (*51*) highlighted the grape juice as the superior radical scavenger; the value corresponded to 36.42 mmol/g of the extract, which is nearly 7-times higher than observed in the current study. Reported differences are due primarily to the nature of the samples themselves, the content of biologically active compounds and, to a lesser extent, fruit processing conditions and sample preparation methods.

As reported earlier, 5-Hydroxymethylfurfural (HMF) in the sample may indicate partial or complete dehydration of sugars, mainly hexoses, under acidic conditions or if the sample was exposed to heat treatment. It is widely used as a marker of quality deterioration, thermal processing and other adulteration practices. Following this statement, the HPLC analysis was done to specify the level of HMF in the prepared JQJC due to heat and time exposure. The HPLC analysis revealed no presence of HMF in prepared JQJC, and overall, the selected conditions for developing JQJC were found appropriate. However, the presence of HMF in boiled juice samples (*52*) and lemon juice and its concentrate (*53*) was reported as the leading cause of thermal exposure. Moreover, during 120-day storage, the concentration of HMF content in lemon juice concentrate nearly doubled, corresponding to 503.20 μg/L. Burdurlu *et al.* (*50*) reported that HMF accumulation in citrus juice concentrates increased as a function of storage temperature, and eight weeks of product storage at 45 °C was equivalent to about 2.7 times longer storage at 37 °C. Higher storage temperatures also negatively affected the vitamin C content of citrus fruit concentrate, which decreased more rapidly during storage. The importance of HMF control during storage is still relevant and must be considered at the product development stage.

The Analytical Hierarchy Process (AHP) was used to evaluate the effect of enzymatic treatment on the quality characteristics of JQJC. Importance vectors were calculated by AHP, considering the clarity (TR) of the concentrate (after juice clarification) and the most important indicators characterizing the chemical content: TA and PE content, antioxidant activity and vitamin C content. According to AHP, the lowest priority coefficient value is the most important criterion. The calculated vectors showed the following values: TR=0.04; TA=0.18; PE=0.21; $DPPH^{\bullet}$=0.25; and vitamin C=0.32. The results of the AHP are summarized in Fig. 3. Evaluating the quality indicators of the JQJC according to the importance of the AHP principle, one can conclude that the treatment of juice with the pectin-degrading enzyme 1000 S positively affected the TR of the concentrate (priority coefficient 0.12). Similar to TR indices, the enzymatic treatment of JQJ samples with 1000 S preparation led to increased $DPPH^{\bullet}$ radical scavenging activity, as indicated by the corresponding priority coefficient 0.19. The most apparent effect of 1000 S is revealed compared to the non-treated control sample. The AHP analysis highlights the importance of RS and EZ enzymatic preparations in effectively removing pectic substances from JQJ, indicating priority coefficients corresponding to 0.13 and 0.12, respectively. According to the summary of AHP, considering all the priority criteria selected, the EnartisZym enzyme preparations EZ and RS, those declared to have both cellulolytic and pectinolytic activities, ensured the lowest priority coefficient values, corresponding to 0.18. The presence of cellulose-degrading enzymes along with pectinases delivered the most efficient removal of high-molecular-mass compounds from colloid systems of JQJ and can be considered suitable for JQJ

clarification purposes. However, in light of the results obtained, further studies need to be undertaken to understand the effect of cellulolytic enzymes on JQJ clarification. The process optimization and operational conditions during fruit juice clarification can deliver the desired quality of the final product with the relative stability of nutrients.

CONCLUSIONS

The present study elucidated the impact of three pectin-degrading enzymes and one bentonite/ activated charcoal-containing clarifying agent on the quality characteristics of Japanese quince (*Chaenomeles japonica* L.) juice and its concentrate. The study results demonstrated that all applied clarifying preparations can be considered by the industry as suitable solutions to improve the appearance of fruit juice along with the ability to reduce such antinutrients as proanthocyanidins. Based on the data obtained by the Analytical Hierarchy Process (AHP), the superiority of enzymatic preparation EnartisZym 1000 S containing polygalacturonase as a sole pectin-degrading enzyme was highlighted. However, considering generalized scores acquired from AHP analysis, the enzyme EnartisZym EZ Filter, possessing both cellulolytic and pectolytic activities, was found to be the most efficient, ensuring the quality characteristics of Japanese quince juice concentrate nearly similar to those commercially available on the market such as lemon juice concentrate. According to the demonstrated clarifying solution of Japanese quince juice done by sequential processing, i.e., enzymatic treatment, filtration, and concentration/ evaporation, the obtained values of the final product were as follows: titratable acidity 29.94 %, pH 2.56, and vitamin C content 236.12 mg/100 g. Overall, the developed product can be considered an alternative to lemon juice concentrates that, along with the preservative and acidifying properties, could also ensure the nutritional benefits of the products to which it is added. Further research should focus on an in-depth study of Japanese quince juice concentrate, including its use in preparing various products.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The manuscript is original. No part of the manuscript has been published before, nor is any part of it under consideration for publication in another journal.

AUTHORS' CONTRIBUTION

D. Segliņa was in charge of the conception of work and writing the original draft of manuscript. I. Krasnova was in charge of performing analyses, data analysis and interpretation. V. Radenkovs was in charge of the article critical revision. K. Juhnevica-Radenkova was in charge of the formal analyses. I. Cinkmanis was in charge of the formal analyses. L. Kļaviņš was in charge of the data collection. D. Lazdiņa was in charge of the final approval of the version to be published.

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REFERENCES

1. Kaufmane E, Ruisa S. Breeding of new cultivars of the fruit crop Japanese quince (*Chaenomeles japonica*) in Latvia. *Acta Hortic.* 2020;1281:51–7.
2. Rural support service Republic of Latvia. Area payment statistics. 2023. Available from: https://www.lad.gov.lv/lv/platibu-maksajumu-statistika
3. Radenkovs V, Krasnova I, Cinkmanis I, Juhnevica-Radenkova K, Rubauskis E, Seglina D. Comparative analysis of Japanese quince juice concentrate as a substitute for lemon juice concentrate: Functional applications as a sweetener, acidifier, stabilizer, and flavoring agent. Horticulturae. 2024;10(12):1362. https://doi.org/10.3390/horticulturae10121362
4. Urbanavičiūtė I, Viškelis P. Biochemical composition of Japanese quince (*Chaenomeles japonica*) and its promising value for food, cosmetic, and pharmaceutical industries. In: Kahramanoğlu İ, Wan C, editors. Fruit Industry. Rijeka, Croatia: InTech Open; 2022. Chapter 19, pp. 365–83.

https://doi.org/10.5772/intechopen.102361

5. Marat N, Danowska-Oziewicz M, Narwojsz A. *Chaenomeles* species—characteristics of plant, fruit and processed products: A review. Plants. 2022;11(22):1–14. https://doi.org/10.3390/plants11223036
6. Thomas M, Guillemin F, Guillon F, Thibault JF. Pectins in the fruits of Japanese quince (*Chaenomeles japonica*). Carbohydr Polym. 2003;53(4):361–72. https://doi.org/10.1016/S0144-8617(03)00118-8
7. Klimek-Szczykutowicz M, Szopa A, Ekiert H. *Citrus limon* (lemon) phenomenon-A review of the chemistry, pharmacological properties, applications in the modern pharmaceutical, food, and cosmetics industries, and biotechnological studies. Plants. 2020;9(1):e119. https://doi.org/10.3390/plants9010119
8. Ros JM, Laencina J, Hellín P, Jordán MJ, Vila R, Rumpunen K. Characterization of juice in fruits of different *Chaenomeles* species. LWT - Food Sci Technol. 2004;37(3):301–7. https://doi.org/10.1016/j.lwt.2003.09.005
9. Bhattarai A, Shrestha N, Shrestha S. Determination of ascorbic acid in different citrus fruits of Kathmandu Valley. J Med Biol Sci Res. 2016;2(1):9–14. Available from: http://pearlresearchjournals.org/journals/jmbsr/index.html
10. Lorente J, Vegara S, Martí N, Ibarz A, Coll L, Hernández J, *et al.* Chemical guide parameters for Spanish lemon (*Citrus limon* (L.) Burm.) juices. Food Chem. 2014;162:186–91. https://doi.org/10.1016/j.foodchem.2014.04.042
11. Hajimahmoodi M, Aliabadipoor M, Moghaddam G, Sadeghi N, Oveisi MR, Jannat B. Evaluation of *in vitro* antioxidant activities of lemon juice for safety assessment. Am J Food Technol. 2012;7(11):708–14. https://doi.org/10.3923/ajft.2012.708.714
12. Adnan A, Mushtaq M, Islam T ul. Fruit juice concentrates. In: Rajauria G, Tiwari BK, editors. Fruit juices: Extraction, composition, quality and analysis. London, UK: Academic Press (Elsevier); 2018. pp. 217–40. https://doi.org/10.1016/B978-0-12-802230-6.00012-6
13. Choudhary A, Kumar V, Kumar S, Majid I, Aggarwal P, Suri S. 5-hydroxymethylfurfural (HMF) formation, occurrence and potential health concerns: Recent developments. Toxin Rev. 2021;40(4):545–61. https://doi.org/10.1080/15569543.2020.1756857

14. Barrosi STD, Mendes ES, Peres L. Influence of depectinization in the ultrafiltration of West Indian cherry (*Malpighia glabra* L.) and pineapple (*Ananas comosus* (L.) Meer) juices. Ciência e Tecnol Aliment. 2004;24(2):194–201. https://doi.org/10.1590/S0101-20612004000200006
15. Heena V, Lokesh K N, Jyoti S J. Pectinase: A useful tool in fruit processing industries. Nutr Food Sci Int J. 2018;5(5). https://doi.org/10.19080/NFSIJ.2018.05.555673
16. Uçan F, Akyildiz A, Ağçam E. Effects of different enzymes and concentrations in the production of clarified lemon juice. J Food Process. 2014;2014:1–14. https://doi.org/10.1155/2014/215854
17. Hellín P, Jordán MJ, Vila R, Gustafsson M, Göransson E, Åkesson B, et al. Processing and products of Japanese quince (*Chaenomeles japonica*) fruits. In: Rumpunen K, editor. Japanese quince – potential fruit crop for Northern Europe. Uppsala, Sweden: Swedish University of Agricultural Sciences; 2003. pp. 169–76. Available from: https://pub.epsilon.slu.se/5201/
18. Rabadán A, Nieto R, Bernabéu R. Food innovation as a means of developing healthier and more sustainable foods. Foods. 2021;10(9):8–11. https://doi.org/10.3390/foods10092069
19. Patel VB, Chatterjee S, Dhoble AS. A review on pectinase properties, application in juice clarification, and membranes as immobilization support. J Food Sci. 2022;87(8):3338–54. https://doi.org/10.1111/1750-3841.16233
20. Sin HN, Yusof S, Hamid NSA, Rahman RA. Optimization of enzymatic clarification of sapodilla juice using response surface methodology. J Food Eng. 2006;73(4):313–9. https://doi.org/10.1016/j.jfoodeng.2005.01.031
21. United States Department of Agriculture. Technical procedures manual. Standard laboratory procedures documents and index. 2020. Available from: https://www.ams.usda.gov/sites/default/files/media/TechnicalProceduresManual.pdf
22. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by me. Methods Enzymol. 1999;299(2):152–78.
23. Paaver U, Matto V, Raal A. Total tannin content in distinct *Quercus robur* L. galls. J Med Plants Res. 2010;4(8):702–5. https://doi.org/10.1016/S0076-6879(99)99017-1
24. Kyriakidis NB, Psoma E. Hydrocolloid interferences in the determination of pectin by the carbazole method. J AOAC Int. 2001;84(6):1947–9. https://doi.org/10.1093/jaoac/84.6.1947

25. EN 14130:2003 European Standard. Foodstuffs - Determination of vitamin C by HPLC. 2003. Available from: https://standards.iteh.ai/catalog/standards/cen/15d21027-d891-4ab0-aaea-5d87c7cc39b7/en-14130-2003

26. Floegel A, Kim DO, Chung SJ, Koo SI, Chun OK. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J Food Compos Anal. 2011;24(7):1043–8. https://doi.org/10.1016/j.jfca.2011.01.008

27. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem. 1996;239(1):70–6. https://doi.org/10.1006/abio.1996.0292

28. Gökmen V, Acar J. Simultaneous determination of 5-hydroxymethylfurfural and patulin in apple juice by reversed-phase liquid chromatography. J Chromatogr A. 1999;847(1–2):69–74. https://doi.org/10.1016/S0021-9673(99)00133-8

29. IBM Corp. IBM SPSS Statistics for Windows, v. 25.0. Armonk, NY, USA: IBM Corp; 2022. Available from: https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-25

30. Saaty TL. Decision making with the analytic hierarchy process. Int J Serv Sci. 2008;1(1):83. https://doi.org/10.1504/IJSSCI.2008.017590

31. Hmid I, Elothmani D, Hanine H, Oukabli A, Mellal T. Effects of enzymatic clarification of pomegranate juice by protease and pectinase treatments. J Bio Innov. 2016;5(4):506–15.

32. Amobonye A, Bhagwat P, Ruzengwe FM, Singh S, Pillai S. Pear juice clarification using polygalacturonase from *Beauveria bassiana*: Effects on rheological, antioxidant and quality properties. Polish J Food Nutr Sci. 2022;72(1):57–67. https://doi.org/10.31883/pjfns/145704

33. Nowak D, Gośliński M, Kłębukowska L. Antioxidant and antimicrobial properties of selected fruit juices. Plant Foods Hum Nutr. 2022;77(3):427–35. https://doi.org/10.1007/s11130-022-00983-2

34. Chevalier LM, Rioux LE, Angers P, Turgeon SL. Study of the interactions between pectin in a blueberry puree and whey proteins: Functionality and application. Food Hydrocoll. 2019;87(July 2018):61–70. https://doi.org/10.1016/j.foodhyd.2018.07.038

35. Valdhuber J, Pulko B. The impact of bentonite fining on apple juice clarity and sodium content. Acta Agric Slov. 2022;19(2):21–8.

36. Martino KG, Paul MS, Pegg RB, Kerr WL. Effect of time-temperature conditions and clarification on the total phenolics and antioxidant constituents of muscadine grape juice. LWT - Food Sci Technol. 2013;53(1):327–30. https://doi.org/10.1016/j.lwt.2013.03.001

37. Candrawinata VI, Blades BL, Golding JB, Stathopoulos CE, Roach PD. Effect of clarification on the polyphenolic compound content and antioxidant activity of commercial apple juices. Int Food Res J. 2012;19(3):1055–61.

38. Radziejewska-Kubzdela E. Effect of ultrasonic, thermal and enzymatic treatment of mash on yield and content of bioactive compounds in strawberry juice. Appl Sci. 2023;2(7)(4268):1–13. https://doi.org/10.3390/app13074268

39. Tangarfa M, Semlali Aouragh Hassani N, Alaoui A. Behavior and mechanism of tannic acid adsorption on the calcite surface: Isothermal, kinetic, and thermodynamic studies. ACS Omega. 2019;4(22):19647–54. https://doi.org/10.1021/acsomega.9b02259

40. Youn KS, Hong JH, Bae DH, Kim SJ, Kim SD. Effective clarifying process of reconstituted apple juice using membrane filtration with filter-aid pretreatment. J Memb Sci. 2004;228(2):179–86. https://doi.org/10.1016/j.memsci.2003.10.006

41. Mazrou S, Messaoudi M, Begaa S, Innocent Ch, Akretche D. Clarification of the Algerian grape juice and their effects on the juice quality Soraya. J Chem Inf Model. 2020;34(1):1–11. https://doi.org/10.4314/bcse.v34i1.1

42. Oszmiański J, Wojdyło A. Effects of various clarification treatments on phenolic compounds and color of apple juice. Eur Food Res Technol. 2007;224(6):755–62. https://doi.org/10.1007/s00217-006-0370-5

43. Dey TB, Banerjee R. Application of decolourized and partially purified polygalacturonase and α-amylase in apple juice clarification. Brazilian J Microbiol. 2014;45(1):97–104. https://doi.org/10.1590/s1517-83822014000100014

44. Culbert JA, Jiang WW, Bilogrevic E, Likos D, Francis IL, Krstic MP, et al. Compositional changes in smoke-affected grape juice as a consequence of activated carbon treatment and the impact on phenolic compounds and smoke flavor in wine. J Agric Food Chem. 2021;69(35):10246–59. https://doi.org/10.1021/ACS.JAFC.1C02642

45. Liu C, Liu X, Tian X, Zhang J, Zhang Z, Shi J, et al. Determination of volatile profiles inside apple fruit storage facilities using Monotrap™ monolithic silica adsorbent and GC–MS. Hortic Plant J. 2021;7(4):267–74. https://doi.org/10.1016/j.hpj.2020.12.003

46. Radenkovs V, Juhnevica-Radenkova K, Górnaś P, Seglina D. Non-waste technology through the enzymatic hydrolysis of agro-industrial by-products. Trends Food Sci Technol. 2018;77:64–76. https://doi.org/10.1016/j.tifs.2018.05.013

47. Chen X, Xu Y, Wu J, Yu Y, Zou B, Li L. Effects of pectinase pre-treatment on the physicochemical properties, bioactive compounds, and volatile components of juices from different cultivars of guava. Foods. 2023;12(2). https://doi.org/10.1016/j.lwt.2020.109929

48. Jiang X, Lu Y, Liu SQ. Effects of pectinase treatment on the physicochemical and oenological properties of red dragon fruit wine fermented with *Torulaspora delbrueckii*. LWT - Food Sci Technol. 2020;132(May):109929. https://doi.org/10.1016/j.lwt.2020.109929

49. Tamer CE, Yekeler FZ, Çopur ÖU, İncedayi B, Suna S. A study of fortification of lemonade with herbal extracts. Food Sci Technol. 2017;37(1):45–51. https://doi.org/10.1590/1678-457X.06016

50. Burdurlu HS, Koca N, Karadeniz F. Degradation of vitamin C in citrus juice concentrates during storage. J Food Eng. 2006;74(2):211–6. https://doi.org/10.1016/j.jfoodeng.2005.03.026

51. Oikeh EI, Omoregie ES, Oviasogie FE, Oriakhi K. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. Food Sci Nutr. 2016;4(1):103–9. https://doi.org/10.1002/fsn3.268

52. Kus S, Gogus F, Eren S. Hydroxymethyl furfural content of concentrated food products. Int J Food Prop. 2005;8(2):367–75. https://doi.org/10.1081/JFP-200060257

53. Uçan F, Ağçam E, Akyildiz A. Bioactive compounds and quality parameters of natural cloudy lemon juices. J Food Sci Technol. 2016;53(3):1465–74. https://doi.org/10.1007/s13197-015-2155-y

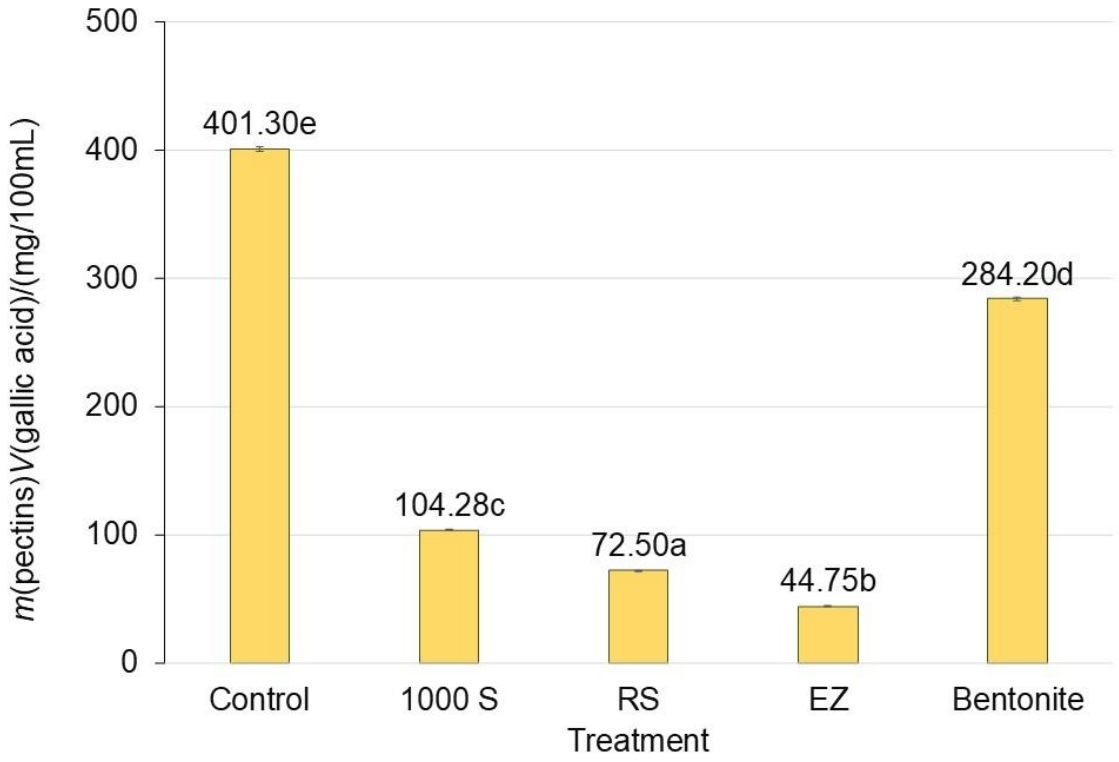

Fig. 1. The content of pectins in Japanese quince juice after enzyme and Neoclar AF treatment. Values are mean±S.D. of triplicates (*N*=3). Bars with different letters show significantly different values ($p<0.05$). 1000 S=EnartisZym 1000 S, RS=EnartisZym RS, EZ=EnartisZym EZ Filter

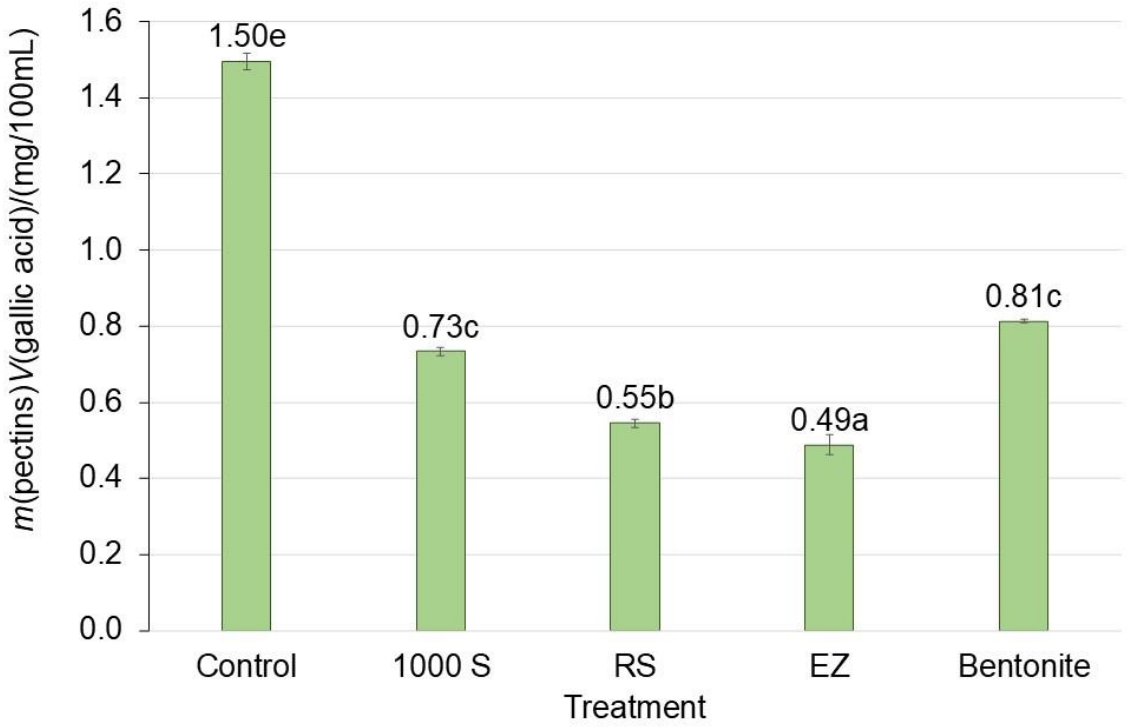

Fig. 2. The content of pectins in Japanese quince juice concentrates after enzyme and Neoclar AF treatment. Values are mean±S.D. of triplicates (*N*=3). Bars with different letters show significantly different values ($p<0.05$). 1000 S=EnartisZym 1000 S, RS=EnartisZym RS, EZ=EnartisZym EZ Filter

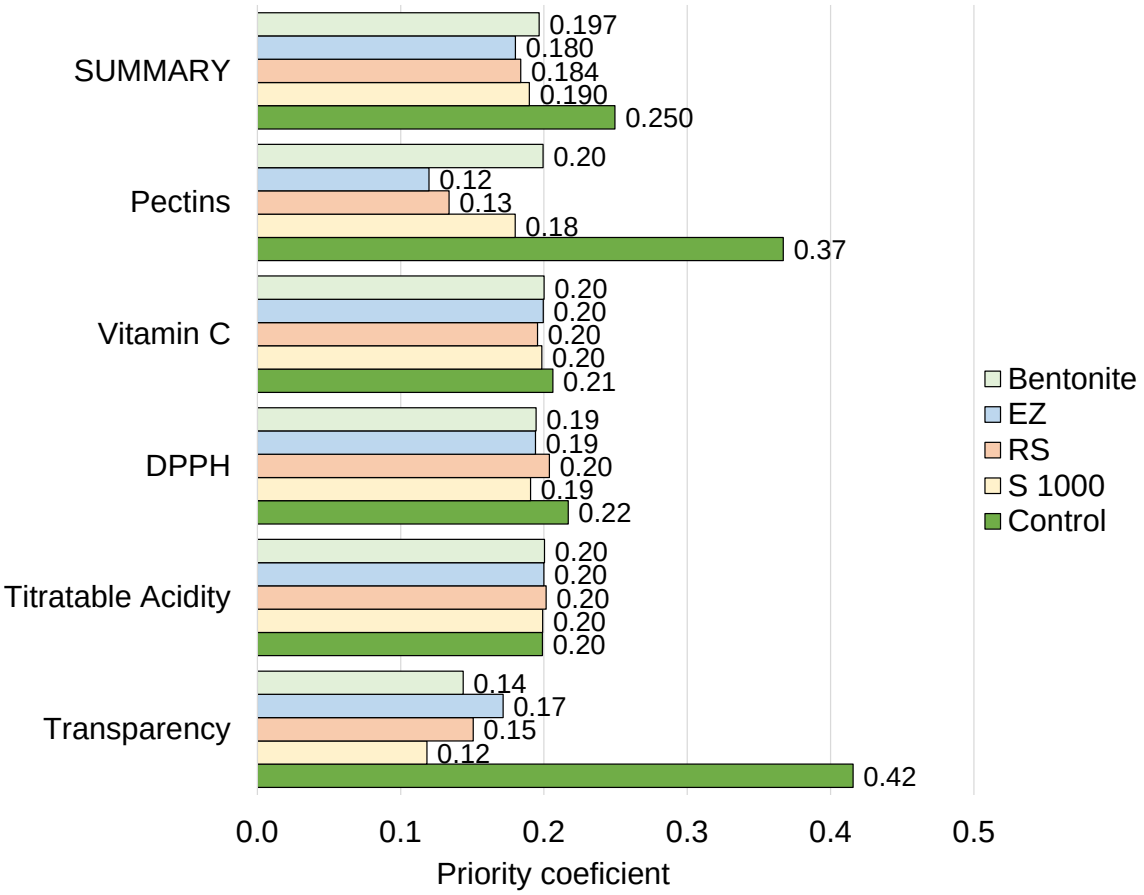

Fig. 3. Analytical hierarchy process analysis evaluation results. 1000 S=EnartisZym 1000 S, RS=EnartisZym RS, EZ=EnartisZym EZ Filter

Table 1. Enzyme and clarifying agent characteristics used for the treatment of Japanese quince juice

Enzyme (abbreviation)	Component	*w*/%	*t*(treatment time)/h	Temperature/°C	Amount of added enzyme/clarifying agent
EnartisZym 1000 S (1000 S)	Polygalacturonase	12.5–15	2	50±0.5	0.02 g
EnartisZym RS (RS)	1,3(4)-β-endo-glucanase	5–7	2	50±0.5	0.03 mL
	Pectin lyase	1–3			
	Pectinesterase	0.5–1			
	Polygalacturonase	0.5–1			
EnartisZym EZ Filter (EZ)	1,3(4)-β-endo-glucanase	5–7	2	50±0.5	0.04 mL
	Pectinesterase	5–7			
	Pectin lyase	3–5			
	Polygalacturonase	3–5			
Neoclar AF (Bentonite)	Bentonite powder	70–80	48	4±1	1.5 g
	Activated carbon	5–7			
	Gelatine powder	not indicated			

Table 2. Chemical composition, antioxidant activity and clarity of Japanese quince juice after treatment with enzymes and clarifying agent

Parameter	Sample				
	Control	1000 S	RS	EZ	Bentonite
TA/%	(2.35±0.06)^{ab}	(2.30±0.00)^{a}	(2.35±0.06)^{ab}	(2.30±0.00)^{a}	(2.45±0.06)^{b}
w(soluble solid)/Brix %	(7.80±0.12)^{a}	(7.78±0.15)^{a}	(7.65±0.06)^{a}	(7.80±0.12)^{a}	(7.65±0.06)^{a}
Clarity ($A_{660\ nm}$)	(0.24±0.01)^{c}	(0.021±0.00)^{a}	(0.053±0.00)^{b}	(0.033±0.00)^{a}	(0.028±0.00)^{a}
w(vitamin C)/(mg/100 g)	(72.80±0.58)^{b}	(68.95±0.98)^{a}	(68.35±0.40)^{a}	(69.45±0.87)^{a}	(69.08±0.85)^{a}
w(total phenolics as GAE)/(mg/100 g)	(326.25±2.94)^{b}	(319.45±3.64)^{ab}	(320.35±2.14)^{ab}	(316.15±5.72)^{a}	(316.55±3.18)^{a}
w(total proanthocyanidins as CE)/(mg/100 g)	(314.31±5.17)^{d}	(287.45±4.76)^{c}	(281.23±2.68)^{c}	(258.20±2.66)^{b}	(242.40±2.13)^{a}
DPPH$^{\bullet}$ scavenging as (*n*(TE)/*m*(FM))/(mmol/100 g)	(2.60±0.02)^{a}	(2.70±0.01)^{b}	(2.75±0.01)^{b}	(2.70±0.06)^{b}	(2.75±0.06)^{b}
FRAP as (*n*(TE)/*m*(FM))/(mmol/100 g)	(92.95±5.33)^{b}	(87.00±1.60)^{a}	(91.75±3.83)^{b}	(87.20±1.15)^{a}	(87.58±2.75)^{a}
pH	(2.83±0.00)^{d}	(2.78±0.00)^{b}	(2.71±0.00)^{a}	(2.82±0.00)^{c}	(2.83±0.00)^{d}

Values are mean±S.D. of quadruplicates (*N*=4). Values with different letters in the same row are significantly different ($p<0.05$). TA=titratable acidity, GAE=gallic acid equivalent, CE=catechin equivalent, TE=Trolox equivalent, FM=fresh mass, 1000 S=EnartisZym 1000 S, RS=EnartisZym RS, EZ=EnartisZym EZ Filter

Table 3. Chemical composition, antioxidant activity and clarity of Japanese quince juice concentrate after treatment with enzymes and clarifying agent

Parameter	Sample				
	Control	1000 S	RS	EZ	Bentonite
TA/%	(29.78±0.00)^{a}	(29.82±0.31)^{a}	(30.17±0.00)^{a}	(29.94±0.00)^{a}	(30.01±0.20)^{a}
w(soluble solid)/Brix %	(50.40±0.12)^{a}	(50.25±0.22)^{a}	(50.50±0.100^{a}	(50.30±0.18)^{a}	(50.40±0.09)^{a}
Clarity ($A_{660\ nm}$)	(1.16±0.00)^{e}	(0.33±0.00)^{a}	(0.42±0.00)^{c}	(0.48±0.00)^{d}	(0.40±0.00)^{b}
w(vitamin C)/(mg/100 g)	(244.95±0.63)^{c}	(235.08±2.25)^{ab}	(231.45±1.35)^{a}	(236.12±1.68)^{b}	(236.90±0.91)^{b}
w(total phenolics as GAE)/(g/100 g)	(3.34±0.05)^{b}	(3.07±0.11)^{a}	(3.16±0.12)^{ab}	(3.13±0.08)^{ab}	(3.20±0.11)^{ab}
w(total proanthocyanidins as CE)/(g/100 g)	(2.93±0.01)^{e}	(2.32±0.01)^{d}	(2.29±0.01)^{c}	(1.90±0.01)^{b}	(1.75±0.01)^{a}
DPPH$^{\bullet}$ scavenging as (*n*(TE)/*m*(FM))/(mmol/100 g)	(6.11±0.29)^{a}	(5.37±0.39)^{a}	(5.74±0.21)^{a}	(5.46±0.80)^{a}	(5.48±0.36)^{a}
FRAP as (*n*(TE)/*m*(FM))/(mmol/100 g)	(0.55±0.02)^{a}	(0.51±0.04)^{a}	(0.51±0.01)^{a}	(0.51±0.00)^{a}	(0.51±0.00)^{a}
pH	(2.55±0.01)^{b}	(2.56±0.01)^{b}	(2.53±0.01)^{a}	(2.56±0.01)^{b}	(2.56±0.01)^{b}

Values are mean±S.D. of quadruplicates (N=4). Values with different letters in the same row are significantly different ($p<0.05$).
TA=titratable acidity, GAE=gallic acid equivalent, CE=catechin equivalent, TE=Trolox equivalent, FM=fresh mass, 1000 S=EnartisZym 1000 S, RS=EnartisZym RS, EZ=EnartisZym EZ Filter