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Biotechnology

Effect of Heat Treatment on the Physicochemical Properties of Wine and Alcohol Derived from Persimmon

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The study is focused on cultivar persimmon fruits (*Diospyros kaki* L.) of "Khachia" variety, which is characterized by high sugar concentration, rendering it a viable candidate for the production of alcoholic beverages. During the wine material preparation phase, persimmons were meticulously sectioned into fragments measuring between 0.8 and 1.2 cm. Subsequent to this partitioning, citric acid at a concentration of 5 g/l was added and a pure strain of *Saccharomyces Cerevisiae* yeast was added representing 4% of the total volume of the mixture. This mixture was then subjected to heat treatment under various conditions. The experimental outcomes demonstrated that macerated persimmon pulp, after being subjected to a thermal hold at 80°C for a duration of 20 minutes and subsequently cooled to 24°C, facilitated the initiation of alcoholic fermentation in the extracted juice (referred to as 'persimmon sweet'). This enhancement in fermentability can be attributed to the observed reduction in pectin and tannin levels, as well as a decrease in the viscosity of the liquid phase. Moreover, a significant increase in juice yield was recorded, rising from 40.50% in the unprocessed state to 63.40% post-treatment. The wine substrate produced exhibited favourable attributes, including reduced levels of soluble solids (3.52%), sugars (0.20%), and volatile acidity (0.24%). The persimmon-derived alcohol contained only negligible concentrations of methanol. The study ascertained that to achieve alcohol with optimal quality parameters from persimmon fruits, preliminary treatment protocol is essential. This protocol necessitates a sequence of operations: mechanical maceration, thermal application, holding period and subsequent cooling. *© 2024 Bull. Georg. Natl. Acad. Sci.*

persimmon "Khachia", heat treatment, wine, persimmon alcohol

The persimmon (*Diospyros kaki* L.), a fruit within the Ebenaceae family, holds significant cultural and economic value. Globally, persimmon production reaches approximately five million tons annually [1]. A diverse range of products, including dried fruits, jellies, jams, natural colourants, and vinegar, are derived from this versatile fruit. Despite its utility, it is noteworthy that less than 10% of the total persimmon yield undergoes processing [2]. The persimmon fruit, scientifically recognized for

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its abundant tannin and dietary fibre composition [3], is categorized into two primary types: astringent and non-astringent varieties. The astringent varieties are noted for their significant levels of soluble tannins. These tannins can be removed both naturally and artificially [4]. Relative to alternative fruit varieties, persimmons are characterized by an elevated sugar concentration, rendering them a viable raw material for vinification [5]. Researchers found significant levels of acetaldehyde, methanol, and fusel oils in alcohols produced from persimmons, substances known for their detrimental effects on human health. Methanol formation occurs through the demethoxylation of pectin, mediated by pectinolytic enzymes, during the process of alcoholic fermentation [6]. The application of pectinase in the preliminary treatment of persimmon must is essential. This enzymatic intervention facilitates the hydrolysis of pectin, softening of the pulp tissue, diminution of viscosity, and increasing juice yield [7].

In Georgia, the industrial use of persimmon fruit remains suboptimal. The utilization rate of persimmons can be increased by processing them into alcoholic beverages. This study aims to optimize the technological methodology for extracting alcohol from persimmons. This refinement encompasses the diminution and equilibration of pectin and tannin content through preliminary thermal processing of the raw materials, the decrease in juice viscosity, the minimization of methanol content in the alcohol, and the enhancement of the overall yield.

Materials and Methods

For the purpose of this investigation, mature persimmons of the Khachia variety, known for their astringency, were harvested from Akhalsheni, a village situated in the western region of Georgia. Selection criteria for the fruits included a pronounced yellow-orange hue and a robust texture, with individual specimens exceeding 220 grams in weight and presenting a sugar content surpassing 14% by weight. Saccharomyces cerevisiae (IOC Harmonie) was the yeast strain employed for the fermentation process.

Production of persimmon alcohol. The persimmon fruits underwent a thorough cleansing process before being macerated into fragments measuring between 0.8 and 1.2 cm. Subsequently, the resultant biomass was apportioned into three distinct segments. The first segment, A1, remained unprocessed as a control; the second, A2, was subjected to thermal treatment at 80°C for a duration of 20 minutes; and the third, A3, received a similar heat treatment but incorporated a subsequent cooling phase to 24°C. The juice was extracted from each of the three experimental groups. The expressed juice was then filtered through a fabric with a porosity of 0.5 mm, after which citric acid was introduced at a concentration of 5 g/l. A pure yeast culture, Saccharomyces cerevisiae, was cultured into the must, base at 4% of the total volume, and fermentation was conducted at a constant temperature of 26°C. The fermentation was deemed complete upon reaching a plateau in the dry matter content within the wine. The alcohol was then procured through a process of double distillation, yielding a persimmon spirit with an alcohol concentration of no less than 60.20 vol. %.

Analytical techniques for physicochemical properties. The quantification of total soluble solids (TSS) was performed utilizing a digital refractometer (Carl Zeiss, Germany), with measurements expressed on the Brix scale. Titratable acidity was assessed via titration using 0.1 N NaOH solution, with results calibrated to equivalent malic acid concentrations, employing an automated titrator (Mettler-Toledo, Switzerland) [8]. Ethanol and volatile compound content were determined in accordance with the AOAC Official Method (1990) [9], while sugar and pectin concentrations were measured following the AOAC Official Method (2005) [10]. Methanol content was analyzed through gas-liquid chromatography, specifically using a TRACE™ 1310 Gas Chromatograph (Thermo Scientific). Detection of the analytes was conducted using a flame ionization detector with an aluminium column.

Determination of soluble tannins. For the quantification of soluble tannins, a 10.0-gram portion of the sample was accurately weighed using a precision analytical balance. This sample was then transferred to a 250-millilitre volumetric flask. To this flask, 200 millilitres of distilled water were added, and the sample was subjected to an extraction process in a water bath at 80°C for 30 minutes. Post-extraction, the volume of the extract was adjusted to 250 millilitres with additional distilled water, and the solution was filtered. A 10-millilitre aliquot of this filtered extract was used for the analysis. To the aliquot, 25 millilitres of indigo carmine solution was added. The mixture was then titrated with 0.1N solution of potassium permanganate ($KMnO_4$) until the solution's colour shifted from blue to amber. The amount of tannins present was quantified using a conversion factor of 0.004157.

Statistical analysis. The statistical analysis was conducted by calculating the standard error for each set of data utilizing the Microsoft Excel software. A confidence level was established, with a significance threshold set at $p \leq 0.05$.

Results and Discussion

The data presented in Table 1 elucidate the physicochemical properties of the persimmon pulp subjected to varying processing conditions. It was observed that the quantity of water-soluble solids experienced a marginal reduction, from 18.60% to 17.60%, throughout the thermal treatment. Conversely, the sugar levels remained relatively stable across the different samples. In terms of pectin and tannin concentrations, there was a notable decrease from 0.86% to 0.51% for pectin, and a slight reduction from 0.68% to 0.58% for tannins.

Table 1. Impact of heat treatment on the physicochemical characteristics of persimmon juice

Parameters	Control (without processing)	After 20 min at 80°C	After 20 min at 80°C followed by cooling to 24°C
Dry matter content, %	18.60	18.04	17.62
Total sugars, g/100g	15.60	15.52	15.45
Titratable acidity, g/L	6.01	6.05	6.10
Tannin content, %	0.41	0.37	0.12
Pectin content, %	0.56	0.54	0.15
Viscosity, centipoise	2.4	1.92	1.5
Juice yield, %	40.5	48.24	63.40

In the resultant juice derived from the thermally processed persimmon pulp, which was initially subjectted to a temperature of 80°C and subsequently cooled to 24°C, a marked reduction in specific constituents was observed. The content of pectin diminished to 0.15%, and tannin levels contracted to 0.12%, representing a substantial decrease relative to other tested samples. Concurrently, the viscosity of the juice exhibited a decline from 2.4 to 1.5 centipoise, and the juice yield experienced an augmentation, reaching 63.40%. A similar effect is achieved by treating persimmon juice with pectinase [11].

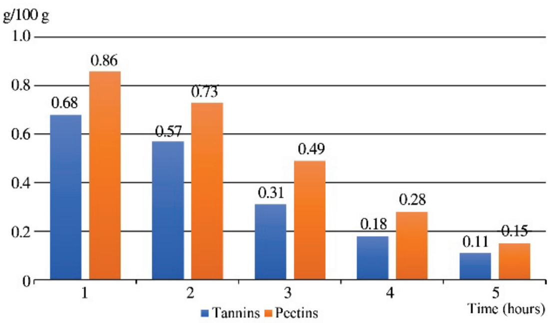

Fig. Temporal variations in tannin and pectin content in thermally processed persimmon mash during the cooling phase.

In the present study, the fluctuations in tannin and pectin concentrations within the macerated persimmon tissue, which underwent thermal treatment at 80°C with a subsequent holding period of 20 minutes, were investigated. This analysis was conducted during the post-cooling phase at discrete one-hour intervals. The results of the study are given in the Figure.

Following a five-hour period, a significant reduction in the concentration of tannins was observed, diminishing by approximately 6.1-fold from an initial value of 0.68% to 0.11%. Concurrently, pectin substances exhibited a comparable decline, decreasing by roughly 5.7-fold from an initial concentration of 0.86% to 0.15%. Notably, after this five-hour interval, the levels of these substances stabilized, showing no further change. The observed decrease in monomeric tannins can be attributed to their interaction and subsequent complex formation with high molecular weight compounds such as pectins and proteins.

In each experimental condition, the total soluble solids (TSS) content exhibited a marked decrease at the commencement of fermentation, most of the sugars were converted to ethanol, but in the terminal phase yeast had an inhibitory effect depending on the increase in alcohol content [12].

Notably, the most substantial diminution was recorded in the A3 variant, where fermentation concluded at 168 hours. Conversely, the A1 variant demonstrated a more extended fermentation duration, culminating at 240 hours. These discrepancies in fermentation kinetics can be attributed to the differential thermal processing modalities applied to the persimmon substrates. The preliminary thermal treatment of the raw materials exerted a pronounced influence on the principal chemical parameters of the resulting persimmon wine and the ethanol derived therefrom. The findings are presented in Tables 2 and 3.

Table 2. Chemical characteristics of persimmon wine (wine material)

Characteristics	Processing options		
	A1	A2	A3
Soluble dry matter, %	4.5	3.54	3.52
Residual sugar, g/100g	1.02	0.56	0.22
Ethanol (vol.%)	8.4	8.94	9.60
Titratable acidity, g/l	6.64	6.58	6.53
Tannin substances, %	0.56	0.28	0.24

The data presented in Table 2 elucidate that the wine base, from which persimmon alcohol is derived, exhibits a significant concentration of ethyl alcohol (9.6 vol.%) when processed using option A3. This high ethanol presence correlates with a minimal residual sugar content (0.22%). Additionally, the volatile acid content stands at 0.24%, serving as an indicator of the wine potency.

Analysis of the persimmon alcohol (Table 3) reveals that in the case of option A3, methanol is present only in trace amounts. Comparable observations were recorded for ethyl acetate and 3-methyl isoamyl alcohol concentrations.

Table 3. Chromatographic analysis of persimmon alcohol (values recalculated to absolute ethyl alcohol percentage)

Peak N	Peak name	Retention time (minutes)	A1	A2	A3
1	Methanol, %	11.633	0.048	0.057	<LOQ
2	Ethanol, %	13.78	99.838	99.77	99.98
3	Ethyl acetate, %	19.217	0.131	0.056	<LOQ
4	3-Methyl-1-butanol, %	24.73	0.029	<LOQ	<LOQ
5	Unknown peak	33.66	0.029	0.039	0.017

<LOQ - the limit of quantitation.

This phenomenon may be elucidated by considering that during thermal processing, pectic substances undergo interactions with tannins. Consequently, the methoxyl (-CH3) groups dissociated from the pectinic compounds lack an appropriate medium to facilitate their attachment to the hydroxyl (-OH) groups present in the vicinity, potentially leading to an elevated synthesis of methyl alcohol. Methanol is produced as a result of demethoxylation of pectin by pectinolytic enzymes during alcoholic fermentation [13].

Conclusions

The results of the conducted research suggest that heat treatment leads to a decrease in pectin and tannin substances, as well as a decrease in the viscosity of the juice. The yield of bran increases. In addition, this process helps to speed up alcoholic fermentation, efficiently convert sugars into ethanol, and reduce methanol and volatile acid levels, ultimately leading to increased alcohol yields. The technology developed from the obtained results has significant potential for commercial exploitation.

ბიოტექნოლოგია

თბური დამუშავების გავლენა ხურმის ღვინისა და მისგან მიღებული სპირტის მახასიათებლებზე

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(წარმოდგენილია აკადემიის წევრის ვ. პაპუნიძის მიერ)

კვლევები ჩატარდა ხურმის (*Diospyros kaki* L.) ჯიში „ჰაჩია“-ს ნაყოფებზე, რომელიც გამოირჩევა შაქრის მაღალი შემცველობით, რითაც შესაძლებელია მისი გამოყენება ალკოჰოლური სასმელების დასამზადებლად. ღვინომასალის მისაღებად მწიფე ნაყოფები დაქუცმაცდა 0,8-1,2 სმ ზომის ნაწილაკებად, დაემატა ლიმონმჟავა (5გ/ლ) და ტკბილში დათესილი საფუარის წმინდა კულტურა ნიმუშის მოცულობის 4% რაოდენობით, ჩაუტარდა თბური დამუშავება სხვადასხვა რეჟიმით. შედეგებმა აჩვენა, რომ ხურმის დაქუცმაცებული მასა 80°C-ზე 20 წთ. დაყოვნებული, გაცივებული 24°C-ზე, შემდგომ მისგან მიღებული წვენი(ხურმის ტკბილი) ადვილად ექვემდებარება სპირტულ დუღილს, რადგანაც მასში შემცირდა პექტინოვანი და მთრიმლავი ნივთიერებების შემცველობა, დაიკლო მადუღარა სითხის სიბლანტემ, გაიზარდა წვენის გამოსავლიანობა 63,40%-მდე, როცა დამუშავების გარეშე იგი 40,50%-ია. გარდა ამისა, მიღებული ღვინომასალა გამოირჩევა სასურველი მახასიათებლებით: მინიმუმამდე დაყვანილი ხსნადი მშრალი ნივთიერებები (3,52%), შაქრები 0,20%, მქროლავი მჟავები 0,24%. ორმაგი დისტილაციით მიღებულ ხურმის სპირტში მეთანოლის შემცველობა აღმოჩენილია კვალის სახით. დადგინდა, რომ ხურმის ნაყოფიდან სასურველი ხარისხობრივი მახასიათებლების მქონე სპირტის მისაღებად საჭიროა დადგენილი რეჟიმების შესაბამისად მოხდეს ხურმის ნაყოფის წინასწარი დამუშავება: დაქუცმაცება, გაცხელება, დაყოვნება და გაგრილება.

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