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A New Process for Wine Production by Penetration of Yeast in Uncrushed Frozen Grapes

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Abstract Different types of red and white wines were prepared by fermentation of the juice which was naturally separated from uncrushed frozen grapes during thawing (A) and from the residual juice by fermentation inside the berries (B). Yeast penetrated the skin of uncrushed grapes and fermented the content completely. The new types of wines were compared with wines prepared conventionally from the whole material of frozen grapes. Chemical and chromatographic analysis (gas chromatography (GC) and solid-phase microextraction–GC/mass spectrometry) showed similar profiles of the aroma volatiles but with significant quantitative differences among the new types of wines, which reflected to the differences observed during the sensory evaluations. The majority of identified compounds were esters, with higher amounts found in (A) wines due to the higher concentration of the must which was separated during thawing. The proposed process is new and of industrial interest for the production of different types of wines from the same raw material in one fermentation batch.

Keywords Wine · Frozen grapes · Yeast · Fermentation · Volatiles · Phenolics · Sensory evaluation

Introduction

The only case of wine making using uncrushed grapes is the carbonic maceration (CM), during which alcoholic fermentation advances due to anaerobic metabolism induced by

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CO_2 [1–3]. During the first stage of CM, yeast cannot penetrate the berries and only low amounts of alcohol are produced (1.2–1.9%, *v*/*v*). Complete conversion of sugars to alcohol is accomplished in a second stage after crushing of grapes. The CM technique is used for dry wine making, with main features the reduced color intensity due to shorter contact of the juice with the grape skins and the production of characteristic aromas [4, 5]. It is a technique traditionally applied in the area of Beaujolais (France) leading to fruity and less tannic and acidic red wines, ready for immediate consumption. Another case of fermentation using uncrushed grapes is after treatment with alkali, which injures the skin and allows yeasts to penetrate, grow in the berry, and ferment sugars to ethanol [6, 7]. Freezing techniques (cryoextraction or supraextraction) are used either for the abrupt crushing of grapes or the enhancement of white wine aromas [8] and red wine colors, according to the vacuum flash cooling (*Flash détente*) technique that is already commercially applied.

Freezing of grapes is naturally applied in cold climate regions in order to produce sweet wines such as ice wines [9–11]. Ice wines have distinct sensory characteristics and differ from those made from fresh grapes as the freezing and thawing process concentrates sugars and flavors and modifies the composition of the must [9, 10]. Cryoconcentration occurs due to freezing and subsequent separation of part of the frozen water in the form of pure ice crystals without retaining solids of the liquid product. The purity of the crystals depends on viscosity and the rate of crystallization [12]. Freezing is also accompanied by changes in pH, total acidity, viscosity, osmotic pressure, and oxidation–reduction potential [11]. These effects on must composition and wine quality are of interest to the wine industry and research. Freezing also damages the tissues of the grape flesh permitting reactions among components that normally are isolated in the berries. In practice, an antioxidant such as SO_2 is usually added in the must from frozen grapes to prevent the development of undesirable flavor or color that may accompany the freezing and thawing cycle [9]. In both cases of induced or natural freezing, crushing of grapes is done before the initiation of fermentation, which is then solely carried out by yeast.

The aim of this study was to evaluate the possibility of yeast penetration in uncrushed frozen red or white grapes during thawing, due to partial damages caused to grape skins by ice crystals. The proposed technique is novel since fermentation of the juice by yeast inside uncrushed grapes has not been reported. Also, when frozen grapes are allowed to thaw slowly, the amount of naturally extracted juice has a different composition from the juice that remains inside the berries, due to separation of water as ice crystals. Fermentation of these two different types of musts in different fermentation micro-environments leads to simultaneous production of different types of wines from the same raw material.

Materials and Methods

Materials

Red grapes of the Greek variety *Agioritiko* (year 2001) and white grapes of the variety *Chardonnay* (crop 2002), from the area of Nemea in Greece, were harvested at the ripeness stage and were frozen at −8°C in plastic bags of 1 kg (20 bags of each variety). The time for complete freezing of the bag contents was 8 h. The commercial strain *Saccharomyces cerevisiae* "Uvaferme 299", which is widely used in industrial wine making, was used in dried form for must fermentation.

Red Wine Making

Amounts of 2 kg of frozen grapes, after stem removal, were transferred into a cylindrical vessel and allowed to defreeze at 25°C. About 700 ml of juice (12.8°Be) was naturally extracted from the uncrushed grapes during thawing. The remaining juice inside the berries had a density of 12.2°Be; 0.2 g of potassium metabisulfite and 1 g of yeast were added. This amount of yeast (instead of about 0.1 g/2 kg of grapes used in common practice) was added in order to aid the penetration of yeast into the uncrushed grapes and to conduct fermentation exclusively with the commercial strain and not with wild yeasts that could potentially grow during fermentation. The time of fermentation outside the grape berries was 8 days, and inside the grapes it was 11 days. When the fermentation started, the intact grapes were drifted by the formed CO_2 toward the top of the vessel (Fig. 1). After the end of fermentation, the wine (A) was collected and the grapes were then crushed, yielding 650 ml of fermented must, having a density of 3°Be (~5.5°Brix), which was left to ferment until all residual sugar was utilized to obtain the second type of wine (B). Both wines were kept at 5°C for 12 h for chill haze precipitation and were then stored at −18°C until further analysis. The above process was repeated with slight modifications, i.e., allowing the system (must and uncrushed grapes) to ferment for three more days until the fermentation inside the grapes was also completed. The small values of SDs indicate that there were no substantial differences between the wines produced either way; therefore, no substantial interchange between the liquid inside and outside the berries occurred.

For comparison reasons, wine was also made using 2 kg of the same stock of frozen grapes, which were crushed after thawing, and the whole material (12.5°Be) was mixed and fermented under the same conditions as described above. In this case, two types of red wines were also prepared; one by keeping the skins and seeds in contact with the

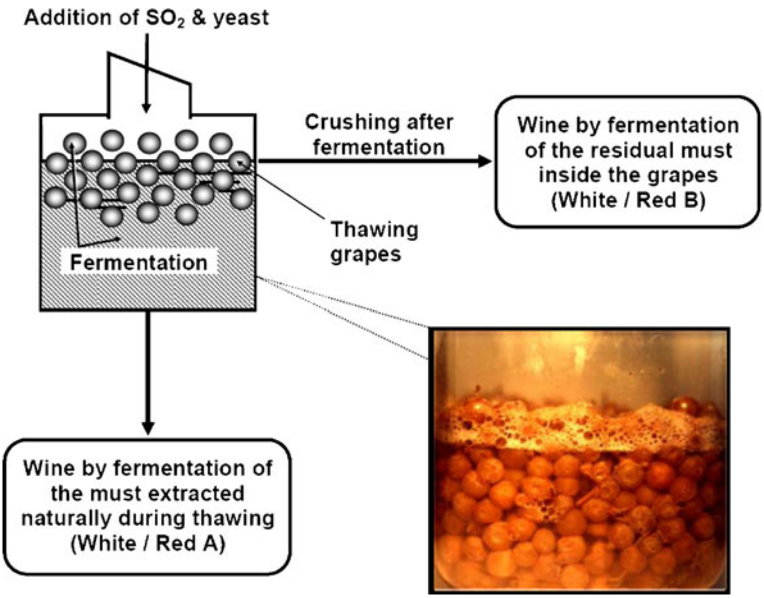

Fig. 1 Schematic representation of the processes for the production of different types of white and red wines (A and B) from frozen–thawed grapes. Photo of the fermentation vessel

fermenting liquid without disturbing them throughout the process (C) and a second by stirring the whole material occasionally for better color extraction (D).

White Wine Making

Two types of white wines were also produced using 2 kg of frozen grapes, following the same procedure as above. The first type was prepared from the juice (~700 ml, 11.7°Be) naturally extracted from the uncrushed grapes during thawing (A; 8 days of fermentation) and the second by fermentation of the residual must (~650 ml, 11.2°Be) inside the grape berries (B; 11 days of fermentation). Wine was also produced by the conventional method for white wine making using 2 kg of the same stock of frozen grapes (C; ~1,350 ml, 11.4°Be). In all cases, 0.2 g of potassium metabisulfite and 1 g of yeast were used, and fermentations were carried out at 25°C. The experiments were carried out twice without modifications, and the results are given as average values (Tables 1, 2, and 3).

Analysis

The color intensity of wines was calculated as the sum of optical densities at 420, 520, and 620 nm [13]. The determination of tannins and total phenolics was done after sample preparation according to the methods described by Riberau-Gayon et al. [13] and measurement of the optical densities at 550 and 260 nm, respectively. The SDs were ≤0.1 for color intensity and tannins and ≤0.5 for total phenolics. Residual sugar and ethanol were determined on a Shimadzu LC-9A HPLC system with a Shim-pack (SCR-101 N) column, a refractive index detector, three times distilled and filtered water as mobile phase (0.8 ml/min), and 1-butanol (0.05%, *v*/*v*) as internal standard (IS). Column temperature was 60°C. Sample dilution was 1% (*v*/*v*) and injection volume 40 μl. Total acidity of the wines (as grams of tartaric acid per liter) was determined by titration with 0.1 N NaOH. Volatile acidity (as grams of acetic acid per liter) was determined after steam distillation of the

Table 1 pH, ethanol, residual sugar, and acidity in red and white wines produced from uncrushed frozen–thawed grapes.

Type of wine	Must			Wine				
	pH	Total acidity (g tartaric acid/l)	°Be density	pH	Ethanol (%, *v*/*v*)	Residual sugar (%, *w*/*v*)	Total acidity (g tartaric acid/l)	Volatile acidity (g acetic acid/l)
Red A	4.3a	3.5a	12.8a	3.6a	12.9a	0.19b	4.7a	0.30
Red B	3.9b	4.5b	12.2b	3.7a	12.5a	0.21b	5.4b	0.28
Red C	3.6c	5.4c	12.5b	3.6a	12.7a	0.19b	5.2b	0.34
Red D	3.6c	5.4c	12.5b	3.6a	12.6a	0.17b	5.3b	0.34
White A	4.5a	3.8a	11.7a	3.3a	11.8a	0.16a	5.2a	0.43
White B	4.0b	4.7b	11.2b	3.5a	11.6a	0.16a	5.7a	0.47
White C	3.6c	5.6c	11.4ab	3.2a	11.7a	0.13a	5.4a	0.45

Different letters indicate significant differences ($p<0.05$) among mean values in the same row and for the same type of wine (red or white)

A fermentation of must extracted naturally during thawing, *B* fermentation of must inside the grapes, *Red C*, *D* fermentation of whole material of frozen grapes, without disturbing the skins during fermentation (C) or with occasional mixing of skins (D), *White C* fermentation of whole material of frozen grapes

Table 2 Color, tannins, and total phenolics in red and white wines produced from uncrushed frozen grapes.

Type of wine	Color intensity $A_{420}+A_{520}+A_{620}$	Tannins g/l	Total phenolics A_{280}
Red A	3.1a	1.4a	35.5a
Red B	6.1b	2.9b	41.0b
Red C	3.9a	1.8a	33.0a
Red D	8.2c	3.3b	54.5b
White A	0.09a	–	6.4a
White B	0.12b	–	8.6b
White C	0.06c	–	5.5c

Different letters indicate significant differences ($p<0.05$) among mean values in the same row and for the same type of wine (red or white)

A fermentation of must extracted naturally during thawing, *B* fermentation of must inside the grapes, *Red C*, *D* fermentation of whole material of frozen grapes, without disturbing the skins during fermentation (C) or with occasional mixing of skins (D), *White C* fermentation of whole material of frozen grapes

wines and titration of the distillates with 0.1 N NaOH. The SDs were ≤0.1 for pH, total acidity, and ethanol, ≤0.03 for residual sugar, and ≤0.03 for volatile acidity. One-way analysis of variance was used to test the significance of differences among results (Tables 1, 2, and 3).

Volatiles

The major volatiles in wines (ethyl acetate, 1-propanol, isobutyl alcohol, and amyl alcohols) were determined by gas chromatography (GC) on a Shimadzu GC-8A chromatograph, with a stainless steel column packed with Escarto-5905 (consisting of 5% squalene, 90% Carbowax-300, and 5% bis(2-ethylhexyl) sebacate), with N_2 as the carrier gas (20 ml/min) and a flame ionization detector (FID) detector. The injection port and detector temperatures were 210°C, and the column temperature was 70°C. The IS was 1-butanol (0.1%, *v*/*v*). Samples of 4 μl of wine were injected directly in the column, and the

Table 3 Major volatiles in red and white wines produced from uncrushed frozen grapes.

Type of wine	Ethyl acetate (mg/l)	1-Propanol (mg/l)	Isobutyl alcohol (mg/l)	Amyl alcohol (mg/l)	Isoamyl acetate (mg/l)	2-Phenyl ethanol (mg/l)	Methanol (mg/l)	Total (methanol excluded; mg/l)
Red A	53a	15a	13a	77a	0.5a	12a	76a	170.5a
Red B	43b	9b	8b	55b	0.3b	11a	150b	126.3b
Red C	61c	22c	8b	98c	0.3b	13a	180b	202.3c
White A	48a	18a	21a	130a	1.4a	44a	14a	262.4a
White B	39a	10b	9b	86b	1.1a	35b	19b	180.1b
White C	51a	22a	14c	130a	1.2a	33b	25c	251.2a

Different letters indicate significant differences ($p<0.05$) among mean values in the same row and for the same type of wine (red or white)

A fermentation of must extracted naturally during thawing, *B* fermentation of must inside the grapes, *Red C*, *D*: fermentation of whole material of frozen grapes, without disturbing the skins during fermentation (C) or with occasional mixing of skins (D), *White C* fermentation of whole material of frozen grapes concentrations of the above compounds were determined using both standard curves and the IS method. Methanol was determined on a similar Shimadzu GC-8A system consisting of a column packed with Porapac-S, N_2 as the carrier gas (20 ml/min), and an FID detector. The injection port and detector temperatures were 210°C, and the column temperature was programmed at 140–180°C (10°C/min). 1-Butanol (0.1%, *v*/*v*) was used as IS, and samples of 2 μl of wine were injected directly into the column. The SDs were ≤3 for ethyl acetate and 2-phenyl ethanol, ≤4 for 1-propanol, ≤2 for isobutyl alcohol, ≤5 for amyl alcohol, ≤0.1 for isoamyl acetate, and ≤12 for methanol.

Wine aroma was also analyzed by headspace solid-phase microextraction (SPME) GC/mass spectrometry (MS). The SPME fiber used was a 2-cm–50/30-mm DVB/Carboxen/PDMS StableFlex for manual holder (Supelco, USA) [14, 15]. The conditions of SPME sampling used were as follows: 10 ml sample and 0.3 g NaCl were transferred into a sealed 10 ml glass vial. The SPME needle was injected through the septum, the fiber was exposed to the headspace, and the vial was heated (waterbath) at 60°C for 45 min for absorption of volatiles. Desorption of volatiles took place in the GC injector in splitless mode, at 280°C for 3 min. GC/MS analysis was performed on a Shimadzu GC-17A chromatograph coupled to a GCMS-QP5050A mass spectrometer. A Supelcowax-10 column (60 m, 0.32 mm i.d., 0.25 μm film thickness) was used. The GC temperature program was initially 35°C held for 6 min, then increased by 2°C/min to 60°C, where it was held for 5 min, then increased by 5°C/min to 200°C, and then to 250°C by 25°C/min, where it was held for 6 min. The carrier gas was helium with a column flow of 1.8 ml/min. The interface temperature was 250°C. Mass spectra were recorded by electronic impact at 70 eV. Compounds were scanned in the range *m*/*z* 29–400. Identification of compounds was done by comparing the retention times and MS data with those of standard compounds and by MS data obtained from NIST107, NIST21, and SZTERP libraries and by determining Kovats retention indices and comparing with those reported in the literature [15]. Kovats retention indices were determined by injection of a standard mixture of normal alkanes (C_8–C_{22}) in pure hexane under the same experimental conditions. Quantification of volatiles was carried out as described recently [15, 16]. Specifically, 4-methyl-2-pentanol (Sigma-Aldrich, Poole, UK) diluted in pure ethanol was used as IS at various concentrations (0.016 M, 0.16 M, and 1.6 M). Quantitative determinations were carried out by dividing the peak areas of the compounds of interest by the peak area of the IS and multiplying this ratio by the initial concentration of the IS (expressed as milligrams per liter). The peak areas were measured from the full scan chromatograph using total ion current. Each determination was carried out in triplicate, and the mean data are presented (SD for all values was ±5% in most cases).

Sensory Evaluation

Sensory evaluations were conducted by 15 trained testers who evaluated separately the taste and aroma of the produced wines, comparing pairs of samples according to the triangle test method (one-tailed, $p=1/2$) proposed by Amerine and Roessler [17].

Results and Discussion

Different types of red and white wines were prepared from uncrushed frozen grapes, by fermentation of the juice naturally extracted during thawing, and by fermentation of the remaining juice inside the berries. The differences between the two types of wines were examined by analysis of aroma volatiles, color, phenolics, and by sensory evaluations, and comparisons were made with wines produced conventionally, using the whole material of frozen grapes. The formation of ice crystals during freezing is responsible for the different composition of the liquids remaining in the berries or extracted during thawing, as in the case of ice wine making. These compositional differences though are smaller compared to ice wines, where the juice is obtained by pressing the grapes while still frozen and is more concentrated than the liquid that remains in the berries. In this study, the remaining juice had lower pH ($p<0.05$), higher total acidity ($p<0.05$), and lower °Be density ($p<0.05$) than the juice that was extracted during thawing (Table 1). Also, due to partial damage of grape skins and tissues caused by freezing, the yeast was able to penetrate and grow inside the berries. Therefore, fermentation of the juice was possible inside the uncrushed grapes, at a different microenvironment compared to the surrounding liquid, and leading to different types of wines with distinct sensory characteristics.

Red and White Wine Making

The analytical parameters of the new types of red and white wines, such as ethanol, acidity, residual sugar, and pH, did not differ substantially ($p<0.05$) from those of the corresponding wines produced by the conventional method (Table 1) [18]. The high levels of alcohol and low levels of residual sugar and volatile acidity indicated a good vinification process [18] showing that fermentation was indeed feasible inside the uncrushed grapes. The organoleptic properties of wine, especially color and taste, are largely related to the tannins and total phenolics that are extracted from grapes during the wine making process and whose concentrations are affected by yeast metabolism [19, 20]. Among them, flavonoids such as anthocyanins, flavonols, and flavanols are particularly important to wine quality. The red wine that was prepared by fermentation of the must inside the berries (B) had higher color intensity (about double; $p<0.05$) and higher amounts of tannins ($p<0.05$) and total phenolics ($p<0.05$) than wine (A; Table 2). This can be attributed to the fact that the juice inside the uncrushed grapes was in longer contact with the seeds and tissues, which are the main sources of tannins in wine [21]. On the other hand, the extracted must was not in contact with all the amount of grape berries, which were drifted to the top of the vessel by the formed CO_2 during the nondisturbed fermentation process. Therefore, fewer phenolic substances were extracted. The higher values for color, tannins, and total phenolics ($p<0.05$) were recorded in the case of red wines prepared by the conventional way from the whole material of grapes but with occasional mixing of skins during fermentation (D) that facilitated extraction of pigments and phenolic compounds from the outer layer of the skins where most of these substances are located [1]. Tables 1 and 2 also include the analytical results obtained when the above processes were repeated allowing the system (extracted must mixed with uncrushed grapes) to ferment for three more days until the fermentation inside the grapes was also completed.

Similar results were obtained in the case of white wine making. The new types of wines (A and B) had higher color intensities ($p<0.05$) and higher amounts of total phenolics ($p<0.05$) than the wine produced by the traditional method (C; Table 2), due to the longer contact of the fermenting musts with the grape skins, tissues, and seeds.

Volatiles

Wine flavor is affected by the amounts of volatiles produced during fermentation [22, 23]. Determination of the major volatiles, such as higher alcohols (1-propanol, isobutyl alcohol, and amyl alcohols), isoamyl acetate, 2-phenyl ethanol, and methanol revealed differences between the studied types of wines, indicating differences in their organoleptic properties (Table 3). The concentrations of higher alcohols in all the produced types of wines were very low (77–130 mg/l), which is desired as they are considered off-flavors above certain concentrations [24, 25]. The lowest amounts of amyl alcohols were found in the wines produced by fermentation of the must inside the grape berries. This is an important observation regarding wine flavor and quality of wine distillates. Various control strategies have been adopted for the reduction of amyl alcohols to improve wine quality, including immobilized cell technologies and low temperature fermentation processes [6, 7, 26, 27]. Isoamyl acetate, a fermentation ester with intense banana flavor [25, 28], was also found in low levels (0.3–1.1 mg/l). It is a characteristic metabolite of CM, and its formation is promoted in limited oxygen and excess CO_2 conditions [29, 30]. Therefore, the low levels of isoamyl acetate in the wines produced by fermentation inside the berries indicated that the fermentation was conducted by yeast and not by the enzymes of the CM pathway. 2-Phenyl ethanol, a characteristic volatile compound of wines with a desirable rose aroma at concentrations 10–183 mg/l [28], was found in higher amounts in the white wines than in the red ones (11–12 and 35–44 mg/l, respectively; Table 3). Among the two types of red or white wines, higher 2-phenyl ethanol amounts were found in those produced from the must extracted from grapes during thawing (A). Methanol is also one of the significant components that determine the quality of wines, originating from the pectin substances of grapes. Therefore, as expected, higher amounts of methanol (150 mg/l; Table 3), but within accepted limits [24], were found in the red wines produced by fermentation inside the grapes (B). To maintain the concentrations of methanol and amyl alcohols at low levels is off critical importance to the quality of wines and their single distillation products, such as the traditional Greek products *tsipouro* and *tsikoudia* [31]. In total (methanol excluded), higher amounts of the major volatiles were found in red or white wines produced from the must extracted from grapes during thawing (A), the highest being those found in white wines (Table 3).

SPME-GC/MS Analysis

Headspace SPME-GC/MS analysis of aroma volatiles revealed differences in the aroma of the different types of the prepared red wines. Specifically, 69 compounds were identified in the headspace of red wines made by fermentation of the juice that was naturally extracted from the uncrushed grapes during thawing (A), 62 in wines made by fermentation of the juice inside the berries, and 59 compounds in wines made by the conventional method, using the whole material of frozen grapes. The majority of compounds identified in all cases were esters, mainly acetic acid esters of the C2–C8 alcohols and ethyl esters of the C2–C16 fatty acids of the aliphatic series (Table 4). Totally 45 esters in 81 compounds were identified. The major esters identified were ethyl acetate, isoamyl acetate, the hexanoate, octanoate, decanoate, and dodecanoate ethyl esters, and 2-phenyl ethyl acetate. All the identified esters are grape or fermentation-derived esters commonly found in wine, known to impart fruity and floral hints to wine aroma. No significant qualitative differences were observed among the tested wines, but the amounts of esters found in wine A were almost 4-fold higher than those of wine B, while the sum of both these amounts was almost equal to the sum of esters found in wine C made from the whole juice of frozen grapes. It is therefore obvious that the juice extracted from grapes during thawing was concentrated compared to the whole juice of frozen grapes. The alcohols identified in all the studied wines included the C2, C4, C6–C8 primary alcohols of the aliphatic series. Fusel alcohols like methyl isopropyl carbinol and 1-butanol were identified in traces (Table 4), like most of

Table 4 SPME-GC/MS analysis of volatiles in red wines produced from uncrushed frozen grapes.

Compound	Identification method	Wine A	Wine B	Wine C
Esters				
Ethyl acetate	RT, KI, MS	4.66	1.58	4.83
Ethyl propanoate	RT, MS	0.07	Nd	Nd
Propyl acetate	MS	Tr	Nd	0.06
Ethyl butanoate	RT, KI, MS	0.83	0.21	0.71
Ethyl 2-methyl butanoate	RT, MS	Tr	Tr	Tr
Ethyl 3-methyl butanoate (ethyl isovalerate)	RT, MS	Tr	Tr	Tr
Butyl 2-methyl-2-propenoate (butyl methacrylate)	MS	Nd	Tr	Nd
3-Methyl-1-butyl acetate (isoamyl acetate)	RT, MS	24.1	5.70	16.50
Ethyl pentanoate (ethyl valerate)	MS	Tr	Tr	Tr
Butyl 2-methyl butanoate	MS	Tr	Nd	Nd
Ethyl-2-butenoate	MS	Tr	Tr	Tr
Methyl hexanoate	RT, MS	0.06	Tr	Tr
Ethyl hexanoate	RT, KI, MS	47.10	16.40	28.70
Hexyl acetate	RT, KI, MS	0.63	0.15	0.22
Ethyl 3-hexenoate	MS	0.10	Tr	0.07
4-Hexenyl acetate	MS	Tr	Tr	Tr
Propyl hexanoate	RT, MS	0.08	Tr	0.07
Ethyl heptanoate	RT, MS	0.89	0.52	0.73
Ethyl 2-hexenoate	MS	Tr	Tr	Nd
Ethyl 2-hydroxy propanoate (ethyl lactate)	RT, MS	Tr	Tr	0.13
Heptyl acetate	MS	0.11	Nd	Nd
Methyl octanoate	RT, MS	0.38	0.13	0.18
Butyl 2-methyl propanoate	MS	Nd	Tr	Nd
Ethyl octanoate	RT, MS	136	34.80	78.10
Isopentyl hexanoate	MS	0.76	0.21	0.50
Octyl acetate	MS	Tr	Tr	Tr
Ethyl 7-octenoate	MS	0.12	0.10	0.11
Propyl octanoate	MS	0.16	Nd	0.19
Ethyl nonanoate	RT, MS	0.32	0.08	0.20
Butyl octanoate	MS	0.14	0.06	0.30
Methyl decanoate	RT, MS	0.14	Tr	0.20
3-Hexenyl butanoate	MS	Tr	Nd	Nd
Ethyl decanoate	RT, KI, MS	32.1	4.76	26.2
3-Methylbutyl octanoate	MS	0.67	0.12	0.79
Diethyl butanedioate (diethyl succinate)	RT, MS	1.23	0.84	3.75
Ethyl 9-decenoate	MS	4.2	0.78	2.49
Propyl decanoate	MS	Tr	Nd	Nd
Methyl 2-hydroxy benzoate (methyl salicylate)	MS	Tr	Nd	Nd
2-Phenylethyl acetate	RT, KI, MS	1.48	0.37	2.26
Ethyl dodecanoate	KI, MS	2.09	Tr	2.86
3-Methylbutyl pentadecanoate	MS	0.08	Tr	0.14
Ethyl 3-hydroxy hexanoate	MS	0.06	Tr	Nd

Table 4 (continued).

Compound	Identification method	Wine A	Wine B	Wine C
Ethyl tetradecanoate	KI, MS	0.07	Tr	0.14
Ethyl hexadecanoate	MS	0.19	0.07	0.47
Ethyl 9-hexadecenoate	MS	0.17	0.08	0.53
Organic acids				
Octanoic acid	RT, KI, MS	8.65	Nd	12.10
Alcohols				
Ethanol	RT, KI, MS	>10,000	>10,000	>10,000
2-Propyn-1-ol	MS	Tr	Nd	Nd
2-Amino-2-methyl-1,3-propanediol	MS	Nd	Tr	Nd
1-Butanol	RT, MS	Tr	Tr	0.08
3,3-Dimethyl-2-butanol (pinacolyl alcohol)	MS	Nd	Nd	Tr
Cyclopentyl methanol	MS	Nd	Tr	Nd
3-Methyl-2-butanol (methyl isopropyl carbinol)	RT, MS	Nd	Nd	Tr
4-Methyl-1-pentanol	RT, MS	Tr	Tr	Tr
2-Heptanol	RT, MS	Tr	Tr	Tr
3-Methyl-1-pentanol	MS	0.06	Tr	0.09
1-Hexanol	RT, KI, MS	0.37	0.20	0.51
1-Octen-3-ol	RT, KI, MS	Nd	Tr	Nd
1-Heptanol	RT, KI, MS	0.13	0.15	0.26
2-Ethyl-1-hexanol	RT, KI, MS	0.06	0.21	0.26
3,7-Dimethyl-1,6-octadien-3-ol (β-linalool)	MS	0.06	Tr	0.37
1-Octanol	RT, KI, MS	0.14	0.11	0.46
2-(4-Methyl-3-cyclohexen-1-yl)-2-propanol (α-terpineol)	MS	Nd	Tr	Nd
3-Methylthio-1-propanol	MS	Tr	Tr	0.32
3,7-Dimethyl-6-octen-1-ol (β-citronellol)	MS	Tr	Tr	0.26
Phenyl methanol (benzyl alcohol)	RT, KI, MS	Tr	Tr	0.1
2-Phenyl ethanol	RT, KI, MS	6.49	3.69	28.9
Carbonyl compounds				
2-Heptanone	RT, KI, MS	Tr	Nd	Nd
2-Nonanone	KI, MS	Tr	Nd	Nd
Decanal	RT, KI, MS	Tr	Nd	Nd
Phenyl methanal (benzaldehyde)	RT, KI, MS	Tr	Tr	0.07
2-Methyl-4,5-dihydro-3(2H)-thiophenone (blackberry thiophenone)	MS	0.08	Tr	0.15
1-[2,6,6-Trimethyl-1,3-cyclohexadien-1-yl]-2-buten-1-one	MS	0.09	Tr	0.14
Acetals				
1,1-Diethoxy ethane (diethyl acetal)	RT, KI, MS	Nd	Nd	3.77
1-(1-Ethoxyethoxy)-pentane	MS	0.11	0.13	0.23
Miscellaneous compounds				
2,4,5-Trimethyl-1,3-dioxolane	MS	Nd	Nd	Tr
Ethoxy ethene (vinyl ethyl ether)	MS	Tr	Nd	Nd
2,3,4-Trimethyl-pentane	MS	Nd	Tr	Nd
Piperidine	MS	Tr	Nd	Nd

Table 4 (continued).

Compound	Identification method	Wine A	Wine B	Wine C
Total (ethanol excluded)		275.2	71.5	219.5

RT positive identification by comparison of retention times and MS data with those of standard compounds, *KI* tentative identification by Kovats retention indices, *MS* positive identification by MS data obtained from NIST107, NIST21, and SZTERP libraries, *Nd* not detected, *Tr* traces (<0.05 mg/l)

the identified alcohols. Comparing the tested samples, the relative amounts of alcohols identified in the new types of wines (A and B) were much lower than those found in wine C. The complexity and increased ratios of esters to alcohols is known to affect wine quality positively, emphasizing its floral and fruity character. Among the tested alcohols, the floral terpene alcohols (β-linalool, β-citronellol, and α-terpineol) and 2-phenyl ethanol were also identified, the latter one, which is characteristic of floral, rose, and honey aromas, was found in big amounts, higher than the sum of all the identified alcohols, excluding ethanol. Unlike esters, the opposite was observed in the case of alcohols, whose amounts were lower in the new types of wines, compared to the wines made conventionally. 3-Methylthio-1-propanol, which is known for its vegetable-like (potato, tomato, sweet) flavor usually found in wines [32], was also identified, in higher amounts in wine A. In total, six carbonyl compounds were identified, most in wine B, the major being β-damascenone, the blackberry thiophenone (2-methyl-4,5-dihydro-3(2H)-thiophenone), and benzaldehyde, all commonly found in red wines (fruity, berry, floral aromas). The acetal 1,1-diethoxy ethane, a derivative of acetaldehyde produced during aging [25], was only identified in wine C.

Sensory Evaluation

The flavor and mouth-feel properties, especially bitterness and astringency, of wines are related to the presence of tannins, polyphenolic compounds, and volatiles [33]. As expected, the sensory evaluations (Table 5) confirmed the differences found by the chemical analysis among the different types of wines. Evaluations were conducted by 15 trained testers according to the paired method (one-tailed, p=1/2). Specifically, three pairs of different combinations of wine samples were tested on the basis of a single characteristic. The samples were covered to avoid influence of tester assessment by the differences in color, and the

Table 5 Sensory evaluation of the red and white wines produced from uncrushed frozen grapes.

Test	Tested pairs of wines	Probability (%)
Astringency	Red A<red B	1
	Red B<red D	5
	Red B=red C	5
Aroma	Red A≠red B	5
	White A≠white B	1

A fermentation of must extracted naturally during thawing, *B* fermentation of must inside the grapes, *Red C*, *D* fermentation of whole material of frozen grapes, without disturbing the skins during fermentation (C) or with occasional mixing of skins (D) testers were asked to reply to single questions such as: “which wine of each pair is more astringent” (probability of a right answer by chance 1/2). Astringency is a term of crucial importance for describing the organoleptic properties of wine. This property is mainly attributed to the presence of tannins and other phenolic compounds. The red wine produced by the traditional method with occasional mixing of grape skins during fermentation (D) was found to be more astringent than the one produced by fermentation inside the grape berries (B; probability level 1%), which was more astringent than the wine produced from the must extracted from grapes during thawing (A; probability level 5%). On the other hand, no differences in astringency were recorded between the red wines produced by fermentation inside the grapes (B) and by the traditional method, without disturbing the skins during fermentation (C; probability level 5%). The test also revealed differences between the aroma of the wines produced from the extracted must (A) and the must inside the grapes (B) for both red and white wines (probability level 5% and 1%, respectively).

Conclusions

The two types of wines, prepared using the same raw material (frozen–thawed grapes), by fermentation of the juice naturally extracted during thawing, and by fermentation of the remaining juice in the uncrushed grapes, had distinct sensory characteristics. Yeast was able to penetrate the uncrushed thawing grapes, without any other specific treatment, and totally ferment their content. The new types of wines, compared to wines produced by the traditional method using the whole material of frozen grapes, were found to contain lower amounts of compounds that contribute to bitterness and astringency, which was also proved by the sensory evaluations. The major volatiles were within desirable limits, but differences were observed among the prepared wines, which were also revealed by the sensory evaluations. The white wines presented more differences concerning their aromatic profile compared to the red ones. These new types of wines could be produced using frozen, stored grapes out of the harvest period. The industrial application of such process is feasible since the freezing costs could be accepted for a product whose market prices differ significantly depending on quality. Also, important from an industrial point of view is the possible simultaneous production of two different products of fine quality by the proposed process, using the same amount of raw material in one fermentation batch. Additionally, the low amounts of amyl alcohols and methanol in both types of wines is highly important to the potential commercialization of the process since these compounds downgrade the quality of wines as well as their single distillation products. The described process is simple and feasible for application in wine making to produce wines with improved quality.

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