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Instrumental analysis of volatile and other compounds of Greek kiwi wine; sensory evaluation and optimisation of its composition

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Abstract

The production of kiwifruits is a dynamic agricultural activity in Greece. The biggest part (nearly 60%) of the total quantity produced is exported. The remainder is locally consumed, with the exception of a percentage which is not marketable due to its appearance or its being in excess of demand. To exploit this surplus of non-attractive, small-sized Greek kiwifruits, there is the possibility of wine production, which is applied in this research study. Various classic and instrumental methods (GC, HPLC, ion chromatography) and sensory analysis, in conjunction with statistical analysis, were developed in an attempt: (a) to maximize the quantity of the juice extracted from raw material, (b) to evaluate kiwi wine composition (volatile compounds, organic acids, inorganic ions, sugars, glycerol) and (c) to optimise its quality according to consumer preference. The yield of the juice is increased up to 75% in weight by using riper kiwifruits and by processing them with pectolytic enzymes. Kiwi fruits left to ripen for 3 months showed a 3 g/l acidity decrease, expressed as citric acid, and an increase in sugar content of about 2 Brix degrees. Kiwi juices were weaker in sugar than the grape musts and the produced kiwi wines were poor in alcohol content but rich in titritable acidity. Citric, galacturonic, lactic and malic acids are the dominating organic acids. In most cases, the kiwi wines' contents of aromatic components are lower in grape wine, but methanol content is higher because of the use of pectolytic enzymes. After sensory evaluation, the analysis of variance for sweetness proved significant only in the sugar effect and just marginally so in that of alcohol and CO_2. Sensory alcohol is significantly influenced by sugars, alcohol and CO_2, while carbon dioxide, viewed as a sensory indicator, was not found to be statistically influenced by any chemical factor examined. The statistical analyses also show that the acceptability of kiwi wines is higher if they contain 10% vol alcohol, more than 30 g/l sugars and 0.5 bar CO_2.

Keywords: Kiwi; *Actinidia chinensis*; Kiwi wine; Kiwi wine composition; Volatile compounds; Sensory evaluation; GC; HPLC; Principal Component Analysis (PCA)

1. Introduction

Kiwi or kiwifruit vine, botanically known as *Actinidia chinensis* Planch, originates in the Yantze River Valley of China (Luh & Wang, 1984). In this country, the gathering of kiwifruits has been reported as early as 2000 years ago. Nevertheless, the systematic cultivation of kiwifruit is recent (Dimoulas, 1988; Noussis, 1978; Spartsis, 1981).

In 1845, R. Fortune introduced the plant to Europe (London) and the French botanist, J.E. Planchon, studied it 2 years later. More recent imports of plants from China to England took place in 1900 and, in the same period, to France (1903), California (1904), New Zealand (1906), and later to Italy. The plant came to Greece from France in 1971 or 1973 (Brousovanas, 1987; Dimoulas, 1988; Paloukis & Ntinopoulos, 1989). The commercial exploitation of this plant was initiated in the 1950s (Luh & Wang, 1984).

The fruit of the *Actinidia plant* is known more commonly as *kiwifruit;* in Greece it is also called *actinidio* (rad = aktis) or *"fruit of Mount Olympus"*. It is cylindrical

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or pear-shaped and has fuzzy brown skin. The interior of the fruit is bright green with tiny black seeds radiating from a central core. The outer part of the flesh has a dark green colour.

Howard, Abbot, Monty, Bruno, and Allison are the cultivars most widely cultivated all over the world as well as in Greece.

Kiwifruits constitute the raw material for the wine production in this project; its composition is important for the product's quality and profitable manipulation.

Luh and Wang (1984) reported that starch is the main carbohydrate that is stored in the tissue of kiwifruit; it is hydrolysed during the fruit's ripening and consequently sugars are increased. The most important kiwifruit sugars are glucose, fructose and sucrose. The glucose levels increase rapidly at the beginning of starch hydrolysis and reach 10% during the collection stage. The fructose content increases gradually from the earliest stage of fruit development until harvest.

The most important organic acids contained in the kiwifruit are citric, quinic and malic. The high concentration of quinic acid, even in the ripe fruit (in comparison with other fruits), is the main characteristic of kiwifruit (Heatherbell, 1975). Kiwifruit also has 3–5 times more ascorbic acid (vitamin C) than citrus fruits. The ascorbic acid content varies significantly (from 57 to 380 mg/100 g of edible flesh) and depends on the variety (Selman, 1983). It also contains gluconic, galacturonic, oxalic, succinic, fumaric, oxalacetic and p-coumaric acids (Luh & Wang, 1985), which cause a pH value ranging from 3 to 4.

Kiwifruit has important quantities of proteins (Wilson & Burns, 1983), carotenoids, phenyloic compounds, sufficient quantities of minerals (P, K, Ca, Mg) and aromatic components (mainly esters, alcohols, aldehydes, and ketones) (Patterson & Burns, 1983).

The cultivation of *Actinidia* is considered to be very significant in Greece. The economic importance is due to the fact that the major proportion (nearly 60%) (cultivating area: 2000–2500 ha, production: 40 000–50 000 t yearly) produced in Greece is exported, as the fruit is reported to be very durable and withstands transport. Simultaneously, it has good many prospects as it may also be produced under conditions of biological cultivation.

Kiwifruits, because of their composition, their sensory characteristics, and resistance during preservation, have great potential for industrial exploitation (Cano, 1991; Fischböck, Plannhauser, & Kellner, 1988; Reid & Harris, 1977). They are eaten fresh in fruit salads, can be canned and/or frozen and dehydrated as a whole fruit or in slices. They are also used for making nectar, jam, preserves, and wine (Luh & Wang, 1984).

In Greece, in an effort to utilise the surplus of kiwifruits, which are unattractive and of small size, several applications have been studied, one of which is the possibility of wine production.

Graebener produced the first kiwifruit wines in 1894 and later, in 1950, Zukovskij mentioned that kiwifruits of the species "*Actinidia arguta* produce an excellent wine" (Lodge, 1981). According to Vitkovskij (1972), kiwifruit wine "has the characteristics of champagne", but none of these authors gave details of the vinification techniques. Numerous researchers (Heatherbell et al., 1980; Lodge, 1981; Paloukis & Ntinopoulos, 1989) reported production of wine from kiwifruit, var. *Actinidia chinensis,* that had similar characteristics to wines made from grapes of Riesling and Sylvaner varieties. In more recent reviews, Craig (1988) indicated that kiwifruit wines differ significantly from wines made from other fruits, but are similar to wines made from Müller–Thürgau grapes.

The present research had a threefold aim: (1) to maximise the amount of high quality juice extracted by improving the production techniques; (2) to study, in detail, the kiwi wine composition; and (3) to improve the composition of the produced wine, based on the results of sensory evaluation of the tasted samples, in conjunction with statistical analysis. The use of data collected on consumer preference kiwi wines is very important. This is of particular interest to Mediterranean populations, for example, who are not familiar with wines having high acidity levels, such as kiwi wines.

2. Materials and methods

2.1. Kiwifruits

Kiwifruits constitute the raw material used for the wine production in this research. Small-sized fruits that did not meet marketing standards and which belonged to Abbot (40%), Monty (30%) and a mixture of Hayward and Bruno (30%) cultivars were used. This percentage of the various cultivars was chosen because this is the proportion of kiwifruit varieties commercialised by the Meliki Agricultural Cooperative, whose fruits were supplied to a large extent. Meliki is situated in the region of Pieria, in the north of Greece; it yields the biggest part of the total Greek kiwifruit production.

Fruits were harvested between the end of November and the beginning of December, and were sorted for size and stored for about 1 month in refrigerated rooms (4–7 °C) of the above-mentioned Cooperative before processing.

2.2. Maturity control

For the process and vinification, fruits of various degrees of maturity were used: (a) fruits taken directly from the cool storage rooms, (b) fruits after 3 months storage in rooms of controlled temperature (16–18 °C), (c) fruits after 3 months storage under environmental conditions (8–10 °C).

During the period of post harvest maturation, total acidity and soluble solids content (Brix) were determined. The results are given in Table 1.

2.3. Processing

The kiwifruits were sorted for size and washed in plenty of running water in order to remove foreign material from the skin (pesticides, hairs, particles). The other procedures and methods of processing (Fig. 1) were adapted from Lodge (1981) and Withy and Lodge (1982).

2.3.1. Pulping

Kiwifruits were pulped in a manual hammer mill grape-cracker in order to facilitate and accelerate the action of pectolytic enzymes added later. After pulping, 30–80 mg/l SO_2 was added, in accordance with the degree of maturation and hygienic state of the kiwifruits. The treated pulp was pressed in a hydraulic grape press to increase the yield of juice (Lodge, 1981).

2.3.2. Enzymatic processing

This was achieved by adding a pectolytic enzyme (Lafazym extract—Activite: 4000 FDU 20/g, J. LAFFORT & Cie: 126, quai de la Souys, 33015 Bordeaux Cedex-France) in various quantities (0.05, 0.1 and 0.15 g/l). In order to determine the most effective amount of enzyme, the results were compared to a control sample that contained no enzymes. The process took place in a closed tank with double walls, and a stable temperature was maintained by water circulation from a thermostatic tank. Moreover, the tank was continuously stirred to achieve a uniform temperature. Pulp was heated and left at 45 °C for 15 h. In some cases, the effect of the temperature was tested, where pulp remained on the extracted juice yield, independently of the addition of enzyme.

2.3.3. Juice extraction

The delivered juice was decanted and the treated pulp was pressed in a hydraulic grape press, in order to increase the yield of extracted juice. The inside of the press was covered with an appropriate fabric to efficiently retain the pulp.

The juice was then analysed to determine free and total SO_2, total acidity, pH and soluble solids content (Brix and Bé). Five of the six samples of juice were ameliorated with sucrose syrup addition in order to increase the "potential alcoholic title" and produce "wines from kiwifruit" with different final alcoholic titles (Tables 2 and 3). These kiwi wine samples allowed us to have a total view of their composition and a first sensory evaluation.

2.3.4. Fermentation

This was carried out by inoculation of 10 g/hectoliter selected cultures of yeasts (Saccharomyces cerevisiae, Fermirouge: No. 7303 INRA Narbonne, Gist-brocades, Food Ingredients Division, BP 239, 59472 Seclin cedex, France). The fermentation was stopped by addition of 50 mg SO_2/l and stored at 4 °C. Then the produced kiwi wine was decanted, treated with casein (0.4 g/l) and filtered through filter plates. Samples were kept for a few weeks for future chemical and sensory analysis, stabilized with a further addition of 30 mg SO_2/l and, finally, bottled.

Table 1
Evolution of post harvest ripeness of kiwifruits stored at 16–18 °C (1996)

Days	Brix	Total acidity (meq/l)	Total acidity (g/l citric acid)	Ratio Brix/T.A.[a] (%)
1	11.8	288	18.4	6.40[b]
5	12.0	286	18.3	6.55
7	12.1	284	18.2	6.65
11	12.2	–	–	–
13	12.3	269	17.2	7.15
18	12.4	271	17.4	7.13
40	13.0	253	16.2	8.02
42	13.0	253	16.2	8.02
47	13.1	255	16.3	8.03
90	13.5	249	15.9	8.49
94	13.5	247	15.8	8.54

[a] T.A., Total acidity expressed as g of citric acid per 100 ml of kiwifruit juice.

[b] This value corresponds to fruits stored in a cool room, while the other corresponds to those stored at 16–18 °C during their maturity period.

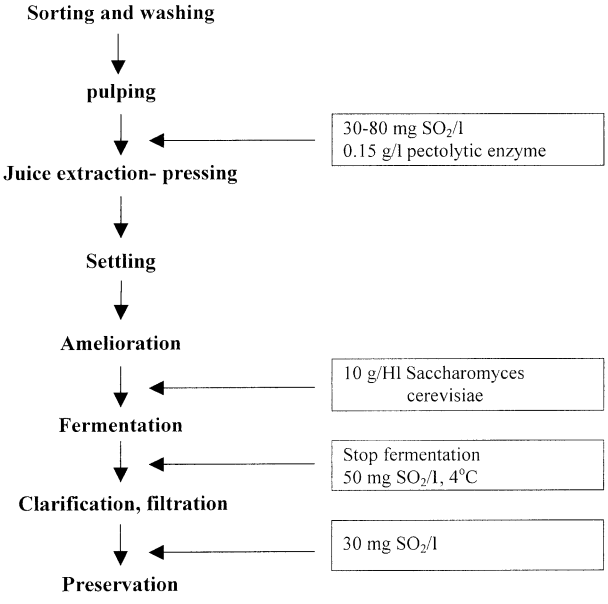

Fig. 1. Flow diagram of kiwifruit wine production.

2.4. Production of several types of kiwi wine

The several samples for sensory evaluation and optimization of kiwi wine composition were produced by additions of various contents of alcohol, reducing sugars and CO_2 to the basic wine. This is not equivalent to making the wine with these levels, but it is the only way to carry out a statistical study of the exclusive influence of each of these chemical factors. The carbonation was made with a post-mix apparatus used for distribution of unpacked refreshments and with a stainless steel tank, resistant to pressure. In the second case, carbonation was achieved by channelling CO_2 through the wine. It was then left in the tank under pressure for some time to assimilate the CO_2.

These kiwi wine types were produced with three levels of alcohol (7, 10, 13% vol.), three levels of sugar (15, 30, 45 g/l) and two levels of CO_2 (0.5 and 2 bars) in numerous combinations. More details are included in the statistical analysis.

2.5. Analytical methods

To determine the composition of the kiwi must and wine samples, several analyses have been done using classical and chromatographic analyses, while sensory evaluation was carried out to evaluate different types of kiwifruit wines.

By using classical analyses, the following were obtained: soluble solids content of kiwifruit juice (Brix, Bé), pH, total acidity (neutralization with 0.1 N NaOH), free and total SO_2 (iodiometric determination with the usual method—OIV), alcoholic title (after distillation with steam flow— official method OIV) and residual reducing sugars (Luff method—official method OIV). CO_2 pressure was measured with a manometer supplied with pin and special mechanism to fit at the mouth of bottles.

Also, the kiwi wines were tested by gas chromatography for 33 volatile compounds (alcohols, esters, volatile acids, carbonyl compounds and the polyalcohol glycerol), by ion chromatography for 13 organic acids and inorganic ions and by liquid chromatography for the following sugars: fructose, glucose and sucrose.

Higher alcohols were determined after the injection of 0.5 μl of a wine sample on a CP Wax 57CB capillary column (0.22 mm×50 m) (Chrompack). Before the injection, 50 μl of 4-methyl pentan-2-ol (10 g/l in 40% ethanol), as internal standard, was added to 5 ml of the wine sample. Detection was carried out using a FID detector. The temperature program employed for this analysis was set at 40 °C for 5 min and then gradually increased to 200 °C, at a 4 °C/min gradient. Nitrogen was used as a carrier gas.

For carbonyl compound determination, 50 μl octan-3-ol solution (408.6 mg/l) was added, as an internal standard, to 1 ml of wine sample, and a further 2 ml methanol was added to precipitate the tannins present. Of this mixture, 1 ml was injected into a Carlo Erba 4130-GC 600 chromatograph equipped with a Carbowax 20 M capillary column (25 m × 0.25 μm) and a FID detector. The conditions employed were: splitless after 20 s, temperature program from 50 to 110 °C increased at a 2 °C/min, then 5 °C/min until 220 °C. Hydrogen was used as a carrier gas.

Table 2
Main analytical characteristics of kiwifruit must and wine samples

Samples	Year of sampling											
	1993						1996					
	E1		E2		E3		S1		S2		S3	
Must												
Total acidity (g/l citric acid)	16.3		16.4		16.2		14.9		15.0		15.1	
Initial Brix	10.4		10.4		10.4		12.8		13.1		13.0	
Initial Bé	6.3		6.3		6.3		7.5		7.6		7.6	
Addition of sugar (g/l)	80.0 (18th day)		110.0 (from the beginning)		80.0 (18th day)		0.0		30.0 (from the beginning)		55.0 (from the beginning)	
Bé after sugar's addition	10.1		11.7		10.25		7.5		9.0		10.8	
Wine												
Alcoholic title (% vol.)	8.9 Correction		11.4		9.0		6.1		8.3		10.05	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
pH	2.87	3.45	2.90	3.43	2.91	3.40	3.20	3.50	3.30	3.55	3.42	3.42
Total acidity (meq/l)	228	165.30	225	170	224	172	222	161	215	159	206	169
Total acidity (g/l citric acid)	14.6	10.6	14.4	10.9	14.3	11.0	14.2	10.3	13.8	10.2	13.2	10.8

Higher esters were quantified by extraction of 50 ml of wine with a mixture of ether/hexane (1/1 v/v) three times, respectively: 4 ml, 2 ml and 2 ml. Before the extraction, a double internal standard (2 ml octanol: 3 to 4 mg/l in 50% ethanol; 2 ml heptanoic acid: 66.2 mg/l in 60% ethanol) and 0.3 ml of H_3PO_4 30% were added to the wine sample (46). 2.0 μl of this extract was injected into a Carbowax 20 M capillary column (25 m × 0.25 μm). The temperature program was set from 50 °C to 200 °C at 3 °C/min. A FID detector was used for the detection of esters, and hydrogen was employed as carrier gas.

Glycerol was quantified by using a GC equipped with an FID detector. One hundred microliters of hexandiol-1,6 (200 g/l) was added, as an internal standard, into a 5 ml wine sample. Two microliters of the sample were

Table 3
Volatile component analysis of kiwifruit wines' samples concentrations (mg/l)[a]

Samples of kiwi wine	Year of sampling					
	1993			1996		
	E_1	E_2	E_3	S_1	S_2	S_3
Alcoholic title (% vol.)	8.90	11.40	9.00	6.10	8.30	10.05
Methanol	710	716	580	485	717	768
Hexanol	0.69	0.71	0.58	1.63	0.61	0.18
Phenyl-2 ethanole	40.7	32.2	33.70	16.0	26.1	38.6
Higher alcohols						
Butan-2-ol	–	–	–	–	–	–
Propan-1-ol	15.2	22.2	14.1	28.0	27.0	53.0
Methyl-2-propan-1-ol	190	127	133	104	208	352
1-Butanol	–	–	–	–	2.00	–
Methyl-2-butan-1-ol	71.3	46.3	45.7	23.0	47.0	64.0
Methyl-3-butan-1-ol	260	229	198	56	153	224
Sum of higher alcohols	537	425	391	211	437	693
Acetoin	36.2	24.5	21.7	1.89	4.62	13.1
Ethyl acetate	85.2	61.2	38.4	58.0	40.0	52.0
Ethyl lactate	4.88	5.85	4.15	0.01	3.77	2.73
Diethyl succinate	0.75	1.82	1.09	0.05	0.11	0.23
Isoamyl acetate	0.33	0.34	0.17	0.17	0.32	0.79
Hexyl acetate	0.04	0.03	0.03	–	–	–
Phenyl-2-ethyl acetate	0.03	0.02	0.02	0.02	0.04	0.11
Ethyl butyrate	–	–	–	0.10	0.27	0.10
Ethyl hexanoate	0.12	0.30	0.15	0.04	0.06	0.07
Ethyl octanoate	0.10	0.41	0.18	0.33	0.07	0.07
Ethyl decanoate	0.02	0.04	0.03	–	–	–
Ethyl dodecanoate	–	–	–	–	–	–
Isobutyric acid	6.85	4.69	5.73	2.49	4.66	3.84
Butyric acid	7.66	7.20	6.88	1.57	3.98	0.75
Isovaleric acid	3.94	1.79	2.74	1.14	1.76	1.07
Hexanoic acid	1.20	1.98	1.50	1.01	1.27	1.07
Octanoic acid	1.14	3.23	2.07	0.67	1.13	1.32
Decanoic acid	0.14	0.22	0.24	0.02	0.04	0.07
Dodecanoic acid	0.02	0.03	0.04	0.07	0.02	0.03
Acetol	5.30	–	9.40	2.34	4.11	6.43
Butan-2,3-diol	296	250	269.80	49.3	151	225
Butanediol meso	148	125	151.10	27.9	64.7	116
Propanediol	27.5	32.8	29.70	9.34	20.8	41.8
γ-Butyrolactone	11.1	12.8	10.90	1.49	2.15	3.51
Fructose (g/l)	0.79	0.14	0.76			
Glucose (g/l)	0.41	–	1.96			
Sucrose (g/l)	0.19	0.00	0.36			
Total sugars (g/l)	1.39	–	3.08	1.00	1.00	1.00
Glycerol (g/l)	2.90	4.49	3.93	3.98	5.34	6.97

[a] Except if there is a different indication.
–, Negligible quantities.

injected into a Tenax 60/80 mesh classic column (1 m × 2.1 mm × 0.4 μm) at 180 °C. Nitrogen was used as a carrier gas (flow = 30 ml/min).

Organic acids were determined according to the ion chromatography method cited by Soufleros et al. (1998) in a previous publication.

An ion-exchange chromatograph Dionex Series 4500 i (Dionex, American Corporation, PO Box 3603, Sunnyvle, CA 94088-3303, USA), connected to a conductimetric detector, was used. The system was equipped with an anion trap column (P/N 037151), a guard column OmniPac PAX-500, P/N 042153 (length = 50 mm, internal diameter = 4 mm) and an analytical or separation column OmniPac PAX-500, P/N 042152 (length = 250 mm, internal diameter = 4 mm, Dionex, American Corporation).

Three eluants were utilized: eluant 1: mixture of 60% water and 40% methanol. In 1 l of the mixture, 5 ml of 2.0 M NaOH aqueous solution, were added; eluant 2: mixture of 68% water and 32% methanol; eluant 3: mixture of 68% water and 40% methanol in which 5 ml of 0.2 M NaOH, were added; flow rate: 1 ml/min; H_2SO_4 volume for regeneration, 25 ml; pressure: 131–138 bars; temperature: ambient. The gradient program used was as follows:

Time (min)	Eluant 1 (%)	Eluant 2 (%)	Eluant 3 (%)
0	80	20	0
15.1	80	20	0
25	80	20	0
25.5	100	0	0
30	100	0	0
60	55	0	45

The wine samples were diluted 50-fold with water of high purity (milli-Q), filtered through a cartridge of polyvinylpolypyrrolidone and a millipore filter (porosity 0.45 μm), and were directly injected (10 μm) by means of a sample holder to an ion-exchange chromatograph.

The sugars: glucose, fructose and sucrose were determined by an HPLC system equipped with a normal phase NH_2 column (5 μm, 20 cm×4 mm) and an UV detector. A mixture of acetonitrile 80% and milli-Q water 20% was used as eluant with a flow rate 0.6 ml/mn.

2.6. Statistical analysis

The statistical analysis included the following techniques:

(a) An independent two-sample *t*-test on the main analytical characteristics of kiwifruit must samples to detect potential differences between the year 1993 and 1996.

(b) Sensory evaluation of the wine. The sensory properties of the wine were evaluated having first made the needed additions to the basic wine in order to ensure: sugar at three levels, 15, 30 and 45 g/l, alcohol at three levels, 7, 10 and 13% vol., carbon dioxide (CO_2) at two levels, 0.5 bars and 2 bars. The basic wine was not sparkling and consisted of 5% vol. alcohol, 2 g/l sugar and pH 3.4. Thus the experimental design of the wine included 3×3×2 combined levels (treatments) of chemical factors. This design, with the addition of the basic wine treatment, was adjusted to a balanced incomplete block plan (Cochran & Cox, 1957, p. 542) with the following properties: $t = 19$ treatments, $k = 3$ treatments per panelist, $b = 57$ panelists, $r = 9$ replicates per treatment and $\lambda = 1$ similar pair of treatments in the design. Twelve well-experienced panelists from previous wine experiments, members of the academic staff, were used, each participating 4 to 5 times in order to make up the 57 panelists needed experimentally. Tests were conducted from 10:00 to 12:00 in individual booths. A 5-min break was taken between samples, during which the panelists were requested to eat a cracker and rinse the mouth thoroughly with spring water. The panelists were instructed to record each judgement by ticking on a 0 to 15 cm unstructured scaled line. The two opposite ends of the line were characterized as no and plenty of sweetness/alcohol/sparkling. Acceptability of the product was also assessed as a subjective variable using the same scaled line and, as opposite anchors, not and very much acceptable.

After the completion of the experiment, the adjusted means per sensory variable (sweetness, alcohol, sparkling wine and acceptability) were produced. The basic wine treatment was then excluded and the sensory variables were examined by a three-factor analysis of variance with no replicates (sugar, alcohol, sparkling wine) to find potential statistically significant chemical effects on the sensory properties. Whenever statistical significance was detected at 0.05 probability level, the Tukey test of multiple paired comparisons of means was employed (Zar, 1984) to detect the pattern of the differences between the sensory means.

(c) Principal component analysis on the three objective sensory variables for the 19 adjusted means was finally employed to discern patterns between variables and wine samples.

The statistical analysis was performed by the Minitab Statistical Software Version 13.

3. Results and discussion

3.1. General results

A large number of samples was prepared and used for several measurements in an attempt to increase the yield of kiwi juice. Furthermore, six samples of kiwifruit wine were completely analysed (three samples in 1993 and

three in 1996) to give a complete picture of its composition (Tables 2 and 3) and the first approach of sensory evaluation.

3.2. Control of kiwifruits degree of maturity and juice extraction

According to Matsumoto, Obara, and Luh (1983) and Reid, Heatherbell, and Pratt (1982), storage of kiwifruits at 20 °C, in the presence of 5 ppm ethylene gas, causes an important decrease in the total acidity and starch content and increases the soluble solids.

Results (Table 1) have shown that, during kiwifruit storage, in rooms of controlled temperature at 16–18 °C without ethylene for 90 days, the soluble solids content (Brix) values were increased up to 15% and total acidity decreased to 14.3%. The relatively small changes, in contrast to the bibliography, are due to the absence of ethylene gas in the storage rooms. Nevertheless, the fruit softening was significant and made its processing much easier.

According to the above-mentioned conditions of storage and the processing described in Section 2 the higher yield of kiwi juice, up to 75% by weight, came from samples with 0.15 g/l of pectolytic enzymes added.

3.3. Sugars

Findings in Table 2 show that the kiwi juice samples of 1993 had statistically (independent two-sample *t*-test) significant lower mean values for the initial Brix and initial Bé in relation to samples of 1996 and amount to 10.4 Brix (or 6.3 Bé) and 13 Brix (or 7.5 Bé), respectively. This difference in sugar content was mainly due to different degrees of kiwifruit ripeness. Both these categories of samples (1993 and 1996) come from just two different initial lots of kiwifruit musts (1993 and 1996). However, these samples were later differentiated in order to compare different samples of wine.

In the beginning of the alcoholic fermentation or in other samples during its evolution, different quantities of sugars (Table 2) were added, in order to make kiwi wines with different alcohol contents.

The total of residual reducing sugars from these six kiwi wine samples, determined by HPLC, were found (Table 3) to vary from 1 to 3 g/l.

3.4. Alcoholic title

The upper musts gave six wines with the following alcoholic titles: 6.1, 8.3, 8.9, 9, 10.05 and 11.4 vols (Table 2).

3.5. pH value

Table 2 shows that the initial pH of wine in 1993 was quite low, around 2.9, but was higher in 1996 and ranged from 3.2 to 3.4. The mean values of the pH from these two sample groups present a statistically (independent two-sample *t*-test) significant difference mainly due to different degrees of kiwi ripeness. Because of this particularly acidic character of kiwifruit wine, the pH value of samples was corrected to higher levels (3.4–3.55) (Table 2). Luh and Wang (1984) report slightly higher levels of pH, varying from 3.1 to 3.96.

3.6. Acidity

According to Table 2, the titratable acidity, expressed as citric acid, ranged from 14.9 to 16.4 g/l for the pressed kiwi juice, and from 13.2 to 14.6 g/l for the kiwi wine, without any amelioration. The 1993 kiwi wine samples had statistically (independent two-sample *t*-test) significant higher mean values for the total acidity compared to those of 1996 (Table 2). This difference is connected with the fact that 1996 kiwi must samples came from kiwifruits with greater progressing ripeness.

Castaldo, Lo, Trifirio, and Gherardi, (1982) gave a titratable acidity of kiwi purée between 12.5 and 17.9 g/kg expressed as citric acid. According to MacRue, Lallu, Searle, and Bowen, (1989), the total acid content remained at a similar level (17–18 g/kg fresh wt.). Luh and Wang (1984) reported that titratable acids (expressed as citric) amount to 18 g/kg in kiwifruit. Heatherbell et al. (1980) mention that the titratable acidity, as citric acid, amounts to 14.4 g/l in press juice (on average from three samples) and to 7.5 g/l in wine. Results of the above in total acidity of the kiwi wines we produced (Table 2) fall within the limits given by other researchers, with minimal exceptions.

Table 2 shows that kiwifruit must acidity is higher than the acidity of wine produced from it. This difference can be due to a tartaric acid precipitation during alcoholic fermentation.

According to our results (Table 1), the brix/acid ratio ranged from 6.4 to 8.6, with progress during the post harvest ripeness. These results refer only to the raw material of 1996, whose ripeness was more satisfying (Table 2). Castaldo et al. (1992) mentioned that this ratio for the optimum harvesting time of Italian kiwifruits ranged from 7.37 to 11.

In addition, Walton and Jong (1990), referring to the total sugars (glucose, fructose, sucrose and inositol) and the total of the most important organic acids (quinic, citric and malic), expressed as mg/g of fruit, gave sugar/acid ratios varying from 0.7 to 1.3, according to the geographic origin. MacRue et al. (1989) reported results similar to Walton and Jong (1990) with sugar/acid ratios between 0.7 and 1.2.

Independently of kiwifruit degree of ripeness in the year 1993 and 1996, we ascertained that the acidity of produced wines was still high. For this reason, through an important voluntary deacidification process, the

titratable acidity of kiwi wine samples was restored to suitable levels, ranging from 10.2 to 11.0 g/l as citric acid (Table 2); this acidity corresponds to pH 3.55 and 3.4, respectively, similar to grape wine pH.

3.7. Organic acids

The individual organic acids were determined by ion exchange chromatography. These analyses, referring to the samples of 1993 show (Table 4), that the dominant acids of kiwifruit wine are citric (12.4–14.6 g/l), galacturonic (12.3–13.3 g/l), lactic (7.1–7.7 g/l) and at a lower level, malic acid (1.9–2.4 g/l). The remaining organic acids are found at concentrations below 1 g/l.

According to Walton and de Jong (1990), the main organic acids detected and identified in the kiwifruit berry were quinic, citric and malic acids. In full ripeness, the kiwifruits' concentrations of those acids amount to about 30, 28–45 and 8–13 g/kg of fruit, respectively. Luh and Wang (1984) reported that kiwifruits contain about 10 g/kg each of citric and quinic acid and 5 g/kg of malic acid. The remaining organic acids are found at much smaller levels, such as: ascorbic 0.7 g/kg, gluconic and galacturonic together 1 g/kg.

Heatherbel et al. (1980) noticed that the acidic composition of kiwifruit and kiwifruit wine, compared to grapes and wines coming from them, are unusual. More specifically, they report that the pressed juice of kiwifruit contains citric, quinic and malic acid in quantities up to 11.0, 9.0 and 2.7 g/kg, correspondingly, while wines of kiwifruits contain about half the quantities of these acids, which amount to 5.5, 5.0 and 1.5 g/l of wine, respectively. The fact that the concentrations of acids in wine are half those in the fruit justifies the differences existing among our results and those of several researchers. More specifically, Walton and Jong (1990) presented much higher concentrations of organic acids than ourselves. It seems that the degree of kiwifruit ripeness and the method of juice extraction markedly affect its composition and more particularly the organic acid content.

3.8. Volatile compounds

3.8.1. Methanol

In our study in six samples of kiwifruit wine (Table 3) it was found that the content of methanol varied between 485 and 768 mg/l of wine (mean 663 mg/l). Heatherbell et al. (1980) reported a concentration of methanol 181 mg/l in kiwifruit wines, while Craig (1988) reported that certain fruit musts, obtained using enzymes, can contain up to 800 mg/l methanol. Based on these remarks, he tried to explain the high contents of butanoic, hexanoic and salicylic acid methyl esters in kiwifruit wines. This researcher attributes the high methanol content to the use of pectolytic enzymes, which are commonly used in kiwifruit wine production and are responsible for the splitting of pectic substances to galacturonic acid and methanol.

The high concentrations of methanol (Table 3) determined—in comparison to other researchers—accord with the high content in galacturonic acid (Table 4), which shares a common origin with methanol. In any case, these contents prevail over the corresponding concentrations in wines (Heatherbell et al., 1980, Soufleros 1978, 1979). However, these methanol levels are not potentially injurious to health.

3.8.2. Hexanol

Hexanol ranges from 0.18 to 1.63 mg/l (mean 0.73 mg/l) (Table 3). It is an alcohol that comes from raw material. Its concentration is lower than in wines (2–3 mg/l) (Soufleros, 1978). Heatherbell et al. (1980) made similar observations by comparing kiwifruit wine composition with wine from grapes.

With reference to real wines (wine from grapes), which come from specific vinification with carbonic anaerobiosis (maceration carbonique), the mean of concentrations ranges from 0.124 to 0.240 mg/l (Salinas, Alonso, Navarro, Pardo, Jimeno, & Huerta, 1996). These quantities, because of the particularity of this vinification, are relatively low compared to wines made by classical vinification methods. Nevertheless, they have about the same content of hexanol as our kiwi wines.

3.8.3. Phenyl-2 ethanol

Phenyl-2 ethanol ranges (Table 3) from 16 to 41 mg/l (mean 31.3 mg/l) while is higher in the real wines (55–70 mg/l) (Soufleros, 1978). In wines that come from carbonic anaerobiosis, the quantities of phenyl-2 ethanol are very low (0.5–mg/l) (Salinas et al., 1996). This component, in pure form, has a pleasant aroma, resembling that of a rose.

Table 4
Main acidulous components of kiwifruit wines concentrations (g/l)[a]

Samples of kiwi wine	Year of sampling 1993		
	E_1	E_2	E_3
Acetic acid	1.27	0.362	0.334
Lactic acid	7.67	7.15	7.17
Formic acid	0.067	–	0.024
Gluconic acid	–	–	0.044
Pyruvic acid	–	–	–
Cl	0.632	0.635	0.581
Galacturonic acid	12.3	13.4	13.4
Succinic acid	0.803	0.859	0.809
Malic acid	2.15	2.41	1.91
Tartaric acid	0.142	0.080	0.099
SO_4	0.250	0.372	0.363
PO_4	0.280	0.182	0.286
Citric acid	14.63	12.4	13.7

[a] Except if there is a different indication.
–, Negligible quantities.

3.8.4. Higher alcohols

These components positively affect the quality of wines in quantities not higher than 500–600 mg/l. Higher concentrations of these alcohols have a negative effect. In this study (Table 3) quantitatively the most important higher alcohols are: methyl-2 propan-1-ol (104–352 mg/l, mean: 186 mg/l), methyl-3 butan-1-ol (56–260 mg/l, mean: 187 mg/l). Then follows methyl-2 butan-1-ol (23–71.3 mg/l, mean 49.6 mg/l). Salinas et al. (1996) gave concentrations of pentan-1-ol (methyl butan-1-ol) from 70 to 370 mg/l in wines from grapes.

3.8.5. Polyols

Butan-2,3-diol, is known for its stability in bacterial alterations. Glycerol is one of the sweet compounds of wines, which increases their softness. Generally, the concentrations (Table 3) of these compounds are higher in kiwi wines of 1996 rather than the ones of 1993. Although alcohol does not differ, the differences of concentration could be due to higher levels of ripeness in the fruits of 1996. Butanediol and glycerol contents in kiwifruit wines (Table 3) are lower than in wines (Bertrand, 1975; Soufleros, 1978). On the other hand, γ-butyrolactone is found in higher quantities in our kiwi wine samples (1.5–3.5 mg/l) (Table 3) than wines (1–14 mg/l) (Salinas et al., 1996)

3.8.6. Total higher alcohols

Their content in kiwi wine samples is between 211 and 693 mg/l, depending on the sample (Table 3). Generally, the total quantity of higher alcohols in wines from grapes ranges between limits similar to those reported by Soufleros (1978) and Soufleros and Bertrand (1979, 1980). Craig (1988), in a comparative evaluation, found that the concentrations of higher alcohols in wines, from the variety Müller–Thürgau, were generally much higher than in kiwi wine. On the other hand, in our study, the concentration of 2-methyl propanol-1 in kiwi wines is higher in comparison to wines from grapes.

3.8.7. Acetol

This is found in kiwi wine samples in quantities from 2.3 to 9.4 mg/l (Table 3).

3.8.8. Esters

Ethyl acetate, ethyl lactate and diethyl succinate are the esters that are mainly produced from bacterial alterations of various wine components, such as ethyl alcohol, sugars and tartaric acid, respectively. The concentrations of these substances (Table 3) amount to: 38.4–85.2 mg/l for ethyl acetate, 0.01–5.85 mg/l for ethyl lactate and 0.05–1.82 mg/l for diethyl succinate and generally lower than that found in real wine (Soufleros, 1978). Francioli, Guerra, Lopez-Tamames, Guadayoi, and Caixach (1999) reported concentrations of diethyl succinate from 1.1 to 4 mg/l in wines from grapes.

Craig (1988) found that the quantity of ethyl acetate in kiwi wine is nearly double that of white wines produced from the Müller–Thürgau variety.

3.8.9. Higher esters

The ethyl esters with C6, C8, C10 and C12, in spite of their small quantitative participation in volatile compounds, determine, to a high degree, the aromatic character of wines and spirits. They are secondary products of alcoholic fermentation and a large part of them remains bound in yeast cells. Their concentrations in the analysed kiwi wines are quite low with ethyl hexanoate and ethyl octanoate in the highest quantities in both years of sampling, 1993 and 1996 (Table 3). Their concentrations are higher in wines from grapes (Francioli et al., 1999) and similar or slightly higher than those given by Soufleros (1978) and Soufleros and Bertrand (1979, 1980). According to Salinas et al. (1996), ethyl decanoate is the predominant ester over the other three esters in wine vinified by carbonic maceration. Craig (1988), comparing the volatile composition of kiwi wines and wines of Vitis vinifera, variety Müller–Thürgau, found that wines are superior to kiwi wines in terms of ethyl hexanoate and ethyl octanoate concentrations but are inferior to them in ethyl decanoate. Bartley and Schwede (1989) noticed that ethyl hexanoate constitutes 9.5% of kiwifruits' volatile compounds and it is considered one of the most important compounds of this category. On the other hand, they do not mention ethyl esters with 8, 10 and 12 carbon atoms. Bartley and Schwede (1989) have determined some of the above mentioned volatile compounds extracted from kiwifruit.

Ethyl butyrate (ethyl butanoate), concentration (Table 3) ranges from 0.10 to 0.27 mg/l in our kiwi wine samples of 1996, and this was important in relation to other ethyl esters. Craig (1988) reported almost the same concentrations of ethyl butyrate and ethyl pentanoate both in kiwi wines and wines from the white variety of Müller–Thürgau grapes. Bartley and Schwede (1989) noted the existence of ethyl butanoate (or butyrate) in the significant percentage (14.6%) of volatile compounds in ripe kiwi and a too much higher level (69.4%) in very ripe kiwi. The same researchers also noted that ethyl pentanoate (ethyl ester of valeric acid) is present in kiwi at 1.0–1.5% of its volatile compounds.

3.8.10. Acetic acid esters

Isoamyl acetate (methyl-3 butyl), hexyl acetate and phenyl ethyl acetate, were determined in the kiwi wine samples (Table 3); the quantitatively dominant ester is isoamyl acetate whose concentrations range between 0.17–0.79 mg/l. On the other hand, the other two esters have concentrations around 0.02–0.11 mg/l (Table 3). In spite of the low contents, these three acetic acid esters are characterised by the aroma of flowers and fruits, which make their presence favourable. According to

Craig (1988), isoamyl acetate was found in quantities 6–7 times lower than in wines coming from the white variety Müller-Thürgau grapes. Results for 1-hexyl acetate are similar. According to other analytical studies of wines (Salinas et al., 1996; Soufleros, 1978), isoamyl acetate amounts to 0.2–0.9 and 0.3–0.6 mg/l, respectively. These concentrations are equal to those of the kiwi wine examined (Table 3). Similar are the results for the other two esters.

3.8.11. Volatile acids

Short chain fatty acids, e.g. isobutyric, butyric, isovaleric, are minor compounds but often their smell is equally as strong as that of acetic acid. Thus, these acids have important contributions to the aromas of wines and spirits. The effect of long-chain fatty acids (C_6–C_{12}) seems to be smaller.

Table 3 shows that isobutyric and butyric acids prevail over isovaleric acid, as they are found in concentrations 2.5–6.9 and 0.8–7.7, respectively, for the first two and 1.1–3.9 mg/l the latter. On the other hand, fatty acids with C6–C12 are found in smaller quantities (Table 3), i.e. hexanoic acid (1–2 mg/l), octanoic (0.7–3.2 mg/l), decanoic (0.02–0.24 mg/l) and dodecanoic (0.02–0.07 mg/l). These concentrations are equal to those of wines from grapes (Salinas et al., 1996; Soufleros, 1978).

3.9. Sensory evaluation of kiwi wine and optimisation of its composition

The analysis of variance for sweetness proved significant only for the sugar effect ($F=15.83$, $P=0.013$), according to which sweetness increases with sugar addition (Fig. 2). The Tukey's comparison of means indicated overlapping of the inequalities: 15 30 45. The same pattern of increase was depicted with alcohol content increase, although this was marginally not significant ($P=0.081$). The addition of alcohol can potentially enhance the intensity of sweetness because it is one of the sweet components of the wines.

Sensory alcohol was significantly influenced by sugar addition ($F=9.22$, $P=0.032$), alcohol addition ($F=21.43$, $P=0.007$) and carbon dioxide ($F=17.37$, $P=0.014$). Furthermore, the interaction term sugar* carbon dioxide was also significant ($F=12.38$, $P=0.009$). The particular pattern of each factor is depicted in Fig. 3, in which the sensory alcohol peaks at 30 g/l sugar content (although not clearly established due to Tukey's overlapping differences: 15 45 30) and

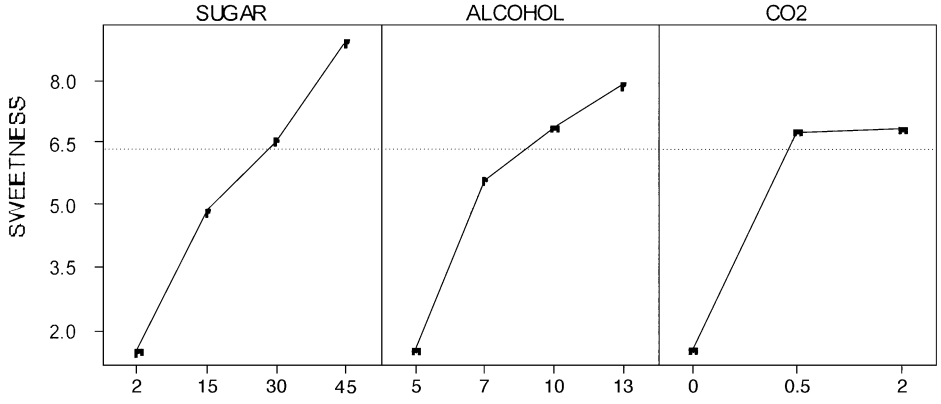

Fig. 2. Influence of chemical factors on the sweetness. The levels of basic wine (2/5/0) were not included in the statistical analysis.

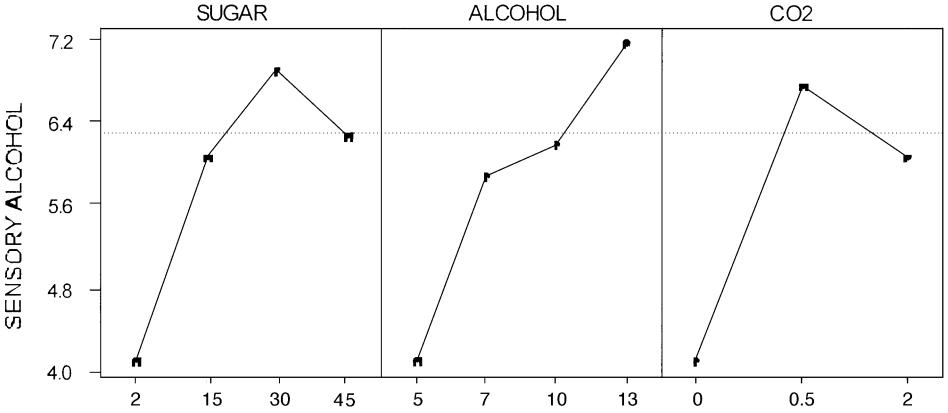

Fig. 3. Influence of chemical factors on the sensory alcohol. The levels of basic wine (2/5/0) were not included in the statistical analysis.

also at 13% vol. alcohol content. Panellists could not detect differences between levels 7 and 10% vol. (Tukey's pattern: $7 = 10 < 13$). Alcohol content is responsible for producing two sensory perceptions: sweetness and sharpness. The sugar addition enhances the feeling of "alcoholic sweetness" up to 30 g/l (Fig. 3). A further sugar increase, however, reduces the intensity of sensory alcohol and the interpretation given is that high sugar quantities mask the sweet taste of alcohol and moderate the sharp effect. On the other hand, alcohol levels of 13% vol. render more distinct the alcoholic character of the wines, whose levels were clearly perceived by the panellists (Fig. 3). Finally, the addition of carbon dioxide resulted in decreasing the sensory alcohol (pattern: $0.5 > 2$ bars). Such an effect can be explained by the fact that the acidic character of carbon dioxide and the pinching organoleptic effect on the panellists drove them to score lower values at higher quantities.

Sugar and carbon dioxide acted antagonistically on the sensory alcohol reducing its performance to 2 bars of CO_2 when the sugar concentration was highest (Fig. 4). This condition actually combines the previous observations about sugar and carbon dioxide effects on the sensory alcohol (alcoholic sweetness, pinching effect).

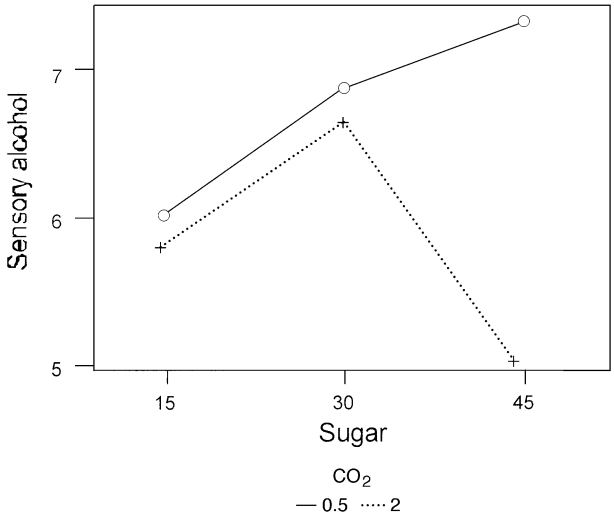

Fig. 4. Antagonistic effect of the interaction between sugar and carbon dioxide on the sensory alcohol.

Carbon dioxide, viewed as a sensory indicator, was not found to be statistically influenced by any chemical factor examined.

Acceptability, however, was significantly influenced by alcohol content ($F = 19.47$, $P = 0.009$) (Fig. 5), and particularly at the higher levels, as Tukey's comparison of means showed: $7 < 13 = 10$. Sugar addition also increased the acceptability, although not significantly but marginally ($P = 0.076$); the optimum sugar content is likely to be higher than 30 g/l. The kiwi wine is characterised by a sour and bitter taste originating from components such as citric and quinic acid. Therefore, sugar and alcohol additions were needed in order to bring that taste to some equilibrium of sensory attributes. This explains why these two sweet components positively affect the intensity of the panellists' acceptability.

The principal component analysis on the objective variables (Fig. 6) and on the wine samples (Fig. 7) revealed that the first two major axes explained 82.9% of the total variation. Sweetness and alcohol prevailed in axis 1 (loading factors, 0.802 and 0.853, respectively) and carbon dioxide in axis 2 (0.907). Comparing the lower right quartiles of Figs. 6 and 7, it appears that samples with high levels of sugar and alcohol are positioned close to the arrows of the corresponding sensory variables. Obviously, sweetness and sensory alcohol correlate strongly and positively and increase their values in the presence of sweeter and highly alcoholic wines.

The analytical study of kiwi wine composition shows that, except for the high acidity, the content in other components is sufficient for the production of wines with low alcohol percentage, with enough freshness as a result of their acidity, fine aroma and full taste. However, it is presumed that an appropriate reduction of acidity should take place, by neutralisation of the main

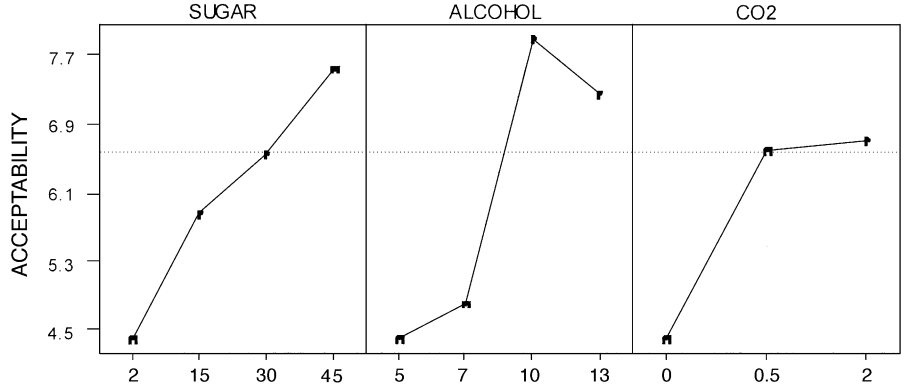

Fig. 5. Influence of chemical factors on the acceptability. The levels of basic wine (2/5/0) were not included in the statistical analysis.

(quantitative) acids: citric, galacturonic, lactic and malic.

Nevertheless, according to results of the sensory evaluation, after their statistical analysis, the acceptability of kiwi wines from panellists is higher if they contain 10% vol. alcohol, higher than 30 g/l sugars and 0.5 bar CO_2. These three sample characteristics create statistically significant differences and are detected by the panellists.

Greater amounts of those characteristics are not indicated as the panellists do not reveal any preference for alcohol contents above 10% vol. ($7 < 13 = 10$), because the results did not identify significant sweetness (just marginally, $P = 0.081$) but only sensory alcohol. Furthermore, sugar addition marginally affects ($P = 0.076$) the preference of panellists, while more than 30 g/l reduces sensory alcohol. CO_2, contents higher than 0.5 bars have negative effects on sensory alcohol and enhance the already high acidity.

Therefore, the production of a new type of kiwi wine, balanced at a higher level in the fundamental characteristics: sweetness, acidity and bitterness, would be a satisfying solution for making good use of small-sized kiwi-fruits. In conclusion, these wines should contain 10% vol. alcohol, more than 30 g/l residual sugars and 0.5 bar CO_2, while their pH values should be not lower

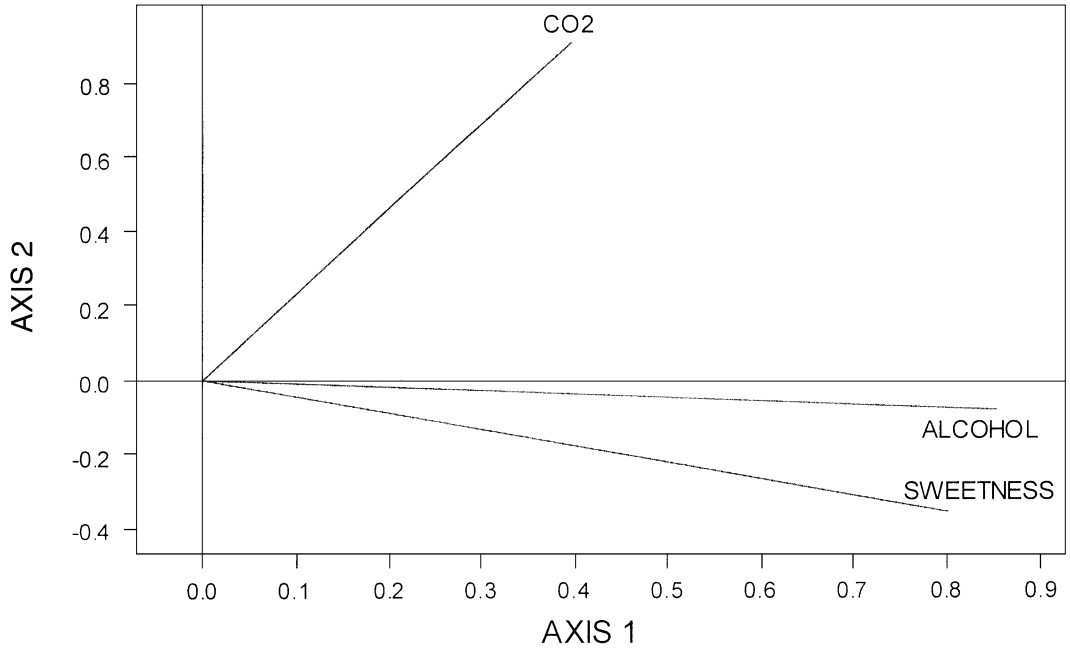

Fig. 6. Loading plot of the chemical variables against the first two major component axes.

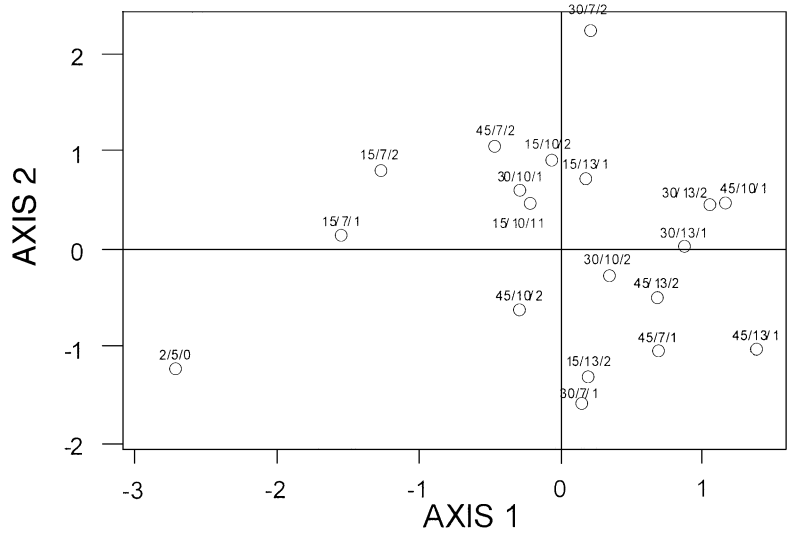

Fig. 7. Arrangement of samples according to the principal component axes 1 and 2. The coding of the sample follows the levels of the chemical factors in the order: sugar/alcohol/carbon dioxide.

than 3.4. According to our results, no preference for sparkling wines was indicated. Possibly the relatively high acidity of the basic wine (pH value 3.4 or acidity 13g/l expressed as citric acid) is responsible for these results.

4. Conclusions

Kiwifruits are important for Greece, as significantly exported products and the effort to use small-sized fruits by producing kiwifruit wine or other products, e.g. kiwifruit juice, will contribute considerably to Greek kiwifruit producers.

The processing and vinification of kiwifruits is more complicated than those of grapes and demand good ripeness and use of pectolytic enzymes for higher juice yield (75% in weight).

The composition of kiwifruits and kiwi is characterised by high acidity (13–15 g/l, expressed as citric acid), pH that range between 2.9 and 3.4 and low content in sugars (10–13 Brix).

The dominating acids in kiwi wines from well-ripened kiwifruits are citric, galacturonic, lactic and malic acids. The content of these acids in kiwi wines amount to the half of those contained in kiwifruits.

Kiwi wine samples are richer than wines (from grapes) in methanol because of the use of pectolytic enzymes, while their concentrations of higher alcohols are more or less at the same as those of wines (from grapes). However, in most cases these are lower than those of wines.

Esters, which come mainly from bacterial alterations, are found at lower concentrations than in grape wines. The concentrations of ethyl esters are lower in kiwi wines than grape wines. Qualitatively, the most important of the esters contained in kiwi wines are ethyl hexanoate and ethyl octanoate, and the most important acetic ester is isoamyl acetate. Some researchers claim that these quantities are equivalent to those contained in wines (from grapes) while other researchers claim that they are quite inferior.

Isobutyric and butyric acids prevail quantitatively in kiwi wines while, in general, the concentrations of these fatty acids, including those with C_6–C_{12}, are equal to those from grape wines or show no significant difference.

The sensory evaluation of kiwi wines and the statistical analysis of the results show that:

- Only the quantity of sugars significantly affect the sweetness of kiwi wines, while the role of alcohol is marginal.
- The sensory alcohol is statistically affected by the contents of sugars, alcohol and CO_2.
- The interaction between sugars and CO_2 is statistically significant.
- The high contents of sugars and CO_2 mask the sensory alcohol and have an influence on it.
- The acceptability of kiwi wines is higher if they contain 10% vol alcohol, more than 30 g/l sugars and 0.5 bar CO_2.

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