

Influence of *Saccharomyces cerevisiae* strains on fermentation and flavor compounds of white wines made from cv. Emir grown in Central Anatolia, Turkey

C Nurgel, H Erten, A Canbaş, T Cabaroğlu and S Selli

Department of Food Engineering, Faculty of Agriculture, University of Cukurova, Adana 01300, Turkey

The effect of inoculation with selected *Saccharomyces cerevisiae* strains was studied on fermentation and flavor compounds of wines made from *Vitis vinifera* L. cv. Emir grown in Central Anatolia, Turkey. Flavor compounds were analysed and identified by GC-FID and GC-MS, respectively. The total concentrations of flavor compounds did not increase with the addition of indigenous and commercial wine yeasts, but differences were noted in individual volatile compounds. Cluster and factor analyses of flavor compounds also showed that wines produced were different depending on the wine strain used. Wines were completely fermented to less than 1.4 g/l residual sugar. Yeasts other than *S. cerevisiae* survived longer than previously reported. Inoculation with selected strains increased the ethanol level.

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Introduction

Yeasts, key microorganisms in winemaking, conduct alcohol fermentation. They derive from the surfaces of grapes, surfaces of winery equipment and from inoculum cultures [11,15]. Wine fermentation is either carried out naturally without inoculation or by inoculation of grape juice with selected wine yeasts [4,17].

In natural fermentations, indigenous yeasts such as *Kloeckera apiculata*, *Hanseniaspora uvarum* and *Candida* spp. commence the alcohol fermentation of grape juice. These yeasts progressively die off with an increase in ethanol concentration, leaving more-ethanol-tolerant *Saccharomyces cerevisiae* to complete the fermentation [9,16]. More controlled fermentations are also performed by inoculating with a starter culture of *S. cerevisiae* [18,27]. The use of local selected and/or commercial starter yeasts offers rapid start of fermentation with a minimum lag phase and wines with acceptable sensory properties [12,15,16,28,32].

The composition and quality of wine are closely related to yeasts [16,15,20]. Besides the main products ethanol and CO₂, yeast produces many flavor compounds as secondary products during the alcohol fermentation. Small amounts of these volatile compounds, depending on the odor threshold, contribute positively to wine quality, while excessive concentrations have detrimental effects [2,16,15,26].

Emir, a native grape variety of *Vitis vinifera* L., is grown in the Nevsehir-Ürgüp region (ancient Cappadocia) of Turkey and gives good-quality white wines [5,8]. Limited studies have been carried out to improve the flavor potential of cv. Emir wines by using selected local and commercial strains of *S. cerevisiae*. The aim of this study was to investigate the effects of addition of *S. cerevisiae*

strains in unpasteurized and pasteurized grape juice on fermentation and flavour compounds to obtain more aromatic cv. Emir wines.

Materials and methods

Must

About 350 kg of healthy grapes of cv. Emir was harvested from Nevsehir-Ürgüp province in 1999. The grapes were transported to the Pilot Winery of the Department of Food Engineering, Faculty of Agriculture, University of Cukurova.

Grapes were crushed and then subjected to a horizontal press (Sarksan, Ankara, Turkey). Free and pressed grape juices were combined and 50 mg/l sulfur dioxide was added. The grape juice was allowed to settle at 15°C for 24 h and then racked.

Fermentation

Fermentations were carried out in unpasteurized and pasteurized musts at 18°C in a controlled environment. Unpasteurized fermentations were performed in 50-l stainless steel vessels (Kavaklıdere, Ankara, Turkey) containing 40 l of grape juice under fermentation locks. Pasteurized fermentations were carried out in 20-l sterile glass vessels containing 16 l of cv. Emir grape juice under aseptic conditions to investigate the influence of selected cultures in pure fermentations. For this reason, grape juice was pasteurized at 70°C for 15 min, then cooled to 20°C and aseptically added to chemically sterilized glass vessels. Glass fermentation vessels were closed with chemically sterilized fermentation locks.

The fermentations performed were: (a) spontaneous fermentation (without inoculation), (b) fermentation inoculated with an indigenous strain of *S. cerevisiae* in unpasteurized grape juice, (c) fermentation inoculated with a commercial strain of *S. cerevisiae* in unpasteurized grape juice, (d) fermentation inoculated with an indigenous *S. cerevisiae* in pasteurized grape juice and (e) fermentation inoculated with a commercial *S. cerevisiae* in

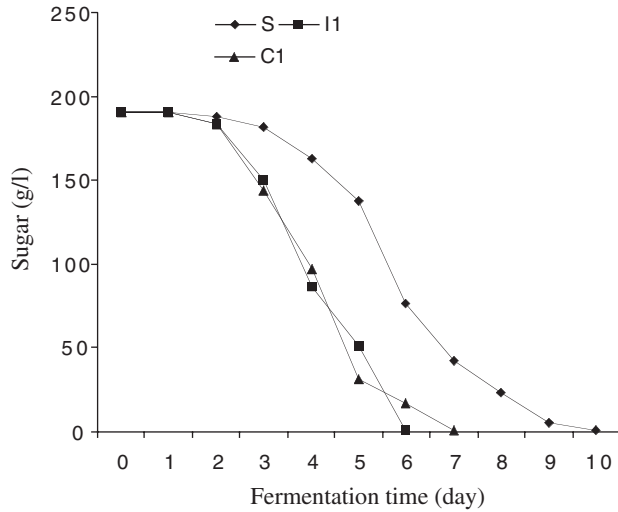


Figure 1 The change in sugar content of unpasteurized cv. Emir grape juices. S: Spontaneously fermented wine; I₁: wine inoculated with indigenous yeast; C₁: wine inoculated with commercial yeast.

pasteurized grape juice. Spontaneous fermentation was used for comparison.

Inocula

An indigenous strain of *S. cerevisiae* was selected among *S. cerevisiae* strains isolated and identified from cv. Emir fermentations in 1998. Inocula of the indigenous strain were prepared by plating a loopful of stock culture on malt extract agar (Difco, Detroit, MI), incubating for 48 h at 25°C and inoculating a single colony into a 250-ml sterile conical flask containing 100 ml of sterile grape juice. The flask was fitted with cotton and incubated for 48 h at 25°C with orbital shaking at 160 rpm. The cells were harvested by centrifugation at 2000×g for 10 min at 4°C and washed once with cold sterile distilled water. The pellet was resuspended in sterile grape juice and cells were counted using a counting chamber [13]. The inoculum size was 5×10⁶ cells/ml.

The commercial yeast used was Zymoflore VL1 (Zymoflore, Bordeaux, France). This yeast was suspended in warm sterile water at 35°C according to the producer's instructions and then added to the fermentation vessels at the level of 5×10⁶ cells/ml.

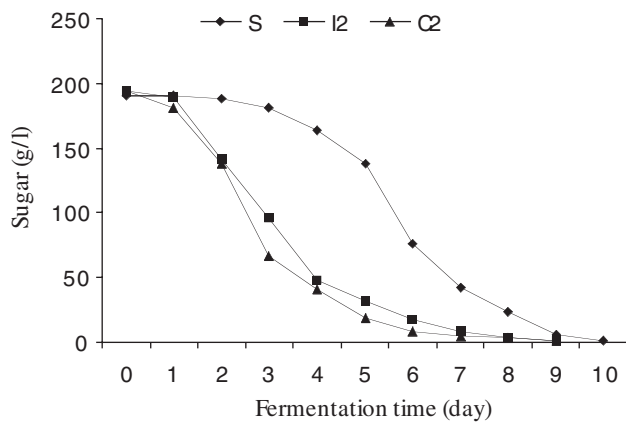


Figure 2 The change in sugar content of pasteurized cv. Emir grape juices. S: Spontaneously fermented wine; I₂: wine inoculated with indigenous yeast; C₂: wine inoculated with commercial yeast.

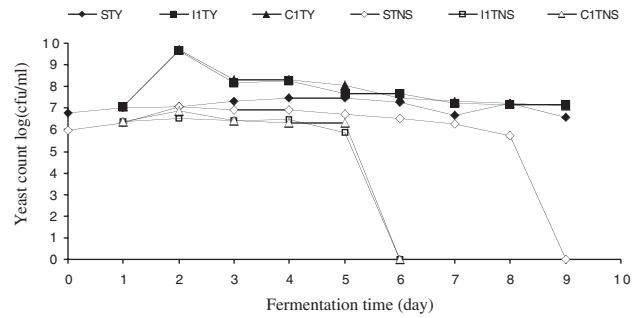


Figure 3 Development of the total yeasts and the total non-*Saccharomyces* yeasts in unpasteurized cv. Emir grape juices during the fermentation. STY: Total yeast of spontaneously fermented wine; I₁TY: total yeast of wine inoculated with indigenous yeast; C₁TY: total yeast of wine inoculated with commercial yeast; STNS: total non-*Saccharomyces* yeasts of spontaneously fermented wine; I₁TNS: total non-*Saccharomyces* yeasts of wine inoculated with indigenous yeast; C₁TNS: total non-*Saccharomyces* yeasts of wine inoculated with commercial yeast.

Analyses

Enumeration of yeast population

Samples were taken aseptically for yeast counts during fermentation. One milliliter of sample was serially diluted as required in 0.1% peptone water and 0.1 ml of diluted sample was spread on malt extract agar and lysine agar (Difco). Lysine agar was used to count non-*Saccharomyces* yeasts because it is a synthetic medium with glucose, vitamins, inorganic salts and L-lysine as the sole nitrogen source, and *Saccharomyces* spp. are unable to grow on it. Plates were incubated at 25°C for 3–5 days and total yeasts and non-*Saccharomyces* yeasts were counted [7,15,16].

General wine composition

General wine composition was determined according to Ough and Amerine [24].

Analysis of flavor compounds

Flavor compounds of wines were extracted in the Biotechnology Laboratory of the Department of Food Engineering of the University of Cukurova, Adana, Turkey. Identification and quantification of flavor compounds were performed in the Aroma Laboratory of INRA-IPV de Montpellier (France).

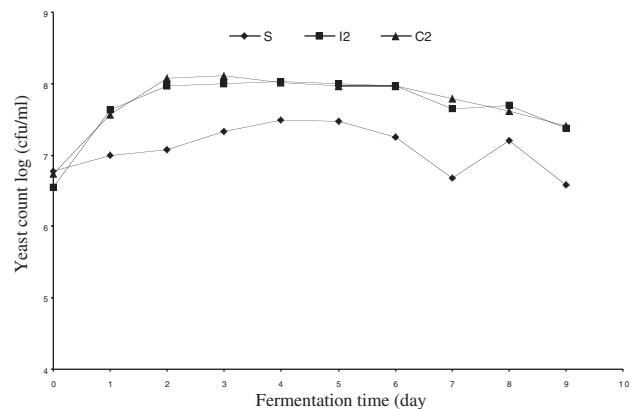


Figure 4 Development in the total yeast in pasteurized cv. Emir grape juices during fermentation. S: Spontaneously fermented wine; I₂: wine produced with indigenous yeast; C₂: wine produced with commercial yeast.

Table 1 Chemical analysis of cv. Emir wines

	Samples*				
	S	I ₁	C ₁	I ₂	C ₂
Density (20°C)	0.9920	0.9927	0.9925	0.9911	0.9912
Ethanol (% vol/vol)	9.90	9.90	9.99	10.87	10.70
Extract (g/l)	18	20.9	20.8	16.96	17.74
Total acidity (mEq/l)	63	62	67	67	59
pH	3.4	3.3	3.3	3.2	3.3
Volatile acidity (mEq/l)	6	2	2	3	3
Acetaldehyde (mg/l)	40	48	50	36	29
Residual sugar (g/l)	0.24	0.73	0.50	1.4	1.2
Free SO ₂ (mg/l)	15	10	9	10	9
Total SO ₂ (mg/l)	91	79	65	95	94

*S: Spontaneously fermented wine (control wine); I₁: wine made from unpasteurized grape juice inoculated with indigenous yeast; C₁: wine made from unpasteurized grape juice inoculated with commercial yeast; I₂: wine made from pasteurized grape juice inoculated with indigenous yeast; C₂: wine made from pasteurized grape juice inoculated with commercial yeast.

Extraction: Wine samples were analysed after alcoholic fermentation was completed. Extraction of flavor compounds was performed according to Blanch *et al* [3] and Schneider *et al* [30].

Before extraction, 10 µl of 4-nonanol (34 µg/l) and then 40 ml of dichloromethane were pipetted into a 500-ml flask containing 100 ml of wine. The content was magnetically stirred for 30 min under nitrogen gas at 4–5°C. Then, the mixture was centrifuged at 9000×g for 15 min at 0°C. The organic phase was recovered, filtered through glass wool with anhydrous sodium sulfate and concentrated to a volume of 1 ml with a Vigreux distillation column. The process was performed in duplicate. The samples were stored at –18°C until GC-FID and GC-MS analyses.

GC-FID analysis: Flavour compounds were measured using a Varian 3300 GC with flame ionization detector at 250°C and a fused capillary column coated with DB-Wax (30 m×0.32 mm i.d. and 0.5 µm film thickness; JW, Folsom, CA). The carrier gas was hydrogen at a flow rate of 2 ml/min. On-column injector temperature was programmed from 20 to 250°C at 180°C/min. The oven temperature was kept at 60°C for 3 min, and then increased to 220°C at 2°C/min. It was then increased from 220 to 245°C at 3°C/min and kept at 245°C for 20 min. One microliter of sample was injected for each analysis. The injection mode was on-column.

GC-MS analysis: The flavor compounds were identified by GC-MS. A Hewlett-Packard 5890 Series II chromatograph was used with the column mentioned above. The injection system was on-column. The temperature programmes of the injector and oven were as described above. The flow rate of helium gas as carrier was 1.5 ml/min. A Hewlett-Packard 5989A mass spectrometer equipped with a quadrupole detector was used for electron impact (EI). The source temperature was 250°C. EI was recorded at 70 eV in the range *m/z* 29–350 at 1-s intervals.

The compounds were identified by comparing their retention times with those of reference compounds, and by mass spectrum matching with database library spectra. Concentrations of flavor compounds were calculated using 4-nonanol as internal standard and expressed as the means of duplicate analytical assays.

Statistical analysis

Factor analysis (principal component method) and cluster analysis (Ward's method) were performed on wine flavors using the SPSS

(Chicago, IL) statistical version 9.0 package programme for Windows (SPSS) [10,25].

Results and discussion

Fermentation rate

Figures 1 and 2 show the utilization of total sugar during the fermentations. An increased rate of fermentation was observed with inoculated cultures. The sugar level dropped to less than 1 g/l in fermentations of unpasteurized grape juices inoculated with indigenous and commercial yeasts by days 6 and 7, respectively. The fermentation was completed on day 10 in spontaneous fermentation (without inoculation). Pasteurized grape juices showed an initial increase in sugar utilization by day 6, but sugar concentration was below 1.4 g/l on day 9. In the present study, inoculation led to faster completion of fermentations compared to spontaneous fermentation as stated in the literature [15,16,27].

Yeast count

The counts of total yeasts and total non-*Saccharomyces* spp. yeasts during fermentations of unpasteurized musts inoculated with selected yeasts are given in Figure 3. At the beginning of the spontaneous fermentation, the fresh grape juice exhibited a total yeast count of 6.77 log cfu/ml, then had a maximum number of 7.47 log cfu/ml on day 4. Grape juices inoculated with indigenous or commercial wine yeast strains reached 9.6–9.7 log cfu/ml. Counts of total yeasts at the end of fermentation ranged from 6.57 to 7.15 log cfu/ml (Figure 3). Initial levels of total non-*Saccharomyces* spp. yeasts were high at 5.95 log cfu/ml. Amounts of these yeasts increased slightly and then they had a long stationary phase in grape juices inoculated with selected *S. cerevisiae* strains. The non-*Saccharomyces* species, however, died off by day 6. During spontaneous fermentation (without inoculation), although dominated by *S. cerevisiae*, the non-*Saccharomyces* spp. rose to a maximum of 7.09 log cfu/ml on day 2 and survived until the end of fermentation (Figure 3). The growth of indigenous and commercial *S. cerevisiae* in pasteurized fermentations is shown in Figure 4. Spontaneous fermentation was used for comparison. Selected yeasts remained in a stationary phase at approximately 7 log cfu/ml during the alcohol fermentation, after reaching maximum populations of 8 log cfu/ml.

Table 2 Flavor compounds of cv. Emir wines inoculated with selected yeast strains

Compounds ($\mu\text{g/l}$)	RRT ^a	Samples*				
		S	I ₁	C ₁	I ₂	C ₂
<i>Higher alcohols</i>						
Isobutanol	5.18	5884	2122	6363	2911	2308
1-Butanol	6.13	2828	2038	3100	73	49
Isoamylalcohol	9.05	101,327	69,498	83,836	70,861	82,670
1-Pentanol	10.17	16	20	19	12	78
2,3-Butandiol	20.98	946	278	292	486	468
Benzylalcohol	32.58	72	24	32	63	40
2-Phenylethanol	33.96	29,434	27,688	27,195	26,977	29,355
Total of alcohols		140,507	101,668	120,837	101,383	114,968
<i>Esters</i>						
Ethyl hexanoate	9.53	814	650	665	724	874
Hexyl acetate	10.83	119	161	231	205	323
Ethyl lactate	13.48	6537	518	1491	563	705
Ethyl octanoate	16.90	671	629	832	796	956
Ethyl decanoate	24.64	282	239	365	305	361
2-Phenylethyl acetate	30.50	376	417	489	699	701
Dodecanoic acid ethyl ester	32.03	103	90	104	74	144
Ethyl 3-(4OH phenyl) propanoate	76.48	431	259	243	865	208
Total of esters		9333	2963	4420	4231	4272
<i>Fatty acids</i>						
Propanoic	20.70	33	19	31	30	32
Butyric	23.76	650	305	375	541	474
Isovaleric	25.48	51	58	106	76	172
Hexanoic	31.66	2807	2474	3232	3213	3510
Octanoic	38.61	5711	5443	7021	7075	7024
Nonanic	41.66	53	54	50	172	102
Decanoic	44.84	2033	1688	2420	2659	2551
Dodecanoic	51.88	151	55	130	39	95
Hexadecanoic	60.95	478	313	401	477	402
Octadecanoic	66.41	401	395	384	406	541
Total of fatty acids		12,368	10,804	14,150	14,688	14,903
<i>Phenols</i>						
4-Vinylguaiacol	42.30	125	134	13	<1	6
4-Vinylphenol	47.83	341	233	51	53	40
Total of phenols		466	367	64	54	46
<i>Carbonyl compounds</i>						
Acetoin	11.21	910	54	73	62	41
Total carbonyl compounds		910	54	73	62	41
Total		165,584	115,856	139,544	120,418	134,230

^aRRT: relative retention time.

*S: Spontaneously fermented wine (control wine); I₁: wine made from unpasteurized grape juice inoculated with indigenous yeast; C₁: wine made from unpasteurized grape juice inoculated with commercial yeast; I₂: wine made from pasteurized grape juice inoculated with indigenous yeast; C₂: wine made from pasteurized grape juice inoculated with commercial yeast. Maximum standard deviation of data was $\pm 10\%$.

Total yeast counts range from 4 to 6 log cfu/ml in freshly extracted grape juice, reaching a maximum of 8–9 log cfu/ml during fermentation. After attaining maximum cell numbers, stationary and decline phases are observed [15,16]. The non-*Saccharomyces* can survive up to 6–7 log cfu/ml in spontaneous fermentations. These yeasts can have a significant effect on wine fermentation and wine flavor [11]. Addition of wine yeast *S. cerevisiae* during fermentation does not eliminate the growth of indigenous yeasts, which contribute to wine character [18].

General wine composition

The general wine composition is shown in Table 1. Fermentations performed with inoculation of selected yeasts in pasteurized musts produced higher concentrations of ethanol. The wine made with

spontaneous fermentation had higher amount of volatile acidity. Wines were fermented to dryness and concentrations of reducing sugar were less than 1.4 g/l. Acetaldehyde levels were lower in wines obtained under sterile conditions. The results in this study are in good accord with those in other studies [28,32], which showed that selected strains influence the wine composition.

Flavor compounds of wines

Flavor compounds give the wine its typical odor and taste. The main origin of these compounds is yeast metabolism during the fermentation, although some compounds are present in some grapes, e.g., Muscat, and also some form during wine maturation. Higher alcohols, esters and volatile acids are the main constituents of wine, whereas carbonyl compounds, phenols and sulfur

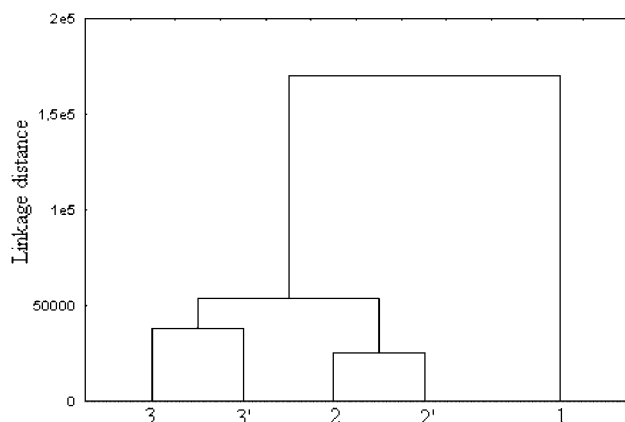


Figure 5 Cluster analysis of wine samples. (1) Spontaneously fermented wine; (2) wine produced with inoculation of indigenous yeast in unpasteurized grape juice; (2') wine produced with inoculation of indigenous yeast in pasteurized grape juice; (3) wine produced with inoculation of commercial yeast in unpasteurized grape juice; (3') wine produced with inoculation of commercial yeast in pasteurized grape juice.

compounds are present in smaller concentrations [21]. The concentrations of flavor compounds produced during fermentation in this study are given in Table 2. In general, the total concentrations showed that spontaneous fermentation produced higher levels than other fermentations performed with inoculation of selected *S. cerevisiae* strains in unpasteurized and pasteurized grape juices. Survival of *K. apiculata*, *H. uvarum* and *Candida* spp. affects final chemical composition and, consequently, wine quality [15,16]. In the present study, a notable growth of non-*Saccharomyces* yeasts was observed until the end of spontaneous fermentation.

The amount of total higher alcohols varied from 101 to 140 mg/l, with a higher level in spontaneous fermentations. Isoamyl alcohol and 2-phenylethanol were formed at the highest concentrations compared to other higher alcohols in wines. Addition of selected *S. cerevisiae* decreased the concentration of these alcohols. López et al [22] stated that 2-phenylethanol is especially responsible for the rose flavor. The flavor threshold in wine is between 60 and 180 mg/l [29,31] for isoamyl alcohol and between 25 and 105 mg/l for 2-phenyl ethanol [1]. These two higher alcohols contribute sensory quality to the wine [14]. Their concentrations in cv. Emir wines as shown in Table 2 are between these flavor thresholds.

Overall low concentrations of esters were produced in wines in which spontaneous fermentation took place, particularly ethyl lactate (Table 2). Ethyl lactate is formed during malolactic fermentation [19,23]. This fermentation is not generally carried out in Turkey for white wines as in other winemaking countries. Moreover, samples were collected in the absence of malolactic fermentation following completion of the usual alcohol fermentation. Ethyl lactate was probably formed during the fermentation by yeasts in agreement with the results of Antonelli et al [2]. Addition of commercial yeast increased the amounts of 2-phenylethyl acetate and ethyl octanoate (Table 2). Esters are important compounds and exhibit a fruity odor [4,14]. The concentrations of esters in cv. Emir wines did not exceed the flavor threshold levels given by Simpson [31] and Etiévant [14], with the exception of ethyl hexanoate. Similar results were also reported in Emir wines by Cabaroğlu et al [6]. Etiévant [14] stated that the low ester content during alcoholic fermentation is mainly affected by yeast strain.

Concentrations of fatty acids in Emir wines were low. Fermentations performed with commercial yeast resulted in low amounts of the long-chain fatty acids hexanoic and octanoic. Fatty acid levels were lower than the threshold levels indicated by Etiévant [14]. Higher amounts of the volatile phenols 4-vinyl guaiacol and 4-vinyl phenol were measured in wines made by spontaneous fermentation and by unpasteurized grape juice inoculated with indigenous *S. cerevisiae* (Table 2). These phenols are produced mainly during the alcohol fermentation by wine yeast [14] or may arise from the activity of *Brettanomyces* spp. and *Dekkera* spp. during storage [4]. They can also be formed from phenolic acids by nonenzymatic reactions [14].

Figure 5 shows the results of cluster analysis (Ward's method) on wine flavor compounds. The dendrogram revealed two clearly defined main groups of wines. One group was divided into two subgroups in which wines resulted from grape juices fermented with commercial and indigenous *S. cerevisiae*. The other main group consisted of wines from spontaneous fermentations.

Factor analysis using the principal component method is given in Figure 6. The first two principal components accounted for 44.90% and 25.37% of the variance. The first principle component of spontaneously fermented wine was located on the positive side of the factor, characterized mainly by higher alcohols, fatty acids and ethyl ester of lactate. The higher alcohols are isobutanol, isoamyl alcohol, 2,3-butandiol, benzyl alcohol and 2-phenyl ethanol, whereas the fatty acids are butyric acid, dodecanoic acid and hexadecanoic acid. The effect of addition of a commercial yeast is on the negative side of the factor, distinguished by the production of the fatty acids hexanoic, octanoic, decanoic, octadecanoic, isovaleric and the esters hexyl acetate, ethyl octanoate, ethyl decanoate and 2-phenyl ethyl acetate. Pentanol from higher alcohols is also found on the negative region of the factor. Factor 2 can be defined by the effect of adding an indigenous wine yeast with ethyl 3-(4OH phenyl) propanoate on the positive side of the vector. Wines produced by spontaneous fermentation and by addition of commercial yeast were located on the negative region of

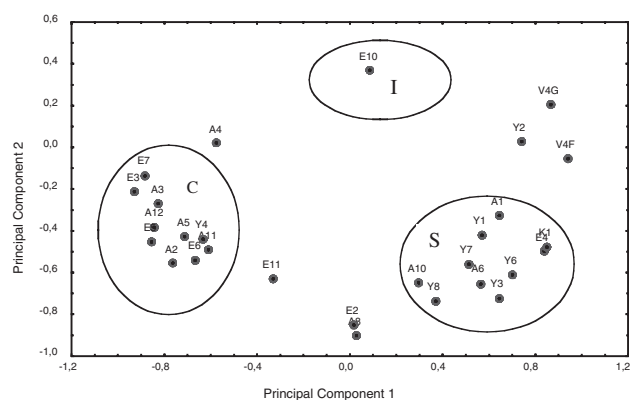


Figure 6 Factor analysis of flavor compounds according to yeast strain. S: Spontaneous wine; I: indigenous yeast; C: commercial yeast; A1: butyric acid; A2: hexanoic acid; A3: octanoic acid; A4: nonanoic acid; A5: decanoic acid; A6: dodecanoic acid; A8: propanoic acid; A10: hexadecanoic acid; A11: octadecanoic acid; A12: isovaleric acid; Y1: isobutanol; Y2: 1-butanol; Y3: isoamyl alcohol; Y4: pentanol; Y6: 2,3-butandiol; Y7: benzylalcohol; Y8: 2-phenylethanol; E2: ethyl hexanoate; E3: hexyl acetate; E4: ethyl lactate; E5: ethyl octanoate; E6: ethyl decanoate; E7: 2-phenylethyl acetate; E10: ethyl 3-(4OH phenyl) propanoate; E11: dodecanoic acid ethyl ester; K1: acetoin; V4G: 4-vinylguaiacol; V4F: 4-vinylphenol.

factor 2. Thus, it can be concluded from factors 1 and 2 that this effect is different for various fermentations.

Conclusion

The influence of adding indigenous and commercial yeasts was studied on fermentation and flavor compounds of *V. vinifera* L. cv. Emir in unpasteurized and pasteurized grape juices. Non-*Saccharomyces* yeasts grew until the end of the fermentation in spontaneously fermented wine, but they were reduced by inoculation of selected *S. cerevisiae* strains. Data obtained from gas chromatography and cluster and factor analyses showed that differences were present in wine volatiles, whereas predictions on the improvement of the Emir wine quality by the inoculation of wine strains remained inconclusive.

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