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Research Note

General and polyphenolic composition of unripe grape juice (verjus/verjuice) from various producers

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Seven unripe grape juices ("verjus") were analyzed for their general and polyphenolic content. Depending on the sugar content, i.e. picking date, the verjus show different chemical composition. In those cases, where the grapes had been picked before sugar accumulation, especially sugar-free extract and polyphenol content are very high. In these products high concentrations of gallic acid and caffeic acid, catechin, and quercetin glucoside have been found by means of HPLC. The other samples, picked at the vegetative stage of sugar accumulation, generally show lower polyphenol concentrations, but are richer in grape reaction product (GRP) content, which might be an indicator for a more oxidative production technique. Most of the polyphenolic fraction seems to be of polymeric nature, since large discrepancies were found between Folin value and HPLC polyphenol content.
Key words: unripe grape juice, verjus, verjuice, phenolics, HPLC

Verjus is a product, which is made from unripe green grapes, the name "verjus" is derived from the French expression "vert jus" ("green juice"). It possesses a tart taste and has a strong acidity. Verjus has been used as a food ingredient and as a medicine as early as the ancient days of the Greeks. Already Hippocrates of Kós (460 - 370 B.C.) wrote on the use of unripe grape juice for the treatment of ulcers (http://etext.library.adelaide.edu.au/h/hippocrates//ulcers/index.html). In the medieval and the early modern times verjus was used as an acidifying agent in cooking, as medicine and as a digestive agent, e.g. in the "Tuhfat al-Muminin" ("Gift of True Believers") verjus is recommended as a digestive agent after the ingestion of fatty foodstuff, such as brain (MU'MIN, 1669). It is still widely used in the oriental cuisine, e.g. in Persian and Turkish cooking, regionally rather known as "Abe ghureh" (Persian: "unripe grape water") and "Koruk suyu" (Turkish: "unripe grape juice"), respectively.

Recently, verjus has received more and more interest in the western countries and is currently "re-discovered" as a savory flavoring (KÖGLER, 2007). Yet, despite this new interest in this product, hardly any information is currently available on the chemical composition of this food stuff. Furthermore, with regard to the medical use of verjus only few studies have been conducted, which analyze the effect of verjus ingestion on health. KARAPINAR and SENGUN (2007) have investigated the impact of verjus on *Salmonella typhimurium* strains on salad vegetables. According to their results the antimicrobial effect of verjus is dependent on the culture strain and the product used. AMINIAN et al. (2006) have studied the effect of verjus on plasma lipid levels in rabbits after egg yolk ingestion. Their results show that verjus has no preventive or therapeutic effect in hypercholesterolemia. In human studies AMINIAN et al. (2003) did not find a lipid-lowering effect, yet an antioxidative effect on LDL cholesterol was observed. The authors conclude that the polyphenol content in verjus might be responsible for this effect, which they suppose is lower than in red wines. Yet, virtually no information is available on the polyphenolic content of this product. Furthermore, there is also a lack of information on the general chemical composition of verjus. Thus, a small project was started to investigate these parameters in various verjus from different producers.

Table 1: General chemical composition of the verjus samples (n.d. = not detected)

Country of origin	France	Germany	Germany	Germany	Germany	Iran	Iran
Total alcohol (g/l)	31.2	23.3	13.5	45.5	21.5	0.0	15.5
Total alcohol (%vol.)	3.95	2.95	1.71	5.77	2.72	0.01	1.97
Alcohol (g/l)	1.7	0.5	n.d.	n.d.	0.4	n.d.	9.7
Alcohol (%vol.)	0.22	0.07	n.d.	n.d.	n.d.5	n.d.0	1.23
Total extract (g/l)	94.7	84.6	64.8	124.6	83.3	38.1	51.7
Sugar-free extract (g/l)	31.9	36.2	36.0	27.7	38.4	38.0	39.3
Sugar content (g/l)	56.6	38.2	22.8	95.1	40.3	0.1	0.2
Acidity (as tartaric acid, g/l)	28.7	32.4	31.4	19.6	29.4	25.0	39.6
Free SO_2	5	7	6	4	n.d.	n.d.	n.d.
Total SO_2	5	13	14	17	1	6	3
Polyphenols (Folin, mg/l)	315	346	783	442	200	1330	780
Density	1.0362	1.0325	1.0250	1.0480	1.0321	1.0148	1.0181
Volatile acidity (g/l)	0.07	0.11	0.22	0.11	0.05	0.43	0.20
OD 420nm	0.343	0.227	0.342	0.363	0.365	0.780	0.628
OD 520nm	0.149	0.124	0.151	0.272	0.168	0.367	0.324
OD 620nm	0.082	0.089	0.083	0.109	0.103	0.120	0.138

OD = optical density

Material and Methods

Verjus samples

The verjus samples were bought from different stores and from our own winery.

General chemical parameters

The general chemical parameters of wine composition, such as sugar, alcohol etc., were analyzed according to the official methods of wine analysis (Commission Regulation (EC) No1293/2005 of 5 August 2005 amending Regulation (EEC) No2676/90 determining Community methods for the analysis of wines)

Polyphenol content was measured using the Folin method as published by Ritter (1997).

Color

Color of unfiltered samples was measured in 10 mm plastic cuvettes by means of a Spectronic 1001 spectrophotometer (Milton Roy Company, Dreieich, Germany) at 420, 520 and 620nm.

Chemicals

All reagents used were of analytical grade unless otherwise stated. HPLC water, acetonitrile (gradient grade), and phosphoric acid were from Merck (Darmstadt, Germany).

Polyphenol standards

Gallic acid, tyrosol, catechin, epicatechin, procyanidin B_2, p-coumaric acid, caffeic acid, ferulic acid, quercetin-3-rutinosid (rutin), trans-resveratrol, and quercetin were from Extrasynthese (Genay, France). Hydroxycinnamic acid esters were calculated as their respective free acids: caftaric acid, p-coutaric acid, fertaric acid, p-coumaroyl-glucosyl-tartrate (p-CGT), and Grape Reaction Product (GRP, 2-*S*-glutathionyl caffeoyl tartaric acid). Hydroxycinnamic acid esters were identified by their photodiode array (PDA) spectra and retention time.

Equipment

A Dionex (Germering, Germany) UltiMate 3,000 HPLC system consisting of degasser, autosampler, pump, column oven, and photodiode array (PDA) detector was used. Best selectivity by means of a PDA detector was achieved at 280nm for benzoic acids; at 310nm for trans-resveratrol; at 320nm for hydroxycinnamic acids and their respective esters and GRP; and at 360nm for flavonols.

HPLC method

Separation of the polyphenolics was performed using the method published by Rechner et al. (1998).

Sample preparation

Before analysis by HPLC, all samples and standards were filtered through a Sartorius (Göttingen, Germany) Minisart RC 15® 0.45µm filter. A volume of 20µl was injected.

Statistics

All statistical analyses were performed using Excel® (Microsoft Corp., Redmond, USA) and XLSTAT® (Addinsoft SARL, Paris, France).

Results and Discussion

General chemical composition

The general chemical composition of the verjus samples is given in Table 1. As can be seen those samples having low to no sugar content show also high contents in total acidity, sugar-free extract, and polyphenol content. Presumably, these samples were made from grapes before sugar accumulation started or when sugar concentration was still very low. Thus, their content on acidity and polyphenols is much higher. It is well known from literature that the polyphenol content of grapes reaches two maxima during ripening: one at or shortly after véraison and the other at maturity (Fernandez De Simon et al., 1992). Some of the samples show polyphenol contents higher than 780mg/l. Thus, the presumption made by Aminian et al. (2003) that verjus could also provide high polyphenol concentrations can be confirmed. The samples from Iran also show only very low alcohol contents, which supports the thesis that these products were made from grapes picked at low sugar levels. Yet, these products showed high contents on volatile acidity, which was also noted during sensory evaluation (data not shown). Presumably, these products either had low sugar or alcohol contents, respectively, and experienced a slight microbial spoilage during production.

Polyphenolic profile (HPLC)

With regard to polyphenolic composition also large differences were found between the samples. Especially in those samples with low sugar content high concentrations on gallic and caffeic acid, catechin, and quercetin glucoside were found. These higher contents are also probably due to the earlier ripeness stage, at which the grapes were picked.
The samples from Germany showed higher contents on GRP, while the other samples were practically GRP-free. This might be an indicator for the more oxidative production technique in Germany compared to the samples from Iran and France, since GRP is formed under oxidative conditions from caftaric acid and glutathione (Cheynier et al., 1990).
The total polyphenol content as estimated by HPLC

Table 2: Polyphenolic composition (mg/l) of the analyzed verjus samples (F = France, G = Germany, IR = Iran; results are mean values of two independent injections; n.d. = not detected)

Origin	F	G	G	G	G	IR	IR
Caffeic acid	n.d.	n.d.	n.d.	n.d.	n.d.	6.6	19.8
Caftaric acid	36.4	15.6	76.3	52.2	24.7	16.7	54.7
Catechin	4.8	6.5	2.1	n.d.	9.8	21.6	n.d.
Epicatechin	2.2	0.8	1.7	1.1	0.4	n.d.	4.0
Fertaric acid	1.1	1.9	1.7	2.7	2.5	n.d.	1.6
Gallic acid	n.d.	n.d.	n.d.	n.d.	n.d.	70.6	36.6
GRP	0.4	2.0	1.5	3.7	2.8	n.d.	0.1
p-CGT	0.2	n.d.	n.d.	0.4	0.3	2.1	n.d.
p-Coumaric acid	n.d.	n.d.	n.d.	n.d.	n.d.	2.1	1.8
p-Coutaric acid	3.3	1.0	7.0	4.5	1.9	5.0	6.6
Protocatechuic acid	0.3	n.d.	0.4	1.4	0.2	2.3	1.1
Quercetin	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	n.d.
Quercetin-glucoside	0.7	0.5	1.1	0.8	0.5	4.4	2.1
Tyrosol	22.1	14.5	28.8	15.9	12.0	32.0	23.8
Total	71.5	42.8	120.6	82.7	55.1	156.9	132.4

ranges from 43 to 156mg/l. Therefore, in some cases the Folin value is about 8.5-fold higher than the total polyphenol amount measured by means of HPLC. This leads to the supposition that most of the polyphenolic fraction within verjus is of polymeric nature and, thus, is not detected during monomeric polyphenol analysis by HPLC. Since polymeric phenols are well-known for their astringent and bitter taste (Vidal et al., 2003), this would also explain the sensory characteristics generally associated with verjus.

Conclusion

Verjus samples were analyzed on their general chemical composition as well as their polyphenolic fingerprint. The results show that the verjus samples contained different concentrations of acidity, sugar-free extract, and total polyphenols as determined by means of the Folin-Ciocalteu method. Early harvested samples generally contained much higher contents in gallic acid and caffeic acid, catechin, and quercetin glucoside, while later harvested samples showed lower polyphenol concentrations, but were richer in GRP content, which might be an indicator for a more oxidative production technique. Most of the polyphenolic fraction of verjus seemed to be of polymeric nature, since large discrepancies were found between Folin value and HPLC polyphenol content. More detailed studies are necessary to investigate the influence of cultivar and ripeness stage on composition and sensory quality of this interesting product.

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