Effect of the Stone Content on the Quality of Plum and Cherry Spirits Produced from Mash Fermentations with Commercial and Laboratory Yeast Strains

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To evaluate the influence of stone content on spirit quality from stone fruit, cherry and plum mashes were prepared and fermented with a commercial and a diploid laboratory yeast strain. Fermentation parameters such as sugar content and ethanol production were followed. Despite an initial lag phase in cherry spirits, both yeast strains performed similarly, as substantiated by the determination of specific flavor compounds, ethyl carbamate, and methanol in the mashes and after distillation. The spirits produced were subjected to sensory analyses by trained panels of at least 25 judges. Although mashes retaining the stones could be clearly distinguished from those where the stones had been removed, no significant preference could be attributed to either spirit, indicating that qualities added by the presence of stones during fermentation are largely a matter of personal taste. Interestingly, the yeast strain used for fermentation seemed to have little influence on the spirit quality.

KEYWORDS: Fermentation; flavor compounds; fruit mashes; sensory analysis; *Saccharomyces cerevisiae*

INTRODUCTION

Traditionally, alcoholic beverages and spirits are produced from a variety of fruits by yeast-based fermentations. Fermentation and distillation technologies have been especially improved in the course of the last century, and refined methods are continuously developed. As a result of the increasing competition in the spirit production business, consumer's interests shifted from "low cost" commodities to high quality beverages. Although the definition of high quality is somewhat prone to personal preferences, there are certain legal requirements to be fulfilled and also some rules in production to be followed to ensure a widely excepted spirit quality. Nevertheless, obeying all of these rules does not necessarily guarantee a high quality and commercially successful product. In addition, attributes such as social acceptability, healthiness, and enjoyment in the consumption are values that can be at least partially influenced by the producer (e.g., by reducing the concentrations of potentially hazardous compounds). Moreover, the judgment of sensory attributes by expert panels is necessary for the development of production schemes that will result in beverages with reproducible quality and good consumer acceptance.

Sensory performance is dependent on the concentration of flavor compounds. These have their origin in the fruit employed as raw material, in the fermentation process itself with substances coming from yeast metabolism or from the degradation of fruit ingredients, and from chemical reactions between these compounds during fermentation, distillation, and storage (*1*).

Besides the aspects concerning the raw material employed, market-orientated yeast strains are currently being developed for the cost competitive production of alcoholic beverages with minimized resource inputs, improved quality, and low environmental impact (*2*). Thus, *Saccharomyces cerevisiae* strains are developed, showing improved fermentation, processing, and biopreservation abilities, as well as improved sensory qualities of the beverages. Different yeast strains will usually produce individual quality profiles (*3*). Therefore, genetically well-defined or even modified yeast strains are more and more constructed for the alcoholic beverage industry (*2, 4*).

Regarding stone fruit as raw materials, consumers often desire the typical "bitter-almond" character in the final spirits. However, such positive flavor compounds introduced from the stones may be accompanied by detrimental influences and even health risks. Thus, fermentation of stone fruit and subsequent spirit production have been claimed to frequently result in the formation of the carcinogenic compound ethyl carbamate (EC) (also referred to as urethane; *5−7*). It was proposed that this compound can form when amygdalin from the stones is degraded to cyanide and exposed to light (*8−10*). Another possible source of EC may be yeast metabolism and secretion of urea into the medium, as an intermediate of arginine

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metabolism (*11*, *12*). In this context, we are testing a diploid laboratory strain since it can be more easily genetically altered. For example, if the laboratory strain performs as well as the commercial yeasts, it is feasible to introduce a modification, so that it does not produce urea and thus reduce the EC content in fermented fruit mashes and the resulting spirits.

As stated above, removal of stones remains another option for the production of spirits with different flavor and a "healthier" spirit. In this work, we tested the effect of such a removal prior to fermentation on the sensory quality and the concentration of several flavor compounds within the spirits produced.

MATERIALS AND METHODS

Yeast Strains Employed. In this work, a commercially available yeast strain named Uvaferm CGC62 (manufacturer's trade name; purchased from Begerow GmbH & Co., Langenlonsheim, Germany) and the laboratory strain HHD1 (MAT**a**/α ura3-52/URA3 leu2-3,112/LEU2 MAL2-8^c/MAL2-8^c SUC2/SUC2; *4*), which is closely related to the *S. cerevisiae* CEN.PK122 (*13*), were used. The commercial strain was packaged as dried yeast in 500 g aliquots.

Media and Culture Conditions. Rich media were based on 1% yeast extract and 2% bacto peptone and supplemented with 2% glucose (YEPD). All strains were incubated at 30 °C. For standardized conditions, all strains were grown in 5 mL of YEPD overnight on a rotor shaker (30 °C, 140 rpm), transferred into 500 mL shake-flask cultures with fresh YEPD, incubated for 12 h, and harvested by centrifugation (3500*g* for 5 min at room temperature). Cell pellets were washed twice with 25 mL of NaCl/peptone (0.85% NaCl, 0.05% peptone), resuspended in 25 mL of the same medium, and transferred to 1.5 L of YEPD in 3 L shake flasks. After 24 h of incubation at 30 °C at 140 rpm, yeasts from each culture were again harvested by centrifugation (3500*g* for 10 min at room temperature), washed twice as described above, and resuspended in 100 mL of NaCl/peptone solution. The cell density was calculated from optical measurements at 578 nm in appropriate dilutions, assuming that 1 OD$_{578}$ equals 10^7 cells/mL. From this, the yeasts were added to the mashes at a final density of 10^6 cells/mL each.

Raw Material and Mashing Process. On the basis of former results, fermentations on a technical scale (90 kg) were initiated (*4*). The studies were performed with two different stone fruit mashes: cherries (cv. Dollenseppler) and plums (cv. Ersinger Frühzwetschge). Cherry and plum mashes, as well as the remaining stones, were inoculated and fermented with the Uvaferm strain and HHD1. The cherries were in an excellent condition; no bruised or decayed fruits were present. The plums contained approximately 10% rot that had to be sorted out by hand prior to mashing.

Mashes were prepared according to standard procedures. Thus, the fruits without the peduncles were washed and chopped using a drill machine attached to a beater so that the stones remained undamaged and then were divided into equal lots. One fraction was not treated any further, and the other portion was passed through a pulping machine and destoner (filter width, 4 mm; capacity, 50–250 kg/h; Bockmeyer, Nürtingen, Germany) for the total removal of the stones. Immediately after comminution or pitting the fruit, the pH value was adjusted to 3.0 with technical sulfuric acid (technical grade). The remaining stones were collected and fermented separately without the addition of sulfuric acid.

The mash was divided in 90 kg lots each and separated in 120 L vessels. For fermentation, the vessels were sealed with a fermentation bung and incubated with the two different yeast strains. All experiments were performed in triplicate, and different parameters such as ethanol yields, extract, sugar content, yeast metabolites, and pH were determined over the fermentation period. The stones were only fermented without checking any of these parameters, distilled, and finally used for sensory assessment.

Fermentation. The fruit mashes (90 kg each) were fermented in 120 L plastic barrels. The mashes were inoculated with the selected commercial yeast strains Uvaferm, Freddo, Forte, the laboratory strain

HHD1 (all standardized to be in the same physiological state and cell density as described above) and fermented to completion at 15–17 °C. During fermentation, mashes were agitated at times and samples were collected and analyzed at the same time for the different parameters indicated.

Distillation. After 8 weeks of fermentation, the mashes were distilled using a 200 L copper pot (Jacob-Carl, Göppingen, Germany) fitted with an enrichment section consisting of three bubble plates, a dephlegmator, and a cyan catalyst (Holstein, Markdorf, Germany). This modern plant facilitates distillation under technical and standardized conditions. The dephlegmator was run with a flow rate of 120 L/h, and the catalyst was used. The fermented mashes were distilled with two plates in operation. The distillates were collected in fractions with a volume of 250–300 mL each. In the vicinity of the switching points (heads to product fractions and product fractions to tailings), smaller volumes of 100–150 mL were collected. The heads were identified with the detaching test determining acetaldehyde according to Pieper et al. (*14*). The tailings were screened by detachment at 72% vol and partly by organoleptic assessment. The stones were distilled on a 19 L plant with three plates, a dephlegmator, and without a catalyst. Fractions of 100 mL each were collected, and the heads and tailings were discarded as described above.

Spirit Fractions. The product fractions were stored for at least 1 week at 17 °C, then diluted with deionized water to an alcohol content of 40% (v/v), cold filtered at 4 °C (Macherey Nagel, Düren, Germany), and kept for another 4 weeks at 17 °C prior to further analysis and sensory assessment.

Analytical Methods. As a preliminary indication to observe the fermentation process, the pH was followed using a pH meter (WTW521, Weilheim, Germany), and the decrease of fermentable carbohydrates (% sugar) was determined with a hand refractometer (Carl Zeiss, Jena, Germany).

The exact decrease of the fermentable sugars (glucose and fructose), the ethanol content, as well as the formation of the volatile compounds acetic acid, propionic acid, and lactic acid were determined by high-performance liquid chromatography (HPLC) (Bischoff model 2200 HPLC using a Bischoff model 728 Autosampler; Bischoff, Leonberg, Germany), using an Aminex HPX-87H column (Biorad, Munich, Germany), a RI detector ERC7510 (ERC, Altegolfsheim, Germany), and a McDAcq15 Integrator (Bischoff, Leonberg, Germany). Sulfuric acid (0.1 N, technical grade) was used for elution.

Quantitative gas chromatography–flame ionization detection (GC-FID) analyses were performed to determine methanol and various yeast metabolites and aroma components such as acetaldehyde, methyl- and ethyl acetate, 3-methyl-butyl acetate, 2-methyl-1-propanol, 1-propanol, and the isoamyl alcohols (3-methyl-1-butanol and 2-methyl-1-butanol). Therefore, we employed a headspace gas chromatograph from Perkin-Elmer (model HS40, GC 8420) equipped with a packed crossbond phenylmethyl-polysiloxane column (Rtx-volatiles; 60 m by 0.32 mm, film thickness 1.5 μm; Resteck GmbH, Bad Homburg, Germany), a flame ionization detector, and a CLASS VP 4.2 integrator (Shimadzu, Duisburg). As an internal standard, *n*-butanol (200 mg/L; Merck, Darmstadt, Germany) was used. For mashes, the method described in Brautechnische Analysenmethoden (*15*) according to Boettger and Pieper (*16*) was used. All gases were supplied by Sauerstoffwerk GmbH (Friedrichshafen, Germany).

The analysis of EC was done using tandem mass spectrometry (GC/MS/MS) as described previously (*17*). Total hydrocyanic acid (HCN) in the spirits was photometrically determined after hydrolysis with potassium hydroxide and the reaction with chloramine-T and pyridine/barbituric acid reagent as described in ref *18*. For the determination in mashes, HCN was separated from the matrix by distillation before the photometric analysis.

Sensory Analyses. The fruit spirits produced in different technological ways and fermented with different yeast strains were analyzed by both sensory and physical methods. They were assessed for their characteristic flavor quality using order-of-precedence and triangle tests (*19*, *20*).

Before sessions, panelist training (staff and graduate students from the University Hohenheim, Department of Food Technology) was accomplished. The participants were trained in evaluation of the basic

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Table 1. Sugar Content and Alcohol Yield during Mash Fermentations

| | % plato[a] | | | | theoretical alcohol yield[b] | observed alcohol yield in mashes[c] | |
| | Uvaferm | | HHD1 | | | | |
mash	initial	final	initial	final		Uvaferm	HHD1
cherries with stones	26.0	14.3	25.4	14.5	9.85	14.00	12.73
cherries w/o stones	23.9	11.6	24.1	12.4	9.04	14.42	15.12
plums with stones	17.2	9.5	16.7	10.6	6.42	11.64	10.30
plums w/o stones	17.1	10.9	16.2	9.5	6.28	11.64	8.83

[a] % Plato = g sucrose per 100 g mash liquid. [b] The theoretical alcohol yield was calculated as follows: L alcohol/100 L mash = (% plato − nonfermentable matters) × 0.56 × TF (with nonfermentable matters for cherries = 5% and for plums = 4% and TF for cherries = 0.850 and for plums = 0.885). [c] Observed alcohol yield = alcohol content of the spirit (v/v) × L spirit per L mash; for the mashes without stones, an average loss of weight of 20% for cherries and 24% for plums was assumed; w/o, without.

flavors (salty, bitter, sweet, and sour) and in detecting differences between typical ingredients of heads and tailings in spirits. To enhance statistical significance, larger panels of at least 25 judges were employed.

Statistical Analyses. Data were analyzed by the statistical software SigmaStat (Jandel Scientific) using "one-way ANOVA" on ranks. This nonparametric test compares several different experimental groups, which received different treatments. To isolate the group or groups that differed, all pairwise multiple comparison procedures (according to the Student−Newman−Keuls method) were performed at the 5% significance level (*21*).

RESULTS

Analyses of Fermentation Parameters in Mashes with and without Stones. To investigate how the presence of stones affects spirit quality, cherry and plum mashes were fermented with different yeast strains and a set of fermentation parameters was followed. For this purpose, 90 kg each of the fruit mashes with and without stones were inoculated in triplicate with 10^6 cells/mL either of the commercial Uvaferm yeast or the laboratory diploid yeast strain HHD1. Fermentation was accomplished under semianaerobic, nonsterile conditions at low temperatures. Samples were taken weekly for microscopic examination, and it was confirmed that no excessive bacterial contaminations were present in the mashes. However, complete mashes (with stones) developed a layer of wild yeast contaminants on the surface, due to exposure to oxygen during sampling. Interestingly, this layer of wild yeasts did not occur on the mashes where stones had been removed (i.e., stoneless mashes).

Table 1 summarizes the general fermentation parameters, including the theoretical and practical alcohol yields. As expected from the higher initial sucrose content, higher alcohol yields were obtained in the cherry mashes than from those of plums. Regarding the use of different yeast strains for fermentation, the presence or absence of stones did not affect the final alcohol yield in the mashes fermented with the Uvaferm strain. The laboratory strain produced slightly more ethanol when stones were removed from the cherry mashes. In contrast, slightly less ethanol was produced from the stoneless plum mashes than from those of the complete mashes.

As a more accurate measure of yeast metabolic activity, we determined the kinetics of glucose and fructose degradation as well as the production of ethanol over a period of 50−60 days by HPLC (**Figure 1**). In the initial phase of fermentation, we noted a difference in the performance of the Uvaferm strain in comparison to the laboratory strain within the cherry mashes: HHD1 displayed a longer lag phase in the onset of fermentation as judged from all three parameters measured. Nevertheless, after a maximum of 10 days of fermentation, all mashes, regardless of their stone contents, were equally well-fermented by both the commercial and the laboratory yeast strains. In these determinations, the laboratory strain initially produced higher

amounts of ethanol from the stoneless plum mashes than the Uvaferm strain. However, this difference diminished later on during fermentation.

Organic Acids and Glycerol. Some organic acids and glycerol play an important role in the quality of the mashes and the spirits produced from them (*22*). We therefore proceeded by determining the concentrations of acetic, propionic, and lactic acids and of glycerol in the mashes by HPLC (**Table 2**).

Acetic and propionic acid concentrations ranged below detectable levels in the plum mashes. For the cherries, slightly lower values were found in the stoneless fermentations than in complete mashes. For the two yeast strains employed, no significant differences were detected. Glycerol production did not vary significantly either under all conditions tested. Only the lactic acid concentrations were increased in the cherry mashes as compared to the plum mashes. Furthermore, the stoneless cherry mashes showed a 2−3-fold increase in the amount of lactic acid as compared to the complete mashes. The latter observation indicates a higher load of bacterial contamination.
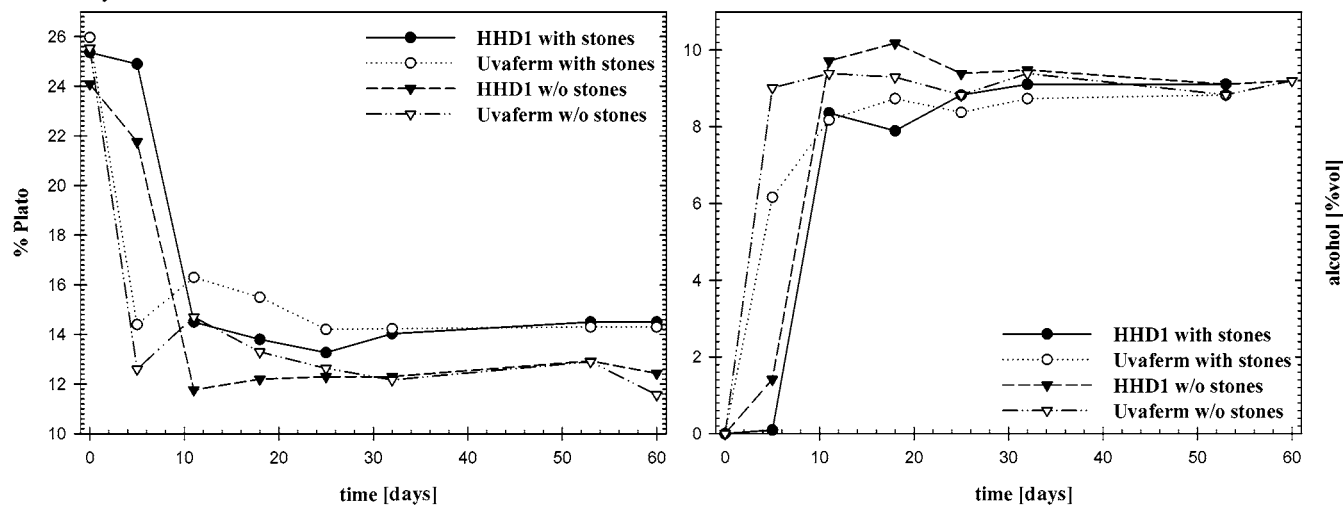
Secondary Fermentation Products and Methanol. Other volatile compounds such as esters, aldehydes, methanol, and higher alcohols present in the mashes after fermentation are of crucial importance for the quality of the final spirits. Therefore, we also quantified some of these key compounds in the mashes by headspace gas chromatography (**Table 3A**).

The methanol content of the cherry mashes containing stones was higher than in the stoneless mashes regardless of the yeast strain employed. From the plum mashes fermented with the Uvaferm yeast, slightly more methanol could be detected than from those fermented with the laboratory strain. Invariably, the concentrations remained below critical thresholds (i.e., 1000 mg/L).

Acetaldehyde concentrations were higher in the mashes fermented without stones than in those with stones. Likewise, the concentrations of 1-propanol were generally higher in the stoneless mashes, with the exception of the plums fermented with the Uvaferm strain. Vice versa, the ethyl acetate content was higher when plums were fermented with stones than in the stoneless mashes. This difference was not observed for the cherry mashes. The other compounds tested did not differ significantly between the different fermentation sets, although a high variability was found within the plum mashes.

Distillation and Spirit Analyses. Although the quality and treatment of the mashes play a key role, distillation conditions still have an influence on the performance of the final spirits (*23*). Thus, through the process of distillation, many volatile compounds can be either removed or concentrated and thermal reactions will produce further compounds. We therefore first also examined the distillates for some aromatic compounds (**Table 3B**). As expected for a successful distillation process,

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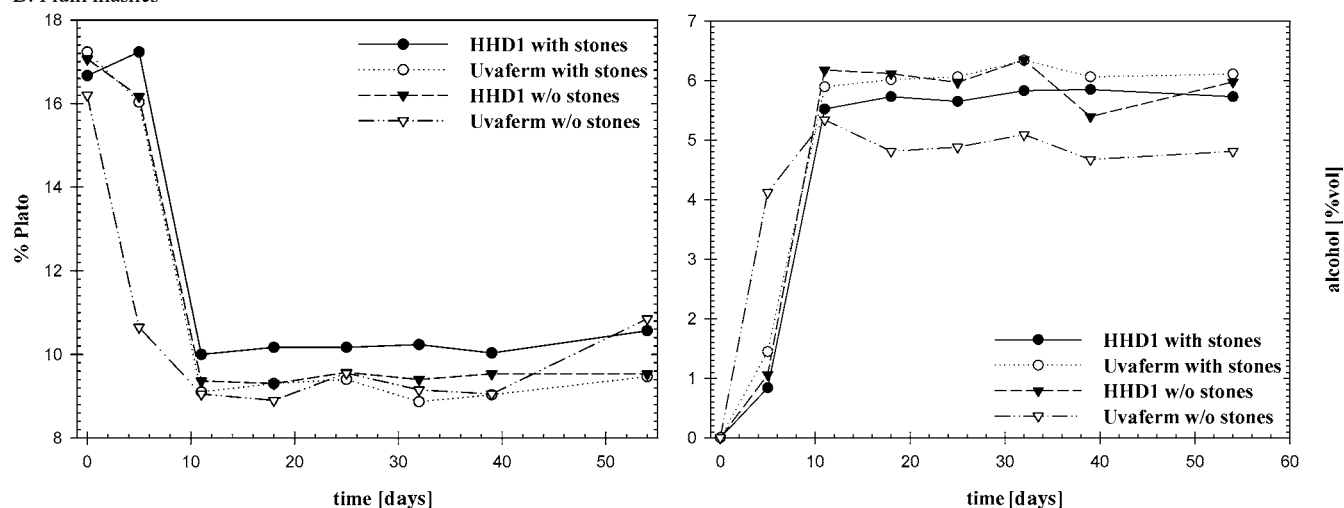
A. Cherry mashes

B. Plum mashes

Figure 1. % Plato and alcohol (v/v) content in mashes during fermentation. The commercial Uvaferm strain and the laboratory yeast strain HHD1 were employed for fermentations. Mashes were prepared and inoculated with approximately 10^6 cells/mL of precultured yeasts as described in the Materials and Methods. Fermentation at 17 °C was followed for up to 60 days. All experiments were performed in triplicate.

Table 2. Organic Acids and Glycerol Contents in Mashes after Fermentation (50–60 Days)[a]

		yeast strain and stone content			
		Uvaferm		HHD1	
mash	compound	with stones	w/o stones	with stones	w/o stones
cherries	acetic acid	0.41 ± 0.28	0.35 ± 0.23	0.45 ± 0.28	0.42 ± 0.34
	propionic acid	1.31 ± 0.26	0.86 ± 0.22	1.20 ± 0.33	0.81 ± 0.14
	lactic acid	1.24 ± 0.57	4.95 ± 1.26	2.86 ± 0.44	5.49 ± 0.99
	glycerol	8.07 ± 0.37	8.33 ± 0.49	9.58 ± 0.68	9.10 ± 0.37
plums	acetic acid	<0.05	<0.05	<0.05	<0.05
	propionic acid	<0.05	<0.05	<0.05	<0.05
	lactic acid	0.47 ± 0.26	0.53 ± 0.12	0.62 ± 0.44	0.58 ± 0.22
	glycerol	6.29 ± 0.87	5.37 ± 0.21	6.77 ± 0.75	6.13 ± 0.66

[a] Concentrations of all compounds are given in g per L mash.

acetaldehyde levels were all generally low in the spirits (note that concentrations in this case are given per 100 mL of total alcohol).

The differences in methanol concentrations discussed above for the mashes were abolished by the distillation process. We suspect that this is due to the separation of the head fractions, where some methanol may be discarded. For the plum spirits

produced from mashes fermented with Uvaferm, methanol concentrations approached the critical limits of 1000 mg/100 mL alcohol. The spirits produced from mashes fermented with the laboratory strain stayed clearly below that concentration. Because methanol is not produced from yeast metabolism, these differences are most likely due to varying esterase activities in the mashes after pH adjustments.

For the amounts of the other compounds tested, substantial variabilities were observed. Because these are mainly volatile substances that are discarded to a major extent as heads during distillation, variations may be attributed to slight differences in the cutoff point for the heads, rather than inherent features of the differently fermented mashes. Generally, differences observed in the mashes for these compounds were therefore not carried over into the spirits. However, it should be noted that the concentrations did not differ significantly comparing stone content or the employed yeast strains.

Formation of EC. Mashes and spirits were also analyzed for their EC and HCN contents (**Table 4**). To simulate effects of light-avtivated EC formation, a 4 h irradiation with UV light prior to the determinations was also included. As evident from our data, the EC concentrations in mashes generally remained below detectable levels (i.e., <0.01 mg/L mash, with the exception of the cherry mashes fermented with the laboratory

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Table 3. Metabolites in Mashes after 50–60 days of Fermentation (Section A) and Spirits after Distillation (Section B)[a]

	Mashes			
	yeast strain and stone content			
	Uvaferm		HHD1	
compound	with stones	w/o stones	with stones	w/o stones
	cherries			
methanol	398.58 ± 23.4	320.83 ± 20.89	440.08 ± 10.97	342.00 ± 22.86
acetaldehyde	27.73 ± 1.7	107.20 ± 35.24	17.52 ± 1.48	88.68 ± 37.93
1-propanol	2.90 ± 0.6	3.45 ± 0.20	5.39 ± 1.46	11.25 ± 1.11
2-methyl-1-propanol	21.07 ± 1.5	18.08 ± 1.35	19.42 ± 1.23	15.04 ± 1.47
3-methyl-1-butanol	98.63 ± 5.5	86.47 ± 11.96	91.16 ± 3.27	89.32 ± 17.37
ethyl acetate	137.65 ± 3.1	147.18 ± 13.13	157.30 ± 0.77	225.95 ± 24.25
3-methyl-butyl acetate	0.21 ± 0.05	0.32± 0.02	0.23 ± 0.05	0.27 ± 0
	plums			
methanol	530.78 ± 49.27	539.37 ± 80.60	413.48 ± 67.59	442.87 ± 85.72
acetaldehyde	22.83 ± 5.32	27.85 ± 2.17	6.92 ± 5.74	45.22 ± 3.92
1-propanol	67.95 ± 32.01	66.28 ± 30.25	8.18 ± 5.99	124.25 ± 17.89
2-methyl-1-propanol	22.57 ± 4.76	16.35 ± 16.07	11.14 ± 7.07	13.83 ± 1.46
3-methyl-1-butanol	76.99 ± 14.11	66.53 ± 5.35	37.13 ± 21.27	43.10 ± 5.34
ethyl acetate	53.58 ± 3.00	28.81 ± 6.09	144.52 ± 62.18	29.91 ± 0.96
3-methyl-butyl acetate	0.30 ± 0.04	0.33 ± 0.31	0.15 ± 0.09	0.30 ± 0.04
	Spirits			
	yeast strain and stone content			
	Uvaferm		HHD1	
compound	with stones	w/o stones	with stones	w/o stones
	cherries			
methanol	562.27 ± 2.76	568.05 ± 176.75	590.31 ± 6.68	582.32 ± 94.04
acetaldehyde	57.46 ± 0.16	40.55 ± 32.84	55.20 ± 0.39	86.32 ± 17.02
1-propanol	195.86 ± 1.07	204.92 ± 68.49	192.70 ± 1.36	170.35 ± 41.86
2-methyl-1-butanol	307.82 ± 2.11	284.94 ± 97.28	276.43 ± 1.26	296.01 ± 36.27
3-methyl-1-butanol	417.57 ± 2.16	450.27 ± 134.98	377.02 ± 2.05	416.82 ± 74.69
ethyl acetate	119.47 ± 0.64	117.13 ± 42.79	115.88 ± 0.75	142.30 ± 19.53
methyl acetate	0.85 ± 0.12	2.46 ± 0.36	0.92 ± 0.02	2.57 ± 0.13
3-methyl-butyl acetate	1.19 ± 0.75	2.41 ± 1.48	1.39 ± 0.30	2.63 ± 5.19
	plums			
methanol	891.24 ± 79.31	1010.63 ± 69.68	732.05 ± 50.41	876.90 ± 3.25
acetaldehyde	13.48 ± 1.29	31.39 ± 1.93	18.81 ± 1.11	31.26 ± 0.15
1-propanol	185.85 ± 13.72	181.93 ± 1.42	198.76 ± 15.96	274.29 ± 11.74
2-methyl-1-butanol	449.48 ± 20.11	341.08 ± 2.07	306.25 ± 41.39	216.77 ± 16.90
3-methyl-1-butanol	477.49 ± 16.55	376.64 ± 2.30	252.93 ± 43.77	197.04 ± 27.15
ethyl acetate	251.67 ± 10.52	138.41 ± 0.59	151.41 ± 22.19	110.18 ± 9.50
methyl acetate	0.22 ± 0.23	1.88 ± 0.02	3.78 ± 0.04	2.88 ± 0.01
3-methyl-butyl acetate	2.75 ± 0.13	0.73 ± 0.11	2.30 ± 0.69	2.58 ± 0.07

[a] w/o, without; concentrations are given in mg per 100 mL alcohol.

Table 4. EC and HCN Concentrations in Stone Fruit Mashes and Spirits

fruit	mash type	yeast strain	content in mashes			content in spirits		
			EC (mg/L)	EC after UV (mg/L)	HCN (mg/ 100 mL)	EC (mg/L)	EC after UV (mg/L)	HCN (mg/ 100 mL)
cherries	complete	Uvaferm	<0.01	<0.01	0.57	<0.01	<0.01	0.05
		HHD1	0.1	0.09	0.47	0.16	0.07	0.14
	w/o stones	Uvaferm	<0.01	<0.01	0.14	<0.01	<0.01	0.05
		HHD1	<0.01	<0.01	<0.01	<0.01	<0.01	0.04
	stones	Uvaferm	<0.01	<0.01	1.87	<0.01	<0.01	0.04
		HHD1	<0.01	<0.01	1.87	<0.01	<0.01	0.04
plums	complete	Uvaferm	<0.01	<0.01	0.13	<0.01	0.06	0.04
		HHD1	<0.01	<0.01	<0.01	<0.01	<0.01	0.03
	w/o stones	Uvaferm	<0.01	<0.01	0.04	<0.01	<0.01	0.04
		HHD1	<0.01	<0.01	<0.01	<0.01	<0.01	0.04
	stones	Uvaferm	<0.01	<0.01	<0.01	3.10	6.30	2.29
		HHD1	<0.01	<0.01	<0.01	0.79	1.80	0.14

strain). On the other hand, HCN concentrations ranged from nondetectable levels to approximately 1.9 mg/100 mL of mash. Generally, levels were higher in the cherry mashes than in the plum mashes.

After storage of the spirits at 17 °C for 45 days, we again determined the concentrations (**Table 4**). There, significant amounts of EC were observed in the spirits produced from the plum stone fermentations. As expected, the concentrations

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Table 5. Sensoric Analyses ("Triangle Test") of Spirits Produced from Mashes with and without Stones by Different Yeasts[a]

			differences detected				preference		
fruit	comparison	no. of tasters	recognized	χ_{theor}^2	χ_{calcd}^2	significance[b] ($\alpha = 5\%$)	χ_{theor}^2	χ_{calcd}^2	preferred spirit[c]
cherries	Uvaferm with vs w/o stones	64	37	3.84	16.17	yes	3.84	0.02	none
	HHD1 with vs w/o stones	44	24	3.84	7.23	yes	3.84	0.46	none
	Uvaferm vs HHD1 w/o stones	70	26	3.84	0.30	no	3.84	0.35	none
plums	Uvaferm with vs w/o stones	64	31	3.84	5.91	yes	3.84	1.16	none
	HHD1 with vs w/o stones	44	22	3.84	4.78	yes	3.84	0.05	none
	Uvaferm vs HHD1 w/o stones	70	35	3.84	8.02	yes	3.84	0.03	none

[a] See the Materials and Methods for details on the triangle test. [b] The number of tasters detecting a difference were subjected to statistical analysis and differences are given (yes = significantly different; no = not significantly different). [c] If differences were detected the tasters were asked to judge their preference; w/o, without; vs, versus.

increased upon UV irradiation. In this experiment, the laboratory strain produced lower concentrations of EC and HCN as compared to the Uvaferm yeast. We believe this to be within statistical variations, since in another set of fruit fermentations, similar amounts were obtained for both strains (data not shown).

Sensoric Evaluation. Despite the highly sensitive detection equipment employed above, it is not yet possible to predict the quality of spirits merely by their known chemical composition (*19, 24−26*). Therefore, two different evaluation methods were employed to determine the sensory properties of the spirits.

In the first series of sensory evaluations, we performed "triangle tests" to determine the influence of stone content on flavor and taste of the spirits. By simply asking each taster to identify the different sample, even small differences in taste or flavor of a spirit can be detected by this method. Up to 70 tasters participated in evaluating the effect of the two yeast strains employed and the different production schemes, i.e., fermentation with or without stones. The tasters were also asked to judge which of the samples was of better quality. For statistical reasons, only the answers of those able to identify the differing sample were used in the latter calculations (*25*). **Table 5** shows the results and statistical analyses of these tests. Spirits produced from mashes with stones could always be distinguished from those of the stoneless mashes. Yet, neither was preferred. Spirits produced from stoneless mashes with the laboratory yeast strain and with the Uvaferm strain could only be distinguished in the case of plums but not in the cherry spirits. Again, no preference was given in this test.

Second, we made "order-of-precedence tests" in different combinations. At least 25 trained tasting panelists were asked to place the spirits in an order of decreasing quality. As shown in **Figure 2A**, no significant difference for Uvaferm or HHD1 in cherry spirits produced without stones could be shown (also not with other commercial strains, data not shown). In the same test, spirits produced with HHD1 from mashes with stones were given a significantly worse ranking than those produced with the Uvaferm strain.

In the case of the plums, for the spirits from mashes with stones, no differences between Uvaferm and HHD1 were found in the overall quality, i.e., combining the ranking of smell and taste (**Figure 2B**). Where stones had been removed, the laboratory strain performed better than the Uvaferm strain.

Finally, to evaluate the effect of stone contents in an independent experiment, we also produced spirits from the stone fractions themselves. For plums, such pure stone distillates earned the worst rank sums in the order of precedence test, as might have been expected (with an average rank of 4.1). Surprisingly, mixing cherry spirits from mashes without stones (obtained with the Uvaferm strain) gradually with up to 40%

of the respective pure stone distillate did not alter preferences consistently (data not shown).

DISCUSSION

This work was aimed to determine the influence of stone content on the fruit spirits produced from cherries and plums as raw materials. In addition, the performance of a genetically defined, diploid laboratory yeast strain (HHD1) as a fermentation agent opposed to commercially available yeasts was further characterized.

As observed previously, neither the speed of fermentation nor the general quality of spirits produced from mashes without stones was significantly different when we used the laboratory strain in comparison to a set of different commercially available yeast strains (with special attention paid to the Uvaferm strain). However, cherry mashes always showed a short lag phase in the onset of fermentation with the laboratory strain, which was even more pronounced in mashes from which the stones had not been removed. Yet, this difference to the use of commercial yeasts was not found in fermentations of plum or pear mashes (this work; *4*). It can be concluded that cherry mashes contain some growth inhibitory compound(s) like sulfur compounds (forming because of acidifying with sulfuric acid), which show antifungal activity to which the laboratory strain may be more sensitive (*27, 28*). In this context, an inadequate supply of nitrogen can crucially influence the growth of the yeast and initiate malolactic fermentation (*29*). It should be noted that despite this initial disadvantage, the laboratory strain adapts within the first 5 days of fermentation and then rapidly reaches the performance of the commercial yeasts. Because mash fermentations are usually carried out for a period of 50−60 days, in terms of sugar consumption and alcohol production, the initial lag phase thus has no practical consequences (*4*).

Regarding the microbial environment, we found that mashes without stones were largely devoid of wild yeast growth on the surface, in contrast to the mashes retaining the stones. This could possibly be a result of the treatment of the mashes: The removal of stones produces a kind of mechanical sieve composed of the stones themselves and residual fruit material such as skin fragments. Because wild yeasts found on the fruit surface frequently form hyphae, they may be retained more readily than single-cell yeast species that stay in suspension during the fermentation process. Given the large amount of *Saccharomyces* yeast added to start the fermentation (which will deplete the medium rapidly of nutrients) and our rigid fermentation and distillation schemes, these wild yeasts will not notably contribute the final spirit composition. Nevertheless, one of the main purposes of this work was to detect differences in the performances of the *S. cerevisiae* strains employed, and this should include secondary effects of wild yeasts present.
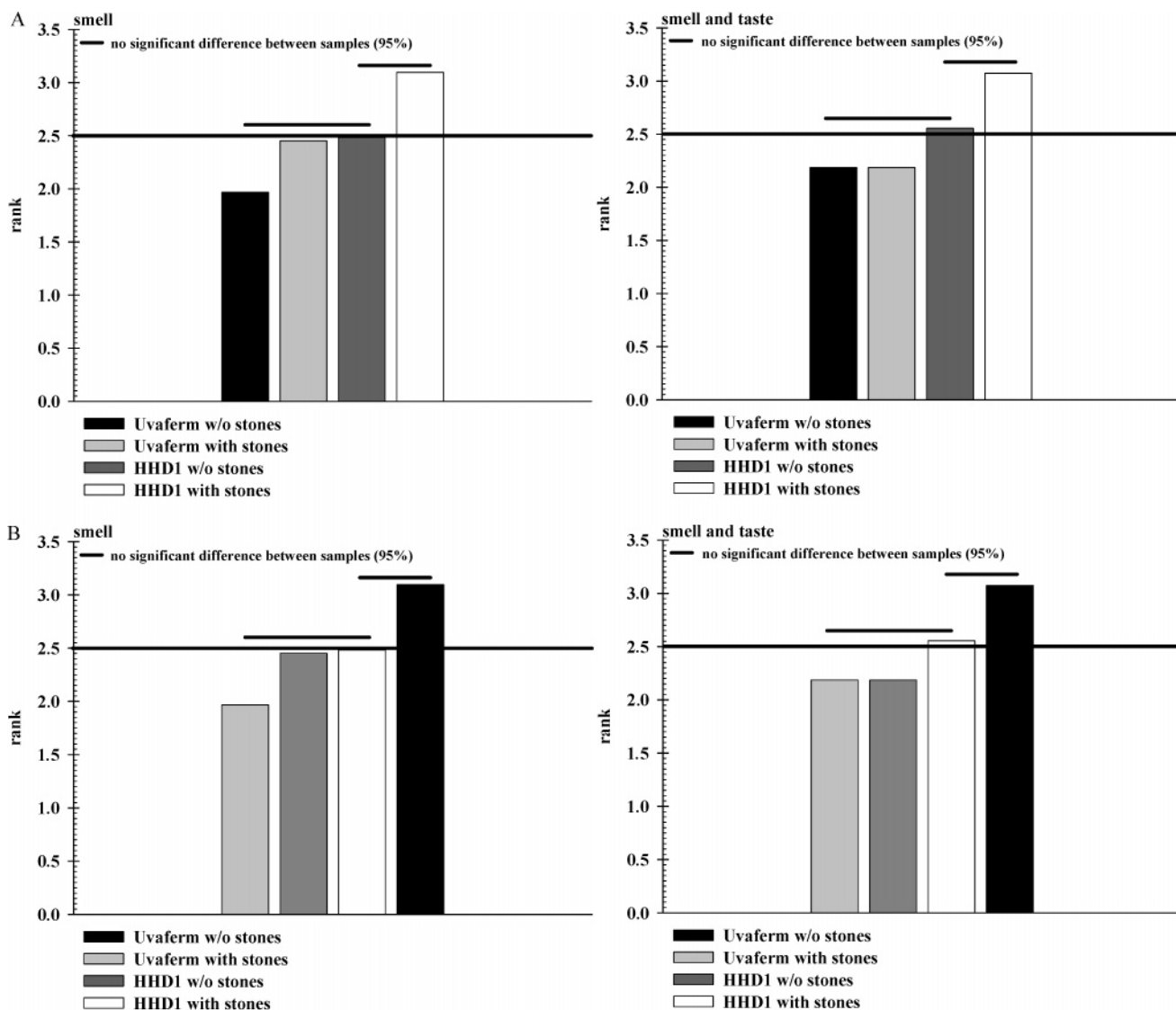
Figure 2. Order of precedence tests. Spirits produced from the mashes as indicated (**A**, cherry spirits; **B**, plum spirits) were judged by a panel of 25 trained tasting panelists. They were asked to give rankings from 1 to 5 to each spirit judging either smell alone or smell and taste in conjunction. The data obtained were statistically analyzed as described in the Materials and Methods. In general, the lower the final ranking number, the better the quality of the spirit. The average rank is indicated by a continuous line. Short bars above the columns indicate those spirits that did not show a statistically significant difference.

Regarding the other microbial environment, we found that lactic acid concentrations, which indicate a higher load of bacterial contamination, were generally increased in the mashes fermented without stones as compared to those retaining the stones. In fruit fermentations, lactic acid bacteria constitute the prevalent prokaryotic genera (*30, 31*). A special feature of these bacteria is their ample need for amino acid and vitamin supplies. One can assume that such compounds are usually scarce in mashes due to the rapid depletion by the yeasts added for fermentation. A further shortage produced from the wild yeasts growing at the surface of the mashes retaining the stones may therefore explain a certain level of protection against such bacteria.

Corresponding to the longer lag phase of the laboratory strain in cherry mashes, which is enhanced by the presence of stones, the overall alcohol yield using this yeast strain was higher in the stoneless cherry mashes. For the plum mashes, where the lag phase was absent, the opposite behavior was found. Because no significant differences were found for the Uvaferm strain with regard to alcohol yields from all mashes, one can conclude that the stone content does not affect sugar degradation or final

alcohol yields. Interestingly, no significant differences in the concentrations of organic acids and glycerol were found between the different mashes fermented with different yeasts (with the exception of lactic acid, which is due to a higher bacterial load as discussed above). This indicates that (i) yeast carbohydrate metabolism (of which acetic acid and glycerol may form as byproducts) is not influenced dramatically by the stone content of the mashes and (ii) that both yeasts perform equally well in this respect. The same holds true for secondary fermentation products such as esters, aldehydes, and higher alcohols. The concentration of these compounds was generally within the normal limits, although the amount of acetaldehyde was higher in stoneless mashes of both fruits tested. Although not detectable in the long-run fermentation kinetics, this may reflect a certain inhibition in yeast stationary phase metabolism, which contributes to the degradation of acetaldehyde, e.g., in beer production (*32*). On the other hand, methanol is produced from fruit specific enzymes and not from yeast metabolism (*33, 34*). Accordingly, a fruit dependence is prevalent in that more methanol is produced from plum mashes than from cherry mashes, regardless of the stone content and yeast strain employed. The observation

Stone Content on the Quality of Plum and Cherry Spirits

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that fermentation by the Uvaferm strain produced slightly more methanol from plum mashes than those fermented with the laboratory strain may thus be due to minor variabilities in the fermentation conditions rather than the yeasts themselves. It should be noted that the experimental setup with "real" fermentations does not allow a large sample number and a judgment on statistical variations in this respect. Nevertheless, methanol contents remained within acceptable limits in all experiments performed in this work.

Regarding the health risk, EC has been proposed to have a carcinogenic effect (*5*). It can be either formed from yeast metabolism or can derive from HCN present in stone fruit mashes (*6*). One of the aims of this work was to establish a laboratory yeast strain that is amenable to genetic techniques, so that the contribution of the yeast to EC formation can be minimized. It is important to note that using state of the art production schemes the EC content in spirits can be usually kept below detectable levels (*35*, *36*). Nevertheless, further reductions of this compound in the fermentation process may be useful to minimize health hazards, when less optimal conditions are employed in distillation. In our experiments, EC and HCN levels were generally quite low, with the exception of spirits derived from fermented stones. It would be interesting to use genetically engineered yeast strains to determine the contribution of yeast metabolism to EC formation under such conditions.

The minor differences in measurable quality-determining compounds within the mashes as discussed above were further diminished during the distillation process, as expected. These can only be judged by sensoric evaluations as the final and most important test. Despite personal preferences, the experimental setup employed in this work and the number of tasters involved allow for a statistically significant assessment of spirit quality (note that further chemometric sensory techniques such as PCA, HCA, or LDA are not applicable here, since they would require a panel of more than 20 experienced tasters, which was not available to us. Therefore, only common sensory assessments were applied). Nevertheless, some general conclusions can be drawn from these data: (i) Invariably, spirits produced from stoneless mashes could always be distinguished from those produced from complete mashes, regardless of the yeast strain used for fermentation. (ii) For the plum mashes without stones, spirits produced from fermentations with the Uvaferm strain were recognized as different from those of the laboratory strain. (iii) Even if differences as discussed in (i) and (ii) were detected, no preference could be assigned to either spirit. This indicates that the quality of the spirits is similar in all cases and preferences for either are a matter of personal taste. (iv) As expected, spirits distilled from pure stone fermentations were always judged to be worse in the order of precedence test. Surprisingly, however, mixing up to 40% of these spirits with those from stoneless cherry mashes did not result in a change of preferences. Thus, whatever ingredients render the pure stone spirits less acceptable in taste and/or smell are near the sensory threshold so that the components of the traditionally produced spirit prevail in the mixture. (v) For the cherry spirits produced from complete mashes, the laboratory strain performs worse than the Uvaferm strain. This may again be attributed to the enhanced lag phase in the onset of fermentation, allowing other microbial contaminants to produce a certain amount of deleterious compounds, before being inhibited by yeast growth and metabolism. This lack of performance of the laboratory strain is not observed in fermentations of stoneless cherry mashes, presumably due to the less pronounced lag phase in the onset

of fermentation. Supporting this view is the absence of strain-dependent quality differences in plum spirits, where fermentation kinetics in the mashes are similar between the two yeasts employed.

In summary, this is the first experimental work comparing simultaneously the influence of stone content in fruit mashes and the employment of different yeast strains on the quality of spirits that can be produced from such mashes. We find that in contrast to the general believe, the presence or absence of stones in the mashes cannot be used as a general quality criterion. Rather, our data provide strong evidence that the preference for one or the other spirit will remain a matter of personal taste. Nevertheless, although the differences cannot be assigned to a specific flavor compound, sensory analyses can clearly distinguish between these two kinds of spirits. Moreover, with little differences in fermentation performance, our results offer the possibility to apply metabolic design techniques to a genetically defined yeast to be employed in large-scale fermentations. One may for instance reduce the health risk of spirit consumption implemented by substances such as the carcinogen EC. We are in the process of testing this hypothesis for fruit mash fermentations.

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