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UPLC analysis of free amino acids in wines: Profiling of *on-lees* aged wines☆

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ABSTRACT

The evolution of free amino acid (FAA) profiles intrinsic to *on-lees* aged white wines was determined by ultra performance liquid chromatography (UPLC™). On basis of the AccQ.Tag™ method as a commercialized amino acid analysis solution for HPLC, a new protocol for dedicated amino acid analysis using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) for pre-column derivatization was established by method transfer onto UPLC™ conditions. Since AQC derivatives enable both fluorescence (AccQ.Tag™ method) and UV detection, the performed method transfer additionally included changing to a more versatile UV detection. Emphasizing enhanced performance of UPLC™, the newly established protocol facilitated rapid and reliable separations of 24 amino acids within 23 min, hence proved to be superior compared to the original HPLC protocol due to significant improvements in resolution and reduced runtime. Applying UV detection enabled adequate quantifications of AQC amino acid derivatives at μM level (LOQs from 0.12 to 1.10 μM), thus proved sufficient sensitivity for amino acid profiling in wine samples. Moreover, this compiled methodology was successfully applied to monitor the changes of FAA concentrations in four distinct sets of *on-lees* aged white wines (fermented with different yeasts) at three progressing ripening periods, each (control, 3 and 6 months aging). For the control wines, the applied winery yeast significantly affected total FAA amounts (1450–1740 mg L^{-1}). During maturation, the proceeding yeast autolysis implied a rather complex impact on FAAs, yielding total FAA excretions up to 360 mg L^{-1}. However, the magnitude for increases of specific FAAs (up to +200%) highly depended on the individual amino acids as well as on the applied fermenting yeast. Given the overall complexity of yeast autolysis in winemaking, the application of efficient LC techniques such as UPLC™ may indeed contribute as a valuable tool in wine research for product monitoring and characterization of intrinsic developments during wine maturation.

1. Introduction

On-lees matured wines, also referred to as *Sur-lie,* imply a specific manufacturing technique in which a base-wine, either after primary or secondary alcoholic fermentation, remains over yeast lees, and consequently develops distinctive organoleptic as well functional properties by a series of biochemical reactions.

In the course of ripening, during yeast autolysis, endogenous biochemical processes catalyze the degradation of yeast intrinsic biopolymers resulting in a substantial excretion of highly variable compounds (e.g., peptides, amino acids, manno-proteins, polysaccharides, lipids, nucleotides) into the maturing product [1,2]. Moreover, the evolution of nitrogenous compounds is characterized by the initial excretion of higher molecular-weight peptides, that are further degraded as maturation prolongs, resulting in subsequent accumulation of free amino acids (FAAs) [3–5]. Consequently, FAA profiles at different stages of maturation represent a valuable marker to follow the proceeding yeast autolysis during ripening of *on-lees* aged wines.

In addition, yeast-excreted amino acids emphasize a crucial class of maturation factors in vinification due to their broad functions as precursor substances for the formation of aromatic compounds.

Derivatization of amino acids in complex biological samples requires selective reactions to ensure specificity of analysis. Commonly applied protocols for quantification of amino acids (and biogenic amines) in wines include derivatization with either ninhydrin, 5-dimethylaminonaphthalene-1-sulfonyl chloride (DANSYL-Cl), 4-dimethylaminoazobenzene-4-sulfonyl chloride (DABSYL-Cl), 1-fluoro-2,4-dinitrobenzene (FDNB), diethyl 2(ethoxymethylidene)propanedioate (DEEMM), phenylisothiocyanate (PITC), *o*-phthalaldehyde (OPA) or 9-fluorenylmethylchloroformate chloride (FMOC-Cl) [6]. However, for all of the above mentioned agents, one or more disadvantages can be found e.g.,: limitation to post-column derivatization (ninhydrin); long derivatization (DANSYL-Cl, DEEMM); photosensitive derivatives (DANSYL-Cl,

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FDNB); the need for the removal of excess reagent (PITC); the inability to derivatize the secondary amino group (OPA); or excess reagent/by-products that, if not properly treated, interfere with the separation (FMOC-Cl) [6,7].

Thus, pre-column derivatization utilizing 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) might represent a feasible alternative as to method attributes that are characterized by: a simple direct derivatization protocol; the rapid derivatization of primary and secondary amino groups; stable derivatives that enable, both fluorescence and UV detection; as well as no interferences due to excess reagent when working with fluorescence detection [8]. Hence, due to its advantageous performance, pre-column AQC derivatization has yet been successfully used in various amino acid analyses [8–17]. Moreover, the combination of AQC derivatization with new, *state-of-the-art* fast-LC techniques, such as ultra performance liquid chromatography (UPLC™) [18], already proved superior performance [19–22] facilitating a broad scope of *high-throughput* applications.

Given the variety of parameters needing to be considered for optimal results, the use of commercialized "*all-in-one*" amino acid analysis solutions may offer some significant advantages indeed (e.g., customized derivatization protocols, ready-for-use solvent systems, dedicated columns, and already optimized gradient elution). One typical example is presented by the Waters AccQ.Tag™ method [23], as a well-established amino acid analysis solution combining AQC derivatization, HPLC separation and fluorescence detection.

Emphasizing the benefits of these commercial "packages", the major objective of this study was to adapt the AccQ.Tag™ method onto a more efficient UPLC™ and changing detection from fluorescence to UV. Moreover, this newly established method was to be further optimized for the reliable application for quantifying free amino acids (FAAs) in wines. Seeing that *Sur-lie* wines exhibit distinct product characteristics which are especially valuable throughout method development (due to the increasing amino acid concentrations at prolonged ripening, thus acting as a "*self-spiking*" matrix), four different sets of *on-lees* aged wines (at 3 successive stages of maturation, each) were to be characterized on the evolution of their FAA profiles.

2. Experimental

2.1. Chemicals and standards

High purity mixed amino acid standard (*type H*, 17 amino acids dissolved in 0.1 M HCl at 2.5 mM; L-cystine at 1.25 mM) was purchased from Pierce (Rockford, IL, USA). Additional amino acids (purity ≥99%) were obtained from a variety of suppliers: DL-alpha-n-amino butyric acid, L-tryptophan and L-asparagine from Sigma (St. Louis, MO, USA); L-citrulline, L-ornithine hydrochloride and gamma-amino butyric acid from Fluka (Buchs, Switzerland); and L-glutamine from Pierce. AccQ.Tag™ Eluent A concentrate as well as pre-column AQC derivatization reagent (AccQ.Fluor™ Reagent Kit) were supplied by Waters (Milford, MA, USA). Supplementary bulk chemicals and solvents exhibited either analytical or HPLC grade and were obtained from Roth (Karlsruhe, Germany) or Merck (Darmstadt, Germany). Ultrapure water from an Elga ultra-high quality (UHQ) system (High Wycombe, Buckinghamshire, UK) was used for the preparation of all solutions.

Fifty millimolar stock solutions of the respective solid amino acids were prepared in 0.1 M HCl, and subsequently combined to result a 2.5 mM intermediate composite solution. By merging with the commercial Pierce standard, mixed amino acid standards (5–160 μM for each of the 23 analytes; constant 40 μM for alpha-amino butyric acid (AABA) as internal standard) were prepared by dilution in ultrapure water. Subsequent to derivatization (resulting in an additional 1/10 dilution), seven standard solutions in the range from 0.5 to 16 μM were analyzed with UPLC™ and further used for system calibration. Final calibration concentrations ranged from 2 to 64 pmol per injection (2, 4, 8, 16, 24, 32, 64 pmol/4 μL injection) for each amino acid and constant 16 pmol for AABA. Non-weighted linear calibration functions were calculated via Waters Empower 2 chromatography software.

2.2. Wine samples

The analyzed wine samples comprised four distinct sets of tailor-made (*non-commercial*), *on-lees* aged, model white wines that were all equally manufactured from *Gruener Veltliner* grapes, but were fermented with four different winery yeasts. The four applied yeasts were *Saccharomyces cerevisiae:* (1) *Oenoferm Veltliner*, (2) *Fermicru 4F9*, (3) *Weiss&Komplex*, and *Saccharomyces bayanus:* (4) *EC1118*. Moreover, each wine set consisted of one control wine and two *Sur-lie* aged wines exhibiting *on-lees* maturation periods of 3 and 6 months, respectively.

2.3. Extraction of free amino acids from wine samples

For the further clarification of wine samples, aliquots (10 mL) were mixed with 0.5 g polyvinylpolypyrrolidone (agent to bind and precipitate organic compounds), stirred for 10 min at room temperature and subsequently centrifuged (16,000 × *g* at 4 °C for 15 min). Considering the alkaline pH that is needed for optimal derivatization, the acidic samples were then neutralized with 0.05 M boric acid buffer (pH 9.0), further diluted (1/10–1/25) to match the calibration range, and subsequently centrifuged once more to remove any precipitations. The obtained cleared supernatants were directly submitted to derivatization procedure utilizing Waters AccQ.Fluor™ Reagent Kit.

2.4. AQC derivatization of standards and samples

Pre-column AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) derivatization of amino acids was accomplished according the Waters AccQ.Tag™ pre-column derivatization procedure [23]. Briefly, for amino acid standards, 5 μL standard solution was mixed with 35 μL AccQ.Fluor™ borate buffer; while for wine samples, 5 μL neutralized sample, 5 μL internal standard (AABA at 40 μM) and 30 μL buffer were combined. Thus, 40 μL derivatization batches were obtained in both cases. To initiate derivatization, 10 μL derivatization reagent (~10 mM AccQ.Fluor™ reagent in acetonitrile) was admixed, mixtures were then immediately vortexed, left to rest for 1 min and finally heated at 55 °C for 10 min.

2.5. Chromatographic conditions

Chromatographic setup consisted of a Waters Acquity™ Ultra Performance LC (UPLC™) system (Milford, MA, USA) equipped with an Acquity™ tunable UV detector (TUV). Reversed-phase separations were performed on an Acquity UPLC™ BEH C_{18} column (1.7 μm, 2.1 × 100 mm) with pre-connected 0.20 μm column in-line filter. Waters Empower 2 chromatography software package was used for data acquisition and management.

Based on the AccQ.Tag™ package [23] as originally designed for HPLC separations on Waters Nova-Pak™ columns, method transfer onto UPLC™ was performed. The applied solvent system consisted of mobile phase A: Waters AccQ.Tag™ Eluent A concentrate diluted 1/11 with ultrapure water and adjusted to pH 4.92 with 10% (v/v) phosphoric acid; and mobile phase B: 60% (v/v) acetonitrile in ultrapure water. Ultraviolet detection was set to 254 nm.

Derivatized amino acid standards or wine samples were injected (4 μL injection volume) onto the column and eluted at 37 °C at a flow rate of 0.4 mL min^{-1} according to the following gradient: initial 0% B; 0.0–0.8 min/0–2% B; 0.8–10.0 min/2–6% B; 10.0–12.6 min/6–10% B; 12.6–22.0 min/10–33% B; 22.0–23.0 min/33–33% B; 23.0–24.0 min/33–100% B; 24.0–26.0 min/100–100% B; 26.0–28.0 min/100–0% B. The column was then further re-equilibrated for 8 min at initial conditions yielding net separations for all 24 amino acid derivatives within 23 min and an overall cycle time of 36 min till the next injection (including an intense column cleaning purge and re-equilibration with special regard to the high backpressure and column lifetime).

3. Results and discussion

3.1. Method performance

A protocol for dedicated amino acid analysis was established by adapting the corresponding HPLC-based AccQ.Tag™ method onto UPLC™ conditions. Since UV detection already proved excellent suitability for UPLC™ analysis of AQC derivatives of biogenic amines [21], method transfer was performed from fluorescence (AccQ.Tag™ method) to UV detection. Additionally, to further improve separation efficiency, UPLC™ was performed using a 100 mm BEH C_{18} column. However, the use of an extended column compromised separation speed, since flow rate was restricted to 0.4 mL min^{-1} only, in order to maintain column backpressure at an acceptable level (peak pressure <12,000 psi).

Moreover, the original HPLC protocol was transferred to yield a shallower gradient profile (e.g., for gradient segment 10–33% B: AccQ.Tag™ HPLC 3.17%/column volume *vs.* UPLC™ 2.12%/column volume) so as to overall optimize resolution and facilitate the integration of additional wine intrinsic amino acids (e.g., gamma-amino butyric acid) within the former elution pattern.

During derivatization, AQC excess reagent hydrolyzes with water to form 6-aminoquinoline (AMQ) as a fluorogenic side compound. Although present at an excessive concentration, AMQ does not interfere in fluorescence detection of amino acid derivatives (excitation 248 nm/emission 395 nm) due to the different emission maxima found at 520 nm. However, when using UV detection at 254 nm, AMQ implies an equal response as amino acid derivatives and elutes as a major peak prior to the polar amino acids at early retention time [8,24]. Since eluent pH significantly affects the retention of AMQ (and the polar amino acids) [24], the pH of mobile phase A (AccQ.Tag™ Eluent A) was decreased to 4.92 (original pH 5.05) in order to facilitate further AMQ segregation, and to ensure a complete return to baseline prior to the elution of aspartic acid.

Capitalizing on *sub-2 μm* particles and proper system optimization, the newly established UPLC™ method enabled appropriate separations of 24 AQC amino acid derivatives (AABA included) within 23 min (Fig. 1), hence pointing out improvements in separation time compared to the original (35 min) AccQ.Tag™ HPLC protocol [15,23,25]. Moreover, decreasing eluent pH facilitated a large gap (>2 min) between AMQ and the polar amino acids, thus ensuring a full return to steady baseline prior to aspartic acid (Fig. 1). Using UPLC™, the derived elution pattern yielded baseline separations (Rs >1.5) for all analytes, except for the critical peak pairs serine/asparagine and histidine/glutamine that implied partial co-elutions (Rs 0.8 and 0.6, respectively). Besides, for the former HPLC method, asparagine and glutamine were not included due to the designated application for analyses of hydrolyzates only. Regarding separation capacity, the obtained peak widths (widths$_{50\%}$ from 6.8 to 2.2 s) implicated theoretical maximum peak capacities up to 200 resolvable peaks per gradient time (91–213 depending on the referenced amino acid).

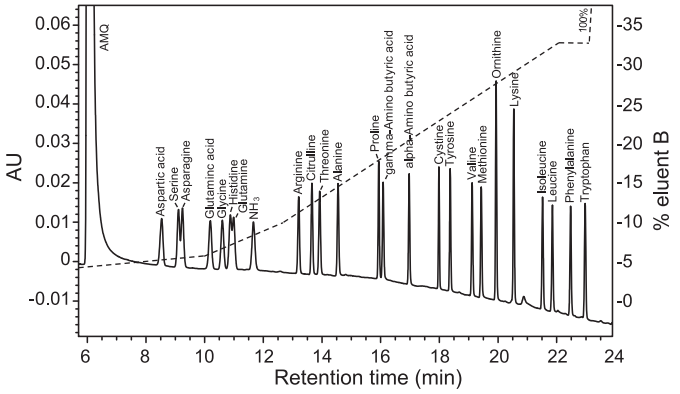

Fig. 1. UV-UPLC™ chromatogram of AQC derivatives of amino acids in a standard solution (16 pmol each) using the established gradient separation. Chromatographic conditions as in Section 2.5.

For quantifying AQC amino acid derivatives using UV detection at 254 nm, the performed method validation indicated appropriate linearity ($R^2 \geq 0.998$) for all analytes within the applied calibration range (2–64 pmol/4 μL injection volume). Detection (LOD) and quantification limits (LOQ) were derived from system calibration according to DIN 32645 (33% uncertainty, 95% significance) [26]. LODs ranged at low μM level (Table 1), varying from 0.03 to 0.31 μM (0.13–1.24 pmol *on-column*) with corresponding LOQs ranging from 0.12 to 1.10 μM (0.48–4.42 pmol *on-column*). Moreover, the obtained sensitivity (LODs and LOQs) implied good accordance with values stated by other authors [19,20,24], even though compared to some studies, where the more sensitive fluorescence detection was used [15]. By converting the analytical μM values to mg amino acids per L wine, and also including sam-

Table 1
Performance parameters of the applied UPLC™ method for the determination of FAAs in wine.

Amino acids	LOD[a]		LOQ[a]		RSD (%)[b]
	μM[c]	mg L^{-1} [d]	μM[c]	mg L^{-1} [d]	
Aspartic acid	0.22	2.9	0.78	10.4	2.9
Serine	0.05	0.6	0.19	2.0	2.5
Asparagine	0.18	2.3	0.63	8.4	3.3
Glutamic acid	0.06	0.9	0.21	3.1	2.5
Glycine	0.08	0.6	0.30	2.2	2.4
Histidine	0.07	1.0	0.24	3.7	3.1
Glutamine	0.05	0.8	0.20	2.9	4.1
Arginine	0.06	1.1	0.22	3.8	2.6
Citrulline	0.06	1.1	0.22	3.9	4.7
Threonine	0.05	0.5	0.17	2.0	3.1
Alanine	0.14	1.2	0.49	4.4	2.4
Proline	0.04	0.5	0.16	1.8	2.6
γ-Amino butyric acid	0.31	3.2	1.10	11.4	2.7
Cystine	0.03	0.8	0.12	2.9	5.2
Tyrosine	0.11	1.9	0.38	6.9	2.7
Valine	0.05	0.5	0.16	1.9	3.0
Methionine	0.05	0.8	0.20	2.9	2.7
Ornithine	0.10	1.3	0.35	4.6	3.3
Lysine	0.10	1.5	0.36	5.3	2.9
Isoleucine	0.11	1.4	0.39	5.1	4.0
Leucine	0.05	0.6	0.18	2.3	2.7
Phenylalanine	0.06	1.0	0.21	3.5	3.0
Tryptophan	0.05	1.1	0.19	3.9	2.3

[a] Limits of detection (LOD) and quantification (LOQ) calculated according to DIN 32645.
[b] RSD for 6 independent sample preparations analyzed within 1 month (test sample was the wine *Weiss&Komplex* at 3 months of *on-lees* aging; the corresponding FAA concentrations are listed in Table 2; for cystine and tryptophan, the intrinsic amounts were below the LOQ).
[c] Assay concentration of AQC amino acid derivatives.
[d] Including complete sample work-up and derivatization procedure.

ple preparation and derivatization procedure, the hereby deduced LOQs (Table 1) ranged from 1.8 to 11.4 mg L^{-1}, thus proved to be sufficient for amino acid profiling of wine samples.

Between run variability (intra-day; 16 pmol amino acid standard injected 6-times within one day) varied ≤1.5% for all analytes, whereas inter-day repeatability (including multiple derivatizations; amino acid standard derivatized 10-times over a period of one month) ranged between 0.4% (cystine) and 2.9% (histidine, lysine). Moreover, RSD for multiple sample work-up (6 independent sample preparations) ranged from 2.3% to 5.2% (Table 1), thus proved to be appropriate regarding the diverse amino acid concentrations in wine.

3.2. Evolution of free amino acid profiles during the on-lees maturation of wines

Endogenous autolysis of winery yeast during *on-lees* maturation primarily implicates the excretion of nitrogenous compounds (e.g., free amino acids) into the aging product, hence contributing to a great extent to specific product characteristics. Seeing that FAA levels are gradually increasing during *on-lees* ripening, *Sur-lie* wines (analyzed at different stages of maturation) can be considered a valuable test material in method development to evaluate separation performance. Based on the ongoing autolysis, these wines act as naturally "*self-spiking*" matrices, which might be advantageous in revealing arising co-elutions due to changes in FAA concentrations or by other interfering substances.

Moreover, as a consequence of microbial decarboxylation, the excreted amino acids may be further converted into biogenic amines. Since AQC reagent is also used for derivatization of biogenic amines [21], the FAAs as well as biogenic amines, which are both present in wine, are derivatized simultaneously. However, using gradient elution as suggested by the AccQ.Tag™ method, the major biogenic amines (e.g., tyramine, putrescine, cadaverine, phenylethylamine) elute at higher retention times (after tryptophan; within the column cleaning purge and re-equilibration), consequently don't interfere with amino acid separation. The only exception is histamine, which elutes as an isolated peak between alanine and proline (Fig. 2).

Intrinsic amino acid excretion due to yeast autolysis was monitored on basis of four sets of model white wines each fermented with a different winery yeast (*Oenoferm Veltliner, Fermicru 4F9, Weiss&Komplex, EC1118*). Additionally, each wine set consisted of a control and two *on-lees* aged samples with 3 and 6 months maturation, respectively. Using the established UPLC™ method facilitated adequate FAA profiling within each wine set; e.g., the evolution of FAAs for the wine set fermented with *Fermicru 4F9* is highlighted within the chromatograms in Fig. 2a–c.

The individual FAA concentrations for each wine are summarized in Table 2. Moreover, only cystine and tryptophan showed intrinsically low amounts varying below their respective LOQs (2.9 and 3.9 mg L^{-1}).

For the four control samples (i.e. wines that were not subjected to yeast autolysis), total FAA amounts ranged from 1740 mg L^{-1} for the set fermented with *Oenoferm Veltliner* to 1450 mg L^{-1} for the wine derived from *EC1118*. However, considering the fact that all wines primarily originated from the same must, the applied winery yeast significantly affected variability of the specific FAA levels (RSD 4–45%). Most distinct variations between the control wines were observed for arginine (±137 mg L^{-1}), proline (±54 mg L^{-1}) as well as glutamic acid (±14 mg L^{-1}).

As yeast autolysis caused an increase of FAA levels relative to that of the control wines, the quantitative evolution of the FAA profiles can be deduced from Fig. 3a–d. Six months *on-lees* aging yielded a total FAA excretion of 363 mg L^{-1} for the yeast *Weiss&Komplex* and 278 mg L^{-1} for *Fermicru 4F9*. In contrast, the maxima for *Oenoferm Veltliner* and *EC1118* were found after 3 months of ripening (146 mg L^{-1} and 240 mg L^{-1}, respectively), mainly influenced by the significant decrease of selective amino acids at the longer maturation period (e.g., arginine, proline). Focusing on individual changes, for the majority of amino acids, a prolonging *on-lees* aging yielded in a successive increase in FAA concentrations showing the most distinct progression after 3 months of ripening.

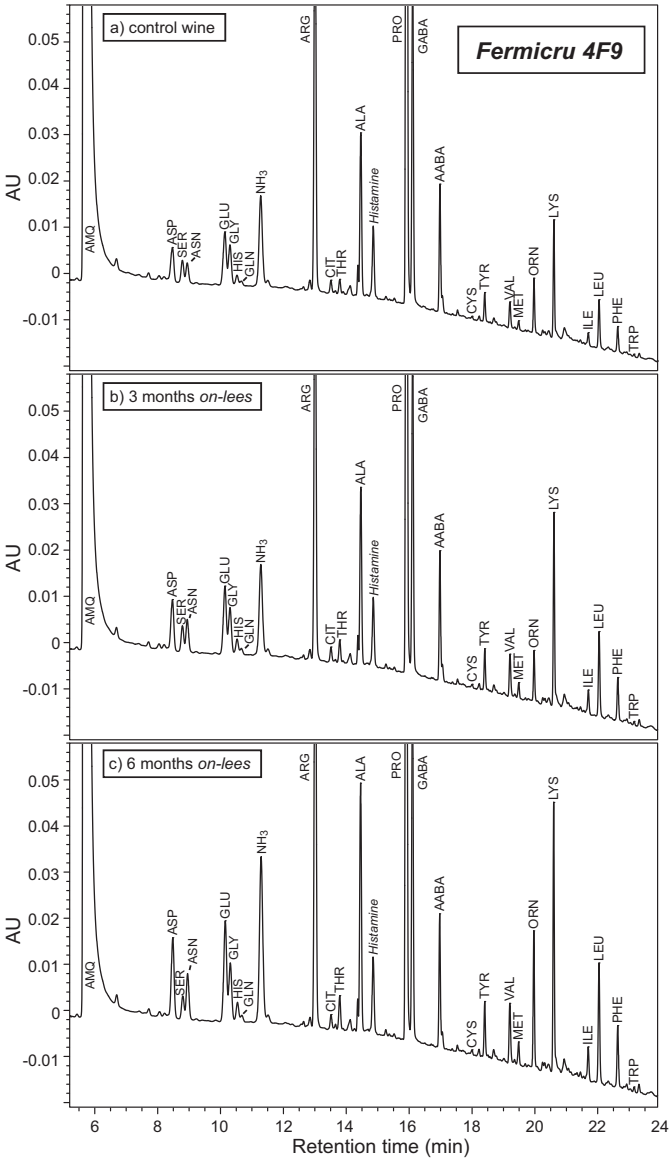

Fig. 2. UPLC™ chromatograms of FAA profiles for the wine set fermented with the yeast *Fermicru 4F9*, (a) control wine, (b) 3 months, and (c) 6 months *on-lees* maturation.

However, as being significantly influenced by the applied winery yeast and the referenced amino acid, the relative increase at the end of maturation ranged up to +200% compared to the control wine concentrations (e.g., lysine: +75% *Oenoferm Veltliner*, +190% *Weiss&Komplex*, +130% *Fermicru 4F9*, +162% *EC1118*; leucine: +86% *Oenoferm Veltliner*, +222% *Weiss&Komplex*, +133% *Fermicru 4F9*, +166% *EC1118*). In contrast, for some amino acids, aging over lees resulted in an initial increase till 3 months of ripening followed by a subsequent decrease in FAA levels (e.g., glutamic acid for *Oenoferm Veltliner*), even below that of the corresponding control wines (e.g., alanine for *Oenoferm Veltliner*, proline for *EC1118*).

Table 2
FAA contents of the four analyzed wine sample sets, comprising wines derived from 4 different fermenting yeasts (*Oenoferm Veltliner, Weiss&Komplex, Fermicru 4F9, EC1118*) at three progressing *on-lees* aging periods (control wine; 3 and 6 months maturation).

Amino acids[a]	Free amino acids concentration (mg L^{-1})											
	Oenoferm Veltliner			*Weiss&Komplex*			*Fermicru 4F9*			*EC1118*		
	Control	3 Months	6 Months	Control	3 Months	6 Months	Control	3 Months	6 Months	Control	3 Months	6 Months
Aspartic acid	40.2	47.4	51.0	24.8	38.5	52.1	31.4	45.2	63.3	25.5	37.3	51.4
Serine	13.0	14.6	11.3	8.1	10.8	10.0	13.6	15.7	12.7	13.4	17.1	16.3
Asparagine	14.7	17.6	18.4	9.2	18.1	24.2	13.7	22.6	28.5	11.3	17.1	21.7
Glutamic acid	69.0	77.9	75.0	42.8	64.8	74.2	55.4	69.8	94.3	38.8	54.9	67.7
Glycine	20.1	21.2	24.2	17.9	23.0	26.3	20.5	23.7	26.8	17.2	20.4	22.9
Histidine	11.2	13.1	15.3	11.1	17.1	21.2	10.8	15.5	17.7	10.4	15.3	19.5
Glutamine	4.8	5.1	4.4	3.1	4.4	4.5	4.9	6.2	6.0	4.3	3.8	4.6
Arginine	451.1	498.0	428.4	280.3	372.8	271.7	359.1	393.0	418.7	128.3	215.8	138.1
Citrulline	7.7	7.9	8.4	8.0	9.5	9.9	8.8	9.5	8.9	6.1	6.6	7.8
Threonine	9.9	12.3	12.8	6.8	11.7	14.9	7.5	11.8	15.8	6.6	10.3	13.6
Alanine	67.9	75.7	63.1	76.4	87.5	92.4	55.9	62.4	80.5	59.6	73.1	78.7
Proline	769.7	774.7	788.5	827.4	838.2	895.7	830.4	771.2	779.3	900.3	893.5	840.0
γ-Amino butyric acid	124.3	130.3	127.5	121.4	122.2	122.8	127.4	123.1	125.8	113.3	122.3	115.7
Tyrosine	18.5	23.9	27.3	14.4	24.8	31.7	16.7	23.0	29.3	16.0	24.2	30.2
Valine	12.1	15.7	18.5	8.8	16.7	22.4	10.1	16.6	21.6	9.4	17.2	21.8
Methionine	8.1	9.9	12.1	7.1	9.6	14.3	4.9	8.8	11.4	5.1	10.2	13.6
Ornithine	8.7	9.1	10.9	22.2	20.7	34.7	11.1	10.5	26.7	12.3	13.3	17.9
Lysine	35.7	53.9	62.6	27.5	55.4	79.8	29.7	51.6	68.5	28.6	55.1	74.7
Isoleucine	6.9	10.9	13.6	5.3	10.5	16.3	5.1	10.0	13.6	5.3	11.2	15.2
Leucine	24.2	36.8	44.9	19.1	41.2	61.4	21.5	38.0	50.1	22.1	43.5	58.8
Phenylalanine	16.9	24.7	29.9	12.2	25.6	36.6	14.0	24.1	31.2	14.2	26.4	35.4
Total	1734.6	1880.6	1848.0	1554.0	1822.9	1917.1	1652.4	1752.5	1930.5	1448.0	1688.4	1665.6

[a] Throughout all wine samples, the amounts for cystine and tryptophan varied below their respective LOQs (2.9 and 3.9 mg L^{-1}).

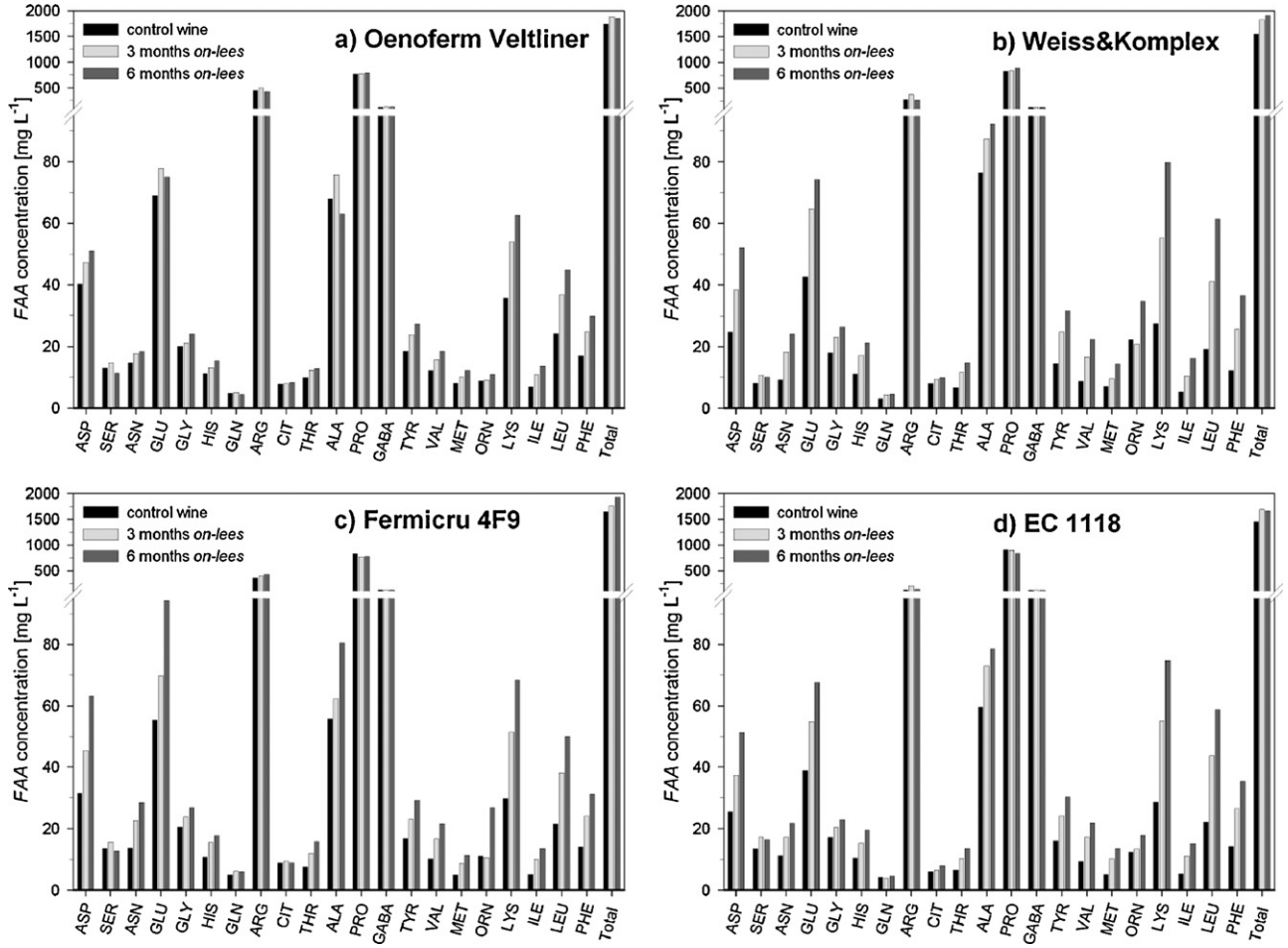

Fig. 3. Evolution of FAA concentrations during *on-lees* maturation (control wine, 3 and 6 months ripening) for the four wine sets fermented (a) *Oenoferm Veltliner*, (b) *Weiss&Komplex*, (c) *Fermicru 4F9*, and (d) *EC1118*.

Despite the rather heterogeneous impact of either yeast or aging, FAA profiling indicated similarities within the four wine sets that were highlighted by most abundant accumulations for lysine, leucine, phenylalanine, and additionally aspartic and glutamic acid for the wines fermented with *Weiss&Komplex, Fermicru 4F9* and *EC1118*.

4. Conclusion

Emphasizing the overall complexity of vinification itself, along with the multitude of influencing factors in wine manufacture, the use of reliable quantification methods may indeed facilitate product monitoring. Combining sensitive AQC amino acid derivatization with a fast-LC technique offered significant advantages compared to conventional HPLC methods, such as a shorter runtime and enhanced chromatographic resolution. Moreover, the hereby newly established UPLC™ protocol enabled an adequate quantification of free amino acid levels in different *Sur-lie* wine samples. Thus, the demonstrated UPLC™ amino acid analysis may contribute a valuable tool for monitoring the complex autolysis processes during the *on-lees* aging of wines.

References

[1] J.A. Peĭrez-Serradilla, M.D.L. de Castro, Food. Chem. 111 (2008) 447.
[2] H. Alexandre, M. Guilloux-Benatier, Aust. J. Grape Wine Res. 12 (2006) 119.
[3] V. Moreno-Arribas, E. Pueyo, M.C. Polo, J. Agric. Food Chem. 44 (1996) 3783.
[4] V. Moreno-Arribas, E. Pueyo, M.C. Polo, P.J. Martiĭn-Aĭlvarez, J. Agric. Food Chem. 46 (1998) 4042.
[5] A.J. Martínez-Rodríguez, M.C. Polo, J. Agric. Food Chem. 48 (2000) 1081.
[6] R.M. Callejón, A.M. Troncoso, M.L. Morales, Talanta 81 (2010) 1143.
[7] I. Molnár-Perl, B. Josefsson, T. Takeuchi, in: I. Molnár-Perl (Ed.), Quantitation of Amino Acids and Amines by Chromatography, Journal of Chromatography Library, vol. 70, Elservier, Amsterdam, 2005, p. 137 (Chapter 1.2.2–1.2.5).
[8] S.A. Cohen, in: I. Molnár-Perl (Ed.), Quantitation of Amino Acids and Amines by Chromatography, Journal of Chromatography Library, vol. 70, Elservier, Amsterdam, 2005, p. 242 (Chapter 1.2.6).
[9] S.A. Cohen, D.P. Michaud, Anal. Biochem. 211 (1993) 279.
[10] D.J. Strydom, S.A. Cohen, Anal. Biochem. 222 (1994) 19.
[11] H. Liu, M.C. Sañuda-Peña, J.D. Harvey-White, S. Kalra, S.A. Cohen, J. Chromatogr. A 828 (1998) 383.
[12] M. Reverter, T. Lundh, J.E. Lindberg, J. Chromatogr. B Biomed. Appl. 696 (1997) 1.
[13] S.A. Cohen, K.M. De Antonis, J. Chromatogr. A 661 (1994) 25.
[14] C. Van Wandelen, S.A. Cohen, J. Chromatogr. A 763 (1997) 11.
[15] L. Bosch, A. Alegriĭa, R. Farreĭ, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 831 (2006) 176.
[16] M. Murkovic, K. Derler, J. Biochem. Biophys. Methods 69 (2006) 25.
[17] P. Hernández-Orte, M.J. Ibarz, J. Cacho, V. Ferreira, Chromatographia 58 (2003) 29.
[18] M.E. Swartz, J. Liq. Chromatogr. Relat. Technol. 28 (2005) 1253.
[19] I. Boogers, W. Plugge, Y.Q. Stokkermans, A.L.L. Duchateau, J. Chromatogr. A 1189 (2008) 406.
[20] W. Liming, Z. Jinhui, X. Xiaofeng, L. Yi, Z. Jing, J. Food Compost. Anal. 22 (2009) 242.
[21] H.K. Mayer, G. Fiechter, E. Fischer, J. Chromatogr. A 1217 (2010) 3251.
[22] H.B. Hewitson, T.E. Wheat, D.M. Diehl, Am. Lab. 41 (2009) 22.
[23] Waters AccQ. Tag Chemistry Package (Instruction Manual), Waters Corp, Milford, MA, USA, April 1993.
[24] H.J. Liu, J. Chromatogr. A 670 (1994) 59.
[25] I. Kabelová, M. Dvořáková, H. Čížková, P. Dostálek, K. Melzoch, J. Food Compost. Anal. 21 (2008) 736.
[26] DIN 32645:1994-05, Chemical analysis; decision limit; detection limit and determination limit; estimation in case of repeatability; terms, methods, evaluation, Beuth Verlag, Berlin, Germany, 1994.