

Quality assessment of grape juice from integrated, organic and biodynamic viticulture using image forming methods

Jürgen Fritz^{1,2}, Miriam Athmann¹, Georg Meissner³, Randolph Kauer³, Uwe Geier⁴, Roya Bornhütter⁴ and Hans R. Schultz³

¹Institute of Crop Science and Resource Conservation, Department of Agroecology and Organic Farming, University of Bonn, Auf dem Hügel 6, D-53121 Bonn, Germany

²Department of Organic Farming and Cropping Systems, University of Kassel, Nordbahnhofstr. 1a, D-37213 Witzenhausen, Germany

³Institute of General and Organic Viticulture, Hochschule Geisenheim University, Von-Lade-Str. 1, D-65366 Geisenheim, Germany

⁴Forschungsring für Biologisch-Dynamische Wirtschaftsweise e.V., Brandschneise 5, D-64295 Darmstadt, Germany

*Corresponding author: j.fritz@uni-kassel.de

ABSTRACT

Aim: The image forming methods biocrystallisation, capillary dynamolysis and circular chromatography are introduced as a complementary tool for grape quality assessment. These methods were used to investigate grape juice samples from a long-term field trial comparing integrated, organic and biodynamic viticultural practices.

Methods and Results: Characteristic changes in structures created by the reaction of metal salts with grape juice were evaluated using biocrystallisation, circular chromatography and capillary dynamolysis image forming methods. In particular, this study tested the effects of cultivation method, aging time and juice concentration on structure formation.

To assess grape quality, the images of the encoded grape juice samples were: i) grouped into pairs with similar image features, ii) characterised based on reference images (e.g., high versus low resistance to degradation, or the amount of substance necessary for structure formation), iii) ranked according to structures associated with grape quality, and iv) assigned to the different production methods (classification). In order for similar structural features in the image forming methods to be expressed, all samples of grape juice harvested from integrated production over four years required higher juice concentrations than samples from organic and biodynamic origin. This was interpreted as the latter two production systems having higher structure formation efficacy. Furthermore, juices produced from the integrated management system exhibited more structures, indicative of a lower resistance to aging. In three out of four harvest years, the biodynamic samples exhibited the highest structure forming efficacy and resistance to aging.

Conclusion: These findings are consistent with enhanced form maintenance and thus higher internal product quality of biodynamic and organic grapes compared to grapes from integrated farming.

Significance and impact of the study: Image forming methods may serve as a valuable tool for grape juice and wine quality assessment to complement compound-specific chemical analyses.

KEYWORDS

biocrystallisation, capillary dynamolysis, circular chromatography, grape quality, picture forming methods

Supplementary data can be downloaded through: <https://oenone.eu/article/view/2548>

INTRODUCTION

Sharp increases in the global production of organic and biodynamic viticulture and the consumption of their products over recent decades (Willer *et al.*, 2013; Castellini *et al.*, 2017) demonstrate the remarkable interest of both winegrowers and consumers in vineyard management systems with a presumed reduced environmental impact. In response to this trend, a long-term and randomised field trial was established in 2006 (Geisenheim, Germany) to compare the influences of integrated, organic and biodynamic management systems on the development of plants, pests and diseases, as well as the impact on soil and the product quality of grapes and wine (Meissner *et al.*, 2010; Meissner *et al.*, 2019). In the first four years of the trial, the two organic management systems exhibited higher soil quality, lower grape yield and lower vegetative growth compared to the integrated management system. In the two organic treatments, grapes were more exposed to light and their compactness was reduced. This different fruit morphology likely contributed to the lower incidence of acetic acid rot in organic versus integrated management systems, and these effects were even more pronounced for biodynamic versus organic management systems (Meissner *et al.*, 2019). These results suggest that organic, and especially biodynamic management practices, promote favourable grape morphology and thereby product quality. However, classical grape quality analyses, such as must density, pH, total acidity and nitrogen by orthophthaldialdehyde (NOPA; Meissner *et al.*, 2019), did not show pronounced differences between the three production systems. Nevertheless, using a sensory image forming analysis, Fritz *et al.* (2017) were able to systematically distinguish the contrasting management systems in the first year following the conversion and implementation of the Geisenheim field trial. The aim of the present study was to further evaluate the ability of image forming methods, including biocrystallisation, capillary dynamolysis and circular chromatography, to distinguish the source (in terms of production system) and to characterise the quality of grape juice samples harvested from the different management systems of the Geisenheim field trial in the years 2007 to 2010.

Previous attempts to differentiate grapes or wines harvested from organic, biodynamic, or conventional management systems via chemical

analyses have yielded mixed results (Plahuta and Raspor, 2007; Yañez *et al.*, 2012; Tassoni *et al.*, 2013; Laghi *et al.*, 2014; Granato *et al.*, 2015; Parpinello *et al.*, 2016). Sensory evaluations of wine may be similarly inconclusive (Bigler *et al.*, 2009; Ross *et al.*, 2009; Meissner, 2015; Parpinello *et al.*, 2016), yet crystal structures formed via the «droplet evaporation method» (Kokornaczyk *et al.*, 2011) and gas discharge visualisation (Bigler *et al.*, 2009) have led to a significant differentiation of management systems. Botelho and Roberti (2016) found no differences between organic and biodynamic management in grape yield and disease indices, but the natural defences of biodynamic grapes seemed to be stimulated. Biodynamic production systems have also been shown to promote microbial community development on the fruit in years with difficult climatic conditions compared to conventional production (Guzzon *et al.*, 2016). While conventional and biodynamic production systems may alter the distribution patterns of fungi in vineyards, no differences in fungal patterns were found in the harvested grapes (Morrison-Whittle *et al.*, 2017). In organic and biodynamic Sangiovese red wines, the yeast microbiota varied independently of the production system (Patrignani *et al.*, 2016). Finally, in a life cycle assessment study in Spain, biodynamic viticulture was suggested to have a lower environmental impact than conventional viticulture (Villanueva-Rey *et al.*, 2014).

One aspect of crop quality is the ability to maintain its form and internal characteristics when aging. This is important for food marketing, because aging rate has a direct impact on storage losses incurred by sellers of products such as potatoes and carrots. In a natural context, the ability to maintain form during aging is often vital for survival. For example, carrots do not flower until their second year and must therefore remain intact throughout winter and into the second year following vegetation. In aging tests with carrots, Samaras (1978) found that storage losses due to microbial decay were lower under biodynamic cultivation than under organic cultivation. Additional comparisons of cucumber (Andersen, 2019), rocket (Athmann, 2011) and carrot (Wistinghausen, 1979) management systems revealed that storage losses were lower in the rank order: biodynamic > organic > integrated. In parallel to these solid foods, the ability of juices to maintain form while aging can be evaluated via the formation of crystal structures that crystallise with copper chloride

(i.e., copper chloride crystallisation: Doesburg *et al.*, 2015).

Biocrystallisation has become the most commonly used and scientifically advanced imaging method. Biocrystallisation is based on the phenomenon that specific crystallisation patterns are formed on a round glass plate when a copper chloride ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) solution is mixed with an organic additive and crystallised (Gallinet and Gauthier-Manuel, 1992; Busscher *et al.*, 2010a). The additive affects the shape of the pattern that arises during the self-organisation process of crystallisation (Kokornaczyk *et al.*, 2011; Busscher *et al.*, 2014).

Recent reviews have documented routine implementation practices for the biocrystallisation method, including all laboratory procedures, properties of the crystallisation chamber and the steps in scientific validation (Busscher *et al.*, 2010a; Busscher *et al.*, 2010b; Kahl *et al.*, 2013). Criteria for evaluating biocrystallisation patterns via both visually defined morphology (Huber *et al.*, 2010; Doesburg *et al.*, 2015) and computer-based structure and texture analysis have also been described (Andersen *et al.*, 1999; Meelursarn, 2007; Doesburg and Nierop, 2013; Kahl *et al.*, 2015a). Additionally, a recent inter-laboratory exercise comparing standardised and non-standardised biocrystallisation approaches revealed consistent classification of decoded wheat samples by five different laboratories (Kahl *et al.*, 2015b).

In practical applications, computerised image analysis has been successfully used to discriminate between samples from organic and conventional production, or from different processing steps (e.g., Kahl *et al.*, 2008; Kahl *et al.*, 2009; Kahl *et al.*, 2015a). Beyond mere discrimination, pattern formation in image forming methods can be linked to the growth conditions of the plant. Physiological processes, such as decomposition and ripening, are reflected by the patterns in a reproducible and characteristic manner (Balzer-Graf and Balzer, 1991; Fritz *et al.*, 2011; Doesburg *et al.*, 2015). These patterns can therefore be used as a reference when investigating the quality of food products. Extensive experimental data for a wide variety of agricultural products has shown that biocrystallisation patterns with low nitrogen fertilisation, or from biodynamic or organic

samples, reveal more image features indicating ripeness or freshness than samples with high nitrogen fertilisation or from conventional production (Mäder *et al.*, 1993; Weibel *et al.*, 2000; Athmann, 2011; Fritz *et al.*, 2011; Fritz *et al.*, 2017). Therefore, these specific evaluation methods are assumed to express aspects of product quality in terms of structural properties by analysing characteristics of the food product as a whole, rather than as concentrations of single chemical compounds.

In general, the growth and quality of agricultural products are considerably influenced by site conditions, and this is particularly true for grapes and wine. Image forming methods have been shown to be sensitive indicators of the effects of growth conditions on the harvested product. Therefore, it was hypothesised that image forming methods could provide a more distinctive characterisation of quality differences in grapes harvested from integrated, organic or biodynamic management than would be possible with chemical analyses alone.

MATERIALS AND METHODS

The field trial in Geisenheim was established in 2006; the experimental setup and management practices have been previously described by Meissner *et al.* (2019) and are briefly reviewed here. More detailed information on experimental design, climate, cover crop type and management, plant protection with product and application time are described for the years 2007-2009 in Meissner *et al.* (2019) and for year 2010 in Döring *et al.* (2015).

1. Site description

The experimental site is a 0.8 ha Riesling (clone Gm 198-30) vineyard at Geisenheim University, Germany (49°59'N 7°56'E), with 10.7 °C mean annual temperature, 535 mm average annual rainfall and 360 mm rainfall from April to October during the vegetation period (DWD – German weather service, Geisenheim station). The soil is a deeply plowed anthrosol, with sandy clayey loam in the topsoil and gravelly sandy loam in the subsoil. Soil pH is 7.1. The site is characterised by high contents of plant available nutrients (P_{CAL} and K_{CAL}) throughout the soil profile (P_2O_5 and K_2O per 100 g soil: 73 mg and 44 mg from 0-30 cm, 74 mg and 38 mg from 30-60 cm, 71 mg and 32 mg from 60-90 cm soil depth, determined according to Schaller, 2000).

2. Experimental setup

The grape scions are grafted onto two different rootstocks *Vitis berlandieri* Planch x *Vitis riparia* Michx, cv. SO4 and *Vitis riparia* Michx x *Vitis cinerea* Engelm. cv. Börner. Grape samples from harvest years 2007 to 2010 were analysed. The vines were planted in 1991 at a spacing of 1.2 m within rows and 2 m between rows. The pruning system comprised a single guyot with about 6–8 buds m⁻². The vineyard was managed according to good agricultural practice (GAP, treatment with plant protection after damage thresholds) until 2005. In January 2006, the vineyard was divided into replicate plots of integrated (GAP), organic and biodynamic viticulture (both EU 834/2007) in a complete block design with four replications. Each plot consisted of four rows with 64 vines each and was divided into two subplots with one rootstock each. Only the inner two rows were considered for measurements. For all parameters, sampling was conducted equally for the two rootstocks.

3. Management

Management was performed according to GAP (integrated treatment INT), the EU 834/2007 and ECOVIN Standard (organic treatment ORG), and the EU 834/2007 and DEMETER Standard (biodynamic treatment BIODYN). The management treatments are summarised in Table 1.

All treatments were fertilised with compost, following the exact mode of preparation and protocol for its application as set out by Meissner *et al.* (2015). For the INT treatment, compost was made from communal green waste with 1.4 and 1.5 % N as dry matter in 2006 and 2007 respectively, and a C/N ratio of 14.7 in

2007 (data on carbon is missing for 2006 due to technical problems). For ORG and BIODYN treatments, compost was made from organic cow manure with 1.9 and 1.0 % N as dry matter in 2006 and 2007 respectively, and a C/N ratio of 13.1 in 2007. The compost from the two organic treatments was prepared from the same batch of manure under equal conditions, except for the biodynamic preparations 502–507 (Koepp *et al.*, 1990).

In all treatments, 50 kg N/ha was applied in 2006 and 2007 respectively, resulting in 6.4 and 5.9 t/ha of compost as fresh matter in INT and 4.7 and 8.4 t/ha in ORG and BIODYN. In 2008 and 2009, compost was not used in any of the treatments following good agricultural practice, since biomass production and wood and leaf nutrient contents were in the optimum range (Tables S7 and S8 in Meissner *et al.*, 2019). In 2006, 25 kg CAN (calcium ammonium nitrate)/ha (15 % N) and 5 kg urea/ha (46 % N) as foliar fertiliser were additionally applied after flowering to INT. All systems also received nitrogen by incorporating the biomass of the legume-containing cover crop mixtures. More detailed information on cover crops is given in Meissner *et al.* (2019).

Canopy management was the same for all treatments. To avoid any inhomogeneity being introduced by canopy management practices, winter pruning was carried out at the beginning of the vegetation period and no further shoot thinning or defoliation was performed during the vegetation period. Tillage was mostly carried out on the same date for all treatments. Weeds were removed from the area underneath the vines by applying herbicides in the integrated treatment,

TABLE 1. Characterisation of the different viticulture management practices (INT=integrated, ORG=organic and BIODYN=biodynamic) used within the experimental trial.

	INT	ORG	BIODYN
Perennial cover crops	Grass mixture	Diverse cover crop mixture (Wolff-Mixture)	
Annual cover crops	Rye + vetch	Diverse cover crop mixtures	
Under vine management	Herbicides	Mechanical	
Fertilisation	Compost + mineral + legumes from annual cover crops	Compost + legumes from perennial and annual cover crops	
Plant protection	Organic fungicides	Copper (max 3 kg/ha and year), sulfur, plant strengtheners	
Biodynamic preparations		Horn manure, horn silica, compost preparations	

and by mechanical removal in the biological treatments (detailed in Table S3 in Meissner *et al.*, 2019).

The plants were protected by controlling for symptoms of fungal diseases and pests and by monitoring climate data. In the integrated treatment, different fungicides and MgSO₄ products (for preventing bunch stem necrosis) were used following the recommendations for integrated plant protection. The organic and biodynamic treatments were applied equally: before flowering the plant strengthening product MycosinVin® was applied; during and after flowering copper products and wettable sulfur and/or potassium hydrogen carbonate was used. Grapevine berry moths (*Lobesia botrana*) were controlled with pheromones, as described in detail by Meissner *et al.* (2019).

4. Samples

The samples were collected from the three management systems INT, ORG and BIODYN. They were mixed together from four field replications for each treatment. Six samples (two repetitions per management system x three management systems) were assessed in the harvest year 2007, and nine samples (three repetitions per management system x three management systems) were assessed in harvest years 2008, 2009 and 2010. Each sample was encoded by co-workers of different laboratories at the University and then analysed in the laboratory of the Institute of Organic Agriculture (Bonn, Germany) using the three image forming methods of biocrystallisation, capillary dynamolysis and circular chromatography. All samples were delivered in juice form (pressed at Geisenheim University with Scharfenberger Europress Px3/2011). The juices were stored at 5 °C and were analysed 1 to 5 days after storage without further processing; juices stored for only one day are referred to as the “fresh” samples for the purposes of the current study. Each juice series was analysed in a sequence with varying juice concentration (Table 2). By varying both aging times (at 5 °C) and mixing ratios of juice and diluted metal salts, the spectrum of images to evaluate for each sample was extended. For each sample and harvest year, 42 images were produced and evaluated: i) circular chromatography four series x four concentrations, ii) capillary dynamolysis two series x four concentrations, and iii) bio-crystallisation six series x three concentrations (Table 2).

TABLE 2. Extract setting times, mixing ratio and reagent composition in the images.

Method	Series	Setting time at		Per Image							
		5 °C	20 °C	Juice [ml]	H ₂ O dest. [ml]	NaOH 0.40 % [ml]	AgNO ₃ 0.25 % [ml]	AgNO ₃ 0.50 % [ml]	FeSO ₄ 0.25 % [ml]	CuCl ₂ 10 % in H ₂ O [ml]	
Circular chromatography	A	1 d	1 h	0.245	0.605	0.40		0.50			
	B	2 d	1 h	0.275	0.575	0.40		0.50			
	C	3 d	1 h	0.300	0.550	0.40		0.50			
	D	4 d	1 h	0.325	0.525	0.40		0.50			
Capillary dynamolysis	A	1 d		0.30	0.30		0.70		2.00		
	B	2 d		0.35	0.25		0.70		2.00		
				0.40	0.20		0.70		2.00		
				0.45	0.15		0.70		2.00		
Bio-crystallisation	A	1 d		0.160	2.840						2.00
	B	2 d		0.200	2.800						2.00
	C	3 d		0.240	2.760						2.00
	D	5 d		0.280	2.720						2.00
	E	6 d									

Standard mixing ratios are given. Depending on juice condition in the different years, the optimum mixing ratio for a differentiated image varied a) for circular chromatography between 0.150 and 0.325 ml juice, and b) for biocrystallisation of juice pressed with the standard procedure in Geisenheim Series A: Juice amount 0.160 ml, 0.200 ml, 0.240 ml; and Series B, C, D, E: Juice amount 0.200 ml, 0.240 ml, 0.280 ml.

5. Circular Chromatography

Filter paper discs (Whatman No. 1) with a total diameter of 15 cm were saturated to a diameter of 8 cm with 0.5 % silver nitrate solution. The filter papers were then dried for 2-3 hours. Juice aliquots of 0.215 ml, 0.245 ml, 0.275 ml, 0.3 ml and 0.325 ml were diluted with distilled water (water distilling devices Muldestor Wagner & Munz) to a total volume of 0.85 ml. The samples were incubated at 20 °C with 0.4 ml of 0.4 % NaOH solution for 1 h. The extract solution migrated through a central filter paper wick and the filter paper discs from the center to a diameter of 12 cm. To maintain sufficient humidity, the paper was covered with a glass container. The images developed to full color formation in diffuse daylight (i.e., not direct sun) within 48 hours.

6. Capillary dynamolysis

In the first phase, 0.4 ml, 0.45 ml, 0.5 ml and 0.55 ml of juice were diluted with distilled water to a total volume of 0.6 ml. These liquids were applied to standard sized chromatography paper (Schleicher & Schuell 2043A) in Kaelin dishes and left to rise (Zalecka *et al.*, 2010). In the second phase, 0.7 ml of a 0.25 % silver nitrate solution rose to 1 cm across the line formerly formed by the juice. In the third phase, 2.0 ml of a 0.25 % iron sulfate solution rose to a total height of 12 cm. During the second and third phase, the chromatograms were covered with tall beakers to maintain sufficient humidity. The drying time between phases was based on the moment that the paper was dry and was therefore set for two hours at 20 °C and 50 % humidity.

7. Biocrystallisation

For the crystallisation method, juice and distilled water (water distilling devices Muldestor Wagner & Munz) were first mixed and then filtered through Schleicher & Schuell No. 604 filter papers (Balzer-Graf and Balzer, 1991). 2 mm thick floatglass plates measuring 10.5 x 10.5 cm were used. Plexiglas rings with an inner diameter of 9 cm were mounted with paraffin on the glass plates. A mixture of juice and a 10 percent copper chloride solution (for mixing ratio see Table 2) were placed into this ring and crystallised in a crystallisation chamber at 30 °C with 50 % humidity. For in-house methodical experiments, the optimal crystallisation time of 12-15 hours was determined.

8. Visual evaluation after harvest 2007-2010 without panel

The visual image evaluation of the grape samples was first carried out after harvest without panel by Jürgen Fritz assisted by Miriam Athmann. Visual image evaluation relied on a catalogue of reference images with grape samples from the same site harvested in 2006 (Fritz *et al.*, 2017) with i) varying amounts of juice amount per plate, and ii) different deterioration stages generated in the laboratory in Bonn. The calibration procedure, also described by Fritz *et al.* (2011), used the criteria listed by Huber *et al.* (2010) to characterise changes in the reference image structures that occurred with i) increasing juice amount per plate, and ii) increasing juice age. These comparison series formed the basis for characterising the form expression of generated images on scales of ‘strong’ to ‘weak’, and ‘fresh’ to ‘aged’. Characteristic changes in form expression caused by the cultivation method were related to the modulation of image structures caused by increasing the amount of juice per plate and by juice aging. For example, if a sample with the same juice quantity per plate had characteristic structures of high juice quantities per plate, then it was described as having ‘strong form expression’. If another sample with the same amount of juice had characteristic structures of low amounts of juice per plate, it was described as having ‘weak form expression’. Qualitative assessments of the generated images were made on the basis of these characterisations. As a result of the qualitative assessment, (a1) juice with strong form expression and (b1) fresh juice were ranked higher than (a2) juice with weak form expression and (b2) aged juice respectively. The encoded grape samples were grouped according to similar form expression and, based on experience from earlier investigations, assigned to management systems (classification).

Statistical Analysis: the agreement between the correct grouping/classification and the grouping/classification based on the results of the image forming methods was tested. The test was based on a 3 x 3 contingency table, which compares a set of given categories to those determined in the investigation (see Tables 4 and 5). For the grouping test, Fisher’s Exact Test was performed. For the classification test, agreement was determined with the simple Kappa coefficient. The corresponding methods

are described by Agresti (2002). The statistical software 'R', version 2.10.1 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria) was used for Fisher's Exact Test. Calculation of Kappa coefficients and exact p-values for testing agreement were determined using PROC FREQ in SAS (SAS Institute Inc., Cary, NC, USA), version 9.3.

9. Visual evaluation harvest year 2010 with a panel

For the evaluation of the pictures from 2010 with a panel, the pictures were arranged in sets as follows. In 2010, nine samples were examined; three management systems with three repetitions each. Pictures were taken on five different days with increasing ageing (1, 2, 3, 5, 6 d; see Table 2). An evaluation set consisted of one sample each from the management systems BIODYN, ORG and INT from the same day of ageing. Three sets were compiled for each day from the three repetitions per management system. Pictures were taken on five days with three sets each, thus a total of fifteen sets were compiled. Each panel member judged the pictures in a separate cabin in the sensory laboratory at the University of Kassel. Each set was judged separately.

A working group of eight researchers from four laboratories for image-forming methods further developed the visual evaluation of biocrystallisation images for "Gestalt" evaluation (Doesburg *et al.*, 2015; Fritz *et al.*, 2018). Four levels of evaluation criteria reflecting a hierarchical complexity were distinguished: (1) single morphological features (e.g., length of side needles), (2) descriptive criteria connected to single features (e.g., regularity of ramifications), (3) gestures or implicit motions in the whole image (e.g., "center coordination" or "integration"), and (4) "Gestalt" criteria (Doesburg *et al.*, 2015). ISO 11035 (1994) and ISO 8587 (2006) were used and adapted for the visual evaluation of biocrystallisation images. The procedure of the adapted ISO 8587 was as follows (Fritz *et al.*, 2018):

I. Formation of the panel: all panelists were experienced in the visual evaluation of biocrystallisation images.

II. Reference: biocrystallisation images of grape juice ranking from fresh to decomposed (one day, five days) were collected. For

characterisation, the relevant features for Gestalt evaluation were prioritised.

III. Training: A process of concept-learning according to supervised classification (Ashby and Maddox, 2005; Galotti, 2014) took place via email and telephone conferences. Encoded training sets were presented in random order to the panel as portable document format (PDF) files. Images of the three mixing ratios of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and additive per sample were shown. The images were evaluated by each panel member independently, then decoded and discussed (concept learning by supervised classification; cf. Fritz *et al.*, 2018).

IV. Exam: Two panel tests (exams) were performed on 10th and 11th September 2013 (Exam I and II). Each panel test occurred over one day in the sensory laboratory at the University of Kassel, Germany. Both panel tests were performed without using the reference images. Each of the eight now further trained panel members simultaneously received encoded evaluation sets in random order. Nine sets included samples with different aging levels (one day and five days) and 15 sets comprised samples collected from the three different management systems at one of the aging times. The panel was asked to rank the biocrystallisation images according to the specified criteria of global intensity of decomposition (Figure 2). On the second day, the same image sets were evaluated again, but all images were vertically reversed - mirrored on the vertical axis - to derive new sets of pictures.

Statistical analysis: SPSS statistical software (version 19.0, SPSS Inc., Chicago, IL, USA) was used. The average rank order of the samples was calculated. As recommended in ISO 8587 (2006), the Friedman test was applied as an overall measure for correct ranking. "In a very general manner, this test gives the maximum opportunities for demonstrating recognition of differences among the samples by the assessors" (ISO 8587, 2006). In addition to testing the ranking order for significance, the Friedman test was also used for pairwise comparisons between adjacent ranks.

RESULTS

In this study, an accurate separation of the encoded samples (grouping) was primarily achieved on the basis of circular chromatography images. Biocrystallisation images were the most

closely connected to the reference series and were therefore predominantly used for quality assessment and classification. The procedure for grouping the encoded samples and for quality assessment are explained using vintage 2010 as an example.

1. Grouping based on circular chromatography images

On the basis of the image composition, three groups of images with similar characteristics were distinguished, each group consisting of three samples (Figure 1):

- Group 1: brown slightly notched outer ring, broad pink inner ring, pale purple lines running from the center outwards. In the center around the hole there was no small purple circle.
- Group 2: brown slightly notched outer ring, thin pink inner ring, no purple lines in the center. In the center around the hole there was a small purple circle.
- Group 3: brown deeply notched outer ring, broad pink inner ring, distinct purple lines

running from the center outwards. In the center around the hole there was no small purple circle.

2. Quality assessment and classification based on biocrystallisation images

In the reference aging series (Figure 2), it was determined that:

- with a decreasing amount of juice per plate, the needle bundles turned from fine to coarse;
- with increasing deterioration of the sample, the needle bundles turned from fine and even from the centre to the outside spacing (fresh juice) to coarse with formation of more chaotic structures and partly dense structures in the center, with a few area-covering structures, more dense structures at the border, and wavy needle courses with undesigned ring zones (juice that had been aged for two days). Chaotic needle bundles are needles that do not have a clear, relatively uniform structure from the center to the outside. They are branching with frequent and abrupt changes in direction.

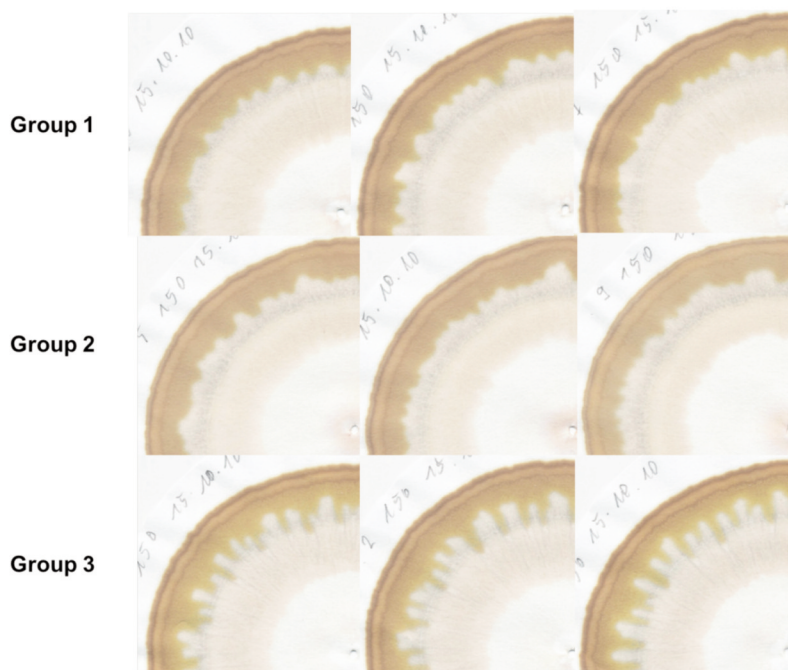


FIGURE 1. Discrimination of 9 samples in 3 groups (n=3) from BIODYN (group 1), ORG (group 2) and INT (group 3) farming systems.

circular chromatography images of grape juice pressed with the standard procedure at Geisenheim harvest year 2010 (juice aged 2 days at 5 °C; all images 0.150 ml juice).

For juice samples from harvest year 2010 pressed with the standard procedure in Geisenheim, the needle bundles were increasingly coarse and chaotic and more wavy needle courses with undesigned ring zones occurred in the following order:

- 1 day aging < 2 days aging < 5 days aging;
- Group 1 < Group 2 < Group 3 (Figure 3).

When applying the capillary dynamolysis method, no differences were observed between the samples of the different management methods (see Supplementary Figure S1). The method could not support the evaluation of the grape juice samples. Earlier investigations on various foods (e.g., wheat: Fritz *et al.*, 2011; lettuce: Athmann, 2011; grapes: Fritz *et al.*, 2017), have revealed that organic and especially biodynamic products usually show fewer signs of aging and stronger form expression than samples from conventional or integrated production. We therefore classified the sample

group with the finest needle bundles (Group 1) as originating from the biodynamic production system, the group with the coarsest and most chaotic needle bundles (Group 3) as derived from the integrated production system, and the intermediate group (Group 2) as originating from the organic production system. The qualitative assessment using image forming methods - ranking the juice samples characterised as 'strong form expression' and 'low age' higher than those characterised as 'weak form expression' and 'advanced age' - showed a clear hierarchy: biodynamic (BIODYN) > organic (ORG) > integrated (INT) (Table 3).

Decoding revealed that two samples were not correctly grouped or classified according to the management method in harvest year 2009 only. In harvest years 2007, 2008 and 2010, all samples were correctly grouped and classified. In all years, the samples from the integrated treatment were correctly grouped and classified (Tables 4 and 5). This means that from the

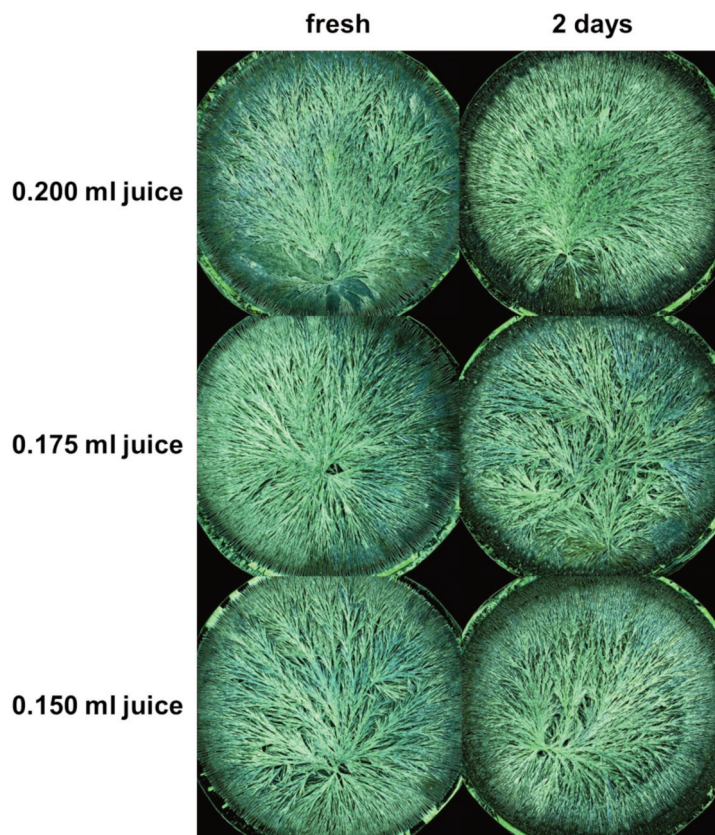


FIGURE 2. Reference series with increasing amount of juice per plate and with juice deterioration. (biocrystallisation images of grape juice harvest year 2006 from INT, the integrated farming system (fresh grape juice and 2 days aged at 5 °C; all images 0.16 g CuCl₂ per plate).

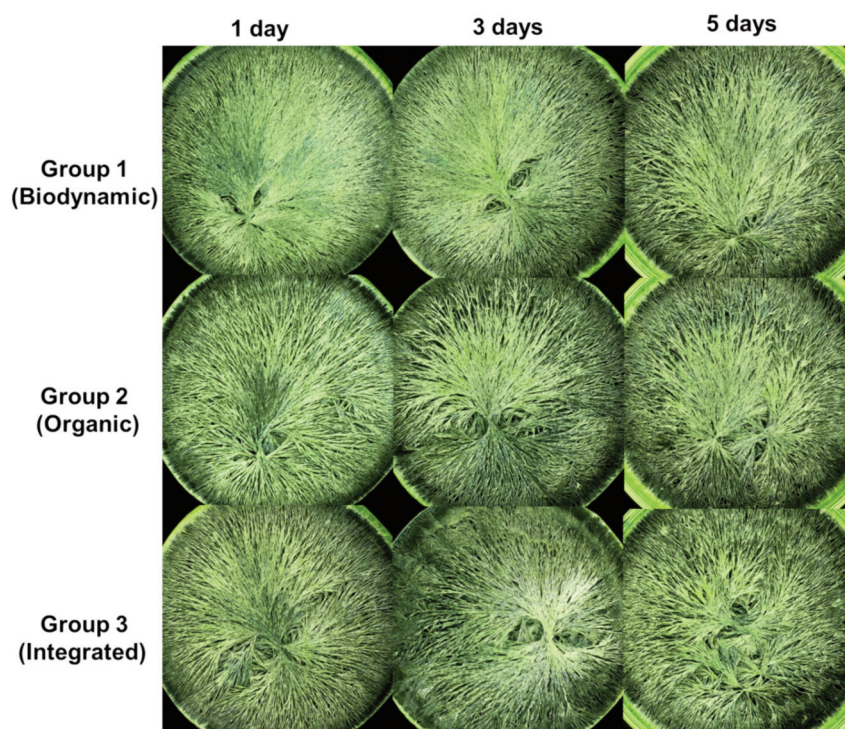


FIGURE 3. Juice deterioration of INT, ORG and BIODYN farming systems. (sample identity was not known at the time of investigation): Biocrystallisation of grape juice samples pressed with the standard procedure in Geisenheim harvest year 2010, aged at 5 °C (all images 0.2 ml juice and 0.16 g CuCl₂ per plate).

33 samples in the four years, 31 were correctly assigned to their cultivation method. Only two samples could not be correctly grouped and classified.

An evaluation of the crystal images from the 2010 harvest year was conducted by a panel of eight experienced evaluators with ranking evaluations. The first test (Exam I, Table 6) monitored the accurate assignment of coded images to the aging treatments (one day or five days) based on the image structures. In Exam I the original pictures were evaluated, while in Exam II (Table 6) the pictures were vertically reversed. In each exam, a total of 72 sets (8 persons x 9 sets) were evaluated. In both exams, 69 sets were evaluated correctly, representing a 95.8 % success rate. The assignment of the coded images to aging levels was therefore highly significant.

In a second test, encoded images of a BIODYN, ORG and INT were evaluated for each set according to the criterion of low aging of the image structures (Table 7). A total of 15 sets were evaluated per person. As above, both the original and their vertically-reversed images were evaluated (Table 7). According to the

criterion of low aging, in both tests the ranking was (mean value of all panel members): BIODYN, Biodynamic>ORG, Organic>INT, Integrated. The ranking values of the two replicate evaluations were redundant with very good agreement. The difference between ORG and INT was not significant in both evaluations. BIODYN differed significantly from ORG in both evaluations. In the 16 exams (8 persons x 2 exams with 15 sets each) of the individual assessors, BIODYN was ranked first in 14 exams.

TABLE 3. Grouped and classified encoded grape samples from different farming systems.

From left to right: i) samples needed more juice per sample for similar form expression, and ii) samples showed more pronounced structural features that indicate enhanced aging/deterioration

Year	Grouping and classification		
2007	D = D	O = O	I = I
2008	D = D = D	O = O = O	I = I = I
2009	D = D = O	D = O = O	I = I = I
2010	D = D = D	O = O = O	I = I = I

D: BIODYN, **O:** ORG, **I:** INT

Red: false classification of sample assigned to production system

TABLE 4. Contingency table of Fisher’s Exact Test (test for grouping of encoded samples).

		Correct classification		
		G1	G2	G3
Harvest year 2007				
Grouping of encoded samples	G1	2	0	0
	G2	0	2	0
	G3	0	0	2
Significance		p=0.067		
Harvest year 2008				
Grouping of encoded samples	G1	3	0	0
	G2	0	3	0
	G3	0	0	3
Significance		p=0.004		
Harvest year 2009				
Grouping of encoded samples	G1	2	1	0
	G2	1	2	0
	G3	0	0	3
Significance		p=0.01		
Harvest year 2010				
Grouping of encoded samples	G1	3	0	0
	G2	0	3	0
	G3	0	0	3
Significance		p=0.004		

G1 – G3: sample group 1 to 3.

TABLE 5. Contingency table of Interrater Agreement (test for classification of encoded samples).

		Correct classification		
		D	O	I
Harvest year 2007				
Classification of encoded samples	D	2	0	0
	O	0	2	0
	I	0	0	2
Significance		p=0.011		
Harvest year 2008				
Classification of encoded samples	D	3	0	0
	O	0	3	0
	I	0	0	3
Significance		p=0.001		
Harvest year 2009				
Classification of encoded samples	D	2	1	0
	O	1	2	0
	I	0	0	3
Significance		p=0.017		
Harvest year 2010				
Classification of encoded samples	D	3	0	0
	O	0	3	0
	I	0	0	3
Significance		p=0.001		

Significance: p=0.001; D: BIODYN, biodynamic system, O: ORG, organic system, I: INT, integrated system.

TABLE 6. Average rank order of the decomposition samples evaluated according to the intensity of the perceived “Gestalt” decomposition level from fresh to degraded.

	1 day	5 days	Correct ranking
Person 1-8			
Exam I	1.04	1.96	95.8 %
Pairw. comp.	L>p<0.001<J		
Exam II	1.04	1.96	95.8 %
Pairw. comp.	L>p<0.001<J		

Exam I: original images. Exam II: vertically reversed images. The Friedman test was significant in both evaluations ($p < 0.001$).

DISCUSSION

1. Grouping and classifying samples with the image-forming methods

Because each method was found to show different aspects of the sample under study, the three image forming methods were performed in parallel. In the present study, circular chromatography was the basis for discriminating the samples, while aspects relevant to quality evaluation and classification (differences in strength of form expression and aging behaviour) were most pronounced in biocrystallisation images. Capillary dynamolysis did not further support the accuracy of the results; however, in other investigations on wheat and other plant foods this method has shown clear differences between the samples investigated (e.g., Fritz *et al.*, 2011). Therefore, the combined application of all three methods is recommended for the investigation of plant food quality. From 33 samples of grape juice harvested from the years 2007 to 2010, it was possible to correctly group and assign (i.e., classify) 31 samples to the cultivation method. The ranking according to the criteria ‘low (slow) aging/deterioration’ and ‘strong form expression’ was: BIODYN, Biodynamic>ORG, Organic>INT, Integrated. In the evaluation of the images from 2010 by a panel of 8 people, ranking was according to image structures that were characteristic of low aging: BIODYN, Biodynamic>ORG, Organic>INT, Integrated.

The results of the present study show the same correctness in the grouping and classifying of samples from different production systems and the same quality ranking as former studies on beetroot (Mäder *et al.*, 1993), apples (Weibel *et al.*, 2000), and wheat (Mäder *et al.*, 2007; Kahl *et al.*, 2008; Fritz *et al.*, 2011; Fritz *et al.*, 2018). These results underline the high accuracy and

high reproducibility of the image forming methods in food quality analysis. Considerable advances have been made in visual image evaluation, with the documentation of both analytical morphological criteria (Huber *et al.*, 2010) and “Gestalt” criteria relating to features of the image as a whole (Doesburg *et al.*, 2015). With a trained panel it has been possible to classify crystal images of coded samples according to aging series, as well as to cultivation methods (Doesburg *et al.*, 2015; Fritz *et al.*, 2018).

2. Relationships between growth conditions, chemical analysis and quality

The measurements of total acidity, NOPA, pH and must density (total soluble solids) of the grape juices from the long-term trial in Geisenheim showed only slight and varying differences between the management systems in the different years (Döring *et al.*, 2015; Meissner *et al.*, 2019). In contrast to the image-creating methods, it was not possible to group or classify the cultivation methods by analysing traditional chemical components.

The management systems of integrated, organic and biodynamic production alter growth conditions. The question of whether grouping and classification of cultivation methods is also possible with chemical measures therefore leads to the questions of i) how the growth conditions were altered by the management systems in the long-term trial in Geisenheim, and ii) how changes in growing conditions can be measured chemically. In the present study, the grapes from biodynamic, organic and integrated production are derived from a long-term field trial, which was established on a fertile sandy loam soil with high levels of plant available nutrients (Meissner *et al.*, 2019). Under these conditions, management practices in the organic and biodynamic

systems led to a reduction in mineral nitrogen in the soil during the first three years of conversion (2006, 2007, 2008) and, probably due to leguminous undergrowth, to higher nitrogen levels in the years 2010 to 2013 (Döring *et al.*, 2015). Management practices in the organic and biodynamic systems led to a reduction in vegetative growth in all years, resulting in a more open canopy and lower compactness of grape clusters (i.e., increasing light exposure of grapes and improved air circulation; Meissner *et al.*, 2019). In 2010, water supply was lower in organic and biodynamic systems, as indicated by lower pre-dawn water potential, lower transpiration rates and stomatal conductivity (Döring *et al.*, 2015). These results support the hypothesis of van Leeuwen and Seguin (2006) that reduced water supply produces moderate vine vigour for quality grape production. In the present study this was the case for organic and biodynamic management.

Apart from management practices, quality parameters such as secondary metabolites, are strongly affected by grape variety and environmental variables such as soil conditions, climate and water availability (Heimler *et al.*, 2017). With decreasing nitrogen availability, morphological and chemical differentiation with production of complex carbon-based defence-related secondary metabolites is favoured over growth ('growth-differentiation balance hypothesis, Herms and Mattson, 1992). Higher contents of these compounds can be generally expected in organic produce than in conventional produce. Meta-analyses that have compared the chemical composition of organically and conventionally produced crops show higher antioxidant activity and higher contents of phenolic acids, flavanones, stilbenes, flavones, flavonols and anthocyanins (Barański *et al.*, 2014), with particularly higher levels of defence-related secondary metabolites (Brandt *et al.*, 2011). However, studies conducted for grapes have yielded ambiguous results: while Malusá *et al.* (2004), Nuñez-Delicado *et al.* (2005) and Dani *et al.* (2007) have found higher polyphenols, polyphenol oxidase, resveratrol and antioxidant activity in organic grapes as compared to conventional grapes, Tassoni *et al.* (2013) have not found any differences in polyphenols, and Vian *et al.* (2006) have detected higher anthocyanin contents in conventional grapes.

TABLE 7. Average rank order of the three management systems evaluated according to the intensity of the perceived Gestalt decomposition level from fresh to degraded.

	BIODYN	ORG	INT
Person 1-8			
Exam III	1.74	2.08	2.16
Pairw. comp.	↳ $p=0.024$ <↳		↳ $p<0.606$ <↳
Exam IV	1.78	2.06	2.17
Pairw. comp.	↳ $p=0.006$ <↳		↳ $p<0.519$ <↳
Person 1			
Exam III	1.60	2.00	2.40
Exam IV	1.60	1.87	2.53
Person 2			
Exam III	1.67	2.20	2.13
Exam IV	1.73	2.20	2.07
Person 3			
Exam III	1.80	2.13	2.27
Exam IV	1.67	2.27	2.07
Person 4			
Exam III	2.00	2.00	2.00
Exam IV	1.87	2.20	1.93
Person 5			
Exam III	1.80	2.33	1.87
Exam IV	1.87	2.13	2.00
Person 6			
Exam III	1.67	2.13	2.20
Exam IV	1.87	2.00	2.13
Person 7			
Exam III	2.00	1.93	2.07
Exam IV	1.67	2.13	2.20
Person 8			
Exam III	1.73	1.87	2.40
Exam IV	1.60	1.93	2.47

Averages for the entire panel and for the individual evaluators are provided. Exam III: non vertically reversed images. Exam IV: vertically reversed images. The Friedman test was significant in both exams $p<0.001$. Additional Friedman pairwise comparisons were performed between adjacent ranks.

According to the growth-differentiation balance hypothesis, the formation of more complex compounds with higher molecular weight should be promoted by the growth conditions of organic and biodynamic management systems in the Geisenheim trial. In this respect, increased nitrogen is thought to delay maturation and thus the formation of sugars, amino acids, organic acids, and flavour and aroma compounds in grapes (Conde *et al.*, 2007). Furthermore, it is

well established that decreased nitrogen supply increases phenolic compounds (Keller and Hrazdina, 1998) and anthocyanin content in grapes (Keller and Hrazdina, 1998; Hilbert *et al.*, 2003; Soubeyrand *et al.*, 2014), likely due to increased light exposure (Mabrouk and Sinoquet, 1998; Keller and Hrazdina, 1998; Koyama *et al.*, 2012) and water stress (Conde *et al.*, 2007; Acevedo-Opazo *et al.*, 2010). The effect of water stress is partly related to smaller berries, because anthocyanins and other phenolic compounds accumulate in the skin (Coombe *et al.*, 1987). This is consistent with the present study, in which organic and biodynamic grapes were found to have smaller berries (Döring *et al.*, 2015; Meissner *et al.*, 2019). However, no studies on the secondary metabolites of the must from the Geisenheim experiment were performed. And as mentioned previously, in studies by Malusá *et al.* (2004), Nuñez-Delicado *et al.* (2005), Dani *et al.* (2007), Tassoni *et al.* (2013), Vian *et al.* (2006), a differentiation of organic and integrated cultivation with secondary plant metabolites was only partially possible.

3. Relations between management systems and sensory analysis

Sensory analysis of the wine from the long-term trial in Geisenheim showed that the biodynamic wine made in 2007 was of better quality than the organic and integrated wines, which did not differ. In 2006 and 2008, the sensory analysis of the wines showed no differences in quality between the management systems (Meissner, 2015). The 2009 vintage was not evaluated. In the sensory analysis studies by Ross *et al.* (2009) and Bigler *et al.* (2009), a differentiation of the organic and biodynamic management systems was possible, whereas a sensory differentiation was not possible in Parpinello *et al.* (2015). From these results the following question arises: how can the image-forming methods accurately group and classify cultivation methods, if this is only partially possible on the basis of chemical and sensory analyses?

4. Relationships between management systems, aging and image-forming methods

In addition to chemical measures of quality, the ability to form and maintain form, reflected in slower aging or decomposition, is an aspect of quality. The instalment of new management systems resulted in detectable changes in aging

velocity during storage (storage losses) of carrots (Wistinghausen, 1979; Samaras, 1978), cucumbers (Ahrens, 1991) and rocket (Athmann *et al.*, 2011; Andersen, 2019). The management systems were consistently ranked Integrated>Organic>Biodynamic in terms of aging velocity, on the basis of storage losses. The image forming methods used in the present study to evaluate grape juice also discerned the same ranking, based on shapes formed as the juice aged.

The major difference among these management systems lies in the growth habits. In the Geisenheim experiment, the organic and biodynamic management systems led to a reduction in vegetative growth, resulting in a more open canopy, lower compactness of grape clusters and thereby increased light exposure of grapes compared to the integrated management system (Meissner *et al.*, 2019). The ranking of the management systems in the main component analysis was the same as that in the present investigation of the image-creating methods: Biodynamic>Organic>Integrated. Furthermore, the grouping and classification of must samples according to the management systems of the Geisenheim experiment was not possible via chemical analyses alone. Together, these results endorsed image-forming methods as good indicators for changes in growth habits resulting from altered nutrition and water status. This conclusion was also supported by the analysis of must samples from the Geisenheim experiment from the harvest year 2006 (Fritz *et al.*, 2017).

Quality ranking by image forming methods has been related to growth conditions in terms of both decomposition (the present study) and ripening (e.g., Fritz *et al.*, 2011; Doesburg *et al.*, 2015). The terroir of a wine producing region is also characterised by its growth conditions. Terroir is difficult to study on a scientific basis because it is influenced by numerous interacting factors including climate, soil, cultivar and agricultural practices. The best expression of terroir is achieved when the precocity of the grapevine variety is suited to the local climatic conditions in such a way that full ripeness is reached by the end of the growing season (van Leeuwen and Seguin, 2006). It is plausible that the complex information ascertained from biocrystallisation, capillary dynamolysis and circular chromatography images reflect the complex property 'ripeness' more completely than chemical analyses alone, and may thus be a

valuable tool for grape and wine quality analysis. As hypothesised by Doesburg *et al.* (2015), Fritz *et al.* (2011) and Fritz *et al.* (2017), the reason for this high accuracy may be that, in contrast to chemical analyses, the structural features and forms evaluated by image forming methods are imprints of the whole sample; i.e., image forming methods reveal characteristics of the whole food matrix that are beyond the scope of compound-specific analyses (Kokornaczyk *et al.*, 2011; Busscher *et al.*, 2014).

5. Hypothesis on aging and shape formation in image-forming methods

It can be hypothesised that compounds with higher molecular weight may persist longer during decomposition than compounds of low molecular weight and may therefore play a supportive role in the maintenance of structure formation in image forming methods. For the polymer polyvinylpyrrolidone such a relationship was established: viscosity and branching of crystal needles in biocrystallisation images decreased with decreasing molecular weight of the polymer (Busscher *et al.*, 2014). Foods are much more complex, and a direct relationship between molecular weight, viscosity and pattern formation in image forming methods has not yet been established.

As indicated by image forming methods, biodynamically or organically produced fruit, vegetable or cereal samples maintain their ability to form crystal structures, thereby indicating slower decomposition than samples harvested from integrated or conventional production. The mechanisms of structure formation remain unknown. Whether the ability to maintain structure formation during juice aging is related to increased chemical differentiation with formation of more compounds of high molecular weight needs further investigation.

It is likely that image forming methods can detect quality differences in both grape juice and wine. Balzer-Graf and Balzer (1991) have shown that it is possible to detect quality differences in cabbage fermentation with capillary dynamolysis. In fact, biocrystallisation is already applied to assess wines and is increasingly demanded by winegrowers (Margaret Chappelle, personal communication). Therefore, wines from the Geisenheim field trial should also be examined using image forming methods to determine the degree to which quality

differences induced by the contrasting grape management systems can be detected after fermentation.

CONCLUSIONS

In four trial years, 31 out of 33 grape samples of different management origin were correctly grouped and classified via image forming methods. A quality ranking based on the strength of form expression in the images and on juice aging behaviour showed a clear hierarchy of Biodynamic>Organic>Integrated. This result is consistent with former quality rankings based on image forming methods and supports the assumption that different management systems influence plant physiology and the product quality of plant foods in a typical and reproducible manner. The evaluation approach realised in the current study adds a new dimension to grape quality research and may considerably advance our understanding of how different farming systems influence plant physiology and therefore product quality. Future research should be directed firstly towards the differentiation and classification of grapes after vinification depending on the management system, and secondly towards linking the results obtained with image forming methods to those from chemical analysis.

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