Grouping and classification of wheat from organic and conventional production systems by combining three image forming methods

Article *in* Biological Agriculture and Horticulture · September 2011

DOI: 10.1080/01448765.2011.648918

CITATIONS
26

READS
416

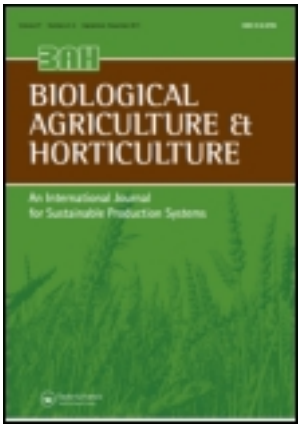
4 authors:

Jürgen Fritz
University of Bonn
25 PUBLICATIONS **158** CITATIONS
SEE PROFILE

Miriam Athmann
Universität Kassel
53 PUBLICATIONS **834** CITATIONS
SEE PROFILE

Timo Kautz
University of Bonn
78 PUBLICATIONS **2,107** CITATIONS
SEE PROFILE

Ulrich Köpke
University of Bonn
167 PUBLICATIONS **4,174** CITATIONS
SEE PROFILE



Biological Agriculture & Horticulture: An International Journal for Sustainable Production Systems

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbah20

Grouping and classification of wheat from organic and conventional production systems by combining three image forming methods

Jürgen Fritz [a], Miriam Athmann [a], Timo Kautz [a] & Ulrich Köpke [a]

[a] Institute of Organic Agriculture, University of Bonn, Katzenburgweg 3, D-53115, Bonn, Germany

Available online: 08 Feb 2012

To cite this article: Jürgen Fritz, Miriam Athmann, Timo Kautz & Ulrich Köpke (2011): Grouping and classification of wheat from organic and conventional production systems by combining three image forming methods, Biological Agriculture & Horticulture: An International Journal for Sustainable Production Systems, 27:3-4, 320-336

To link to this article: http://dx.doi.org/10.1080/01448765.2011.648918

Biological Agriculture & Horticulture
Vol. 27, Nos. 3–4, September–December 2011, 320–336

Taylor & Francis
Taylor & Francis Group

Grouping and classification of wheat from organic and conventional production systems by combining three image forming methods

Jürgen Fritz*, Miriam Athmann, Timo Kautz and Ulrich Köpke

Institute of Organic Agriculture, University of Bonn, Katzenburgweg 3, D-53115 Bonn, Germany

Using image forming methods, products from organic and conventional origin have been discriminated with high accuracy, but the meaning of these differences in terms of product quality is so far poorly understood. The aim of the presented study is therefore to gain further insights into the suitability of image forming methods for food quality evaluation based on wheat samples from a long-term field trial on the comparison of different organic and conventional production systems (DOC-trial). The images of the encoded samples were (1) grouped into pairs with similar image features, (2) characterized based on reference images (e.g. high resistance to degradation – low resistance to degradation), (3) ranked (according to the quality characterization), and (4) assigned to the different production methods (classified). The encoded samples from the production methods mineral fertilization, conventional production (combination of mineral fertilization and farmyard manure) and the class of organic production methods (biodynamic, bioorganic and unfertilized control) could be grouped and classified in both years. Within the class of organic production methods grouping (assigning of similar samples to image categories) and classification (assigning of categories to production methods) was partially possible. The correct grouping and classification of samples from organic and conventional production shows that different fertilization systems influence image structures in a typical and reproducible manner. The evaluation approach followed in the presented research can provide a considerable contribution to advance our understanding of quality differences between products from different farming systems or plant production measures.

Keywords: biocrystallization; food quality; image forming methods; production systems; wheat

Introduction

Authentication of plant foods from organic and conventional origin is still not solved satisfactorily (Siderer et al. 2005; Kahl et al. 2010). Due to their high accuracy in discriminating produce from organic and conventional origin, the image forming methods biocrystallization, capillary dynamolysis and circular chromatography are increasingly used in food quality evaluation (Mäder et al. 1993; Weibel et al. 2000; Mäder et al. 2007; Kahl et al. 2008). The methods are based on the evaluation of structures formed by the reaction of the food matrix with certain inorganic salts. Characteristic qualitative traits of the food, e.g. degree of freshness or ripening stage, result in typical and reproducible image structures. Therefore, samples can be characterized with respect to food quality by linking image structures to physiological processes such as maturation and aging (Keller 1997; Balzer-Graf and Balzer 1991).

*Corresponding author. Email: j.fritz@uni-bonn.de

ISSN 0144-8765 print/ISSN 2165-0616 online

http://dx.doi.org/10.1080/01448765.2011.648918
http://www.tandfonline.com

Using the three image forming methods, different crop samples from the DOC long-term experiment in Oberwil (Switzerland) have been investigated. In this trial, different conventional and organic cultivation methods have been compared in a crop rotation by using identical cultivars in a field plot design since 1978 (Mäder et al. 2002). Samples from this trial provide an excellent basis for authentication of new techniques of quality investigation such as the image forming methods.

Samples from the DOC-trial have been intensively investigated with respect to food quality (Mäder et al. 1993; Mäder et al. 2007; Langenkämper et al. 2006a, 2006b; Zörb et al. 2006). While for beetroot, with a cluster analysis comprising 12 chemical parameters, it was possible to group correctly the unfertilized control, the mineral control, the conventional system and both organic systems as separate groups (Mäder et al. 1993), detailed chemical analysis for wheat detected no clear differences between the cultivation methods (Mäder et al. 2007; Langenkämper et al. 2006a, 2006b; Zörb et al. 2006).

With image forming methods, combination of same/similar individual encoded samples of cultivation and fertilization treatments from the DOC-trial into groups (grouping) was possible with a high probability of correctness (Table 1). Classification (assignment of the groups to the encoded cultivation and fertilization methods) was possible with high accuracy in experimental years 1992 and 1993 with wheat and in 1987, 1990 and 1991 with beetroot (Mäder et al. 1993; Mäder et al. 2007).

In a study comparing apples from integrated and organically managed farms from five different locations in Switzerland, a sensory test, a maturation test, a feed decision test and investigations with three image forming methods were carried out in addition to chemical analysis (Weibel et al. 2000). Among all methods, the clearest differentiation between integrated and organically managed samples was observed with the image forming methods. Fresh and stored samples taken from organically managed farms had a 66% and 132% higher quality index respectively when compared with fresh and stored samples from integrated farms. The ten encoded samples were identified 100% correctly using the image forming methods with fresh as well as with stored samples.

Using three image forming methods on ten encoded carrot samples from five organically and five conventionally managed farms, the samples from organically managed farms were assigned the first five places in quality ranking, while the five samples with lower quality were those with conventional origin (Balzer-Graf 1994). This ranking was determined using

Table 1. Grouping of encoded samples from the various cultivation methods of the DOC trial with image forming methods; quality ranking decreasing from left to right.

Crop/year	Grouping				
Beetroot					
1987[a]	D1 = D2 = D3 = D4;		K1 = K2 = K3 = K4		
1990[b]	D1 = D2;	N1 = N2;	O1 = K1;	O2 = K2;	M1 = M2
1991[b]	D1 = D2;	N1 = N2;	O1 = O2;	K1 = K2;	M1 = M2
Wheat					
1992[b]	D1 = O1;	D2 = O2;	N1 = N2;	K1 = K2;	M1 = M2
1993[b]	D1 = D2;	N1 = N2;	O1 = O2;	M1 = M2;	K1 = K2

Note: D: biodynamic system, O: bioorganic system, N: unfertilized control with application of the biodynamic preparations, K: conventional system (mineral fertilizer and manure), M: mineral fertilization. Samples connected with ' = ' were graded as belonging to the same group, samples separated by ';' as belonging to different groups. [a]: Index 1–4: field replicates of the DOC trial, [b]: Index 1 and 2: double-bulked samples originating from four joint field replicates; according to Mäder et al. (1993) and Mäder et al. (2007).

two independent sets of encoded samples, those from the fall after harvest and those from the spring after a period of storage. Results from a side-by-side chemical analysis of the two sets of samples showed no great differences. In another study, three apple samples from the cultivation methods unfertilized, bioorganic and biodynamic, respectively, were analysed from a long-term trial. All nine encoded apple samples were analyzed using the three image forming methods and correctly assigned to the cultivation method, i.e., appropriately classified (Balzer-Graf et al. 1997).

The qualitative approach in image evaluation has been referred to as questionable from a scientific point of view (Siderer et al. 2005). There are basically two reasons for these doubts on scientific validity. First, the approach has not been properly tested for intersubjectivity as the above mentioned results were all achieved by the same laboratory. Second, the exact procedure of linking image structures to plant physiological processes and thus qualitative traits of the foods investigated is not evident from the publications mentioned above. The aim of the presented study was therefore to provide a detailed layout of the visual evaluation procedure used in image forming methods as a basis for further establishing these methods complementary to chemical analysis in food quality research. This objective was approached by reproducing grouping and classification of encoded DOC-wheat samples through characterization using the combined application of the three image forming methods. Linking this characterization to plant physiological processes by means of connecting the classification to reference images of different maturation and aging stages enables a thorough understanding of the quality evaluation with image forming methods.

Materials and methods

Ten wheat grain samples were taken from a long-term trial comparing different farming systems in Oberwil/Switzerland (so called DOC trial; Mäder et al. 2002) from harvest years 2000 and 2005 (sowing dates: 13 October 1999 and 4 November 2004, harvest dates: 21 July 2000 and 23 July 2005 at full maturity). The samples were each encoded by the Research Institute of Organic Agriculture in Switzerland and then analysed using the three image forming methods. The ten samples consisted of two samples each from five different cultivation methods: biodynamic, bioorganic, unfertilized, mineral and conventional (mineral fertilizer combined with manure). The analysed wheat varieties were Tamaro (harvest year 2000) and Titlis (harvest year 2005). The samples from harvest year 2000 were mixed samples of field replicates 1 and 2 and replicates 3 and 4, respectively. In harvest year 2005 the samples were mixed samples from the four replicates of the DOC trial. This means that in 2000 field heterogeneity was included in the investigation, while in 2005 it was not.

Prior to the analysis of the DOC-samples, series of reference images for the characterization of growth processes with variation of maturity stages (milk stage to full maturity) and aging were created as proposed by Balzer-Graf and Balzer (1991). Wheat cvs. Titlis, Aszita and Wiwa, harvest year 2004 at the maturation stages milk ripe, dough ripe and full ripe, were used for the maturation series; wheat cv. Titlis, harvest year 2005, harvested at full maturity, was used for the aging series. All wheat samples were produced according to the rules of biodynamic farming in Rheinau, Germany.

The grain kernels were ground using a hand grain mill with stone mill work (Schnitzer hand grain mill type ‘Country’) with a standard adjustment. For each method, series with different pre-treatment of the extract were prepared: For capillary dynamolysis and for biocrystallization, the meal was set with distilled water at 28°C for 3.5 h and 14 h,

respectively. Additionally, for harvest year 2005 aging series were carried out: The samples set with distilled water at 28°C for 3.5 h were afterwards stored for 2, 4, 6 and 8 days respectively at 8°C. For circular chromatography, the samples were set at 20°C with a 0.2% NaOH solution for 4 h. A sequence with varying extract concentrations was then constructed from every sample as described below. By varying both extraction times (at 28°C), aging times (at 8°C) and mixing ratios of extract and metal salts, the spectrum of images for the analysis of every sample was extended. For an overview of extract composition, extract setting times and reagent composition in the images see Table 2.

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 (Pfeiffer 1959; Bangert 1994). The filter papers were dried for 2–3 h after saturation. The extract solution migrated through 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. In diffuse daylight, not direct sun, the images developed to full colour formation in two days.

Capillary dynamolysis

In the first phase 0.6 ml extract, if necessary, diluted with distilled water (Table 2) were applied to standard sized chromatography paper (Schleicher & Schuell 2043A) in Kaelin dishes and left to rise (Balzer-Graf 1987; Zalecka 2006). In the second phase 0.7 ml of a 0.25% silver nitrate solution rose to 1 cm over the extract line. 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 chosen for the moment that the paper was dry and was therefore set for two hours at 20°C and 50% humidity.

Biocrystallization

For the crystallization method aqueous grain extract (Table 2) was filtered through Schleicher & Schuell No. 604 filters (Balzer-Graf and Balzer 1991; Pfeiffer 1931; Engquist 1970; Kahl 2006). Floatglass plates 2 mm thick and 10.5 × 10.5 cm were used. The glass plates were cleaned with 2% sodium carbonate solution and a small brush, then rinsed with hot water and sorted into frames, each containing 15 plates, for soaking baths. Subsequently, two soaking baths were carried out with chrome sulfuric acid (10 min each). The plates were then rinsed in six baths with clear Aqua bidest. Plexiglas rings with an inner diameter of 9 cm were mounted with paraffin on the glass plates. Extract and a 20% copper chloride solution (for mixing ratio see Table 2) were placed into this ring and crystallized in a crystallization chamber at 30°C with 50% humidity (Kahl 2006). In in-house methodical experiments, optimal crystallization time was found to be 12–15 h.

Analysis

The calibration procedure of visual image evaluation of the DOC-samples was as follows: First, in the reference images with varying stages of maturation and aging, characteristic changes in the image structures that occurred (1) with increasing maturation and (2) with increasing extract aging were described using the criteria listed by Balzer-Graf and Balzer (1991) and Huber et al. (2010). These comparison series were the basis for the

Table 2. Extract composition, extract setting times and reagent composition in the images.

		Extract			Setting time at			Per Image					
Method	Series	Wheat meal (g)	H_2O dist. (ml)	NaOH 0.2% (ml)	20°C	28°C	8°C	Extract (ml)	H_2O dist. (ml)	$AgNO_3$ 0.25% (ml)	$AgNO_3$ 0.50% (ml)	$FeSO_4$ 0.25% (ml)	$CuCl_2.2$ H_2O 20% (ml)
Circular	A	2.00	22.50	2.50	4 h			1.20			0.50		
chromatography	B	2.25	22.50	2.50	4 h			1.20			0.50		
Capillary	A	5.00	25.00			3.5 h	0 d	0.40	0.20	0.70		2.00	
dynamolysis	B	5.00	25.00			14 h	0 d	0.50	0.10	0.70		2.00	
	C	5.00	25.00			3.5 h	2 d	0.60		0.70		2.00	
	D	5.00	25.00			3.5 h	4 d						
	E	5.00	25.00			3.5 h	6 d						
	F	5.00	25.00			3.5 h	8 d						
Bio-	A	5.00	25.00			3.5 h		0.22	2.98				0.80
crystallization	B	5.00	25.00			14 h		0.24	2.96				0.80
	C	5.00	25.00			3.5 h	2 d	0.26	2.94				0.80
	D	5.00	25.00			3.5 h	4 d						
	E	5.00	25.00			3.5 h	6 d						
	F	5.00	25.00			3.5 h	8 d						

characterization of the generated images as 'mature – immature' and 'fresh – aged' by relating characteristic changes in the image structures, that were identified as being due to the cultivation system, to the typical structural changes brought about by maturation and extract aging.

This way, a qualitative assessment of the generated images could be made based on these characterizations. As a result of the qualitative assessment (a1) mature and (b1) fresh wheat was ranked higher than (a2) immature and (b2) aged wheat. Due to the different growth conditions in cultivation practices and related modified growth processes (Mäder et al. 2002; Athmann 2011), the encoded samples were assigned to cultivation methods (classification). In order to assess the samples for each harvest year, three series of experiments were carried out. Samples from harvest year 2000 were evaluated examining only 3.5 h and 14 h sets in 450 images. In samples from harvest year 2005, aging series of 2, 4, 6 and 8 days were additionally carried out, resulting in a total of 1300 images.

Statistical analysis

For statistical analysis, the agreement between correct grouping / classification and the grouping / classification based on the results of the image forming methods was tested. The test is based on a 5×5 contingency table, which compares a set of given categories to the ones determined in the investigation (Tables 4 and 5). For the grouping test, Fisher's Exact Test was carried out. For the classification test, the agreement was determined with the simple Kappa coefficient. The 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 was carried out using PROC FREQ in SAS (SAS Institute Inc., Cary, NC, USA), version 9.2.

Results and discussion

The visual evaluation procedure is explained based on two examples, one each from biocrystallization and capillary dynamolysis. Circular chromatography in this study did not contribute to the accurate discrimination and characterization of the samples.

Visual evaluation example: Crystallization images – aging

Slightly curved, tightly bundled needle strands were identified as characteristic for 3.5 h wheat samples through long-term empirical evidence from in-house experiments (example shown in Figure 1 left image). In the calibration aging series (Figure 1), it was found that with increasing deterioration of the sample, the needle bundles spread out progressively. In more advanced aging stages this led to loss of needle strands and formation of fuzzy structures.

In the DOC-samples investigated directly after 3.5 h extraction time from harvest year 2005 the needle bundles spread out increasingly in the following order: Biodynamic one group with Bioorganic $<$ Control $<$ Conventional $<$ Mineral (Figure 2).

In the images of the samples that had been aged for two days, the share of fuzzy needle structures in the centre was greater in the conventional and mineral treatments than in the bioorganic and biodynamic treatment (Figure 3).

Since fuzzy structures had already been identified as a sign of deterioration (see above), the conventional and mineral treatments were now characterized as deteriorating faster than the biodynamic and bioorganic treatments.

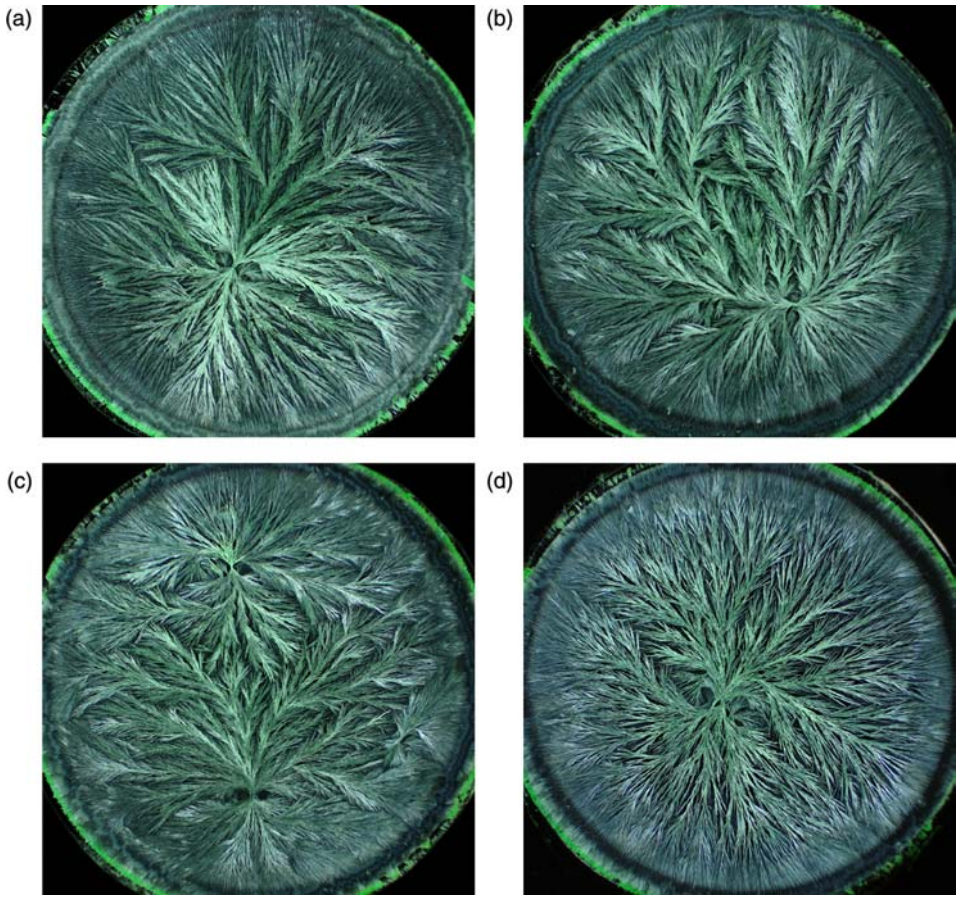


Figure 1. Reference series Deterioration: Biocrystallization of wheat samples cv. Titlis from Rheinau harvest year 2005, 72 mg wheat per image, extraction time 3.5 h at 28°C. From left to right: not aged, aged 3, 6 and 8 days at 8°C.

Visual evaluation example: Capillary dynamolysis – maturation

Two traits in the drop-like structures in the center of the images were identified as characteristics for maturation: (1) the colours became darker, and (2) white spots interspersed within the drop-like structures were reduced (Figure 4).

In the 14 h series on the samples from harvest year 2005, two groups were formed. The samples from the biodynamic, bioorganic and control treatments had darker drop structures with only a few white spots and stripes on the edge of the drop formation. The conventional and mineral samples had lighter drop structures with many white spots and stripes on the edges (Figure 5).

From characterization to quality assessment and classification

The DOC-samples characterized as ‘mature’ and ‘low age’ were ranked higher than those characterized as ‘immature’ and ‘advanced age’ in the qualitative assessment. For all cultivation treatments investigated except for the control treatment, structures indicating incomplete maturation in capillary dynamolysis and structures indicating advanced aging

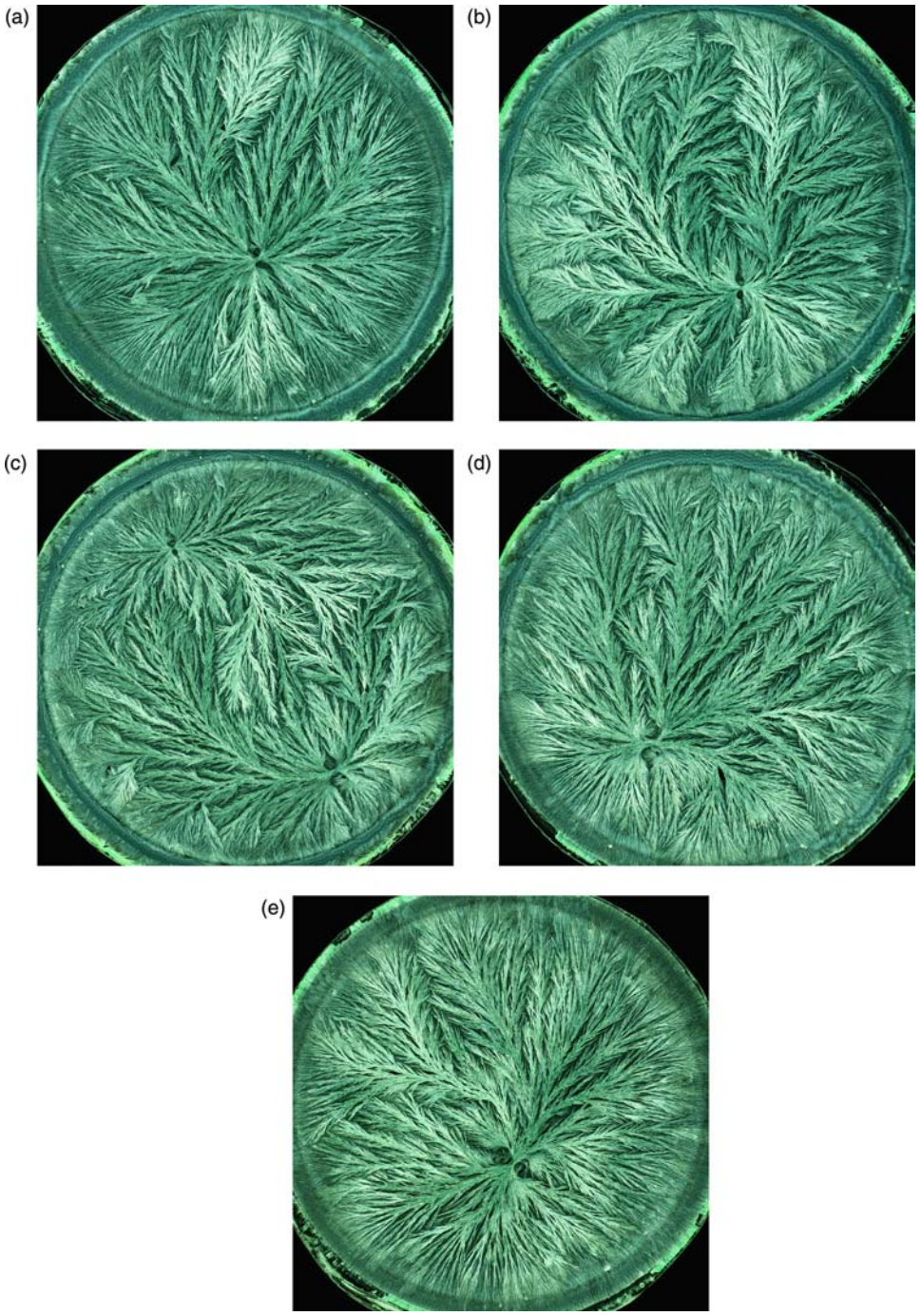


Figure 2. Biocrystallization of DOC-wheat samples cv. Titlis harvest year 2005, 72 mg wheat per image, extraction time 3.5 h at 28°C. From left to right: biodynamic system, bioorganic system, unfertilized control, conventional system, mineral fertilization.

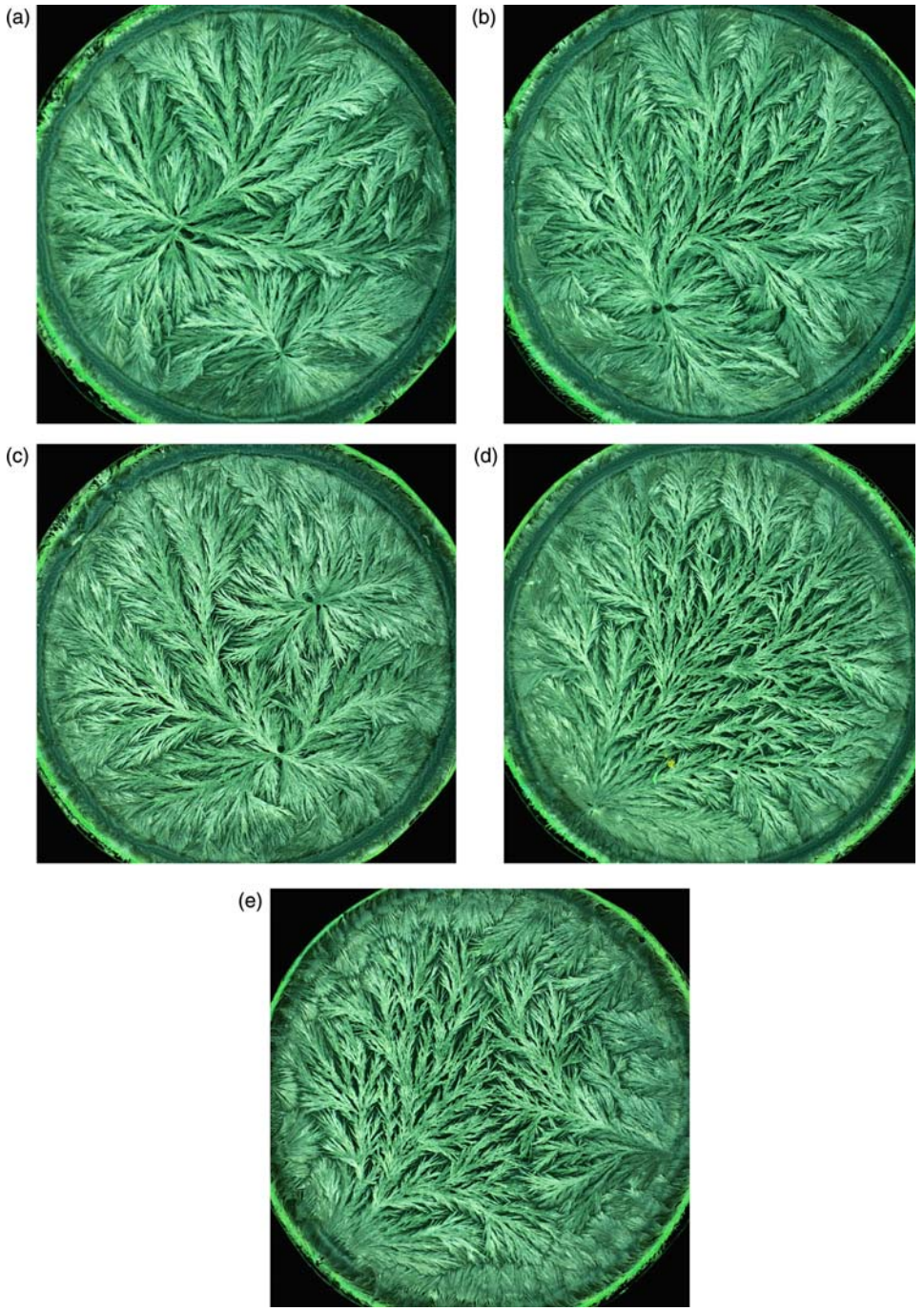


Figure 3. Biocrystallization of DOC-wheat samples cv. Titlis harvest year 2005, 72 mg wheat per image, extraction time 3.5 h at 28°C and aged for 2 days at 8°C. From left to right: biodynamic system, bioorganic system, unfertilized control, conventional system, mineral fertilization.

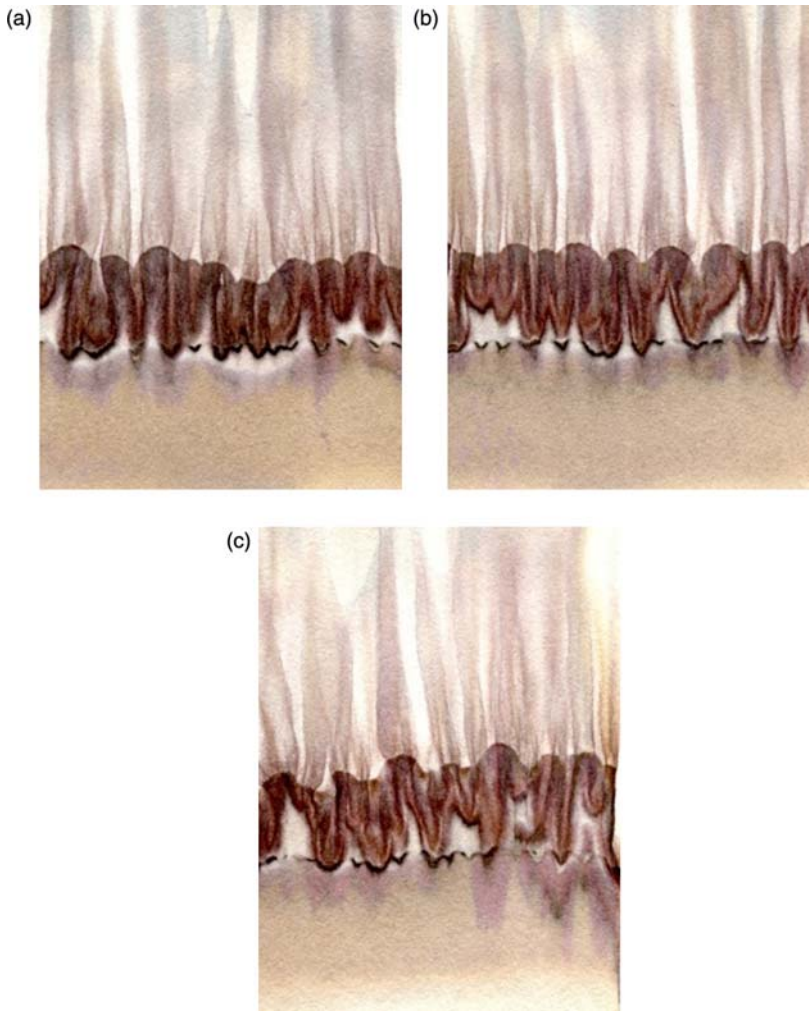


Figure 4. Reference series Ripening: Capillary dynamolysis wheat samples cv. Titlis harvest year 2004, mixing ratio 80 mg wheat per image, extraction time 14 h at 28°C. From left to right: full ripe stage, dough ripe stage, milk ripe stage.

in the biocrystallization images were pointing in the same direction, thus the quality ranking was clear-cut: There was a clear hierarchy from Biodynamic and Bioorganic > Conventional > Mineral.

The two samples from the control treatment showed especially dark and tapered drop forms in capillary dynamolysis (Figure 5). For these samples, a profound characterization was only possible by considering both the biocrystallization images and the images from capillary dynamolysis: While the dark and tapered drop forms in the latter indicated, on first sight, a high degree of maturation, in the biocrystallization images structures indicating aging were much more pronounced than in the samples which were later decoded as the biodynamic and the bioorganic samples. Therefore, for the two samples later identified as the control samples, the dark drop structures were interpreted as an indicator of premature ripening due to growth stress. Using these observations, final sample characterization led to the following ranking of the inner maturation status of the samples: Biodynamic and

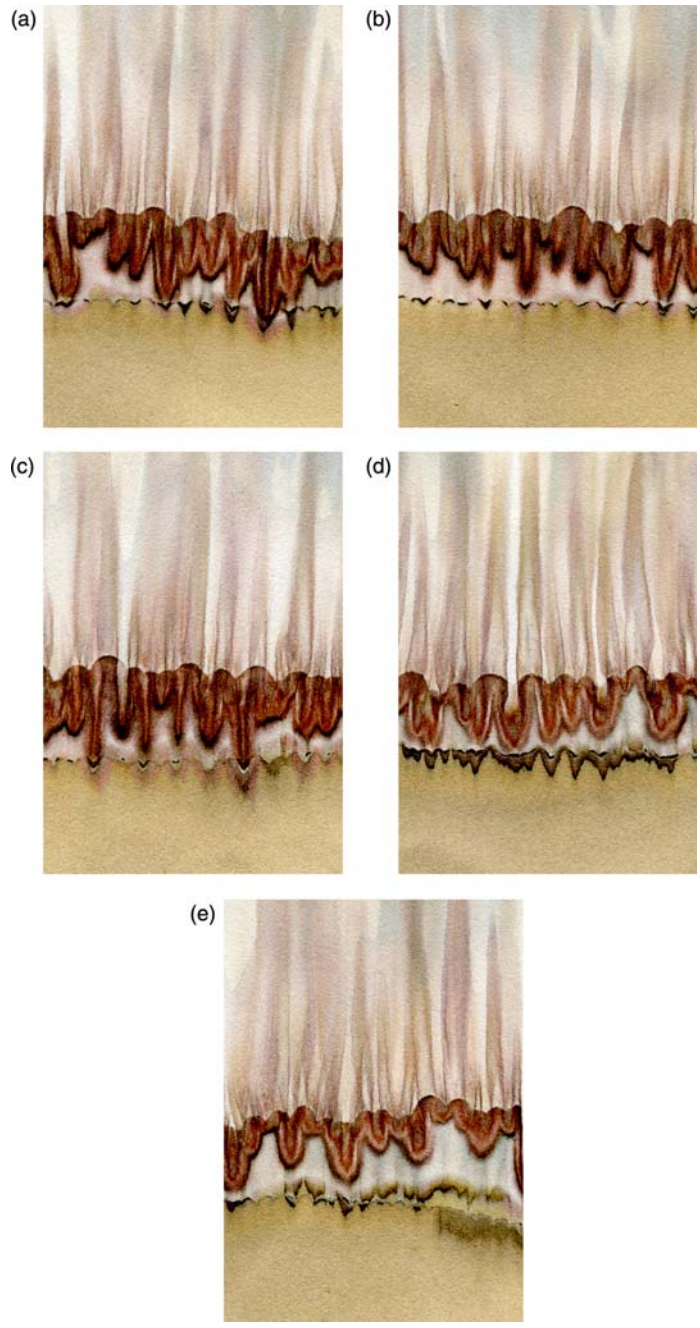


Figure 5. Capillary dynamolysis DOC-wheat samples cv. Titlis harvest year 2005, harvested at full maturity, 80 mg wheat per image, extraction time 14 h at 28°C. From left to right: biodynamic system, bioorganic system, unfertilized control, conventional system, mineral fertilization.

Bioorganic > Control > Conventional and Mineral. The quality ranking for wheat samples from 2005, based on these characterizations, is shown in Table 3. Here, the ranking decreases in quality from top to bottom.

Next it was examined whether the samples labelled using the characterization of growth processes could be assigned to the different production processes (classification). Due to the different growth conditions in cultivation practices, e.g. lower N supply and no mineral fertilizer in biodynamic and organic treatments (Mäder et al. 2002), and the associated modified growth processes, e.g. lower biomass production, earlier onset of maturation with manure as compared to mineral fertilization (Athmann 2011), the samples, which at this point in time were still encoded, were assigned to the respective cultivation method (Table 3). Decoding showed that the samples from the conventional and mineral treatments in both years were correctly grouped and classified into two groups, i.e. each sample was assigned to the group of cultivation method from which it came. Only samples within the organic cultivation method group were not correctly grouped and classified. In harvest year 2000, all samples were correctly grouped, but the samples were not correctly assigned to the cultivation method (classified). In the statistical test for harvest year 2000 grouping was significant, for harvest year 2005 grouping and classification were significant (Tables 4 and 5).

It should be noted that for harvest year 2000, grouping was 100% correct even though field heterogeneity was included since samples were not identical, but mixed samples from two field repetitions each. Likewise, Balzer-Graf (in Mäder et al. 1993) was able to group correctly beetroot samples from four different field repetitions (Table 1). These results support the hypothesis that those differences in image structures which are due to the cultivation method are more characteristic than possible influences of field heterogeneity.

On the other hand, classification was not significant for the samples from harvest year 2000. In this year, no aging series were carried out, i.e. there was no information on the resistance to aging of the samples when evaluating the images. Since the correct classification of the control treatment for the samples from harvest year 2005 was only

Table 3. Evaluation and quality ranking of DOC wheat samples 2005.

	Characterization[1]								
	Biocrystallization: Criteria indicating high resistance to deterioration			Capillary dynamolysis: Maturation criteria					
Sample no.	No spreading of needles 3.5h	No fuzzy needles after aging	No loss of needle strands after aging	High color intensity of droplets	No white spots interspersed	Droplet length	Quality ranking	Assignment to cultivation method	Correct cultivation method
1/10	++	++	++	+	++	+	1st	D/D	D/O
3/7	+	++	++	+	++	+	2nd	O/O	O/D
4/8	0	−	−	++	++	++	3rd	N/N	N/N
6/5	−	−−	−	−	−	−	4th	C/C	C/C
2/9	−−	−−	−−	−	−	−	5th	M/M	M/M

Note: ++: very strong expression, + strong expression, 0: medium expression, −: low expression, −−: very low expression.

[1] Circular chromatography not used for the quality characterization.

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

		Correct grouping						Correct grouping				
Harvest year 2000		G1	G2	G3	G4	G5	Harvest year 2005	G1	G2	G3	G4	G5
Grouping of encoded samples	G1	2	0	0	0	0	G1	1	1	0	0	0
	G2	0	2	0	0	0	G2	1	1	0	0	0
	G3	0	0	2	0	0	G3	0	0	2	0	0
	G4	0	0	0	2	0	G4	0	0	0	2	0
	G5	0	0	0	0	2	G5	0	0	0	0	2
Significance		$p = 0.001$						$p = 0.022$				

Note: G1–G5: sample group 1 to 5

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

		Correct grouping						Correct grouping				
Harvest year 2000		D	O	N	C	M	Harvest year 2005	D	O	N	C	M
Grouping of encoded samples	D	0	2	0	0	0	D	1	1	0	0	0
	O	0	0	2	0	0	O	1	1	0	0	0
	N	2	0	0	0	0	N	0	0	2	0	0
	C	0	0	0	2	0	C	0	0	0	2	0
	M	0	0	0	0	2	M	0	0	0	0	2
Significance		$p = 0.254$						$p = 0.0004$				

Note: D: biodynamic system, O: bioorganic system, N: unfertilized control with application of the biodynamic preparations, C: conventional system (mineral fertilizer and manure), M: mineral fertilization.

possible when considering this aging behavior, for further studies it is recommended to include aging series in the investigation.

The aging series showed clear differences in biocrystallization; in the maturation series, however, differences were more pronounced in capillary dynamolysis. Both aspects were needed for the correct organization of encoded samples into actual cultivation method groups (classification). This demonstrates the special value of combining several image forming methods in quality investigations. Circular chromatography in this case did not aid in characterizing and discriminating the samples. However, in other in-house investigations on wheat and on other plant foods this method showed very clear differences between the samples investigated. Therefore, the combined application of all three methods is recommended for the investigation of plant foods.

Evaluation requirements

A correct characterization of samples using information on growth processes gained from image forming methods demands both a high level of experience in image assessment and a wide knowledge of plant physiology. For a profound quality ranking, the image assessor has to be able to identify the relevant morphological changes in the images induced by a defined sample development, e.g. increasing drop length and color intensity with advanced ripening in capillary dynamolysis, increased spreading of needle bundles in biocrystallization with advanced aging, and to relate them to image changes

in the samples under investigation. As explained earlier, furthermore for the correct assignment of cultivation methods (classification), a thorough understanding of plant physiology and the effect of different plant production methods on plant growth and development is essential.

Visual versus computerized image analysis

As a reaction to the demand for standardized and objective methods to quantify and classify the morphological features of the image forming methods, a validation process and scope has been described for biocrystallization (Kahl 2006). The method is currently standardized for different foodstuffs, e.g. milk and milk products in Kahl et al. (2009) and carrots in Busscher et al. (2010). While image production follows generally the same procedure as described here (Kahl 2006; Zalecka et al. 2010), grouping of biocrystallization images is conducted not only visually, but by a procedure of computerized image analysis (Andersen et al. 1999). In these investigations, samples from different origins (feeding regimes, cultivation systems, cultivars) have been distinguished significantly. Wheat samples from the DOC-trial (harvest years 1999, 2002, 2003, 2005, 2006) could be separated into the group of conventional samples and the group of organic samples (Kahl et al. 2008). With respect to this study, it is remarkable that with parallel visual evaluation carried out for harvest years 2005 and 2006, the authors were able to classify all five cultivation methods for 2005 and three out of five cultivation methods for 2006, that is, with an accuracy similar to that achieved in the studies presented by Mäder et al. (2007) (Table 1) and in the results shown here (Table 2).

Computerized image analysis is a means to quantify the qualitative morphological crystal features. The method is based on the analysis of differences in image texture (grey-level differentiation). Up to now, differences on the structural level as applied in the visual evaluation approach described here are not included in the algorithm. According to the above mentioned results from Kahl et al. (2008), visual evaluation is still superior in discriminating samples from different origins. Furthermore, profound knowledge in visual image analysis is essential for the interpretation of differences detected with computerized texture analysis. Therefore, besides the continued elaboration of computerized image analysis, there are also efforts in further developing visual evaluation procedures. Validation and standardization procedures are underway for visual evaluation both for biocrystallization (Huber et al. 2010) and capillary dynamolysis (Zalecka et al. 2010). Here, the focus is on adapting existing norms for sensory analysis by validating a trained panel for the evaluation of the images with a defined set of morphological descriptors.

Image forming methods versus chemical analysis

Compared with chemical analysis of DOC-samples, the degree of differentiation obtained with image forming methods so far is striking. The reason for this high accuracy may be that in contrast to chemical analysis, the structures evaluated with the image forming methods are an imprint of the whole sample, i.e. characteristics of the whole food matrix otherwise lost in the analysis are included in the evaluation.

However, the complex information obtained in the images is accompanied by a loss of specificity compared with chemical analysis. While for singular chemical compounds, the function and the significance for human health is known, this significance can only be inferred from the complex characteristics indicated in the image structures such as

'maturity' or 'high resistance to deterioration'. Therefore, chemical analysis remains essential for product quality investigation. Image forming methods are seen as a complementary approach to chemical analysis. In future research with joint application of chemical analysis and image forming methods, the focus should be on elaborating a defined set of parameters from chemical analysis connected to ripening and deterioration to further elucidate the nature of the quality differences depicted by the image forming methods.

Conclusions

In both trial years a correct grouping and classification of (1) the pure mineral fertilized treatment, (2) the conventional cultivation method and (3) the organic cultivation methods was possible. This shows that a differentiated evaluation using the three image forming methods is possible, and the results do not depend on just one person. The quality ranking carried out in this experiment using the samples from cultivation treatments in 2005 is consistent with the quality ranking of cultivation treatments of wheat from harvest year 1992 published by Mäder et al. (2007; Table 1). This fact supports the assumption that different fertilization systems influence product quality of plant foods in a typical and reproducible manner.

In both years there was confusion in the assignment of the origin within the organic cultivation methods only. An error free arrangement of samples labelled with the organic cultivation practices compared with conventional and mineral practices, however, was possible.

Visual evaluation as applied in this study requires a high level of experience and is hard to standardize. In addition, image interpretation is very time consuming and laborious. Regarding possible future applications of the image forming methods in food quality investigations, it is unlikely that this approach can meet the demand for a rapid investigation tool for authentication of organic foods; here computerized image analysis provides considerable advantages in terms of rapidity and capability to be standardized. However, both for a thorough interpretation of the results based on physiological processes and for further methodical development of computerized image analysis, the capability for visual evaluation of the images remains essential. Also, since so far no procedure of computerized analysis for capillary dynamolysis and circular chromatography has been developed, the additional information gained by these methods can only be included in a quality evaluation when visually evaluated.

The evaluation approach followed in the presented research can provide a considerable contribution to advance our understanding of quality differences between products from different farming systems or plant production measures. Future research should be directed towards linking the results obtained with image forming methods (1) to established methods like chemical analysis, for a more thorough understanding of quality formation as a consequence of the plant physiological processes during growth and product development under defined environments and (2) to implications for human health.

Acknowledgements

The authors gratefully acknowledge funding from Software AG Foundation, Darmstadt, Germany. We thank Dr. Paul Mäder from the Research Institute for Organic Agriculture in Frick, Switzerland and the Swiss Federal Research Station Agroscope in Reckenholz, Switzerland, for providing the samples. Statistical analysis guidance by Prof. Dr. Hans-Peter Piepho, Bioinformatics department, Institute of Crop Science, Faculty of Agricultural Sciences, University of Hohenheim, and Stefan Mihalache, Institute of Mathematics, University of Cologne, is kindly appreciated.

References

Agresti A. 2002. Categorical data analysis. 2nd ed. Hoboken (NJ): John Wiley & Sons Inc.

Andersen JO, Henriksen CB, Laursen J, Nielsen AA. 1999. Computerised image analysis of biocrystallograms originating from agricultural products. Comput. Electron. Agr. 22:51–69.

Athmann M. 2011. Produktqualität von Salatrauke (*Eruca sativa* L.) und Weizen (*Triticum aestivum* L.): Einfluss von Einstrahlungsintensität, Stickstoffangebot, Düngungsart und Hornkieselapplikation auf Wachstum und Differenzierung [PhD thesis]. [Bonn(Germany)]: Bonn University.

Balzer-Graf U. 1987. Vitalaktivität von Nahrungsmitteln. Elemente der Naturwissenschaft. 46:69–92.

Balzer-Graf U. 1994. Die Qualität ökologisch erzeugter Produkte. In: Mayer J, Faul O, Ries M, Gerber A, Kärcher A, editors. Ökologischer Landbau – Perspektive für die Zukunft. Bad Dürkheim (Germany): Stiftung Ökologie und Landbau. pp. 261–290.

Balzer-Graf U, Balzer FM. 1991. Steigbild und Kupferchloridkristallisation – Spiegel der Vitalaktivität von Lebensmitteln. In: Meier-Ploeger A, Vogtmann H, editors. Lebensmittelqualität – ganzheitliche Methoden und Konzepte. 2nd ed. Karlsruhe (Germany): Verlag CF Müller. pp. 163–210.

Balzer-Graf U, Hoppe H, Straub M. 1997. Vergleichende Untersuchung zur Vitalqualität von Äpfeln aus ökologischem und biologisch-dynamischem Anbau. In: 8. Internationaler Erfahrungsaustausch über Forschungsergebnisse zum Ökologischen Obstbau, Beiträge zur Tagung vom 13.–14. 11.1997. Weinsberg (Germany): Fördergemeinschaft Ökologischer Obstbau.

Bangert D. 1994. Zu den naturwissenschaftlichen Grundlagen des Chroma-Boden-Tests als bildschaffender Methode – Empirische Untersuchungen und theoretische Deutungen. Barsinghausen (Germany): Verlag Edition Zukunft.

Busscher N, Kahl J, Andersen JO, Huber M, Mergardt G, Doesburg P, Paulsen M, Ploeger A. 2010. Standardization of the biocrystallization method for carrot samples. Biol. Agric. Hort. 27(1):1–23.

Engquist M. 1970. Gestaltkräfte des Lebendigen. Frankfurt a.M. (Germany): Vittorio Klostermann.

Huber M, Andersen JO, Kahl J, Busscher N, Doesburg P, Mergardt G, Kretschmer S, Zalecka A, Meelursan A, Ploeger A, Nierop D, van de Vijver L, Baars E. 2010. Standardization and validation of the visual evaluation of biocrystallizations. Biol. Agric. Hort. 27(1):25–40.

Kahl J. 2006. Entwicklung, in-house Validierung und Anwendung des ganzheitlichen Verfahrens Biokristallisation für die Unterscheidung von Weizen-, Möhren- und Apfelproben aus unterschiedlichem Anbau und Verarbeitungsschritten [Habilitation thesis]. [Kassel (Germany)]: Kassel University.

Kahl J, Busscher N, Doesburg P, Mergardt G, Huber M, Ploeger A. 2009. First tests of standardized biocrystallization on milk and milk products. Eur. Food Res. Technol. 229:175–178.

Kahl J, Busscher N, Mergardt G, Mäder P, Dubois D, Ploeger A. 2008. Authentication of organic wheat samples from a long-term trial using biocrystallization. In: Neuhoff D, Halberg N, Alföldi T, Lockeretz W, Thommen A, Rasmussen I, Hermansen J, Vaarst M, Lueck L, Caporali F, Hogh Jensen H, Migliorini P, Willer H, editors. Cultivating the future based on Science. Vol. 2. Livestock, socio-economy and cross disciplinary research in organic agriculture. Proceedings of the 2nd scientific conference of the International Society of Organic Agriculture Research (ISOFAR); 2008 Jun 18–20; Modena, Italy. Bonn (Germany): ISOFAR. pp. 742–745.

Kahl J, van der Burgt GJ, Kusche D, Bügel S, Busscher N, Hallmann E, Kretzschmar U, Ploeger A, Rembialkowska E, Huber M. 2010. Organic food claims in Europe. Food Technol. 3:38–46.

Keller ER. 1997. Bildschaffende Methoden. In: Keller ER, Hanus H, Heyland K-U, editors. Grundlagen der landwirtschaftlichen Pflanzenproduktion. Handbuch des Pflanzenbaues Bd. 1. Stuttgart (Germany): Ulmer. pp. 698–701.

Langenkämper G, Zörb C, Seifert M, Betsche T. 2006a. Mineralstoffkonzentration und Antioxidantien in Weizen aus ökologischem und konventionellem Anbau. Getreidetechnologie. 60:295–300.

Langenkämper G, Zörb C, Seifert M, Mäder P, Fretzdorff B, Betsche T. 2006b. Nutritional quality of organic and conventional wheat. J. Appl. Bot. Food Qual. 80:150–154.

Mäder P, Fliessbach A, Dubois D, Gunst L, Fried P, Niggli U. 2002. Soil fertility and biodiversity in organic farming. Science. 296:1694–1697.

Mäder P, Hahn D, Dubois D, Gunst L, Alföldi T, Bergmann H, Oehme M, Amadó R, Schneider H, Graf U, Velimirov A, Fließbach A, Niggli U. 2007. Wheat quality in organic and conventional farming: results of a 21 year field experiment. J. Sci. Food Agric. 87:1826–1835.

Mäder P, Pfiffner L, Niggli U, Balzer U, Balzer FM, Plochberger A, Velimirov A, Besson JM. 1993. Effect of three farming systems (bio-dynamic, bio-organic, conventional) on yield and quality of beetroot (*Beta vulgaris* L. var. *esculenta* L.) in a seven year crop rotation. Acta Hortic. 339:10–31.

Pfeiffer E. 1931. Studium von Formkräften an Kristallisationen mit besonderer Berücksichtigung landwirtschaftlicher Gesichtspunkte. Dornach (Germany): Naturwissenschaftliche Sektion am Goetheanum.

Pfeiffer E. 1959. Eine qualitative chromatographische Methode zur Bestimmung biologischer Werte. Lebendige Erde. 5:205–215.

Siderer Y, Maquet A, Anklam E. 2005. Need for research to support consumer confidence in the growing organic food market. Trends Food Sci Tech. 16:332–343.

Weibel F, Bickel R, Leuthold S, Alföldi T, Balzer-Graf U. 2000. Are organically grown apples tastier and healthier? A comparative study using conventional and alternative methods to measure fruit quality. Acta Hortic. 517:417–427.

Zalecka A. 2006. Entwicklung und Validierung der Steigbildmethode zur Differenzierung von ausgewählten Lebensmitteln aus verschiedenen Anbausystemen und Verarbeitungsprozessen [PhD thesis]. [Kassel (Germany)]: Kassel University.

Zalecka A, Kahl J, Doesburg P, Pyskow B, Huber M, Skjerbaek K, Ploeger A. 2010. Standardization of the steigbild method. Biol. Agric. Hort. 27(1):41–57.

Zörb C, Langenkämper G, Betsche T, Niehaus K, Barsch A. 2006. Metabolite profiling of wheat grains (*Triticum aestivum* L.) from organic and conventional agriculture. J. Agric. Food Chem. 54:8301–8306.