

Questions on the Validation of Holistic Methods of Testing Organic Food Quality

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ABSTRACT

The regulation of organic production and processing focuses on process-related quality concepts rather than product oriented quality management. Therefore no standards exist that define the product oriented quality aspects of organic food. Methods such as the biocrystallization method seem to be able to classify organic products. Methods that are applied must reflect the history of the sample in growth and processing. These methods can be applied either for authentication or in relation to technical aspects (storage, processing steps) as well as human health. Each quality determination needs a question which will be answered from the laboratory method. The question needs to be specific enough and the method needs validation. Validation means testing if the method can answer the question or not. The process of validation is defined for analytical methods (single compound determination) in international norms such as the ISO 17025 for testing laboratories. The article describes how validation procedures can be applied to biocrystallization methods for quality tests of organic products.

INTRODUCTION

The market for organic food is growing (Hamm *et al.*, 2004; Willer & Yussefi, 2006). More consumers are deciding in favour of organic produce because they believe that these goods are healthy and safe and that they contribute to a form of production that is good for the environment and society (BMVEL, 2004; Torjusen *et al.*, 2004; Siderer *et al.*, 2005). While it is not disputed that the production process of organic foodstuffs also meets process-oriented expectations, the product-oriented aspects are discussed (Tauscher *et al.*, 2003; Siderer *et al.*, 2005). Nevertheless, many consumers are prepared to pay a higher price for organic food, because they expect a 'plus' in quality compared

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with food from conventional production. The growth of the market depends on whether the consumers continue to value the quality of organic produce over that of conventional production (EU Commission, 2004). In order to be able to prove organic produce as such there have to be fixed indicators that identify a product as organic. A test of organic foodstuffs for authenticity requires indicators (or markers) of this sort. Results of some studies on the subject indicate that methods such as biocrystallization are particularly suitable for this question (Meier-Ploeger & Vogtmann, 1991; Mäder *et al.*, 1993; Weibel *et al.*, 2000). Within the framework of the Federal German Programme for Organic Farming, several methods have been tested since 2002 for their ability to differentiate and classify samples from different treatments (Kahl *et al.*, 2003; Kahl *et al.*, 2007). All the methods investigated can be validated. Each method needs its own approach in testing method specific parameters and factors of influence. What can be defined as a standard validation procedure for all different methods is described in this article. Three resulting problems will be dealt with in summary in the following section, which are decisive for further research with these methods on the quality of organic produce: defining product quality, scientific study of methods and the process of validation.

THE QUALITY OF ORGANIC PRODUCTS

The claim of organic farming to produce healthy and authentic foodstuffs of high quality meets the expectations of the consumers (Torjusen *et al.*, 2004; Siderer *et al.*, 2005). More than 50% of the consumers surveyed in a study in Germany (BMVEL, 2004) gave the following reasons for buying organic produce: (1) animal protection, (2) health, (3) fair price for high quality, (4) freshness, (5) good taste and (6) avoidance of synthetic means of plant protection (pesticides).

Only 15% of those surveyed pay attention to the certification label. Most consumers believe that organic food is very important for the health of their children (58%), but for 54% the high price is a problem and 19% do not trust the labelling. The quality of these products, however, is defined by the production process (EC Directive 2092/91 and Directives of the Cultivation Associations). Methods that can be used to verify and demonstrate the result of the process quality in the product are important in order to be able to demonstrate the special product quality expected by the consumer. The consumer of organic produce assumes that they have a higher taste and health value, though this is still a matter of controversy (Clarke, 2001; van Mansveld, 2001; Reaganold *et al.*, 2001; Bourn & Prescott, 2002; Grindler-Pedersen *et al.*, 2003; Tauscher *et al.*, 2003; Finamore *et al.*, 2004), and at least expects a difference from conventionally produced foods that justifies the higher price. So those who sell organic food have started to present to their customers in a

more transparent way the special quality of their product by means of a more or less testable index (cf. www.natureandmore.com).

For quality assessment of organic produce the definition of food product quality is necessary. It has to correspond to the holistic system-oriented approach of organic farming. For this various concepts are presented (James, 1993; Woodward & Meier-Ploeger, 1999; Heaton, 2001). Product-related quality is perceived by the consumer as: (1) direct, visible properties (colour, appearance), (2) simple identifiable properties (smell, taste) and (3) properties which cannot be determined simply and directly (shelf-life, nutritional value) (Meltzer *et al.*, 1992).

The concept of quality has thus developed in recent decades from a product-oriented approach via a process-orientation to a consumer-oriented approach (Huyskens-Keil & Schreiner, 2003). This is evident in the development of quality management from the final control to total quality management (TQM), a holistic way of looking at processes (DGQ, 1992). Each actor in the food products chain, from farmer to consumer, thus has his own demands of quality. To give a generally accepted definition of quality of organic food products is not possible, since each actor has different demands and these demands change, e.g. with social developments and advanced scientific knowledge. Food products quality thus defines itself as the sum total of these demands and six important aspects of quality are definable: (1) authenticity, including labelling, (2) function, including shelf-life, (3) biology/physiology, including health, (4) nutrition, (5) sense (perception) and (6) ethics, including environment and social.

The definitions depend on the perspective from which these aspects are defined (Beck, 2000; Heaton 2001; Spiekermann & Schönberger, 2001; Schmid *et al.*, 2004). Thus, for example, the consideration of the nutrition-related quality of organic products has so far been based on the individual substance. Benchmarks for a balanced and healthy nutrition are thus formulated in relation to the individual substance, for example formulated from the German Society for Nutrition, DGE. The assessment of food products according to individual substances, which are analytically determined separately from their surrounding matrix (the product), is also clear in the definition of food products quality. According to regulation 178/2002 of the EC food products are “all substances or produce which are intended or likely to be consumed by human beings in processed, partially processed or unprocessed condition.”

Consideration of product-related, organic food product quality is complex, because in addition to the evaluation of individual substance concentration, the interaction of substances (cf. Whitworth, 2006) or the specification of newly discovered properties of the product (structural ability, spectral light storage capacity, physiological amino-acid status, sensory analysis, cf. Strube & Stolz, 2004) are required. More recent studies, however, show that a substance works in different ways, according to whether it is consumed with or without the

food product (cf., for example, Whitworth, 2006). The interaction of various substances in a food product is increasingly the subject of research, since health studies, on the basis of the application of individual substances, have shown no effects (Palozza & Krinsky, 1992; Truscott, 2001; Stanner *et al.*, 2003; Milde *et al.*, 2004; Trombino *et al.*, 2004; Bösch-Saadatmandi *et al.*, 2006). Additional, more holistic approaches to evaluation, which complement reductionistic considerations, require an extended concept of quality that goes beyond the definitions used, for example, in the German Food and Feed Products Law (LFGB) (Meier-Ploeger & Vogtmann, 1991; Meier-Ploeger 2001; Tauscher *et al.*, 2003). But organic agriculture does not have a suitable, holistic quality concept for product evaluation (Breda, 1973; Pettersson 1970; 1982; Huber *et al.*, 1991; Meier Ploeger *et al.*, 1991; Meier-Ploeger 1995; Schulz 2000; Meier-Ploeger 2001; Bloksma *et al.*, 2001a, 2001b; Velimirov 2003; Kahl *et al.*, 2005; Schmid *et al.* 2004). This problem has been described in the report Assessment of Food Products from Different Production Methods, submitted to the Senate Working Group by the BMVEL 2001 (Tauscher *et al.*, 2003) and provided with appropriate comments. The recommendations for further research range from studying secondary plant substances via the use of feed selection trials to the use of several holistic methods in an interdisciplinary synopsis. The principal goal is to distinguish organic from conventional produce (authentication).

Studies so far on differentiating cultivation systems have not been able to provide unequivocal answers (Heaton, 2001; Bourn & Prescott, 2002; Tauscher *et al.*, 2003; Siderer *et al.*, 2005). Measurements of individual substances have shown contradictory results. Only differences have been consistently shown with some other methods (Heaton, 2001; Alföldi *et al.*, 2006). It has been possible to show that organic products are less polluted than conventional ones, but positive quality characteristics, in the sense of, for example, the high quality (nutrition and vital quality) promised by IFOAM, cannot be demonstrated consistently. There is so far no product-oriented definition of organic foodstuffs. In order to distinguish organic from conventional food products there must be recognizable features that identify a product as organic. A test of organic food products for their authenticity requires such features (markers). It must be remembered that the regulations of the processes in organic agriculture are not uniform. Since there are different process guidelines, e.g. between the EU Regulation and standards of national cultivation associations, an overlapping of the scatter of features in a comparative, product-related quality assessment between cultivation systems is expected. It remains to be investigated whether the process-label guarantees a clear distinction also in the product. Organic farming is to be understood as a system. The result, the product with its 'biography' is influenced by several factors, such soil, climate, variety, fertilization and mulch regulation. The product-related quality registration has to represent the system, i.e. the result of an interplay of all factors. And this

is where the problem lies: the eco-label is also awarded to those who cultivate and harvest on poor soil an unsuitable variety with insufficient mulch as long as they conform to the requirements of the EU Regulation. The eco-seal is also awarded to those who produce according to EU Directives, but use processing technologies that intensively affect the product, e.g. extruders. A distinction according to cultivation systems is possible only if the system boundaries are defined. Only then will it be possible to estimate the scatter of the system and guarantee the product quality through testing (authentication).

HOLISTIC METHODS

Studies so far on how to distinguish between organic and conventional produce show holistic methods are most suitable for this purpose. These methods have so far not been validated. The results are the subject of controversial discussion among scientists (Meier-Ploeger & Vogtmann, 1991; Woese *et al.*, 1995; Alföldi *et al.*, 2001; Heaton, 2001; Tauscher *et al.*, 2003; Siderer *et al.*, 2005; Alföldi *et al.*, 2006). So that such methods can be used to evaluate organic products they have to be validated for the relevant problem.

Methods such as these have been used for decades, which either leave the product as intact as possible or represent it and are thus able to make holistic statements instead of analytical ones related to the individual substances. The innovation here lies in the possibility of showing the quality of the process in the product and in the process itself, because instead of analytical individual substances, organizational and classificational phenomena can be used to characterize products.

It is thus important to define the product-related quality of organic produce not only by the absence or reduced quantity of harmful substances (e.g. pesticides, nitrates), but to display evidence of the special nature claimed for the product. To determine the enjoyment value the help of the senses is enlisted. The perception of sensory features has become a standard procedure for describing a product through subjective sense perception (taste, appearance, texture etc.) in such a way that, by establishing marginal conditions, objective, reproducible and statistically evaluable results can be achieved (cf. ISO standards). The sensory analysis is a method that yields customer-oriented results, because the first perception of the product in the market is via the senses (Busch-Stockfisch, 2002; Flidner & Wilhelmi, 2003).

A number of analytical methods that register individual substances, e.g. vitamins and minerals are used to determine those ingredients that are able to make statements about plant health and its nutritional-physiological and/or health importance for human beings. Among these are the substance classes of the amino acids and proteins (N-metabolism) and of the secondary plant ingredients, various substance classes and their sum parameters as well as their

anti-oxidative potential. For this, recognized analytical standard procedures, e.g. high performance liquid chromatography, HPLC, with corresponding detectors are used. The holistic nature of these methods lies in the systemic interpretation of individual results with respect to the whole plant and human being.

The physiological amino-acid status (N-metabolism) makes it possible to determine the cultivation system used via the metabolism physiology of the plant. The determination of the physiological amino acid status is made by means of a product-specific combination of methods of various nitrogen and/or amino-acid analysis optimized for high precision. The method makes it possible to register substance changes caused by modifying growth and reproduction processes by various growth conditions in the internal metabolic processes of the plant. N-metabolism designates the totality of the metabolic products formed by the N-metabolism of a cultivated plant, which can be used to characterize the cultivated plant or its cultivation conditions. The method has proved effective so far with certain products for differentiation and identification (Stolz *et al.*, 2000, Kahl *et al.*, 2003).

Determination of secondary plant substances, e.g. the spectrum of the polyphenols and carotenoids and, among others, the sum of the yellow pigments, as well as the anti-oxidative potential are the important variables to estimate plant health (stress, immune system) and thus the holistic consideration of the plant. At the same time, knowledge of their importance for human health is increasing; concentration and form (structure, bonding) of the individual substance is decisive for the effect (Bennett *et al.*, 1994; Watzl & Leitzmann, 1999; Asami *et al.*, 2003). The methods have been used to differentiate food products from organic and conventional cultivation (Reaganold *et al.* 2001; Asami *et al.*, 2003; Alföldi *et al.*, 2006).

Near-Infra-Red Spectroscopy (NIR-Spectroscopy) in principle is between these analytical and holistic methods that register order and structure (see below). It is based on the principle of infra-red spectroscopy in a certain frequency range and yields quick results on individual ingredients, such as starch and dietary fibres. The method has been validated for many applications with individual substances, but so far has been used and interpreted exclusively analytically (VDLUFA, 2000) and has thus not been used as a holistic method that reflects a system. Whether and to what extent this is possible has so far not been studied.

Organic food quality research should include study methods that represent a sample or part of it, e.g. an extract, holistically. Thus, in addition to individual substances, properties of a food product are determined. This can happen either through the relationship to light, e.g. fluorescence-excitation spectroscopy, or organization and structure, e.g. biocrystallization, or redox processes, e.g. electro-chemistry. In all three cases the process is holistic, i.e. individual substances from the matrix (food product) are not identified separately.

The results of biocrystallization (Andersen *et al.*, 2001; Kahl *et al.*, 2003; Kahl, 2007; Kahl *et al.*, 2007) are more or less ordered and product-typical crystal patterns on a round glass dish, which are evaluated and interpreted according to given criteria. The method yields a direct ‘finger print’ of the product and offers also an interesting marketing tool in the form of its visual character. It is used for questions of cultivation, variety selection, storage and evaluation of processing steps.

Unlike thin layer chromatography, where the substance separation is highlighted, Steigbild is a structurally different process. The method has been used for decades for a holistic assessment of plant and foodstuffs quality (Balzer-Graf & Balzer, 1987, 1994). Very recently the Steigbild method has been developed and characterized for distinguishing samples of different treatment methods, especially cultivation systems. The results are documented in a dissertation (Zalecka, 2006).

Fluorescence-Excitation Spectroscopy (FES) examines, using unground samples (original matrix), data in the visible area of the spectrum, which by comparison with known specimens enables one to make inferences about the development status of the plant and/or product, which is distinguished in various ways according to the cultivation system. FES methods show a clear tendency to increased maturation of the plants in organic cultivation. The increased maturation is expressed, for example, as more fruit-typical development of apples or more seed-typical (increased dormancy) of seed produce such as wheat and rye (Strube & Stolz, 2004). With this method it has been possible to differentiate and also identify coded organic and conventional samples (Strube 1996; Strube & Stolz, 1999, 2000).

In electro-chemical measurements three electro-chemical characteristics, pH value, conductivity/electrical resistance and redox potential, are measured in an aqueous environment and the results combined to make a total result. Special importance is attributed to the redox potential, because it is supposed to characterize the reduction capacity of a food product (Hoffmann, 1997). The methods have been used for various problems on a series of products. These methods, biocrystallization, Steigbild, fluorescence-excitation spectroscopy and electro-chemical measurements as a combination, have not yet been validated and so are not scientifically recognized and are commercially of no use.

VALIDATION

Validation, from the Latin *validus*, meaning ‘strong, effective’, in this context means a test of the effectiveness of a scientific method or result achieved by a scientific method. The validation of laboratory methods to register aspects of the food product quality means testing whether the method is suitable for answering questions (Kromidas, 2000). In the concept standard DIN EN ISO

8402:1995 it says: “Confirmation by examining and making available proofs that the special requirements for a special intended use.” A proof is information “whose correctness can be proved, and is based on facts that have been gained by observation, measurement or other examination methods.”

Since March 2000 there has been a European Standard ‘General Competence Requirements of Testing and Calibration Laboratories’ ISO 17025:2000, which stipulates the general competence to carry out tests and/or calibrations, including sampling. It is thus principally a matter of quality assurance in labs that pass on their results to third parties. In section 5.4.5 ‘Validation of Methods’, it is stipulated that the laboratory has to validate methods not stipulated in normative documents and methods developed in the laboratory in order to confirm that the methods are suitable for the use intended. The customer thus determines these requirements and with them the scope. To determine the process features, instruments are named which are to be used either alone or in combination. They are as follows: (1) systematic assessment of the factors influencing the result (characterization of the method), (2) calibration using reference standards or material, (3) comparison of results achieved with other methods, (4) inter-laboratory comparisons, and (5) assessment of the uncertainty of the results based on scientific understanding of the theoretical principles of the method and practical experience.

A method is a part of the process concerned with a particular problem/sample. For example, the determination of carotenoids is a process and high performance liquid chromatography (HPLC) a separating method within this process. With laboratory methods, recognition and avoidance of systematic errors (testing and correctness) and the results scatter (result uncertainty) are in the foreground. For each new sample type, which here means the agriculturally unprocessed or processed product, e.g. wheat, carrots, apples, carrot juice, and for each new problem, suitable methods have to be validated, even if the process, including HPLC, as such has already been validated. The scope of a validation is determined by the problem. Thus, three types of problem can be named: (1) A yes/no needs a qualitative process, in which it is a matter of whether a sample feature is present or not, (2) a comparison with limit values, and (3) a process control (monitoring). Methods used for investigating food products have been classified by the Codex Alimentarius Commission, Codex Committee on Methods of Analysis and Sampling into four types (ALINORM 01/23 or rev. versions CL 2005/44-MAS) (Codex Alimentarius Commission, 2001, 2005).

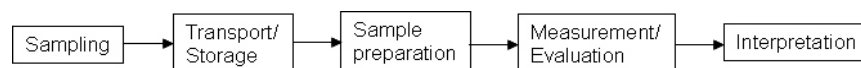


FIGURE 1. The different steps which a sample undergoes from the field to the reported result.

How a validation procedure can be transferred from analytical to holistic methods has been demonstrated by Kahl (2007). This is described in the following using the example of biocrystallization (Andersen 2001; Kahl *et al.*, 2003). Instead of the concept measurement the concept crystallization has been introduced. The method is validated for a nominal or ordinal distinction of samples and thus falls outside the type designations defined for chemical methods by the Codex Alimentarius Commission. It is supposed for evaluation that the method shows the influence of a sample on the crystallization pattern. A crystallization pattern or, for short, pattern is thus the residue of a mixture from samples solution and CuCl_2 crystallized on the glass dish. The 'crystallization' step of the process is thus understood as the 'black box' for the validation. There is no calibration standard in the sense of the EU Directive 96/23/EG. A homogenized material is used, which is permanently usable as standard material and yields patterns comparable with those of the samples examined. Laboratory comparative studies can be carried out with the method, though the concept 'analytical' in describing the effectiveness should be omitted. The laboratory suitability can be tested by means of the differentiation ability of the method to distinguish significantly between two samples of different types. This is a defined process feature for biocrystallization, which cannot be compared with the selectivity/specificity defined for analytical methods. The precision of the method can also be studied for the variables of the texture analysis (Kahl, 2007). Repeatability, reproducibility between laboratories and laboratory-internal reproducibility precision (factors researchers, day, chamber) can be studied. Calculation of a variation coefficient is not possible because of the missing zero point. The robustness of the method can be examined with the fourth instrument of ISO 17025 or the second characterization method according to Kromidas (2000). In this way one can speak only of 'method', not 'analysis method' and analytes can be replaced by samples solution or chamber solution.

The sample preparation results in an aqueous solution, which is the juice of carrots and apples or the extract of wheat and maize samples. The chamber solution is the mixture of sample solution and CuCl_2 , which is pipetted on to the dishes for crystallization in the chamber. The substitution is also true of all other definitions of the process features listed above. In addition to the process features of analytical methods, two factors examined as influence variables have to be able to be defined as process features specific to biocrystallization. These are the mixing ratio of sample solution and CuCl_2 and the region of interest of the scanned patterns for the evaluation with the texture analysis. If one speaks of matrix of different mixing ratios, one does not mean the samples matrix but the arrangement of different quantities of sample per dish with the same amount of CuCl_2 , described as sample vector and different quantities of CuCl_2 per dish with the same amount of sample, the CuCl_2 vector. The quantity of CuCl_2 per dish is related to the weighed amount of

CuCl₂, the quantity of sample in carrot and apple samples to the quantity of juice weighed for the chamber solution, in wheat to the quantity of meal weighed for the extraction.

For validation of holistic methods, various individual processes are validated in a laboratory, dealing with prototypes. One can speak here of a single laboratory validation. For the analytical methods, where only the results can be evaluated holistically, the accuracy of the method can be tested relating to the individual substance through participation in ring trials or with a reference material. Methods that work holistically (biocrystallization, fluorescence-excitation spectroscopy, sensory analysis) to generate results cannot do this. They work comparatively. A reference material for the sample does not yet exist. In each case a method must show that it determines the quality aspect that it claims to determine. According to Siderer *et al.* (2005) scientifically testable concepts are necessary here. This is not yet the case for methods such as biocrystallization or fluorescence-excitation spectroscopy. Concepts like 'vitality', so called in the IFOAM for the processing, or used for the interpretation of some holistic methods (Siderer *et al.*, 2005), are not scientifically described or tested.

CONCLUSIONS

(1) Complex interest areas of the various actors in the organic market have to be broken down into concrete and validated issues. (2) So the quality of organic products has to be defined as determinable (provable or measurable) aspects. (3) The samples on which questions about individual aspects have to be examined must be representative. This is the case not only for the samples quantity, but also the conclusions of the results for norms and standards (e.g. EU Regulations versus national standards). (4) Holistic methods have to show if they yield a 'plus' in information compared with analytical methods. (5) This 'plus' has to be able to be presented as scientifically investigable and make a relationship to the quality aspects possible. (6) Holistic methods have to be validated for new issues and according to product. (7) Scope and depth of the validation are determined according to the question, the customer and the method. (8) To guarantee the comparability of various studies with each other appropriate reference materials have to be developed and used for holistic methods.

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