ELM FARM RESEARCH CENTRE

ELM FARM RESEARCH CENTRE CONFERENCE

DOES ORGANIC FOOD HAVE AN 'EXTRA QUALITY'?
New Research, New Perspectives and New Insights

A record of the Conference held on TUESDAY, 23RD NOVEMBER 2004

This Conference was sponsored by Sheepdrove Trust in collaboration with

FQH (International Network for Food Quality and Health)
Sustain (the alliance for better food and farming)

ELM FARM RESEARCH CENTRE
The Organic Research Centre
'For organic principles and best practice'

HAMSTEAD MARSHALL
NEWBURY
BERKSHIRE, UK
RG20 0HR

TEL: +44 (0)1488 658298
FAX: +44 (0)1488 658503
E-MAIL: elmfarm@efrc.com

www.efrc.com
www.organicresearchcentre.com

PROGRESSIVE FARMING TRUST LTD
t/a Elm Farm Research Centre
.
Registered Charity No. 281276

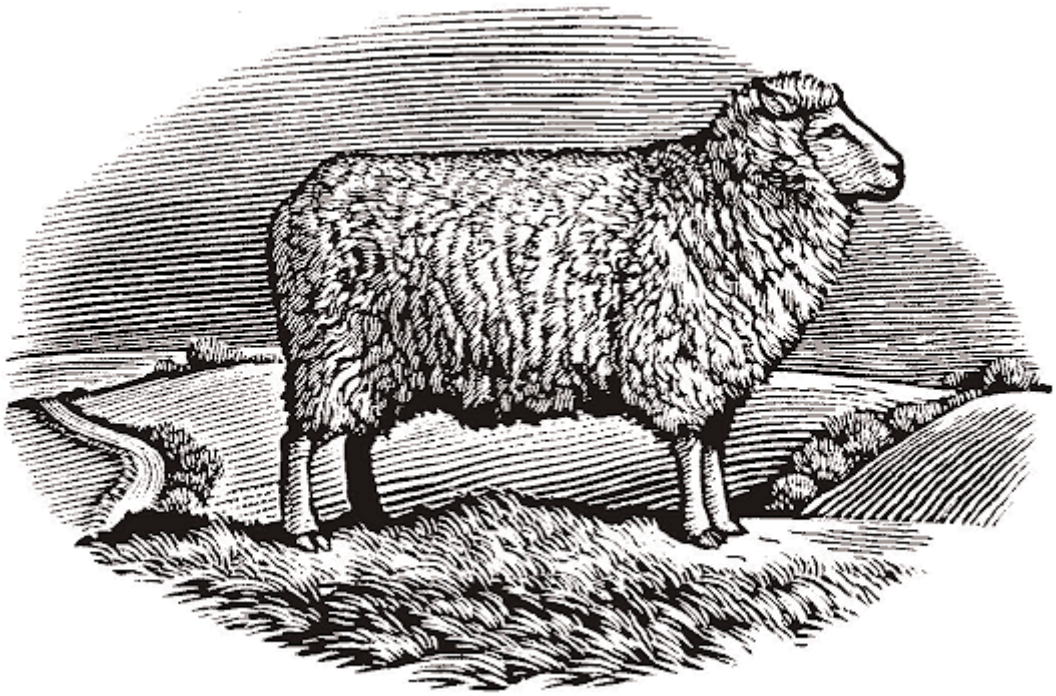
Published April 2005

For further information please contact the Director, Elm Farm Research Centre.

ACKNOWLEDGEMENTS

A special acknowledgement

We acknowledge and thank the Sheepdrove Trust for its financial support towards this Conference which allowed the use of the beautiful setting of the Kindersley Centre and its excellent facilities



ACKNOWLEDGEMENTS

Acknowledgements

We would like to thank **all** those who participated in the Conference on 23rd November 2004 - those who gave presentations, those who chaired, those who responded and those who participated in asking questions and the discussions.

We also thank the team that organised the event so effectively.

The production of these proceedings has been supported by:

promar International

"Stressing the importance of differentiating between accepted dogma: "Organic food is better for you" and what is actually "true", i.e. the scientifically proven, Dr Brandt's interesting and balanced paper highlighted the need for a consistent approach and common understanding if claims about organic food are to be accepted.

The science that proves the "extra qualities" of organic food, or equally that demonstrates the detrimental effects of "conventionally-produced" foods, on our health is still developing, as shown by other speakers. But Dr Brandt concluded that organic farming, which has distinct benefits for the environment and food produced, has that "extra quality" that was the Conference's theme.

For consumers, the key benefit of organic produce may simply derive from the fact that positive choices are made in food purchasing that enhance a sense of individual value and well-being".

Alara Wholefoods

"Projects that give statistically robust nutritional differentiation between organic and non-organic food are very welcome by organic food manufacturers".

Duchy Home Farm

CHARACTERISATION OF THE BIOCRYSTALLIZATION METHOD USING COMPUTERISED IMAGE ANALYSIS

Dr Johannes Kahl

I will try to explain something about the biocrystallization method and I will start by saying that we started 2 years ago from scratch so we had nothing which has built up in the labs in Kassel. We build up a platform with other institutes ready for measuring intermediate precision and reproducibility.

Overview

1. **Why characterization of the Biocrystallization method?**
2. **Description of the Biocrystallization method**
3. **Results from measurements on coded wheat and carrot samples**
4. **Characterization results**
5. **Conclusions**

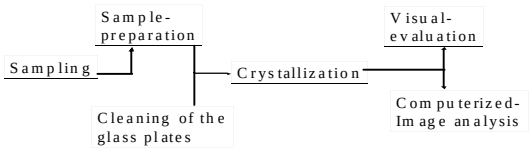
1. Why characterization of the Biocrystallization method?

- **Organic produce is a systemic approach**
- **Analytical methods detect single substances**
- **Biocrystallization method is systemic and makes properties visible**
- **The method has to be validated**

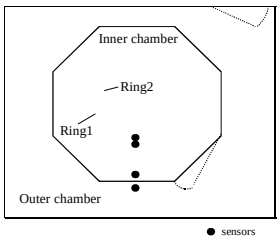
We call our method biocrystallization although it is also known as a copper chloride crystallisaton or just crystallization. I will talk about the characterisation of the biocrystallization method and look at how can we use such a method in analytical science and therefore I start with a question - why we characterise the biocrystallization method? and then I will describe the method.

Description of the Biocrystallization method

Procedure



Crystallization chamber



Why shall a method be characterised or why a method is introduced? Why should we use biocrystallization as the method because we have enough methods already available?

I think we refer to what Lawrence Woodward said earlier, that organic produce is a systemic approach and now we are looking for the analysis of the food and we apply analytical methods so we try to separate single compounds from the matrix. Why do we not introduce methods which are also systemic? The biocrystallization is a systemic approach but the method has to be validated and so validation in terms of the ISO 70 025 means to see if a method is fit for the purpose so can we re-apply the method for a question. We need a question before we start the validation process. But I don't want to talk about the validation but the requirements for validation and this is that we have to document the method or the procedures to come to a standardised operation procedure. Then we have to standardise the whole thing so that we can transfer the method to other places and next we have to make a statistical evaluation of the results.

What is the method about? Here you see a crystallogram.

It is simply that we mix a copper chloride solution together with a plant extract or juice and then we put this on a glass plate and let the water evaporate. Then we get a certain residue of copper chloride crystals and there is an overall ramification showing a structure and a texture, starting from the centre and then going to the outside. The interesting thing is that we cannot easily connect the macroscopic structure over 9 cm on the plate with the microscopic properties of the copper chloride.

CHARACTERISATION OF THE BIOCRYSTALLIZATION METHOD USING COMPUTERISED IMAGE ANALYSIS

Dr Johannes Kahl

That is a very important phenomenon. So we are dealing with crystal patterns and want to know if we can use those crystal patterns to get information about the sample.

When we start we say OK let's take the laboratory method as a normal analytical method for routine analysis and we have several steps to fulfil. We have the sampling, then of course the sample preparation because we need to have a juice or an extract. Then we have some preconditioned steps like cleaning the glass plates and then instead of a HPLC or gaschromotography and mass spectrometry or something like that, we have this crystallization step.

Then we have two different methods to evaluate the patterns. First the visual evaluation technique which was applied over decades and we try to standardise this in that we transfer the knowledge from sensory analysis to the visual evaluation of the patterns, so we just apply morphological criteria and we can come to the statistics of it. Here I want to focus on the second approach, the computerised image analysis because this evaluation method gives us the opportunity to deal with a large amount of patterns.

The crystallization unit is in a chamber and we have two different rings and on these rings there are the glass plates during evaporation and crystallization. It is very important that we control the whole system because the most variation is coming from the crystallization step.

This is the crystallization unit so we build just another chamber around to avoid air turbulence during the evaporation of the water and you see the glass plates on the two different rings 43 per each run and there are sensors to measure and control relevant humidity and temperature in different places inside and out of the chamber.

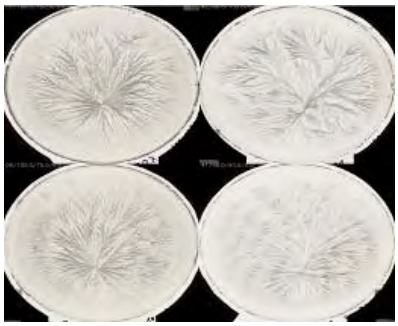
Crystallization chamber

The next slide shows the patterns - on the left side are carrots and on the right wheat.

Carrot

100mg substance
100mg CuCl2

100mg substance
75mg CuCl2



Wheat

90mg substance
135mg CuCl2

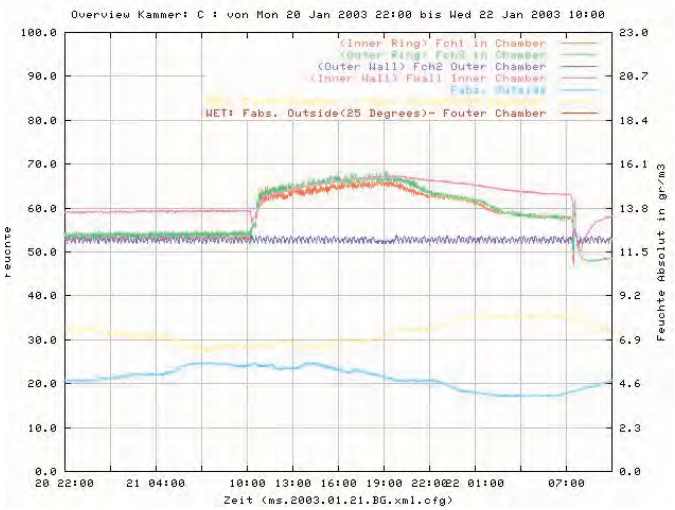
90mg substance
90mg CuCl2

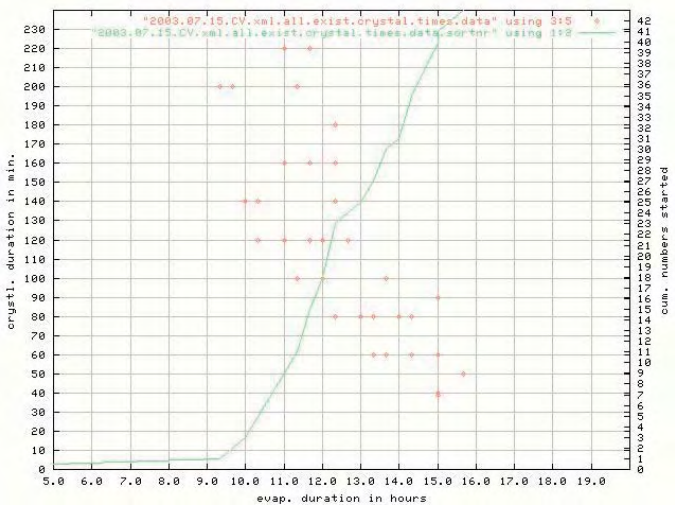
We started to test different mixing ratio of copper chloride and the extracts. You see that there is an influence of the mixing ratio so when we are thinking about characterising the method the climate conditions inside the chamber and the mixing ratio are important factors of influence.

CHARACTERISATION OF THE BIOCRYSTALLIZATION METHOD USING COMPUTERISED IMAGE ANALYSIS

Dr Johannes Kahl

Evaporation and crystallization process





On the left side, you will see on the y axis the relative humidity. The green and red lines are the relative humidity above the plates and, of course, when we pipette the solution in the plates then water starts to evaporate and relative humidity is increasing. It was very important for us to stabilise the whole system and standardise it because in former times people reported that they could reflect the year in that they get summer and winter pictures but this was just due to the relative humidity and after standardising we get the same pictures over the whole year.

On the right side you will see something we cannot standardise because that is random. On the y x axis you see the plates already started with the crystallization as the accumulative number and on the x axis you see the time after pipetting. That means that after about 8 or 9 hours after pipetting the solution the first plate of the 43 starts to crystallise. And after 16 or 17 hours the last, so that means we have a variation inside the chamber just to the random start of the crystallization. Therefore, we introduce a standard control over the year and developed a standard and we use this standard for every chamber to control the whole system.

The different evaluation approaches - we have visual evaluation and we make a methodology out of it by just looking at other methods like the sensory analysis and the computer based image analysis we have two different approaches as structure analysis, but that is very sensitive and the much more robust method of texture analysis and we are working with a grey level distribution, just looking at different patterns.

Evaluation: different approaches

Methodological approach for the evaluation of the patterns

Visual evaluation

Description according to morphological criteria (method adapted from ISO 11035:1994 and developed in the triangle)

Computer based image analysis

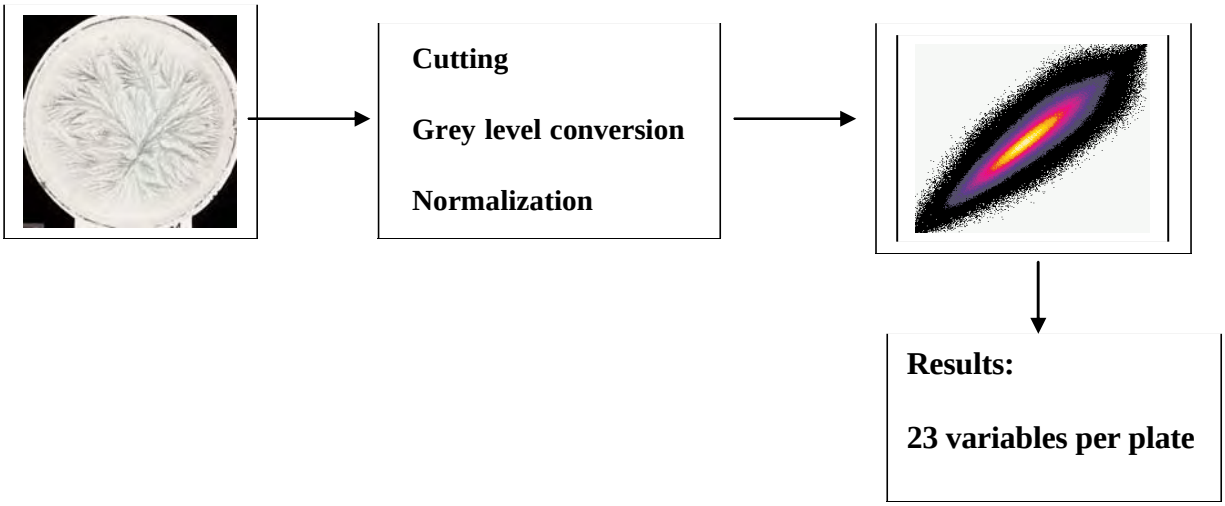
Texture analysis (grey level distribution and GLCM)

Structure analysis

CHARACTERISATION OF THE BIOCRYSTALLIZATION METHOD USING COMPUTERISED IMAGE ANALYSIS

Dr Johannes Kahl

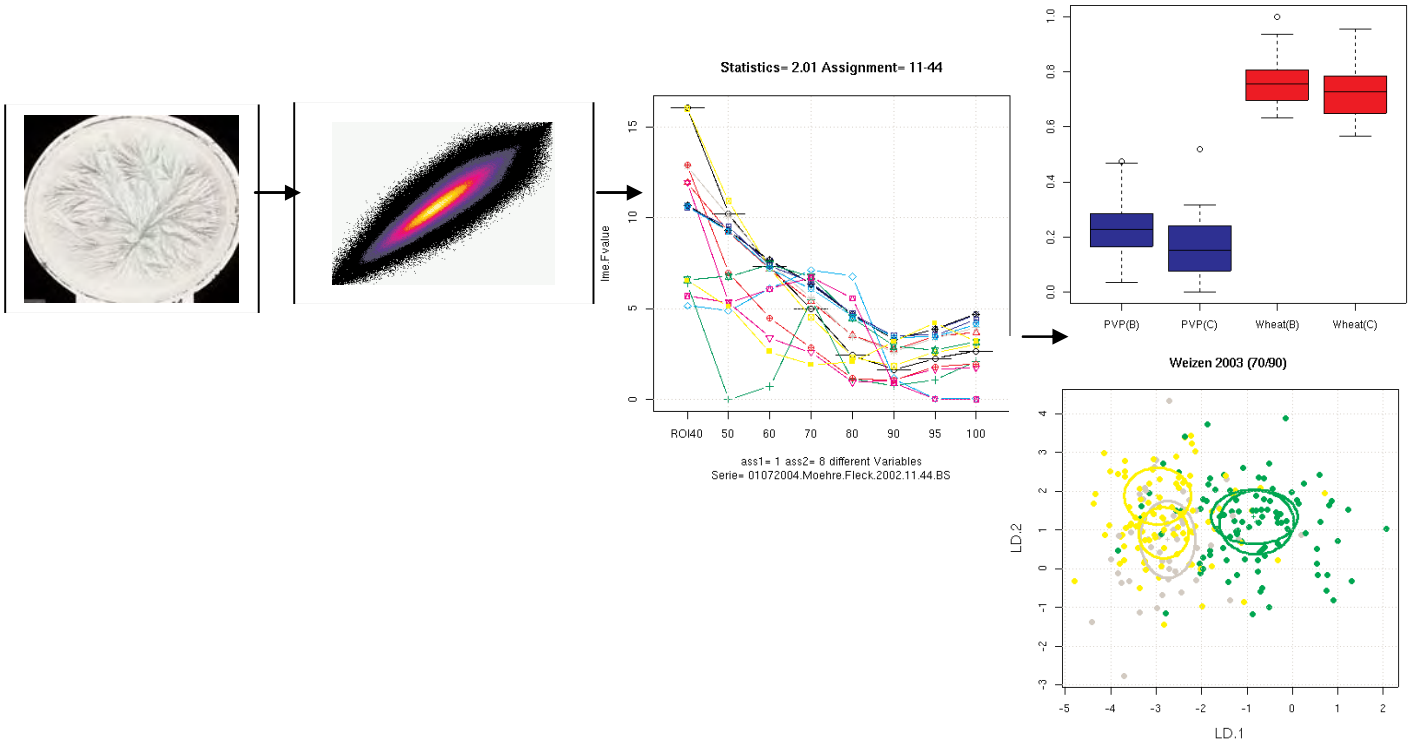
Evaluation: texture analysis



We just want to see can we differentiate the patterns due to the texture analysis results. What we do is we scan the pictures then we cut a part of it so we have a region of interest i.e. we can take the whole picture, 100%, or just the inner part, 10% or between, and we make a grey level conversion, a normalisation and then we have statistics first and second … and we get a result, several variables of this texture analysis programme is just looking for example at the nearest neighbours.

Our goal is to differentiate pictures so we are working with qualitative methods not quantitative. We have no absolute scale until now so we want to differentiate samples. We do this in that we create this single variable of the texture analysis and you will see on this diagram.

Differentiation of samples:



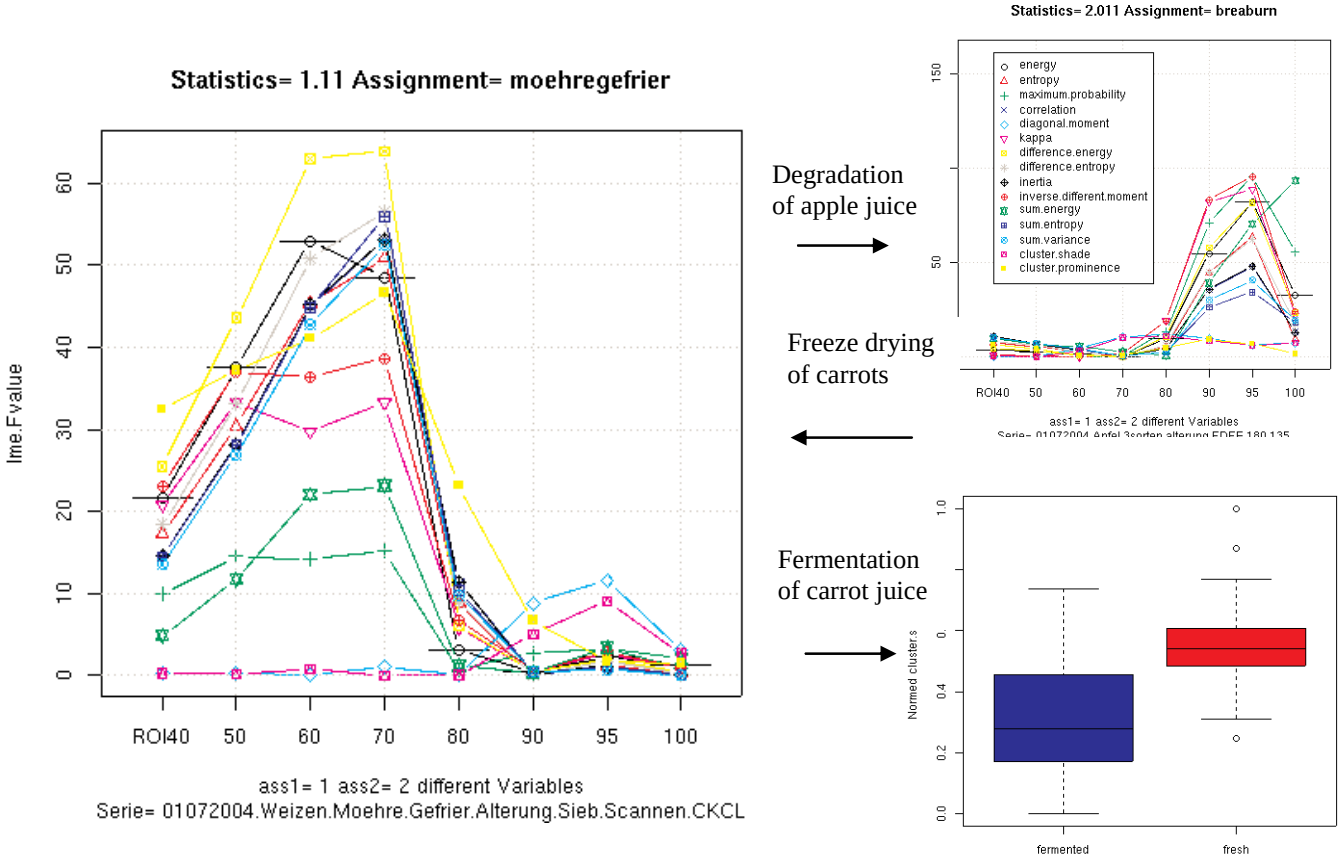
CHARACTERISATION OF THE BIOCRYSTALLIZATION METHOD USING COMPUTERISED IMAGE ANALYSIS

Dr Johannes Kahl

You will see the information when we look at the F value which gives the results, if we can differentiate samples or not, that the F value is - for this example - decreasing when we take the whole picture so that means the information depends on the size of the pattern we are using and using that, we can just take out a single variable, and here we differentiate an organic molecule polybdon and weed samples, we use this as standard as statistically significant independent from the two chambers we are using and the other pictures shows a linear discriminatory analysis so a multivarious statitistic applied.

Now I want to show the results of the linear discriminate analysis because these are much more picture-like.

Texture analysis:
Goal: Differentiation of samples



On the left side you see the F value and on the x y you see the ROI and what we want to test if dry matter has influence on the picture. We know that when we play with the mixing ratio, yes of course there is an influence but what we want to know is the process of freeze drying to a carrot, how it can be best reflected to the method because normally when we look at polyphenols we freeze dry the whole sample and then we make the analysis.

Here, when you look at the F value of freeze drying versus fresh carrots we see that of course there is a tremendous influence of freeze drying on the results of the method. This depends on the path of the pattern we are looking at. On the right upper part we look at degradation of apple juice because we want to look at the stability of the solution and here once more, the F value of an anova of fresh and 6 days degradated apple juices, and of course we find a tremendous influence of the degradation of the product. The other part of this slide shows the fermented and fresh carrot juice compared and of course there is also a statistical significance based on one single variable and making an anova and of course we introduce a repeated measured model.

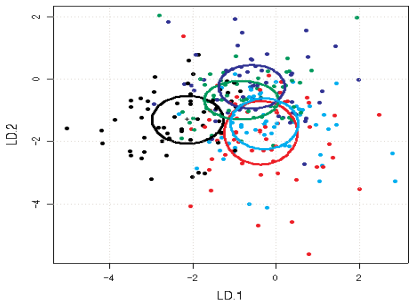
Now in the next slide we look at the first samples. On the left shows 5 DOC samples in 2002, the control, 2 organic and 2 conventional and on the right side, the samples after decoding.

CHARACTERISATION OF THE BIOCRYSTALLIZATION METHOD USING COMPUTERISED IMAGE ANALYSIS

Dr Johannes Kahl

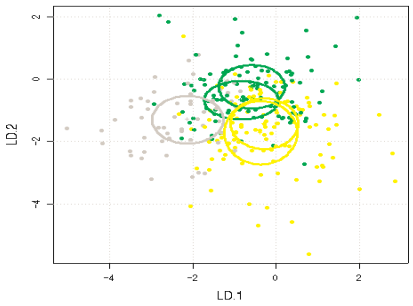
3. Results from measurements on coded samples

Wheat: 5 DOC samples harvest 2002, measurements spring 2003



before decoding: LD2 against LD1

For 5 DOC samples, harvest 2002



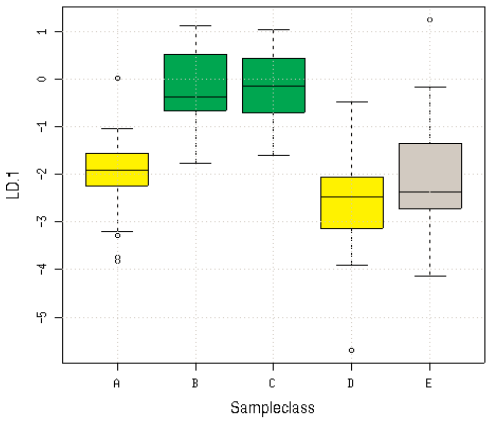
after decoding: grey = control,

conventional = yellow, organic = green

You will see that we can separate the organic and the conventional from the control and this is statistically significant on the basis of the single variable. This is just the linear discriminant analysis results so the circles are reflecting the samples, and the dots the different pictures we make. We can separate the organic from the control and we can separate the organic from the conventional. When we did this in 2003 this is only the first linear discriminant faction and you will see the same - green is the organic, yellow the conventional and the grey is the control.

3. Results from measurements on coded samples

Wheat: 5 DOC samples harvest 2003, measurements fall 2003



LD1 value against sample classes for all

5 DOC-samples harvest 2003

(after decoding: grey = control,

yellow = conventional, green = organic)

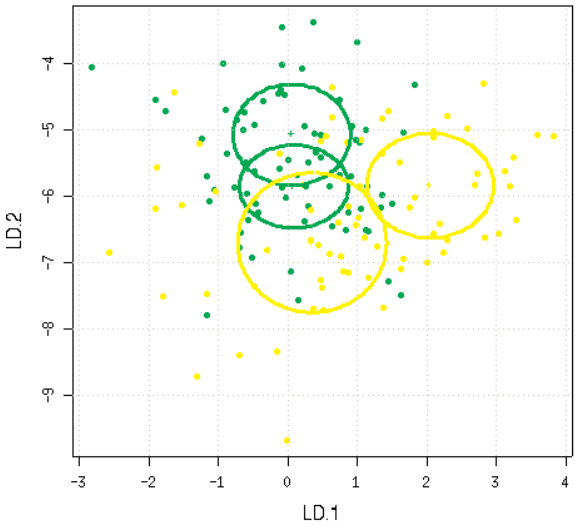
You really need to think about what the picture is saying. As an analytical chemist you start to want to correlate the pictures with other stuff, with amino acids or secondary compounds, dry matter, falling number or whatever - so we start immediately not to stick on the phenomenon and to refer this to concepts like self organisation but to compare. Of course let's start to compare.

CHARACTERISATION OF THE BIOCRYSTALLIZATION METHOD USING COMPUTERISED IMAGE ANALYSIS

Dr Johannes Kahl

3. Results from measurements on coded samples

Carrots:Different varieties, harvest 2003, measurements fall 2003



LD2 against LD1 for all 4 FIBL-samples harvest 2003

(after decoding: yellow = hybrids, green = open pollinating)

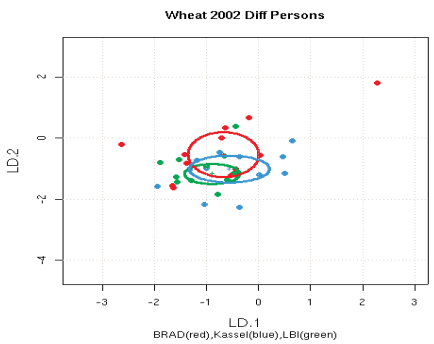
Normally you would put the control to the organic because organic is just a little bit more than control doing nothing. When we look at the total nitrogen and the amino acids, yet it is true. The control belonging to the organic can be separated from the conventional but this method and also Jurgen Stube's fluorescence excitation spectroscopy shows it differently.

We are grouping the control to the conventional and not to the organic. In both years we can separate the organic from the control. This was the question of different varieties, open pollinating versus hybrids and we can group the hybrids and open pollinating together, these are the greens and as Jurgen Strube showed, also here the method one of the hybrids is belonging to the open pollinating.

The repeatability, the intermediate precision and the reproducibility are shown on the next slide.

4. Characterization results

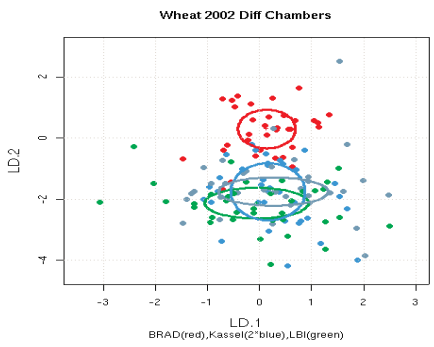
Reproducibility and Repeatability



LD1-value for 1 wheat sample (2002)

3 different people, trained, in 1 chamber

in Kassel (Ks = blue, LBI = green, BRAD = red)



LD1 value for 1 wheat sample (2002)

4 chambers in 3 laboratories, 3 different people

CHARACTERISATION OF THE BIOCRYSTALLIZATION METHOD USING COMPUTERISED IMAGE ANALYSIS

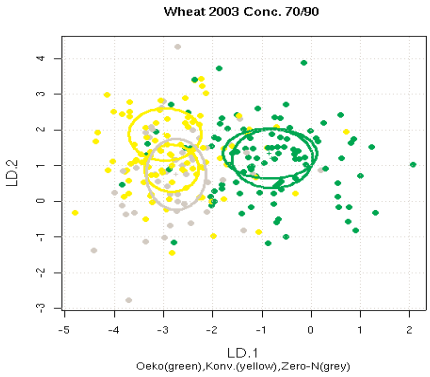
Dr Johannes Kahl

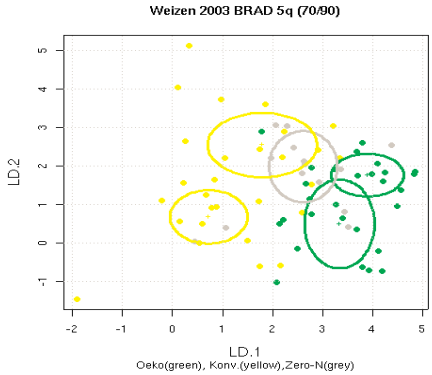
You will see on the left side the result of the LEA when we put different technicians together in one lab with different machines. To look at what is going on - if people are doing it in parallel and you see it is quite the same and we make statistics on the basis of the single variable and there is no statistical significance between the people. On the right side we make reproducibility tests so we sent one weed sample around to three different labs with four chambers and then measured in repeatability tests so 6 times sample preparation. We can see three chambers are belonging together, one is a bit apart and this is of statistical significance so the red lab is a little bit apart but because we have no absolute scale we cannot measure the accuracy of the method and we don't know who is right, and therefore we send the same DOC samples we could differentiate, to that red lab to look at if it depends on the place or if this lab which is in total a little bit apart, can also differentiate.

In the next slide on the left side you see once more the results produced in Kassel and on the right you see the results produced in Denmark and you see also in total that they are different. They can separate the organic from the conventional samples testing the discriminatory ability of the method in different places.

4. Characterization results

Reproducibility and Repeatability III

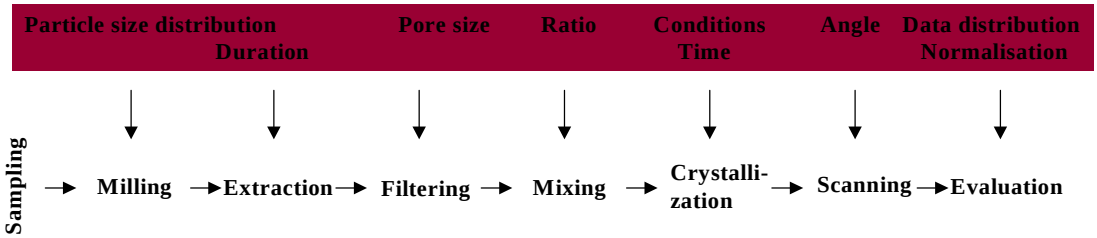




In the next slide of course characterisation of the method means that you really have to know about your methods and the details and therefore we just put the whole chain in the lab together and then we looked at the different factors of influence - how those factors are influencing the variation and the results. We look at repeatability, intermediate precision and reproducibility so as soon as we increase the amount of labs to aid we can fulfil the international requirements for the validation of the method.

4. Characterization results

Characterisation of the method (wheat)



Tested:

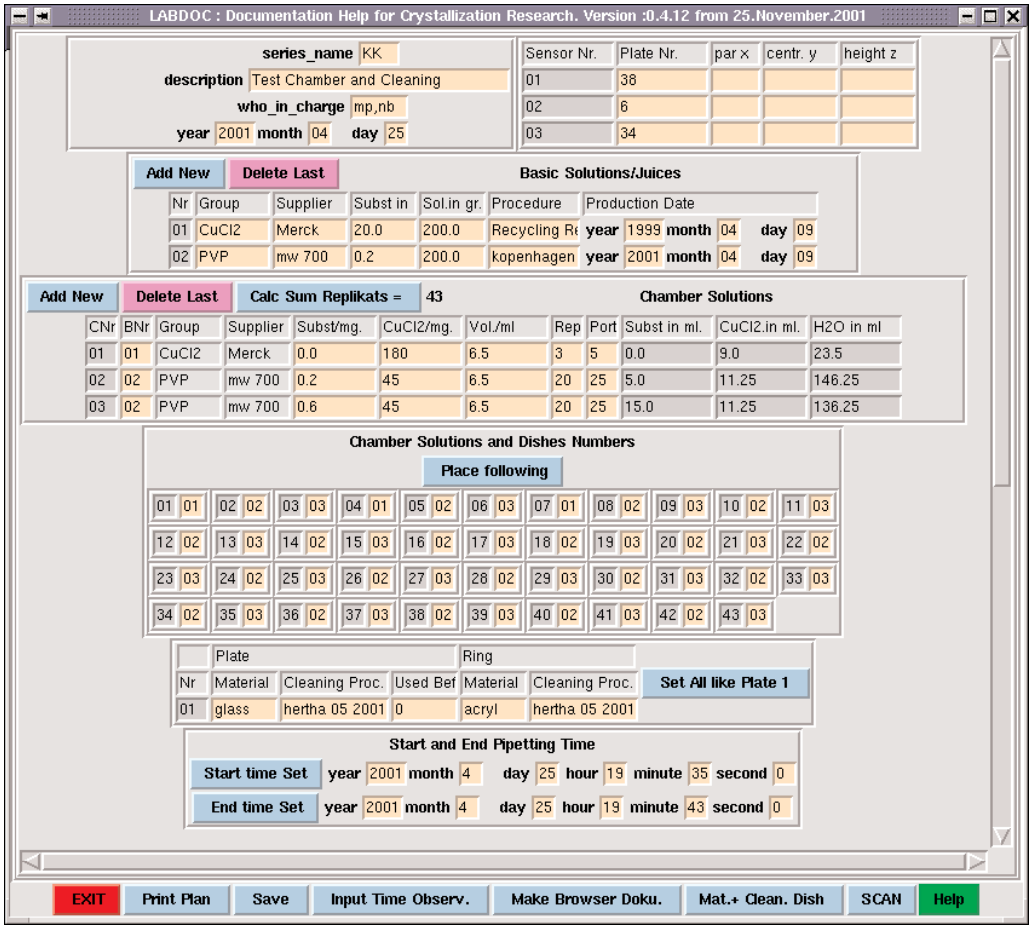
1. **Repeatability**
2. **Intermediate precision**
3. **Reproducebility**

CHARACTERISATION OF THE BIOCRYSTALLIZATION METHOD USING COMPUTERISED IMAGE ANALYSIS

Dr Johannes Kahl

In the ISO 70 025 of course there is the requirement that you need a quality management in all labs that are accredited due to this norm and we are working with a computer assisted lab documentation system. Here you see the front page that every sample entering the lab is connected with the sample preparation procedures with the chemicals used, the place on the crystallization unit and with the climate conditions and the results from the texture analysis or even visual evaluation.

Documentation: computer assisted laboratory documentation



In conclusion, the biocrystallization method including the texture analysis can be documented and used as a lab procedure and a lab method but it is a qualitative method so we are working on a nominal scale. We can group and differentiate the 5 different wheat samples from the DOC trial in a 2 year repetition. We did the same for the 4 variety samples of carrot and the nitrogen fertilisation samples and we can characterise the method according to international standards so that we measure the repeatability, the intermediate precision, reproducibility and look at the factors of influence.

Conclusions

1. **The Biocrystallization method including texture analysis can be documented as a l laboratory procedure**
2. **The 5 wheat DOC-samples can be grouped in control, conventional and organic in a 2 year repetition**
3. **From the 4 varity carrot-samples the open pollinating can be grouped together and one hybrid can be separated**
4. **The Biocrystallization method can be characterized according to international standards and therefore is able to be validated**

CHARACTERISATION OF THE BIOCRYSTALLIZATION METHOD USING COMPUTERISED IMAGE ANALYSIS

Dr Johannes Kahl

The next steps for routine analysis we have to reduce the variation coming from the chamber because of this random crystallization start. Then we have to look if we can use the time information about the different crystallization behaviour to include this in the evaluation. We have to optimise the evaluation tool and that we introduce multivarious statistics and of course then what is the method saying, so we have to develop a model for a scientific understanding of the process.

Here we started with micro molecules like glycogine or some others to look at what is about this self organisation concept. Can we relate that to the method? And then of course we have to correlate the results derived from the copper chloride crystallization or biocrystallization method with results achieved from other methods to know if this is some addition or what is the method about and we do this in the next governmental project we are running and we increase the amount of samples so that we also look at market samples. Then of course we are waiting for questions so we have to look to which extent the method can be applied for different questions in processing and organic farming.

5. Next steps

1. **The conditions during evaporation and crystallization have to be optimised in order to reduce the variation of the patterns.**
2. **The time information has to be included into the evaluation.**
3. **The evaluation tools have to be optimised (multivariate statistics).**
4. **A model has to be developed for the scientific understanding of the process.**
5. **The results from the biocrystallization method have to be correlated with the results achieved from other methods on the same material.**
6. **It has to be proven to which extent the method can be applied for different questions (e.g. influence of processing) on crop and food products.**

When one is working in the fields of organic and holistic methods you know that to get a platform running is a huge amount of work and normally people are not able to work together. They have their own systems and this is right. They are not coming together so we built a platform in this organic field of holistic methods with colleagues from the Biodynamic Assoc in Denmark, from the Louis Bolk Institute and the University of Kassel where we work together in an inter-disciplinary group to be able to measure this reproducibility for example and, of course, without money we can do nothing so I would like to thank our sponsors.

Elm Farm Research Centre, Hamstead Marshall, Nr Newbury, Berkshire RG20 0HR United Kingdom

Tel: +44 (0) 1488 658298 Fax: +44 (0) 1488 658503 Email: elmfarm@efrc.com

www.efrc.com

Charity No. 281276

Elm Farm Research Centre (EFRC) is one of the UK's leading research, development and advisory institutes for organic agriculture.

For more than 20 years EFRC has played a central role in the development of policy and standards for organic farming and food within the UK, EU and internationally.

The Centre's alliance of practice and policy – on-farm and desk research and consultancy and advice is unique.

We acknowledge and thank Sheepdrove Trust for its financial support towards this Conference and the use of the beautiful setting of The Kindersley Centre and its facilities.

Elm Farm Research Centre, Hamstead Marshall, Nr Newbury, Berkshire RG20 0HR United Kingdom

Tel: +44 (0) 1488 658298 Fax: +44 (0) 1488 658503 Email: elmfarm@efrc.com

www.efrc.com

Charity No. 281276