

# Parameters for Apple Quality



## Part **1** Report

JOKE BLOKSMA, MARTIN NORTHOLT, MACHTELD HUBER

LOUIS BOLK INSTITUUT, 2001

# **Parameters for apple quality**

**and an outline for a new quality concept**

**met Nederlandse samenvatting: Parameters voor appelkwaliteit  
en een aanzet tot een nieuw kwaliteitsconcept**

**mit Deutschen Zusammenfassung: Parametern für Apfelqualität  
und ein Entwurf eines neuen Qualitätsbegriffes**

**part 1 report**

**Joke Bloksma, Martin Northolt, Machteld Huber,  
Louis Bolk Institute, 2001.**



## About the Food, Quality and Health programme (FQH)

The FQH programme has been established in 2000 by Louis Bolk Institute, Triodos Bank and organic product trade companies to develop quality concepts based on life processes and parameters for determination the quality of products grown with different agricultural methods.

The programme is developed and carried out by an international group of institutes with experience of traditional and holistic research methods. The programme will be extended by research on the effect on health of conventional and organic grown products.

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## About this first apple project

Directed by Joke Bloksma, Martin Northolt, Machteld Huber from the Louis Bolk Institute in co-operation with Pieterjans Jansonius (LBI), Mirjam Matze (LBI), Monique Hospers (LBI), Anna de Weerd (LBI), Paul Doesburg (LBI), Orchard ter Linde (NL), Roel van Wijk and Jan Soeren (Meluna Bio-photon research, NL), Jürgen Strube and Peter Stolz (Kwalis Qualitätsforschung Fulda GmbH, D), Hartmut Heilmann (Elektrochemische Qualitätsuntersuchungen, Kirchberg/Jagst, D), Jens Otto Andersen (Univ. Kopenhagen, DK), Ruth Manderla (D), Ingo Hagel (Inst. for biodynamic Research, Darmstadt, D) and Franco Weibel (FiBL, CH).

## About the Louis Bolk Institute

The Louis Bolk Institute has been a pioneer in innovative scientific research in organic farming, nutrition and healthcare since 1976. Broadening the basis of scientific research is the aim of the institute's work.

Where conventional research methods do not suffice, new methods are looked for, such as: phenomenology, participatory research, pictomorphological investigations and conscious-intuitive methods, Rudolf Steiners philosophy being a source of inspiration.

You can order a general Annual Report about the Louis Bolk Institute or an annual report only about fruit growing research. These texts and the list of publications are also available on the web-site.

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# Summary and conclusions: Parameters for apple quality and an outline for a new quality concept

## Motivation and framework of the Food, Quality and Health programme

The commonly used quality-concept, with the emphasis on external appearance and nutritional content, is not sufficient for organic products and their market. Organic growers and their consumers strive to a product with 'vitality', 'life-force', 'coherence' or terms of similar wording. These terms are expected to play a role in wholesome nourishment and to distinguish between living food products and a solution of nutrients.

The mentioned terms are interpreted very diverse, mostly based on a single test, while a quality concept with operational parameters was still lacking.

The objective of the international 'Food, Quality and Health (FQH) programme' is to develop a contextual quality concept with testing parameters that corresponds to the needs of the organic producers. The first step to this is the development of a quality concept and parameters. This resulted in the preliminary concept of 'vital quality'. Eventually we strive to examine the effect of high vital-quality food on human health.

During this first FQH-project with apples, we studied the possibility of introducing this new concept, 'vital quality' linked to testing parameters. We stress however the orientation character of this project as the introduction of a new quality concept with experimental parameters entails the lure of circular reasoning, as validation is performed with new and experimental parameters. The new quality concept and parameters are 'preliminary' and evaluation of the consistency and usability requires several years of testing with various products. Eventually this will inspire confidence whether 'vital quality' is a meaningful and operational concept.

In this project the concept will be developed and adjusted in a very limited context. Namely, apples from a single variety that were grown under different conditions, at one orchard and in one season.

## Quality concept 'vital quality'

We mention a number of requirements that apply to a quality concept for organic agriculture. Firstly, a relationship with the central paradigm of organic agriculture: to sustain life processes. Growth and differentiation can be considered the two basic life processes, whether or not balanced and integrated. Therefore growth, differentiation and their integration are the three aspects upon which the new quality concept is based. Secondly, the quality concept must be communicated to both the producer and the consumer, who have a different reference index. The producer, who works with growing plants, thinks in terms of life processes, which can be stimulated, reduced and balanced. While on the other hand, the consumer and retailer think in terms of controllable and recognisable product aspects. Therefore the quality concept has two sides; life processes and product aspects. Both sides are related to one another.

Thirdly, the quality concept must relate to the holistic health view of physicians and dieticians to facilitate the initiation of health assessing research projects during the programmes second phase.

All three requirements combined, resulted in a quality concept indicated as 'vital quality', based on the life processes growth, differentiation and integration processes and the corresponding product aspects; vitality, structure and coherence.

## Vitality is only one aspect of 'vital quality'

In the quality concept 'vital quality'; vitality has been defined as the result of growth processes. Thereby corresponding to the usage of vitality in the sense of growth-full, young and lively. It does not correspond however, to the usage of vitality in the sense of health related due to a harmonious balance between growth and differentiation, and strong resistance and self-regulating abilities. In the new quality concept the latter is incorporated in the integrated or contextual aspect of vital quality.



## Objectives and methods

### Aim

This research aims to develop a coherent quality concept for the organic agriculture including the accessory testing parameters. So, in the first instance this research does not aim to answer whether organic cultivated products are better than conventionally cultivated products, and what we may consider 'better'. In a later stage, when the quality concept has been further developed and validated, different agricultural cultivation methods will be compared.

### The apple as the first research crop

The apple has been chosen as the first crop as the life processes growth and differentiation are well-known phenomena amongst fruit-growers, and because the Louis Bolk Institute has experience with this crop. World-wide research on apple-quality aided to determining which aspects had to be standardised in order to exclude unwanted variations. The Elstar variety has been chosen as one of the most tasty, Dutch apple-varieties.

### Reference series

The basis of this research is formed by sets of 20 apples, specifically cultivated for this research. We attempted to grow the apples in such a way that only one of the life processes would increasingly be affected between the two extremes within each series, whereas all other factors were standardised as much as possible. The series differed in picking date, bearing, sun exposure, Bd-preparations and ageing after storage.

The season 2000 had ideal weather conditions for apple cultivation as a result of which the intended extremes in the series weren't realised, and the optima weren't located in the centre of the series.

### Choice of parameters

We chose traditional parameters that are commonly used to assess the quality of apples, and experimental parameters which we expect to be relevant for vital quality.

The following parameters were evaluated (per series a set of parameters was chosen)

- crop: soil, growth, bearing, diseases and plagues, leaf series, next years budding (LBI).
- traditional parameters: crop size, ground colour, blush colour, shine, firmness, starch, Brix, acid, N, P, K, Mg, Ca and dry matter (Lab. PPO and lab ZVI)
- vitamin C (LBI), phenolic compounds (TU-München, D), amino acids and protein (Kwalis Qualitätsforschung, Fulda, D).
- self-disintegration (LBI), taste (LBI), copperchloride crystallisations (LBI), capillary pictures (LBI + R. Mander).
- two different methods with biophotons (Meluna Biophotone research, Wijk bij Duurstede NL, and Kwalis Qualitätsforschung, Fulda, D).
- electrochemical parameters: pH, redoxpotential, electrical resistance, combined in the P-value (H. Heilmann, D).
- Bovis-value (this is an intuitive observation technique, which was added for evaluation, LBI).

### Partners

- apple cultivation (LBI, bio-dynamic Orchard ter Linde, Oost-kapelle, NL).
- quality concept development: LBI, R. van Wijk (NL), J.O. Andersen (DK), F. Weibel (CH), J. Strube and P. Stolz (D), H. Heilmann (D).
- Funding: LBI, Meluna Biophotone research (NL), Kwalis Qualitätsforschung, (D), Stichting Triodos Fonds (NL), Software AG Stiftung (D), Zukunftsstiftung Landwirtschaft (D), Stichting Klaverblad (NL).

### Repeats and reliability

We collected samples of 120-apples each, originating from minimal 10 different trees, and are divided in subsamples for the different laboratories. To cut back expenses in this preliminary research, we chose not to perform independent repetitions in the field. Per parameter we chose either for subsample analysis or for independent repeats on individual apples. For the experimental parameters we predominantly chose for repetitions within the sample to demonstrate the variation of the method. A number of traditional parameters acted as reference and control for the homogeneity of the sample.

To a certain extent treatment series compensate the lack of independent repetitions. The strict standardisation of apple size and location of growth in the tree aided to minimise the variation within the samples. Practically all parameters showed a consequent course within the series by which means we judged, in retrospect, the uniformity of the series and the reliability of the quality measurements as satisfactory. The only exceptions were the self-disintegration test and the vitamin C analysis, although these analyses might be improved in the near future.

### **Methodical steps**

The risk of reasoning in a circle can not be avoid completely. Our basis lays in the cultivated series of apples with a gradual increase of certain life processes, and assaying of the consistency between concept and results of the parameters.

The strategy was as follows: we attempted to cultivate apples in such a way that only one of the life processes would be affected per series. In this way we depicted the experimental parameters that seemed to be relevant for these life processes. The results were compared to the outcome of the traditional parameters and with literature, indicating the usability of our series. The experimental parameters appeared to supplement the traditional parameters and enrich our vision of the life processes and the quality concept. We also determined the mutual relation between the parameters. Finally, all parameters were reviewed for there relation with the life processes and therefore with the new quality concept.

## **Results of the treatment series**

### **Ripening**

For comparison within one treatment series, we chose to perform the analyses on the same day. For the series with 5 different picking dates (between September the 1<sup>st</sup> and October the 9<sup>th</sup>) this means that early picking is related to a several weeks longer cold storage period. So this series actually shows the difference between ripening on the tree versus ripening in storage. As the conversion of starch into sugar and loss of firmness occur both on the tree and during cold storage. For many other aspects of ripening like colour, size, taste, biophotons, crystallisation, capillary pictures and Bovis-value, ripening on the tree is essential.

Determining all these parameters aided us to regard the process of ripening as a successive process in which continually different compounds change from a solid state into a soluble state or evaporate. For example firm fruit with starch, acid and phenolic compounds ripen into juicy fruit with soluble sugars and aromatic compounds. The holistic methods show an increase in openness and 'facing-outwards'. The ripening process can be characterised as a transition from vitality into structure and coherence. Which is contrary to the ageing process in cold storage, during which also a loss of vitality occurs, but without an increase in structure and coherence. various parameters related to coherence have a maximum (or optimum) value at the second or fourth picking date.

### **Bearing**

In June the trees were pruned to the desired bearing level: 35, 75, 100, 125 and 140 fruit per tree. This corresponds to 14, 30, 40, 50 and 60 tons of apples per hectare. Previously, we estimated the third bearing to be optimal but due to the favourable season for apple cultivation the fourth bearing level turned out also to be optimal for satisfactory taste and flowerbud formation.

A well-known phenomenon, which we also encountered here, are the relations between a higher bearing and: decreased twig onset (visualised with the leaf series), lower leaf/fruit ratios and reduced flowerbud formation for the next season. The impact of high bearing on fruit quality was demonstrated by the dilution of all parameters involved with assimilation and mineral uptake: dry matter, sugar, sourness, aroma and the various minerals. However, the opposite was found for Ca. Ca levels and the Ca/K ratio as a measure for storage potential actually increased at a higher bearing, as is also found in the field.

The copperchloride crystallisation images of apples from low bearing trees gave a powerless and vegetative impression, whereas apples from high bearing trees gave poor, sharply outlined impressions. Average bearing gave rise to the most vital and differentiated images. The capillary pictures were sharper at a high bearing. The taste was more or less constant and only decreased at the highest bearing. The biophotone level decreased directly after excitation (a measure for vitality) and the hyperbolicallity (a measure for the differentiation/growth ratio) increased.

In conclusion, a higher bearing resulted in a decrease in vitality and an increase in structure.

## Sun exposure

During harvest, separate apples were picked, hanging either in full sunlight, complete shadow or in between these two extremes. We harvested two sunlight series, one with and one without Bd-preparations. The sun exposure levels of both series were comparable. Apples grown in the sun gave rise to increased colour, phenolic compound, biophotons (all three a measure for the differentiation/growth ratio), a broader colour spectrum with biophotons (a measure for fruit-typicality), higher protein/amino acid ratios, more coherence and transparency in copperchloride crystallisation images and more round, open shapes in the capillary pictures (all measures for increased integration). Surprisingly, no difference was found in taste, firmness, calcium or acidity. New for us were the much higher levels of N, P, K, amino acids and proteins in the shaden-grown fruit. This resulted in the higher Ca/K ratio for the sun-exposed fruit, which corresponds to the experience that sun-exposed fruit stores better. Seemingly, sun-exposure stimulates differentiation, resulting in an increased structure and coherence.

## Bio-dynamic preparations

A part of the orchard wasn't treated with Bd-preparations this season. The other part was treated twice with a cow-manure preparation and twice with a silica preparation. The sunlight series was gathered from both parts of the orchard.

When testing for homogeneity, the part of the orchard that had preparations administered appeared to be slightly more growthfull, although this might not due to preparation usage.

Remarkable was that traditional parameters like colour, taste, firmness, minerals, starch, acid and pH revealed no difference between apples with and without preparations. However, a couple of experimental parameters linked to either differentiation or integration did reveal differences, e.g. phenolic compounds, protein/amino acid ratio, electrical resistance, broader colour spectrum with biophotons, increased transparent copperchloride crystallisation images and Bovis-value. Albeit the effects were not encountered at all three levels of sun-exposure, making it hard to draw firm conclusions.

The light and preparation series are combined, as we expected preparations to have an effect similar to that of sun-exposure and moreover, would enhance the integration process. The results did not confirm nor deny our expectations.

## Shelf-life

The apples of the fourth picking date remained in cold storage for three months, and were taken out at different time intervals. This resulted in a shelf-life series of 1, 4, 8 and 12 days. As is commonly known, and found here too, firmness and acidity clearly decrease during ageing while the sugar level remains constant for some time due to remobilization. Practically all parameters indicated a limited ageing, but not as severe as we had anticipated. The series wasn't extreme enough and, in retrospect, should have contained longer shelf-lives to obtain a real image of decay. As the apples grew older the needle structure of the crystallisation images developed more and more towards the periphery.

Surprisingly, apples only one day out of cold storage were judged by a lot of parameters as being less good than apples 4 days out of cold storage. Apparently, apples need to acclimatise to altered conditions after cold storage (and/or transport) for a couple of days.

This series wasn't illustrative to assay the quality-concept as besides the vitality, also the differentiation and integration decreased. The changes in life processes aren't distinguishable in shelf-life series.

## Further elaboration of the concept 'vital-quality'

Research indicates that besides the two life processes, growth and differentiation, it is meaningful to add integration as a third aspect between these two processes.

Distinguishing the two life processes, growth and differentiation, can only theoretically be done. As soon as a living organism starts growing, both processes are apparent, albeit in a certain balance. Sometimes with the emphasis on growth (vegetative life stage, luxurious, cancer growth), sometimes with the emphasis on differentiation (generative life stage, poor emergency flowering). Some of the characteristics of 'vital-quality' are described below based of apple parameters. In the following scheme Fig. 11a these concepts are generalised to form the basis for other crop research.

### **Vitality is the result of growth**

Growth can be described as the process of the expansive filling of space with unformed mass, growth of organs, cell division and, in the case of plants, the production of primary metabolites by photosynthesis. A vital tree has a lot of green leaves and a considerable apple yield. A vital apple has a good size, is firm, crisp and juicy. The product contains a high amount of starch, sugar, acid, amino acids and proteins whereby the ratio between the two is determined by the degree of ripeness. A vital apple is still building up biomass, whereby a lot of transportable compounds are formed (amino acids, sugar). The acidity is low, the turgor is high, the amount of biophotons directly after excitation is high and the crystallisation images are filled with a dense needle structure.

### **Structure is the result of differentiation**

Differentiation is the process of specialisation in form and function, cell differentiation, refinement of form, flavour, gloss and colour, ripening into maturity, flowerbud formation, forming pollen and seed formation. Organisation of structure is initiated and secondary metabolites are formed, e.g. the wax on the skin, phenolic compounds, vitamins and aromatic compounds. Well-structured fruit contain a lot of pits, a high calcium level and store well. The amount of biophotons decreases after excitation in a hyperbolic manner. The crystallisation images are clearly arranged, have a high degree of one-centredness and the sharp side-needles have a large angle.

### **Coherence is the result of integration**

The growth processes must take place on a medium level to allow the differentiation process to proceed well. Too luxurious growth will inhibit ripening to full maturity. Whereas with a trifling growth, differentiation will result in poorness, emergency flowering and 'conservation', resulting in hard, dry and small apples. When growth and differentiation progress simultaneously and balanced, possibly in a rhythmic alternation, we refer to this as an integration process. A dynamic balance between growth and differentiation that depends on the context, like species, variety, development stage, season, soil and orchard management. However, integration is not only about a certain balance between growth and differentiation, but also about the degree of interaction. Sufficient integration allows an apple to combine a good taste with a reasonably good storage potential. The taste is aromatic, juicy and crisp, and has a well-balanced sweet/sour ratio. The biosynthesis is completed, which is expressed in the fairly low free-amino acid, and relatively high protein levels. Such apples are resistant to self-disintegration, stress and disease. The flesh is elastic and retains tension a while after picking. The electrical resistance is high and the redoxpotential is low. The amount of biophotons starts off on a high level after excitation, decreases hyperbolically and expresses a broad, fruit-typical colour spectrum. The crystallisations show a coherent and apple-typical image. The Bovis-value is high.

### **The aspect 'coherence' appears to be a relational feature**

When judging vitality and structure, the observer can remain detached and objective. At this level even the crystallisation images can to a certain degree be judged by image analysis software. When judging coherence, the observer is expected to be related to, and be involved with the subject, and to express an aim as a reference for quality. The fruitgrower adds his own aims. He chooses the target values in the relation between growth and differentiation by determining the balance between a high yield and a good taste.

A lot of the tests relate to coherence, depend on an active participation of the researcher, for example the sensory properties and 'empathical observations' when judging the crystallisation images. When developing these parameters, specific attention must be spent on the (inter-subjective) judgements, to allow the tests to be scientifically sound. The development of tests to determine coherence is of great importance for the new quality-concept; because the coherence aspect is the most essential aspect of 'vital-quality'.

### **Assessment of the usefulness of the quality-concept for fruitgrowers**

Recognising and judging the balance is of great importance for growers to deploy cultural measurements which will restore the balance in good time. After presentation of this quality-concept, fruitgrowers were capable of pointing out the cultural measurements they could deploy to limit surplus growth or differentiation. Most fruitgrowers were however uncertain on how to actually stimulate the integration of both processes. Biodynamical growers presume Bd-preparations play a role in this event, although this has not been proven yet.

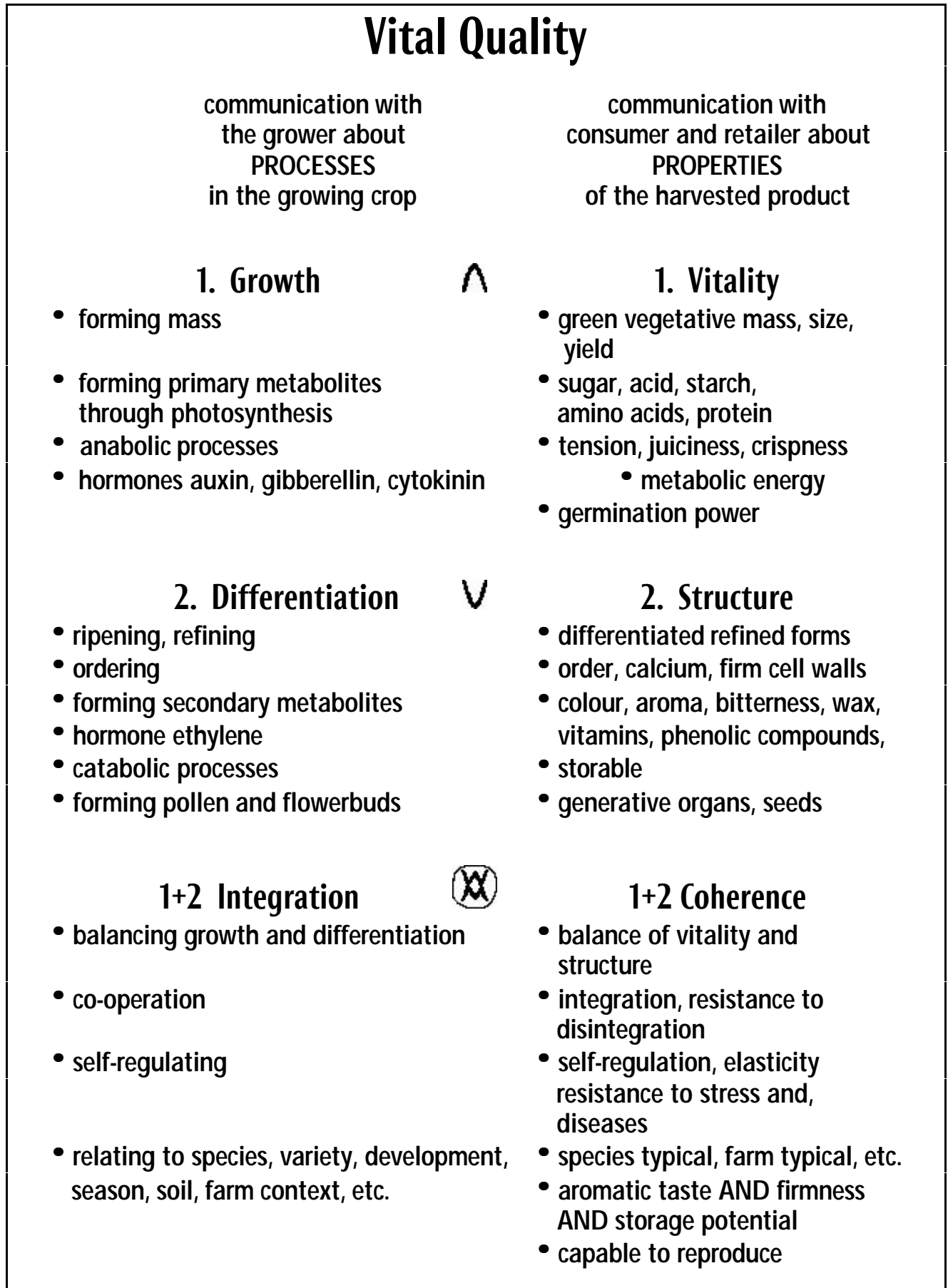
### **Assessment of the used parameters for the quality-concept**

The traditional parameters are indispensable as they allow us to verify the series and the homogeneity, and form a link with literature. Amongst the experimental parameters, notably the crystallisations, biophotons and Bovis-value were of great value for the development of a new and coherent quality-concept. The significance of the capillary pictures has not been established yet. Perhaps in the future, the low cost parameters can be used routinely, albeit interpreted in respect to the entire quality-concept, like for example the sweet/sour ratio and the protein/amino acid ratio.

### **Recommendations for further research for concept- and method-development**

- comparable experimental design with apples in a series of increasing nitrogen fertilisation, and a new series with Bd-preparations, see discussion in §19.3.
- comparable experiments with other products, notably carrot, red beet, potato, tomato and milk and determine whether the three aspects of vital-quality can also be found in these products.
- methodical improvement of the parameters: taste, self-disintegration, crystallisation and Bovis-value.
- further development and interpretation of biophotons and copperchloride crystallisations.
- which cultivation circumstances improve the integration process?
- nutritional research to determine the significance of vitality, structure and coherence for human health.

Fig. 11a: Vital Quality in keywords for various crops



# Samenvatting en conclusies: Parameters voor appelkwaliteit en een aanzet tot een nieuw kwaliteitsconcept

## Aanleiding en het kader van het ‘Food, Quality and Health-programma’

Het veel gehanteerde kwaliteitsbegrip, met nadruk op uiterlijk en inhoudsstoffen, blijkt niet te voldoen voor producten uit de biologische landbouw en het bijbehorende handelskanaal. Biologische telers en hun consumenten streven naar een product met ‘vitaliteit’, ‘levenskracht’, ‘samenhang’ of noemen het nog anders. Van deze begrippen wordt verwacht dat ze een rol spelen bij gezonde voeding en dat een levend voedingsproduct zich onderscheidt van een oplossing van nutriënten.

De genoemde begrippen worden echter door verschillende mensen heel verschillend ingevuld, meestal gebaseerd op één of enkele analyses. Een kwaliteitsconcept op basis van deze begrippen met toetsbare parameters ontbrak nog.

Het internationale meerjaren onderzoek ‘Food, Quality and Health’ (FQH) heeft als doel een samenhangend kwaliteitsbegrip met toetsbare parameters te ontwikkelen dat aansluit bij de behoefte van de biologische producenten en consumenten. De eerste stap is het ontwikkelen van kwaliteitsbegrippen en parameters. Hiervoor is het voorlopige concept van de ‘vitale kwaliteit’ ontwikkeld. Daarna is pas de tweede stap mogelijk, het toetsen of producten met een hoge ‘vitale kwaliteit’ inderdaad gezonder zijn voor (bepaalde groepen) mensen. In dit eerste FQH-project met appels verkenden we de mogelijkheid om een begrip voor ‘vitale kwaliteit’ te introduceren met toetsbare parameters. Het is nadrukkelijk een verkenning omdat het introduceren van een nieuw kwaliteitsbegrip met experimentele parameters het risico van een cirkelredenering met zich meebrengt. Immers een onbekend begrip laat zich moeilijk introduceren door onbekende parameters. Het nieuwe kwaliteitsbegrip en de parameters zijn ‘voorlopig’ en moeten gedurende meerdere jaren en bij verschillende producten op consistentie en bruikbaarheid getoetst worden. Uiteindelijk kan dan het vertrouwen ontstaan of ‘vitale kwaliteit’ een zinvol en toetsbaar begrip is. In dit project is het hier beschreven begrip ontwikkeld en aangepast aan een heel beperkte context, namelijk aan appels van één ras, wel met verschillende behandelingen gegroeid, maar op één bedrijf en in één jaar.

## Kwaliteitsbegrip ‘vitale kwaliteit’

Wij stellen aan een kwaliteitsbegrip voor de biologische landbouw een aantal eisen. Ten eerste dat het aansluit bij het centrale paradigma uit de biologische landbouw, het verzorgen van levensprocessen. Groei en differentiatie kunnen als de twee basis levensprocessen beschouwd worden, die al dan niet in balans en geïntegreerd met elkaar kunnen zijn. Groei, differentiatie en hun integratie zijn de drie aspecten waarop het nieuwe kwaliteitsbegrip is gebaseerd.

Ten tweede moet het kwaliteitsbegrip gebruikt kunnen worden zowel door de producent als door de consument. Beide hebben een heel verschillende belevingswereld. De producent ziet het groeiend gewas en denkt in termen van levensprocessen, die hij of zij kan bevorderen of afremmen en in balans brengen. De consument en handel denken in termen van eigenschappen van het eindproduct die controleerbaar en herkenbaar moeten zijn. Het kwaliteitsbegrip heeft daarom twee kanten, die van de processen en die van de eigenschappen. Beide kanten zijn aan elkaar gerelateerd.

Ten derde moet het kwaliteitsbegrip aansluiten bij de holistische visie op gezondheid van artsen en voedingskundigen, zodat in de tweede fase van het programma onderzoeksprojecten opgezet kunnen worden om gezondheid te toetsen en een theorie voor het werkingsmechanisme ontwikkeld kan worden.

Alle eisen verenigd, ontwikkelden we een kwaliteitsbegrip dat we aanduiden met ‘vitale kwaliteit’, dat gebaseerd is op de processen groei, differentiatie en integratie met de corresponderende producteigenschappen vitaliteit, structuur en samenhang.

## Vitaliteit is maar één aspect van ‘vitale kwaliteit’

Het begrip vitaliteit heeft hierin dus een specifieke invulling gekregen, namelijk als het resultaat van groeiprocessen. Het sluit hiermee aan bij mensen die ‘vitaal’ gebruiken in de zin van groeikrachtig, jong, levenslustig. Het sluit niet meer aan bij de mensen die ‘vitaal’ gebruiken voor de eigenschap dat een organisme

door harmonie tussen groei en differentiatie gezond is, met een sterke weerstand en met sterk zelfregulerend vermogen. Deze laatste toestand is in het nieuwe kwaliteitsbegrip terug te vinden in het geïntegreerde of samenhangende aspect van vitale kwaliteit.

## Doel en werkwijze

### Doelstelling

Het onderzoek richt zich op het ontwikkelen van een kwaliteitsbegrip voor de biologische landbouw en het zoeken naar bijbehorende toetsbare parameters. Dit onderzoek is dus niet in de eerste instantie gericht op de vraag of producten uit de biologische landbouw beter zijn dan producten uit de conventionele landbouw. We weten immers nog niet naar welke kwaliteitsaspecten we moeten kijken en wat we als 'beter' moeten beschouwen. Pas in tweede instantie, als het kwaliteitsbegrip ontwikkeld en getoetst is, kunnen verschillende teeltmethoden met elkaar vergeleken worden.

### De appel als eerste proefgewas

De appel is gekozen als eerste proefgewas omdat voor fruittelers de levensprocessen groei en differentiatie herkenbaar zijn en het Louis Bolk Instituut ervaring heeft met dit gewas. Bij appel is al wereldwijd veel onderzoek naar kwaliteit gedaan zodat we vooraf al wisten waarop gestandaardiseerd moest worden om geen ongewenste variatie te krijgen. Het ras Elstar is gekozen als één van de meest smakelijke, Nederlandse appelrassen.

### Referentieseries

De 20 speciaal voor dit onderzoek geteelde partijen Elstar appels vormen de basis van dit onderzoek. De appels waren zó geteeld dat zo mogelijk slechts één van de levensprocessen in kleine stapjes tussen beide extremen varieerde binnen één serie en verder alle andere factoren zoveel mogelijk gestandaardiseerd waren. De partijen varieerden in pluktijdstip, dracht, zonlicht, Bd-preparaten en veroudering na bewaring. Door het zeer gunstige teeltjaar 2000 bleken achteraf de beoogde extremen in de series te ontbreken en de beoogde optima niet precies in het midden van de serie.

### Keuze van parameters

We kozen parameters uit die een rol spelen in het reguliere kwaliteitsbegrip voor appel en experimentele parameters waarvan we verwachtten dat ze relevant zijn voor 'vitale kwaliteit'.

De volgende parameters zijn onderzocht (per serie is een relevant geacht pakket samengesteld)

- gewas: bodem, groei, dracht, ziekten en plagen, bladreeks, bloei het volgende jaar(LBI)
- gangbare parameters: vruchtmaat, grondkleur, bloeskleur, glans, hardheid, zetmeel, Brix, zuur, N, P, K, Mg, Ca, droge stof (lab. PPO. Lab Zeeuws Vlaanderen).
- vitamine C (LBI), fenolen (TU-München, D), aminozuren en eiwit (Kwalis Qualitätsforschung, Fulda, D), zelfontbindingstest (LBI), smaak (LBI), koperchloride kristallisaties (LBI), stijfbeelden (LBI+ R.Mandera).
- biofotonen bepaald met 2 verschillende methoden (Meluna Biofotonen-onderzoek, Wijk bij Duurstede NL, en Kwalis Qualitätsforschung, Fulda, D)
- electrochemische parameters: pH, redox-potentiaal, elektrische weerstand, tezamen in P-waarde (H. Heilmann, Kirchberg, D)
- Bovis-waarde (dit is een intuïtieve waarneming die ter oriëntatie werd verkend, LBI).

### Partners

- Teelt van de appels: LBI, biologisch-dynamische Boomgaard ter Linde, Oost-Kapelle (NL).
- Ontwikkelen kwaliteitsbegrip: LBI, R. van Wijk (NL), J.O. Andersen (DK), F. Weibel (CH), J. Strube en P. Stolz (D), H. Heilmann (D).
- Financiering: LBI, Meluna-biofotonen-onderzoek (NL), Kwalis Qualitätsforschung (D), Stichting Triodos Fonds (NL), Software AG Stiftung (D), Zukunftsstiftung Landwirtschaft (D), Stichting Klaverblad (NL).

### Herhalingen en betrouwbaarheid

De partijen van 120 appels zijn mengmonsters van minimaal 10 verschillende bomen, die verdeeld zijn in submonsters voor de verschillende laboratoria. Om de kosten te beperken in dit oriënterende onderzoek kozen we niet voor onafhankelijke herhalingen in het veld. Per parameter is gekozen voor bepalingen aan mengmonsters of aan



onafhankelijke herhalingen van individuele appels. Bij de experimentele parameters is zo veel mogelijk gekozen voor herhalingen binnen de partij om zicht op de variatie in de methode te krijgen. Een aantal gangbare parameters dienden als referentie en controle op homogeniteit van partijen.

Het werken met behandelingsseries compenseert enigszins het gemis aan onafhankelijke herhalingen. De strikte standaardisatie van appelgrootte en groeiplaats in de boom hielp de variatie binnen de partijen te verkleinen.

Bijna alle parameters lieten een consequent verloop binnen de series zien waardoor we achteraf de uniformiteit van de series en de betrouwbaarheid van de kwaliteitsmetingen als voldoende beoordeelden. Alleen de zelfontbindingstest, het smaakonderzoek en de vitamine C-bepaling waren onvoldoende nauwkeurig, maar kunnen in de toekomst nog verbeterd worden.

### **Methodische stappen**

Het risico van een cirkelredenering is niet geheel weg te nemen. Ons anker ligt in de geteelde series appels met een geleidelijke toename in bepaalde levensprocessen en het toetsen van consistentie tussen begrip en uitkomsten van parameters.

De werkvolgorde was als volgt: De appels werden geteeld in series die binnen één serie zomogelijk in slechts één van de levensprocessen varieerden. De experimentele parameters die relevant leken voor deze levensprocessen werden bepaald. De uitkomsten werden vergeleken met het verloop van gangbare parameters en met gegevens uit de literatuur. Hieruit bleek dat we goed bruikbare series hadden. De experimentele parameters bleken de gangbare parameters aan te vullen en verrijkten ons beeld van de levensprocessen en van het kwaliteitsbegrip. Ook werd beoordeeld hoe parameters zich onderling verhielden. Tenslotte werden alle parameters stuk voor stuk beoordeeld op hun relatie met de levensprocessen en hun relatie met het nieuwe kwaliteitbegrip.

## **Resultaten van de behandelingsseries**

### **Rijpheid**

Voor de vergelijkbaarheid binnen één behandelingsserie is gekozen voor analyses op dezelfde dag. Voor de serie met 5 verschillende pluktijdstippen (tussen 1 september en 9 oktober) betekent dit dat vroeg plukken gekoppeld is aan enkele weken langer gekoeld bewaren. De serie laat daarom in feite zien wat het verschil is tussen rijping aan de boom en rijping in de koeling. De omzetting van zetmeel naar suiker en verlies van hardheid treden immers zowel aan de boom als in de koeling op. Echter, voor bepaalde aspecten van rijping is rijping aan de boom essentieel, zoals kleur, maat, smaak, biofotonen, kristallisatiebeeld, stijgbeeld en Bovis-waarde.

Het bepalen van al deze parameters hielp ons om het rijpingsproces te leren herkennen als een opeenvolgend proces waarin steeds andere stoffen van vaste vorm in oplossing gaan of verdampen: bijvoorbeeld harde vruchten met zetmeel, zuur en fenolen gaan over in sappige vruchten met opgeloste suikers en aromatische stoffen.

De beeldvormende methoden laten een toename in openheid en naar-buiten-gericht-zijn zien. Het rijpingsproces kunnen we karakteriseren als een lichte afname van vitaliteit en een toename van structuur en samenhang. Dit in tegenstelling tot het verouderingsproces in de koeling, waarin ook verlies van vitaliteit optreedt, maar zonder toename van structuur en samenhang. Veel parameters, die samenhang aangeven hadden een maximale (hier optimale) waarde ten tijde van het derde of vierde pluktijdstip.

### **Dracht**

In juni werden vruchten uit bomen gedund tot de gewenste drachtniveaus: 35, 75, 100, 125 en 140 vruchten per boom. Dit komt overeen met 14, 30, 40, 50 en 60 ton appels per hectare. Vooraf hadden we het derde drachtniveau als optimaal ingeschat, maar door het gunstige seizoen bleek achteraf het vierde drachtniveau dit jaar ook nog mogelijk voor voldoende smaak en bloemknopvorming. Een bekend fenomeen, en ook hier weer gevonden, was dat hogere dracht samen gaat met minder twijggroei (in beeld gebracht met de bladreeks methode), en ook met een lagere blad/vrucht-verhouding en minder bloemknopaanleg voor het volgende jaar. Bij de kwaliteit van de vruchten bleek de hogere dracht een afname door verdunning tot gevolg te hebben in alle parameters die met assimilatie en mineralenopname te maken hebben: droge stof, suiker, zuur, aroma en de verschillende mineralen. Calcium gedroeg zich omgekeerd ten opzichte van de andere mineralen. Ca en Ca/K als maat voor bewaarbaarheid namen juist toe bij hogere dracht, hetgeen aansluit bij de ervaring in de praktijk.

De kristallisatiebeelden gaven bij weinig vruchten de indruk van krachteloos en vegetatief en bij veel vruchten scherpe vormen en indruk van armoede. Bij de middelmatige dracht waren de meest vitale en gedifferentieerde beelden zien. De stijgbeelden werden scherper bij hoge dracht. De smaak was min of meer constant en alleen bij de hoogste dracht minder goed. Bij de hogere dracht nam de biofotonenemissie na excitatie af (maat voor vitaliteit) en de mate van hyperboliciteit van de emissie (maat voor de verhouding differentiatie/groei) toe.

Samenvattend was bij toenemende dracht een afname van vitaliteit en een toename van structuur waarneembaar.

## **Zonlicht**

Bij de oogst zijn uit dezelfde bomen apart appels geplukt die in de volle zon hingen, geheel in de schaduw of daar tussen in. We hadden twee series met zonlicht, één met en één zonder Bd-preparaten. In beide series werden vrijwel overeenkomende resultaten ten aanzien van de zonbelichting gemeten. Bij de appels in volle zon vonden we vooral meer kleur, fenolen, biofotonen (alle drie een indicatie voor differentiatie), een breder kleurenspectrum bij biofotonen (maat voor vrucht-typisch), hogere verhouding eiwit/aminozuren, meer samenhang en transparantie in het kristallisatiebeeld en meer ronde open vormen in de stijgbeelden (allen maat voor meer integratie). Verrassend was dat er geen verschil in smaak was, ook niet in hardheid, calcium of zuurgraad. Nieuw voor ons waren de veel hogere gehalten van N, P, K, aminozuren en eiwit in de schaduwvruchten. De zonvruchten hadden hierdoor een hogere Ca/K-verhouding dat aansluit bij de ervaring in de praktijk dat zonbelichte vruchten beter bewaarbaar zijn.

Samenvattend lijkt zonbelichting dus de differentiatie te stimuleren, waardoor meer structuur en samenhang werd gevonden.

## **Biologisch-dynamisch spuit preparaten**

Een deel van de boomgaard kreeg dit seizoen geen Bd-sputpreparaten. Het andere deel werd twee maal behandeld met koemestpreparaat en tweemaal met kiezelpreparaat. Er werd een zonlicht-serie geplukt in zowel het deel met preparaten als in het deel zonder preparaten.

Bij de controle op homogeniteit bleek het deel zonder preparaten iets groeikrachtiger, waarbij we niet kunnen aangeven of dit door de preparaten komt of door andere oorzaken.

Opvallend was dat met gangbare parameters, zoals kleur, smaak, hardheid, mineralen, zetmeel, zuur en pH geen verschil gevonden werd tussen appels uit het deel met en zonder preparaten. Echter bij een aantal experimentele parameters die differentiatie of integratie aangeven vonden we wel verschillen, zoals bij fenolen, eiwit/aminozuren, elektrische weerstand, breder kleurenspectrum bij biofotonen, transparanter kristallisatiebeelden en Bovis-waarde. De effecten treden echter niet altijd bij alle drie niveaus van zonbelichting op en maken daardoor harde conclusies niet mogelijk.

De series over licht en preparaten zijn gecombineerd omdat we van preparaten een vergelijkbaar effect als van zonlicht verwachtten en bovendien het bevorderen van het integratie-proces. Beide verwachtingen kunnen noch bevestigd noch ontkent worden door deze resultaten.

## **Veroudering**

De appels van het vierde pluktijdstip werden 3 maanden in de mechanische koeling bewaard en op verschillende momenten uit de koeling op de fruitschaal gelegd. Hierdoor ontstond een serie in uitstalleven van 1, 4, 8 en 12 dagen. Bekend, en ook hier gevonden, is dat bij veroudering de hardheid en het zuurgehalte duidelijk afnemen, terwijl het suikergehalte nog een tijdlang op peil blijft. Bijna alle parameters lieten een beperkte veroudering zien, maar minder sterk dan we verwacht hadden. De serie was niet extreem genoeg en had achteraf nog langere uitstalperiodes moeten bevatten om werkelijk verval in beeld te krijgen. Bij de kristallisatiebeelden ontwikkelde de naaldstructuur zich steeds meer in de periferie naarmate de appels ouder waren.

Opmerkelijk was dat de appels die net 1 dag uit de koeling waren, door veel parameters als minder goed werden beoordeeld dan appels van 4 dagen uit de koeling. Ze lijken na koeling (en/of transport) gedurende een aantal dagen te moeten acclimatiseren.

Voor het toetsen van het kwaliteitsbegrip was deze serie geen illustratieve serie omdat waarschijnlijk vooral vitaliteit, maar ook differentiatie en integratie afnamen. De veranderingen in levensprocessen zijn in deze verouderingsserie niet te onderscheiden.

## **Verdere uitwerking van het begrip 'vitale kwaliteit'**

Uit het onderzoek blijkt dat, naast de twee levensprocessen groei en differentiatie, het zinvol lijkt om als derde aspect de integratie tussen deze twee processen toe te voegen.

Het onderscheiden van twee levensprocessen, groei en differentiatie, is slechts een denkbaar onderscheid. Zodra een levend wezen begint te groeien is er al sprake van beide processen, die in een bepaalde verhouding staan.

Soms met de nadruk op groei (vegetatieve levensfase, weelderigheid, kankergezwellen) en soms met nadruk op differentiatie (generatieve levensfase, armetierige noodbloei). Hieronder worden de kenmerken van 'vitale kwaliteit' beschreven aan de hand van de appelparameters. In bijgaand schema fig. 11b worden deze begrippen

veralgemeniseerd als basis voor onderzoek bij andere gewassen.

### **Vitaliteit is het resultaat van groei**

Groei kan worden beschreven als het proces van expansief vullen van de ruimte met ongevormde massa, groei van organen, celdeling en bij planten de productie van primaire metabolieten door fotosynthese. Een vitale boom heeft veel groene bladeren en een flinke appelopbrengst. Een vitale appel heeft een grote maat, is stevig, knapperig en sappig. Het product heeft een hoog gehalte aan zetmeel, suiker, zuur, aminozuren en eiwitten waarbij de verhouding tussen aminozuren en eiwit wordt bepaald door de mate van rijpheid. Een vitale appel is nog bezig met opbouw van biomassa, waarbij nog vele transporteerbare stoffen worden gevonden (aminozuren, suikers). De pH is hoog, de turgor is hoog, de hoeveelheid biofotonen direct na excitatie is hoog en de kristallisatiebeelden zijn goed gevuld met een dichte naaldstructuur.

### **Structuur is het resultaat van differentiatie**

Differentiatie is het proces van specialisatie in vorm en functie, zoals celdifferentiatie, verfijning in vorm, geur, kleur, glans, afrijping, bloemknopvorming, stuifmeelvorming en zaadvorming. Er ontstaat ordening van structuur en vorming van secundaire metabolieten, zoals de was op de schil, fenolen, vitaminen en aromatische stoffen. Vruchten met goede structuur hebben veel pitten, een hoog calciumgehalte en zijn lang bewaarbaar. De hoeveelheid biofotonen neemt na excitatie in een hyperbolische curve af. De kristallisatiebeelden zijn duidelijk geordend, hebben slechts één centrum en de scherpe zijnaalden hebben een grote hoek.

### **Samenhang is het resultaat van integratie**

De groeiprocessen moeten op een middelhoog niveau plaatsvinden om de differentiatie processen goed te laten verlopen. Bij te weelderige groei komt de afrijping niet tot zijn recht. En bij geringe groei, leidt differentiatie tot armetierigheid, tot noodbloei en tot 'conservering' met harde, droge appeltjes als gevolg.

Als groei en differentiatie gelijktijdig en evenwichtig verlopen, eventueel in ritmische afwisseling, dan is sprake van een integratie proces. Er is een dynamische balans tussen groei en differentiatie die mede afhangt van de context, zoals soort, ras, ontwikkelingsstadium, seizoen, bodem en boomgaardmanagement.

Bij integratie gaat het niet alleen om een bepaalde balans tussen groei en differentiatie, maar ook om de mate van in wisselwerking treden. Bij voldoende integratie combineert een appel een goede smaak met een redelijk lange bewaarbaarheid. De smaak is aromatisch, sappig, knapperig en heeft een goede verhouding tussen zoet en zuur. De biosynthese is afgerond, wat herkenbaar is aan weinig vrije aminozuren en relatief veel eiwit. Zulke appels hebben weerstand tegen zelfontbinding, stress en ziekten. Het vruchtvlees is elastisch en behoudt de vochtspanning lange tijd na het plukken. De elektrische weerstand is hoog en de redox-potentiaal is laag. De hoeveelheid biofotonen begint na excitatie op hoog niveau en neemt met een hyperbolische curve af en laat een breed, vrucht-typische kleurenspectrum zien. De kristallisaties tonen een samenhangend en appel-typisch beeld. De Bovis-waarde is hoog.

### **Het aspect 'samenhang' blijkt een relationeel kenmerk**

Bij het beoordelen van vitaliteit en structuur kan de waarnemer nog afstandelijk en objectief beoordelen. Zelfs de kristallisatiebeelden kunnen op dit niveau door een beeldherkende computer tot op zekere hoogte beoordeeld worden. Bij het beoordelen van samenhang wordt betrokkenheid en inleving van de waarnemer verwacht en ook een doelstelling als referentie voor de beoordeling. De fruitteler brengt een eigen regie in. Hij kiest de streefwaarden in de verhouding tussen groei en differentiatie door de afweging tussen streven naar hoge opbrengst en streven naar goede smaak. Bij veel van de testen op samenhang wordt een actieve deelname van de onderzoeker verwacht, bijvoorbeeld smaakproeven en inlevend waarnemen bij de beoordeling van de kristallisatiebeelden. Bij het ontwikkelen van deze parameters moet speciaal aandacht aan de (inter-subjectieve) beoordeling worden gegeven om de testen acceptabel voor wetenschappelijk onderzoek te laten zijn. Het ontwikkelen van testen om integratie te meten is van groot belang voor het nieuwe kwaliteitsbegrip; het aspect samenhang is immers het meest wezenlijk voor 'vitale kwaliteit'.

### **Beoordeling op bruikbaarheid voor fruittelers van het kwaliteitsbegrip**

Het herkennen en beoordelen van de balans is voor telers van groot belang om tijdig met cultuurmaatregelen te kunnen corrigeren. Fruittelers bleken in staat om na presentatie van dit kwaliteitsconcept de cultuurmaatregelen te noemen die ze kunnen inzetten als correctie op te veel groei of op te veel differentiatie. Hoe de werkelijke integratie tussen beide processen te bevorderen is, bleek voor de fruittelers onzeker. Biologisch-dynamische telers nemen aan dat de Bd-preparaten hierbij een rol spelen, maar dat moet nog beter aangetoond worden in volgend onderzoek.

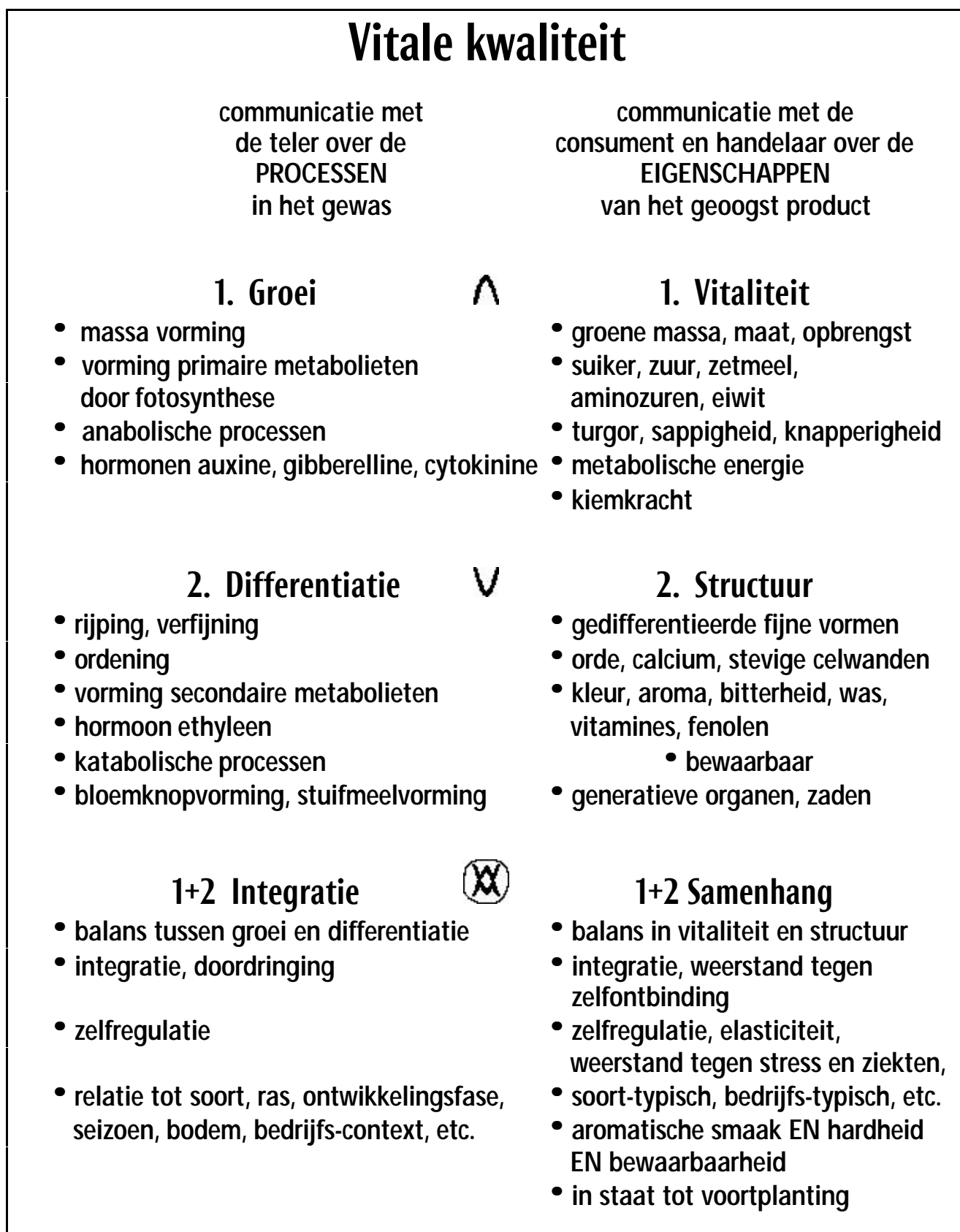
### **Beoordeling van de gebruikte parameters voor het kwaliteitsbegrip**

De gangbare parameters zijn als basis onontbeerlijk en maken controle van series, van homogeniteit en aansluiting op de literatuur mogelijk. Onder de experimentele parameters, lijken met name de kristallisaties, biofotonen en Bovis-waarde van waarde te zijn voor het ontwikkelen van een nieuw en samenhangend kwaliteitsbegrip. De betekenis van stijgbeelden en elektrochemische parameters is nog onvoldoende duidelijk. In de toekomst kunnen wellicht de goedkopere parameters routinematig gebruikt worden, maar dan geïnterpreteerd vanuit het gehele kwaliteitbegrip, zoals bijvoorbeeld de verhouding zoet/zuur en de verhouding eiwit/aminozuren.

### **Aanbevelingen voor verder onderzoek voor de begrips- en methode-ontwikkeling**

- vergelijkbare proefopzet met appels in een serie met toenemende stikstofbemesting en opnieuw een serie met Bd-preparaten.
- vergelijkbare proefopzet met andere producten, m.n. wortel, rode biet, tarwe, aardappel, tomaat, melk en beoordelen of de drie aspecten van vitale kwaliteit hier ook te vinden zijn.
- methodische verbetering van de parameters: smaak, zelf-ontbindingstest en kristallisaties.
- verdere ontwikkeling in interpretatie van biofotonen, koperchloride kristallisaties en Bovis-waarde.
- welke teeltomstandigheden verbeteren het integratieproces?
- voedingsonderzoek om de betekenis van vitaliteit, structuur en samenhang voor de menselijke gezondheid te bepalen.

Fig. 11 b: Vitale kwaliteit in trefwoorden voor diverse gewassen



# Zusammenfassung und Konklusionen: Parametern für Apfelqualität und ein Entwurf eines neuen Qualitätsbegriffes

## Der Grund und der Rahmen vom ‘Food, Quality and Health-Programm’

Der viel verwendete Begriff der Qualität mit Betonung des Aussehens und der Inhaltstoffe, scheint sich bei den Produkten des ökologischen Landbaus und dem dazugehörigen Handel nicht zu bewähren.

Ökologische Bauern und ihre Konsumenten streben ein Produkt an, dem ‘Vitalität’, ‘Lebenskraft’, ‘Ganzheit’, ‘Zusammenhang’ oder wie es auch immer bezeichnet wird, innewohnt. Von diesen Begriffen wird erwartet, dass sie gesunde Nahrungsmittel beschreiben und mit ihrer Hilfe lebendige Nahrung von einer bloßen Nährstofflösung unterschieden werden kann. Hiervon ist die sogenannte Astronautennahrung die extremste Form.

Die genannten Begriffe werden von jedem Menschen unterschiedlich interpretiert, häufig in Bezugnahme auf eine Untersuchung, wobei ein Qualitätskonzept mit prüfbar Parametern bisher immer noch fehlte.

Das internationale mehrjährige Programm ‘Food, Quality and Health’ (FQH) hat zum Ziel einen umfassenden Qualitätsbegriff mit überprüfbar Parametern zu entwickeln, der den Bedürfnissen ökologischer Produzenten und Konsumenten entspricht. Der erste Schritt ist das Entwickeln von Qualitätsbegriffen und Parametern. Hierfür ist das vorläufige Konzept von der ‘vitalen Qualität’ entwickelt worden. Dann erst ist es als zweiter Schritt möglich zu prüfen, ob für bestimmte Gruppen von Menschen die Produkte mit einer hohen ‘vitalen Qualität’ tatsächlich gesünder sind.

In diesem ersten FQH-Projekt mit Äpfeln, suchten wir nach Möglichkeiten einen Begriff für ‘vitale Qualität’ mit nachprüfbar Parametern einzuführen.

Dies ist lediglich ein Versuch, weil die Einführung eines neuen Qualitätsbegriffs mit Hilfe von experimentellen Parametern Gefahr läuft, sich im Kreis seiner eigenen Gedanken zu verfangen. Der hier entwickelte neue Qualitätsbegriff und die Parameter verstehen sich als „vorläufig“ und müssen mehrere Jahre hindurch bei vielen Produkten auf ihre Zuverlässigkeit und Handhabbarkeit hin getestet werden. Dann erst kann man darauf vertrauen, dass ‘vitale Qualität’ ein sinnvoller und nachprüfbarer Begriff ist. In diesem Projekt wurde der hier beschriebene Begriff in einem sehr begrenzten Kontext entwickelt, nämlich anhand von Äpfeln einer Sorte, die innerhalb eines Jahres auf einem Betrieb gewachsen sind und dabei unterschiedlichen Behandlungen ausgesetzt waren.

## Qualitätsbegriff ‘vitale Qualität’

An einem Qualitätsbegriff für den ökologischen Landbau stellen wir einige Ansprüche. Erstens sollte er an das zentrale Paradigma, nämlich das Bewahren von Lebensprozessen, anschließen. Wachstum und Differenzierung als die Grundlagen der Lebensprozesse können miteinander im Gleichgewicht stehen und in den Prozess integriert sein oder auch nicht. Wachstum, Differenzierung und ihre Integration sind die drei Aspekte, auf denen der neuen Qualitätsbegriff basiert.

Als zweites sollte der Qualitätsbegriff sowohl vom Produzenten als auch vom Konsumenten angewendet werden können. Beide haben äußerst unterschiedliche Vorstellungen. Der Produzent sieht die wachsende Pflanze und denkt in Begriffen von Lebensprozessen, die er oder sie fördern, hemmen und ins Gleichgewicht bringen kann. Konsument und Handel denken an die Eigenschaften des Endproduktes, die kontrollierbar und erkennbar sein sollen. Der Qualitätsbegriff hat darum zwei Seiten: Prozesse und Eigenschaften. Beide Seiten stehen miteinander in Verbindung.

Als drittes soll der Qualitätsbegriff anschließen an die ganzheitliche Betrachtungsweise von Ärzten und Ernährungswissenschaftlern sowie über Gesundheit, so dass in der zweiten Phase des Programms Untersuchungsprojekte durchgeführt werden können, die den Nutzen der Produkte für die Gesundheit testen und einer Theorie für die Wirkungsweise zu entwickeln.

Unter Berücksichtigung aller Ansprüche haben wir einen Qualitätsbegriff entwickelt, der mit ‘vitaler Qualität’ bezeichnet wird. Er basiert auf den Prozessen Wachstum, Differenzierung und Integration und beinhaltet die

zugehörigen Produkteigenschaften Vitalität, Struktur und Kohärenz.

### **Vitalität ist nur ein Aspekt von 'vitaler Qualität'**

Die Bedeutung des Begriffes Vitalität ist im Moment vorrangig das Resultat von Wachstumsprozessen. Vitalität in diesem Sinne bedeutet wachstumskräftig, jung, lebensfreudig. Der Aspekt von Vitalität, der die Fähigkeit meint, daß ein Organismus zwischen Wachstums- und Differenzierungsprozessen gesund und in Harmonie bleibt, ein kräftiges Immunsystem und ein starkes selbstregulierendes Vermögen besitzt, dieser Aspekt findet sich in dem neuen Qualitätsbegriff unter den Gesichtspunkten der Integration und Kohärenz wieder.

## **Ziel und Arbeitsmethode**

### **Zielsetzung**

Ziel unserer Forschung ist es, einen Qualitätsbegriff für den ökologischen Landbau zu entwickeln und nach den dazugehörigen prüfbar Parametern zu suchen. Dieses Forschungsvorhaben zielt also in erster Linie nicht auf die Frage, ob Produkte aus dem ökologischen Landbau besser sind als Produkte aus dem konventionellen Landbau. Wir wissen ja noch nicht, welche Qualitätsaspekte wir zu Rate ziehen müssen, und was wir als ‚besser‘ beurteilen sollen. Erst in zweiter Linie, wenn der Qualitätsbegriff entwickelt und geprüft worden ist, können unterschiedliche Anbaumethoden miteinander verglichen werden.

### **Der Apfel als erstes Untersuchungsobjekt**

Der Apfel ist als erstes Untersuchungsobjekt gewählt worden, weil die Obstanbauer die Lebensprozesse Wachstum und Differenzierung des Apfels erkennen und das Louis Bolk Instituut mit diesem Obst Erfahrung hat. An Äpfeln ist weltweit schon viel Forschung bezüglich Qualität betrieben worden, so dass wir vorher schon wussten, worauf wir standardisieren sollten, um unerwünschte Variationen zu vermeiden. Die Sorte Elstar wurde gewählt, da sie eine der schmackhaftesten niederländischen Apfelsorten ist.

### **Referenzserien**

Die 20 speziell für diese Untersuchung angebauten Partien von Elstar-Äpfeln bilden die Grundlage dieser Untersuchung. Die Äpfel wurde so angebaut, dass möglichst nur ein Lebensprozess innerhalb einer Serie in kleinen Schritten zwischen den Extremen variiert und alle anderen Faktoren so gut wie möglich standardisiert bleiben. Die Partien unterscheiden sich durch den Zeitpunkt des Pflückens, den Ertrag, Sonneneinstrahlung, Anwendung der biodynamische Präparate und die Alterung nach der Lagerung. Durch die günstigen Bedingungen, die im Anbaujahr 2000 herrschten, traten die erwarteten Extreme nicht auf, und die zu erwarteten Optima wichen vom Mittelwert in der Serie ab.

### **Die Wahl der Parameter**

Wir haben Parameter ausgewählt, die innerhalb des regulären Qualitätsbegriffes für Äpfel eine Rolle spielen und haben experimentelle Parameter ausgewählt, von denen wir erwarteten, dass sie für die ‚vitale Qualität‘ wichtig sind.

Die folgenden Parameter sind untersucht worden (pro Referenzserie wurde ein für relevant befundenes Paket zusammengestellt)

- Apfelbaum: Boden, Wachstum, der Ertrag, Krankheiten und Schädlingen, Blattfolge, Blüte im nächsten Jahr (LBI).
- Konventionelle Parameter: Fruchtgröße, Grundfarbe, Deckfarbe, Glanz, Festigkeit, Stärke, Brix, Säure, N, P, K, Mg, Ca, Trockenmasse (Lab. PPO Randwijk und Lab. Zeeuws Vlaanderen).
- Vitamin C (LBI), Phenole (TU-München), Aminosäuren und Eiweiß (Kwalis Qualitätsforschung, Fulda, D).
- Selbstzersetzungstest (LBI), Geschmack (LBI), Kupferchloridkristallisation (LBI), Steigbilder (LBI + R. Mandra).
- Zwei unterschiedliche Methoden mit Biophotonen (Meluna Biofotonen-onderzoek, Wijk bij Duurstede NL, und Kwalis Qualitätsforschung, Fulda, D).
- Elektrochemische Parameter: pH, Redoxpotential, elektrischer Widerstand, P-Wert (H. Heilmann, Kirchberg, D).
- Bovis-Wert (dies ist eine intuitive Wahrnehmung, welche zur Orientierung durchgeführt wird, LBI).

## Die Partner

- Für den Apfel-Anbau: LBI, biologisch-dynamische Boomgaard ter Linde.
- Entwickeln des Qualitätsbegriffes: LBI, R. van Wijk (NL), J.O. Andersen (DK), F. Weibel (CH), J. Strube und P. Stolz (D), H. Heilmann (D).
- Finanzierung: LBI, Meluna-biofotonen-onderzoek (NL), Kwalis Qualitätsforschung (D), Stiftung Triodos Fonds (NL), Software AG Stiftung (D), Zukunftsstiftung Landwirtschaft (D), Stichting Klaverblad (NL).

## Die Wiederholbarkeit und die Zuverlässigkeit

Die Partien von je 120 Äpfeln sind Mischproben von mindestens 10 unterschiedlichen Bäumen, die für die verschiedenen Labors auf sogenannte Subproben verteilt werden. Um in dieser ersten Orientierung dienenden Untersuchung die Kosten zu beschränken, haben wir uns nicht für unabhängige Wiederholungen im Feld entschieden. Jeder Untersuchungsparameter wurde jeweils an einer Mischprobe oder an individuellen Äpfeln geprüft. Bei den experimentellen Parametern sind so viele Wiederholungen wie möglich innerhalb der Partien untersucht worden, um so die Varianz der Methode abschätzen zu können.

Einige herkömmliche Parameter dienten als Referenz und als Kontrolle für die Homogenität der Partien. Durch Behandlungsserien wird der Mangel an unabhängigen Partien einigermaßen kompensiert. Die genaue Standardisierung der Apfelgröße und der Wachstumsstelle des Apfels im Baum reduzierte die Variation innerhalb der Partien. Bei fast allen Parametern gab es einen konsequenten Verlauf innerhalb der Serien, wodurch wir hinterher sowohl die Einheitlichkeit der Serien als auch die Zuverlässigkeit der Qualitätsmessungen als ausreichend beurteilten. Nur der Selbstzersetzungstest, die Geschmacksuntersuchung und die Vitamin C-Bestimmung erwiesen sich als ungenau, können aber in der Zukunft noch verbessert werden.

## Methodische Schritte

Das Risiko, sich im Kreis seiner eigenen Gedanken zu befinden, kann man nicht ausschließen. Von diesem Standpunkt aus versuchten wir, innerhalb der angebauten Apfelserien einen Zusammenhang zwischen den sich verändernden Lebensprozesse zu erkennen und diese zu verstehen. Bei dem Experiment wurde folgendermaßen vorgegangen:

Zuerst wurden die Äpfel in Serien angebaut. Die Äpfeln einer Serie sollten möglichst in nur eines Lebensprozesses variieren. Anschließend wurden die experimentellen Parameter, die für den entsprechenden Lebensprozeß aussagekräftig sind, bestimmt. Die Resultate wurden mit herkömmlichen Parametern und mit Aufzeichnungen aus der Literatur verglichen. Hier zeigte sich, dass wir über brauchbare Serien verfügten. Die experimentellen Parameter ergänzten die herkömmlichen Parameter und ergänzten so unser Bild von den Lebensprozessen und dem Qualitätsbegriff. Die Beziehungen der Parameter untereinander wurden untersucht. Schließlich wurden die Beziehungen der Parameter zu den entsprechenden Lebensprozessen beurteilt und in Beziehung zum neuen Qualitätsbegriff gesetzt.

## Die Resultate der Behandlungsserien

### Reife

Für die Vergleichbarkeit innerhalb einer Behandlungsserie, sind die Analysen am gleichen Tag untersucht worden. Für die Serie mit fünf unterschiedlichen Ernteterminen (zwischen dem 1. September und dem 9. Oktober) bedeutet ein früher Pflücktermin immer auch einige Wochen Lagerung bei kühlen Temperaturen. Aus dieser Serie lässt sich deshalb der Unterschied zwischen der Reifung am Baum und der Reifung bei der Lagerung erkennen. Die Umwandlung von Stärke zu Zucker und der Verlust von Festigkeit verlaufen sowohl am Baum als auch bei der Lagerung. Für viele andere Aspekte der Reifung ist die Reifung am Baum essentiell, wie z.B. Farbe, Größe, Geschmack, Biophotonen, Kristallisationsbild, Steigbild und der Bovis-Wert.

Durch die Bestimmung all dieser Parameter verstanden wir den Prozess der Reifung als eine aufeinanderfolgende Umwandlung von festen Stoffen in die flüssige oder gasförmige Zustandsform. So werden z.B. harte Früchte, mit Stärke, Säuren und Phenole, in saftige Früchte mit aufgelösten Zuckern und Aromastoffen umgewandelt.

Die bildschaffenden Methoden zeigen eine Zunahme von Offenheit und sind nach Außen gerichtet. Den Reifungsprozess können wir als einen Übergang von Vitalität zu Struktur und Kohärenz charakterisieren. Das ist ein Gegensatz zum Alterungsprozess, der in der Kühlung stattgefunden hat, wobei auch ein Verlust an Vitalität auftrat, aber ohne Zunahme von Struktur und Kohärenz.

Vor allem die Früchte des dritten oder vierten Erntetermins zeigten optimale Werte bei den



Untersuchungsparametern an.

## Der Ertrag

Im Juni wurden die Früchte im Baum ausgedünnt bis auf die erwünschte Anzahl von: 35, 75, 100, 125 und 140 Früchte pro Baum. Dies korreliert mit einer Erntemenge von 14, 30, 40, 50 und 60 Tonnen Äpfel pro Hektar. Vorher schätzten wir das dritte Ertragsniveau als den optimalsten Wert ein. Aber auf Grund der günstigen Saison erwies sich auch das vierte Ertragsniveau bezüglich Geschmack und Blütenknospenformung als geeignet. Ein bekanntes Phänomen, das sich auch hier wieder gezeigt hat, ist der Zusammenhang zwischen höherem Ertrag und geringerem Wachstum der Äste (bildlich dargestellt durch die Blattfolgemethode) und auch ein niedriges Blatt/Frucht-Verhältnis und weniger Knospenansatz für das nächste Jahr.

Bezüglich der Qualität der Früchte zeigten sich bei dem höheren Ertrag niedrigere Werte bei den Untersuchungsparametern, die mit Assimilation und mit Aufnahme der Mineralien zu tun haben z.B. Trockenmasse, Zucker, Säuren, Aromen und mehrere Mineralien. Bei Calcium zeigte sich als einziges Mineral eine steigende Konzentration. Die Calciumkonzentration und das Ca/K-Verhältnis als Maßstab für die Lagerung, steigt mit höherem Ertrag, was mit der Erfahrung in der Praxis übereinstimmt.

Die Kristallisationsbilder zeigten bei niedrigen Ertragsniveaus ein Bild von Kraftlosigkeit und entsprachen eher einem vegetativen Stadium. Die Kristallisationsbilder des höheren Ertragsniveaus waren verkümmert, besaßen aber schärfere Formen. Bei den mittelmäßigen Erträgen waren meistens vitale und differenzierte Bilder zu sehen. Die Steigbilder wurden mit zunehmendem Ertrag schärfer. Der Geschmack zeigte kaum Unterschiede, verschlechterte sich aber bei dem höchsten Ertrag. Bei den Biophotonen nahm das Niveau gerade nach der Anregung ab (ein Maß für die Vitalität) und die „Hyperbolizität“ nahm zu (ein Maß für das Verhältnis Differenzierung/Wachstum).

Zusammenfassend muß gesagt werden, daß mit zunehmendem Ertrag eine Abnahme an Vitalität und eine Zunahme an Struktur zu beobachten war.

## Sonnenlicht

Der Baum wird in drei Bereiche gegliedert: erster Bereich mit voller Sonneneinstrahlung, zweiter Bereich mit totalem Schatten und der dritte Bereich stellt eine Mischform von beiden dar. Aus jedem der Bereiche werden anschließend Äpfel geerntet.

Es gibt zwei Serien mit drei Varianten Sonnenlicht, eine Serie mit und eine Serie ohne Biodynamische Präparate. In beiden Serien mit Sonnenlicht wurden fast übereinstimmende Resultate hinsichtlich Sonnenbeleuchtung gemessen. Bei den Äpfeln die in der vollen Sonne gewachsen sind, zeigte sich eine bessere Färbung, höhere Gehalte an Phenolen, bessere Werte bei den Biophotonenmessungen (sind alle drei ein Maß für das Verhältnis Differenzierung/Wachstum), ein breiteres Farbenspektrum bei den Biophotonen (ist ein Kennzeichen für den Begriff „Fruchttypisch“), höheres Verhältnis Eiweiß/Aminosäuren, bessere Einheitlichkeit und Transparenz im Kristallbild und rundere, offenere Formen im Steigbild (sie sind alle ein Maß für mehr Integration). Überraschend war, dass kein Unterschied im Geschmack gefunden wurde, so wie auch nicht in Festigkeit, Calcium- oder im Säuregehalt.

Neu für uns waren die viel höheren Gehalte an N, P, K, Aminosäuren und Eiweiß bei den Früchten die im Schatten gewachsen sind. Die Früchte, die in der Sonne gewachsen sind, besaßen ein höheres Ca/K-Verhältnis. Dieser letzter Zusammenhang ist in der Praxis bekannt; sonnebeleuchtete Früchte lassen sich besser lagern. Sonneneinstrahlung scheint die Differenzierung zu stimulieren, wodurch mehr Struktur und Kohärenz gefunden wurde.

## Biologisch-dynamische Spritzpräparate

Ein Teil des Obstgartens wurde in dieser Saison nicht mit biodynamischen Spritzpräparaten behandelt. Der andere Teil wurde zweimal mit dem Kuhmistpräparat und zweimal mit dem Kieselpräparat behandelt. Sowohl aus der behandelten als auch aus der unbehandeltem Fläche des Obstgartens wurden eine Sonnenlichtserien gepflückt. Bei der Kontrolle auf Homogenität erschien der Teil ohne Präparate ein wenig kräftiger im Wuchs, wir können aber nicht genau angeben, ob dies durch die Präparate verursacht wurde oder andere Ursachen hatte. Auffällig war, dass mit den herkömmlichen Parametern, wie Farbe, Geschmack, Festigkeit, Mineralien, Stärke, Säuren und pH-Wert, kein Unterschied gefunden wurde zwischen Äpfeln aus dem Teil mit und aus dem Teil ohne Präparate. Aber bei einigen experimentellen Parametern, die Differenzierung oder Integration angeben, fanden wir schon Unterschiede, z.B. bei Phenolen, Eiweiß/Aminosäuren, elektrischer Widerstand, breiteres Farbenspektrum bei den Biophotonen, transparenterer Kristallisationsbilder und höhere Bovis-Werte. Die Effekte treten aber nicht

bei allen drei Niveaus der Beleuchtung auf und machen deshalb eindeutige Aussagen nicht möglich. Die Licht und Präparatenserien sind miteinander kombiniert, weil wir von den Präparaten einen ähnlichen Effekt wie von dem Sonnenlicht erwarteten und fördern den Integrationsprozess. Beide Erwartungen erwiesen sich durch die Resultate weder als bestätigt noch als falsch.

### **Alterung**

Die Äpfeln des vierten Erntetermins wurden drei Monate lang bei kühlen Temperaturen gelagert und zu unterschiedlichen Zeitpunkten aus dem Lager geholt und in eine Obstschale gelegt. So entstand eine Serie mit 1, 4, 8 und 12 Tagen alten Äpfeln. Hier bestätigte sich die in der Praxis bekannte Tatsache, daß mit zunehmender Alterung die Festigkeit und der Gehalt an Säuren deutlich abnehmen, während der Zuckergehalt durch die Remobilisierung noch eine Weile konstant bleibt. Fast alle Parameter zeigen eine begrenzte Alterung, aber weniger stark als wir erwarteten. Die Serie war nicht ausreichend aussagekräftig. Daher sollten die Lagerungszeiten der Äpfel erhöht werden, um einen wirklichen Verfall beobachten zu können.

Bei den Kristallisationsbildern zeigte sich mit zunehmendem Alter der Äpfel auch eine Zunahme der Nadelstruktur an der Peripherie. Bemerkenswert war, dass die Äpfel die nach einem Tag aus der Kühlung geholt wurden, bei vielen Parametern weniger gute Werte hatten als die Äpfel die vor 4 Tage aus dem Kühlhaus geholt wurden. Es scheint, als ob sich die Äpfel erst vom eventuellen Transportstress oder an die Bedingungen außerhalb der Kühlung anpassen müssen.

Für die Prüfung des Qualitätsbegriffes war diese Serie nicht anschaulich, wir vermuten, dass vor allem die Vitalität, aber auch die Differenzierung und die Integration abnehmen. Die Änderungen in den Lebensprozessen lassen sich in einer Alterungsserie nicht voneinander unterscheiden.

## **Weitere Ausarbeitung des Begriffes 'vitale Qualität'**

Aus der Untersuchung zeigt sich, dass die Integration zwischen den beiden Lebensprozessen Wachstum und Differenzierung eingefügt werden müsste.

Das Unterscheiden von den zwei Lebensprozessen, Wachstum und Differenzierung ist nur ein begrifflicher Unterschied. Sobald ein lebendiges Wesen anfängt sich zu entwickeln, treten beide Lebensprozesse in einem bestimmten Verhältnis gleichzeitig auf. Manchmal überwiegt das Wachstum (vegetative Lebensphase, Üppigkeit, Krebsgeschwulst) und manchmal die Differenzierung (generative Lebensphase, Notreife). Im unterstehenden Text wurden die Merkmale des Begriffes 'vitale Qualität' an Hand der untersuchten Qualitätsparameter bei Äpfeln beschrieben. In dem beiliegenden Schema Fig. 11c werden diese Begriffe verallgemeinert, das als die Basis für weitere Untersuchung an anderen Pflanzen dient.

### **Vitalität ist das Ergebnis von Wachstum**

Wachstum ist das Ausdehnen einer formloser Substanz in einen bestimmten Raum: z.B. das Wachstum von Organen, Zellteilung und die Produktion primärer Pflanzenstoffe durch die Photosynthese bei Pflanzen. Ein vitaler Baum zeichnet sich durch viele grüne Blätter und einen guten Apfelertrag aus. Der entsprechende vitale Apfel ist groß, fest, knusprig und saftig. Das Produkt enthält einen hohen Gehalt an Stärke, Zucker, Säuren, Aminosäuren und Eiweißen, wobei das Verhältnis zwischen Aminosäuren und Eiweiß durch das Maß der Reifung bestimmt wird. Ein vitaler Apfel ist noch mit dem Aufbau der Biomasse beschäftigt, aber es lassen sich noch viele transportierbare Stoffe finden (Aminosäuren, Zucker). Der Säuregrad ist niedrig, der Turgor ist hoch, die Menge an Biophotonen gleich nach der Anregung ist auch hoch und die Kristallisationsbilder sind mit einer dicht besetzten Nadelstruktur gefüllt.

### **Struktur ist das Resultat von Differenzierung**

Differenzierung ist der Prozess von Spezialisierung in Form und Funktion. Das sind z.B. die Zelldifferenzierung, die Verfeinerung in Form, Geruch, Farbe und Glanz, die Reifung, die Blüteknochenformung, die Pollenbildung und Samenformung. Dabei wird die Struktur geordnet und die sekundäre Pflanzenstoffe werden geformt, wie z.B. das Wachs auf der Schale, Phenole, Vitamine und aromatische Stoffe. Früchte mit einer guten Struktur enthalten viele Kerne, haben einen hohen Gehalt an Calcium und die Lagerungszeit ist länger. Die Menge an Biophotonen nimmt nach „Excitation“ in einer hyperbolischen Kurve ab. Die Kristallisationsbilder sind deutlich strukturiert, haben nur ein Zentrum und die spitzen Äste sind in einem großen Winkel angeordnet.

## **Kohärenz ist das Resultat von Integration**

Die Wachstumsprozesse müssen auf einem mittelhohen Niveau stattfinden, um die Differenzierungsprozesse gut verlaufen zu lassen. Bei einem zu üppigen Wachstum erfolgt keine vollständige Ausreifung. Und bei einem geringen Wachstum, z. B. Notreife erfolgt die Differenzierung in verkümmerte, kleine, harte, haltbare getrocknete Äpfel. Wenn sich die gleichzeitig ablaufende Prozesse, Wachstum und Differenzierung in einem Gleichgewicht befinden, oder sich rhythmisch abwechseln, dann wird von einem Integrationsprozess gesprochen. Das dynamische Gleichgewicht zwischen Wachstum und Differenzierung, ist z.B. abhängig von Art, Sorte, Entwicklungsstadium, Saison, Boden und Obstgartenmanagement.

Bei der Integration geht es nicht nur um ein spezifisches Gleichgewicht zwischen Wachstum und Differenzierung, sondern auch um die Wechselwirkung zwischen den beiden Prozessen.

Bei einer ausreichenden Integration vereint ein Apfel einen guten Geschmack mit einer ziemlich langen Haltbarkeit. Der Geschmack kennzeichnet sich als aromatisch, saftig, knusprig und einem optimalen Verhältnis zwischen süß und sauer. Die Biosynthese ist beendet, wenn wenig freie Aminosäuren und relativ viel Eiweiß in der Frucht vorhanden sind. Solche Äpfel besitzen bessere 'Schutzmechanismen' gegen Selbstzersetzung, Stress und Krankheiten. Das Fruchtfleisch ist elastisch und hält lange Zeit nach der Ernte die Feuchtigkeitsspannung. Der elektrische Widerstand ist hoch und das Redoxpotential niedrig. Die Menge an Biophotonen fängt nach „Excitation“ auf einem hohen Niveau an, nimmt danach mit einer hyperbolischen Kurve ab und zeigt dabei ein fruchttypisches Farbenspektrum. Die Kristallisationen zeigen ein einheitliches und apfeltypisches Bild. Der Bovis-Wert ist hoch.

## **Der Aspekt der Kohärenz scheint ein relatives Kennzeichen zu sein**

Bei der Beurteilung von Vitalität und Struktur ist der Beobachter noch in der Lage seine Objektivität zu erhalten und alles von einem gewissen Abstand zu betrachten. Auch die Kristallisationsbilder werden von diesem Standpunkt (Objektivität und Abstand) zum Teil mit Computerprogrammen analysiert. Zur Beurteilung der Kohärenz wird vom Beobachter ein Engagement und mit dem Versuchsobjekt erwartet, sowie eine klare Zielsetzung für die Referenz der Beurteilung.

Der Obstbauer bringt seine eigenen Regie ein. Er entscheidet sich mit seinen Maßnahmen für einen guten Geschmack oder einen hohen Ertrag. Damit legt er die optimalen Werte zwischen Wachstum und Differenzierung fest.

Viele der Tests zum Aspekt des Kohärenzes verlangen eine aktive Teilnahme des Untersuchers (z.B. Geschmackstest) und ein Sich-Hineinversetzen in die Kristallisationsbilder.

Bei der Entwicklung dieser Parameter soll die Aufmerksamkeit auf der intersubjektiven Beurteilung liegen, um die Tests für wissenschaftliche Untersuchungen geeignet zu machen. Das Entwickeln von Tests zur Messung der Integration ist sehr wichtig für den neuen Qualitätsbegriff; der Aspekt der Kohärenz ist das wesentlichste Element für den Begriff der 'vitalen Qualität'.

## **Beurteilung der Brauchbarkeit des Qualitätsbegriffes für den Obstbauer**

Das Erkennen und Beurteilen des Gleichgewichts ist für den Anbauer sehr wichtig, um rechtzeitig mit entsprechenden Kulturmaßnahmen Korrekturen vornehmen zu können. So waren die Obstanbauer in der Lage, nach der Vorstellung des Qualitätskonzeptes Kulturmaßnahmen zu nennen, die bei zu viel Wachstum oder Differenzierung zur Regulation angewendet werden könnten. Wie die tatsächliche Integration zwischen beiden Prozessen sich stimulieren läßt, war den Obstbauern jedoch unklar. Biologisch-dynamisch wirtschaftende Obstbauern vermuten, dass die biodynamische Präparate hierbei eine Rolle spielen, diese Vermutung muß aber noch in weiteren Forschungen nachgewiesen werden.

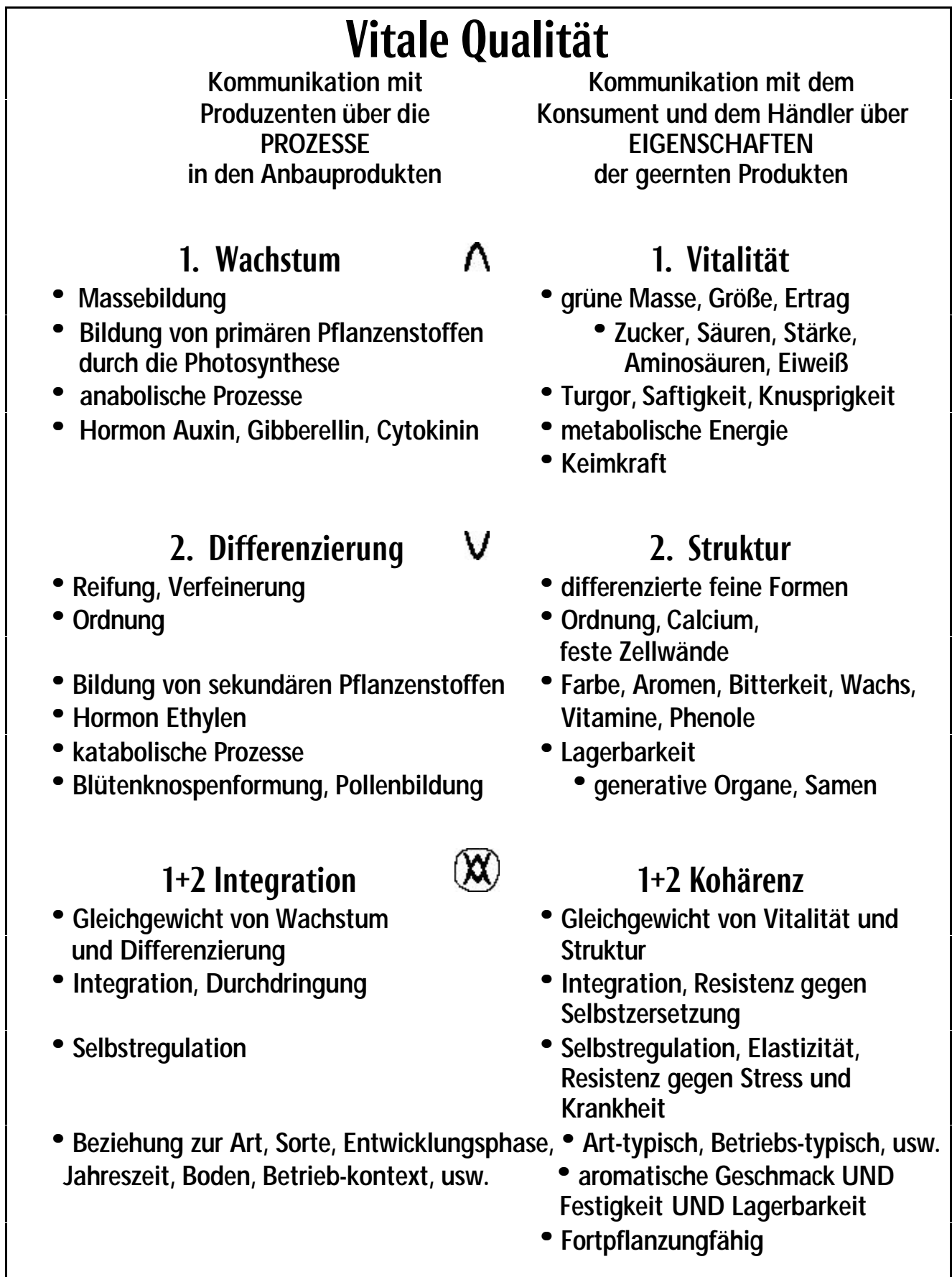
## **Die Beurteilung der verwendeten Parameter für den Qualitätsbegriff**

Die herkömmlichen Parameter sind als Basis unentbehrlich, erst sie machen die Untersuchung von Serien, Homogenität und die Vergleichbarkeit mit Aufzeichnungen in der Literatur möglich. Von den experimentellen Parametern erscheinen die Kristallisationen, die Biophotonenmessung und der Bovis-Wert, für die Entwicklung des neuen, ganzheitlichen Qualitätsbegriffes als brauchbar. Die Bedeutung der Steigbilder und die elektrochemischen Parameter sind noch unzureichend. In der Zukunft können vielleicht die kostengünstigen Parameter routinemäßig angewendet werden, aus der Sicht des neuen Qualitätsbegriffes, wie z.B. das Verhältnis süß/sauer oder das Verhältnis Eiweiß/Aminosäuren.

## **Empfehlungen für weitere Untersuchungen bezüglich der Begriffsentwicklung und der Methodenentwicklung**

- Vergleichbare Versuchsreihe mit Äpfeln in einer Serie bei zunehmenden Stickstoffdüngung und erneut eine Serie mit biodynamische Präparaten.
- Vergleichbare Versuchsreihen mit anderen Produkten vor allem Möhren, Rote Bete, Weizen, Tomate, Milch und die Beurteilung, ob die drei Aspekte des Begriffes „vitale Qualität“ auch hier zu treffen.
- Methodische Verbesserung der Parameter: Geschmack, Selbstersetzungstest, und die Kristallisationen.
- Weitere Verbesserung der Interpretation der Biophotonenmesswerte, der Kupferchloridkristallisation und Bovis-Wert.
- Welche Anbaubedingungen verbessern den Integrationsprozess?
- Die Untersuchung der Nahrung, um die Bedeutung von Vitalität, Struktur und Kohärenz für die menschliche Gesundheit zu erkennen.

Fig. 11c: Vitale Qualität in Stichworten für unterschiedliche Anbauprodukte



# 1 Introduction

*This chapter describes motivation, the FQH-framework, the global objective and method and the hypothesis of the new quality concept.*

## 1.1 Background: search for a quality concept for organic food

The continued development of sustainable agriculture will depend to an important degree on a high stable quality of organic products. To reach this we need a sound operational definition of product quality. In the organic vision of product quality and nutritional value, product quality encompasses the concept vitality.

But the concept 'vitality' is used in several ways by different authors, varying from extreme 'growth full' to 'not growth full at all' and emphasizes on keeping its own form and regulation processes. In practise it is a property that is more expected than really measured. Although there are some people in the world trying to develop tools to measure aspects of this vitality.

In this project we introduce *vitality* just for the *result* of the growth process. Furthermore we introduce the quality aspect *structure* for the *result* of the differentiation or ripening process, and the quality aspect *coherence* for the *result* of the integration of growth and differentiation process. The processes growth, differentiation and integration and the properties vitality, structure and coherence are covered by the term 'vital quality' as an overall concept. With 'vital quality' we associate to the German 'Vital Qualität'. In §1.2 and fig.12 this quality concept is further explained.

This quality concept is different from the conventional view. The quality aspects vitality, structure, and coherence are inextricably linked with an organic philosophy based on the life processes, and the assumption that the processes during development of the crop are reflected in properties of the final product. The conventional nutritional theory, on the other hand, tends to focus only on nutrients and external quality.

The organic concept of product quality requires further substantiation. At this moment, it is difficult to communicate the organic quality concept to the consumers because of lack of illustrative examples of the different aspects of quality and the confusing use of the concept 'vitality'.

In this investigation, therefore, we further develop the organic vision and organic concept of quality, we develop appropriate quality parameters for measuring the quality of products and develop the 'vital quality' concept in a way that it will inform both grower and consumer.

### Working hypothesis

From a scientific viewpoint, the idea that vital quality, or more specific vitality, structure and coherence are important quality aspects of agricultural products and relevant for a healthy nutrition, only qualifies as a working hypothesis. Although a number of positive indications exist to support this assumption, as yet there is not enough sound evidence to substantiate it scientifically. The substantiation of the assumption is difficult. To avoid being caught up in a vicious circle that would prevent any further research into this matter, we have chosen to accept this working hypothesis as a starting point. Once sufficient progress has been made in defining the quality aspects, we will go back and strive to substantiate the working hypothesis. Follow-up research will be necessary with respect to the effect of high-quality foods on health.

### Left-out aspects of quality

Some aspects of quality are left out in this concept; these important aspects must find a place in another context:

1. The aspects that are not seen in the final product such as social-economic aspects, influence on environment, use of energy, influence on personal development and enjoy of labour, fair earnings, communication between producer and consumer.
2. The aspects of storage potential, transportability, suitability for processing.
3. The residue aspects: microbiological, mycotoxins, residues of pesticides.

## 1.2 Life processes as the conceptual background

In organic agriculture the central paradigm is about *life processes*, as they continuously unroll in plants, animals and man, in relationship with the surrounding soil, water and air, and the light and warmth being encompassed in these. In organic agriculture the farmer doesn't manage minerals and nutrients, but he manages life processes of *living organisms*. The results of these life processes are expressed in the quality and quantity of the product that is grown or raised. Quality and quantity, in their turn, have an effect on the life processes of man, the consumer of the product.

How can, in this way of thinking, the *product quality* be described, doing justice to the various processes in the lifecycle in living beings? Can product quality be described in such a way that a bridge can be built between life processes that lead to these products and life processes in man who is eating the product? The hypothesis in the organic thinking is, that also the life processes in food products have to be 'digested' by the consuming organism and that in this active process of interaction the consumer's health is stimulated.

### Growth and differentiation as universal life processes

Such basic and universally found life processes - let it be somewhat adjusted to either the plant-, animal- or human-life situation - are processes of *growth* and of *differentiation*. They are expressed as well in the morphology as in the physiology. We mention some examples in plant and humans to emphasise the universality of these two processes (see Fig.12 for these processes in scheme) and to lay the basis for connecting plant product quality to human health.

**Growth** is associated with increase of mass and size.

**Differentiation** is associated with formation of a complex morphology, blossoming, fruit forming, ripening and synthesis of specific compounds.

**Integration of growth and differentiation** is associated with an integrated structure, resistance to stress, diseases and disintegration.

### Examples of growth and differentiation in human, plant and animal

In morphology growth brings an increase of living substance, material, whereas differentiation brings the form and structure of tissues and organs. In human physiology growth is found in anabolic processes, promoted for example by corticosteroid hormones and differentiation is related to catabolic processes, of which adrenaline is an example. In plant physiology primary metabolites (sugar, starch) are synthesised in growth processes and secondary metabolites (vitamins, phenols) in differentiation processes.

In the human being these processes of growth and differentiation can be easily seen in the embryonic development. A hand is formed out of two principles: first a centrifugal process of growing and proliferation, producing a 'bud', in which then through a centripetal differentiating process form and structure of the fingers are 'modelled' in. When these processes are in balance and well integrated the proper shape appears. Also in adult man a physiologically healthy situation exists by the balance and harmonious integration of growth and differentiation. In a disease like cancer growth of cells has become dominant and differentiation has decreased. Modern cancer therapies therefore try to improve differentiation processes (Leenstra 1996).

Also in plant growth and differentiation are found, but the intensity of either differs at different stages of development (von Kraft 1995). In the vegetative phase of the young plant, growth predominates in the formation of green substance, mainly leaves. Later this leaf growing process is reduced (in fruit trees there is even a leaf-pause in the winter) and a generative phase of blooming, fruit- and seed forming starts with a drastic differentiation of form and substance and the appearance of colour, aroma and taste.

### Integration as additional aspect

Typical for the cultivated nutritional plants is the fact that these two phases are intensified and have become intermingled to a certain degree. Berendt (1983) studied this phenomenon in lettuce. In wild lettuce development starts with a vegetative phase of sprouting and rosette building of small leaves, so the emphasis is on growth processes. This phase is followed by a differentiation phase with 'shooting', development of very fine flowers and seeds, and no leaves anymore, so the emphasis is on differentiation processes.

The head of cultivated lettuce has much more substantial leaves than its wild counterpart, so growth and differentiating processes both have increased and intermingled. At the moment of being 'ready for harvest' these leaves are aromatic and have a typical 'salad-like' taste. These characteristics are a result of integration processes by cultivation (breeding and cultural practises).

In apple, we see the same tendency, the small wild apple fruit has been cultivated into a substantial fruit, which in ripening becomes juicy, coloured and tasteful. Also here growing and differentiating processes are intensified and integrated by cultivation.

In this study we chose to work with a quality concept which is based on the intensity of *growing processes*, of *differentiating processes* and of harmonious and species typical *integration* of these two life processes. They are all part of the *vital quality* of a product. For a farmer who works with growing plants these concepts are recognisable and easily translated into fertilising, pruning, etc. For the consumer or trader, who buys a harvested product and didn't see the growing process, these concepts are less usable. So we need other concepts for the 'ready product' the consumer buys: the result of the growing processes should be found in its *vitality*, the result of the differentiating processes in the refinement of the product, characterised by its fine *structure* (including smell, colour), while a harmonious *integration* of life processes should lead to *coherence* in the product in a context typical way. With 'context' we think of the typical way species, variety, soil, climate, season, development stage, farm and farmer will reflect.

## Communication about quality

So we will use the three quality concepts in process terms for communication with the grower. The grower should be able to manage growth, differentiation and integration to reach a high vital quality.

And for the final product and the communication with the consumer we use the three quality concepts in terms of condition: so products with a high vitality, structure and coherence are assumed to influence the well being in a positive way.

We intend to link the conceptual quality aspects to the living forces and growing conditions known by the grower on the one hand and to the nutritional value, experienced by the trader and consumer on the other hand. This concept also has the possibility to connect to the holistic health view of physicians and dieticians.

This consumer's health hypothesis should be tested later in other FQH-projects, but that is beyond the scope of this study. Here, the aim is to develop tools to describe and judge these quality-aspects.

## The word 'vitality' can be confusing

As overall concept, in which both life processes are present, we chose for 'vital quality' (E), as it is already internationally used in 'Vital Qualität'(D) and 'vitale kwaliteit' (NL), also see figure 12a,b,c in the summaries in three languages.

Realise there is a meaningful difference between this 'vital quality', as a balance between the two life processes (growth, differentiation) and their integration with optimal self-regulation properties and the 'vitality', a concept only at the level of growth, so especially fresh, green, growing, young and lively.

In some groups (e.g. homeopathic healers) the word 'vitality' is used for what we call here 'coherence' and in other groups (e.g. conventional agriculture) the word 'vitality' is used for what we also call here 'vitality'.

## 1.3 The apple as the first research crop

Several projects on apple growth and ripening processes in orchards are already being carried out by the Louis Bolk Institute's fruit section. The polarity of 'growth and ripening' is a well-known and well-documented phenomenon in the literature on conventional fruit growing, as are its effects on product quality. The decision to focus initially on the apple in this project is thus prompted by the fact that this polarity is recognised as relevant to apples which thus provides us an opportunity to tie in with conventional views on quality. Later we will apply this concept to other crops, on which the growth and differentiating processes are more hidden.

Another reason to choose the apple as an example is the comparative study of organic and conventional grown Golden Delicious apples by FIBL to find relevant parameters for organic products (Weibel et al 2000). This study shows a better quality for organic in many parameters (firmness, taste, phosphate, phenols, fibres and 'vitality' by picture developing methods). And no difference in dry matter, Brix, acid, minerals (N, K, Mg, Ca), preference by



rats and self-disintegration test.

In a study which compares whole production systems there is no answer which cultural practices cause the better quality in organic systems (picked riper? no over-bearing trees? less nitrogen? more light exposed? bio-dynamic preparations?). This question is the next step we want to take and leads to our current experimental design with series of apples with one varying factor to teach us how these factors respond in the several parameters. And finally how to improve cultural practices by fruit growers to reach optimal quality.

## 1.4 Choice of quality parameters

Appropriate parameters are necessary to test the vitality, structure and coherence of agricultural products and foods. Different types of quality parameters can be distinguished: sensory tests, component analyses and more complex or holistic parameters.

In sensory tests the taste, aroma, consistency, colour and appearance are determined. Component analyses are used to determine the contents of components such as sugars, acids, minerals, vitamins, amino acids, carotenoids and polyphenols.

Holistic parameters are the copper chloride crystallisation, capillary pictures, biophotons, disintegration test, electro-chemical measurements and Bovis-value. An overview of holistic parameters is given by Meier-Ploeger and Vogtmann (1988). In this report you find each parameter in separate chapters in §5-§17.

The results of the sensory tests, component analyses and the holistic parameters could be related to the growing conditions and ageing conditions. However, an evaluation of these parameters, measured separately or in combination, with respect to the quality aspects vitality, structure and coherence has never been made. To evaluate these parameters we need reference series of products grown under different, sometimes extreme conditions in order to influence their growth and ripening. In each series only one cultural factor is varied in steps.

## 1.5 Reference series

Before quality parameters can be developed, reference values have to be established first on the basis of reference series. Four reference series are prepared: ripening period, fruit-bearing, light conditions with and without bio-dynamic preparations, and finally different ageing by different shelf-lives.

The apples were grown in an organic fruit orchard in series of 5 or 6 with a progressive intensity of the above mentioned conditions. Growing conditions in the orchard are documented. Apple samples have been taken from these series and all mentioned parameters are determined. The series will enable us to identify the appropriate parameters. The intention was to plan the middle intensity of the series treatments as ideal from a fruit grower's perspective for trading.

Apples thus produced should express characteristics in a manner that should enable us to measure extreme values of the parameters, and to relate them to the process of growth, differentiation (here ripening) and the integration.

- Series A, 5 harvest times from unripe to over-ripe, cold stored until the last picking date.
- Series B, 5 levels of fruit bearing from very low to trees laden heavily with fruit (this series is also part of a running project on optimisation of fruit yield and quality).
- Series C, 3 levels of light (shady, moderate, full sunlight) both with and without bio-dynamic field preparations, thus a total of  $2 \times 3 = 6$  treatments.
- Series D, 4 periods of 'on the shelf' after a 3 month period in cold storage. Apples from the 4th harvest date from series A are used for these D-series. D1 is the same as A4, and D2 to D5 have an increased shelf life. Between A4=D1 and D2 the influence of three months of cold storage is seen.

## 1.6 Research questions

The underlying questions were:

1. Is the intensity of the growth processes, the ripening processes and the integration of these in the reference series, reflected in the following parameters: fruit size, colour, shine on skin, firmness, sugar, starch, acid, vitamin C, minerals, amino-acids, phenolic compounds, taste, copper chloride crystallisation, capillary pictures,

bio-photons, electrochemical measurements and self-disintegration? If so, in which degree? Which parameters are so similar that it makes the other redundant? Is it possible to interpret the cheap component analyses in a more holistic view?

2. Can the measured values be used for testing vital quality? Can working hypotheses be set up on the basis of this information regarding consequences for human health and growing methods?

## 1.7 Co-operation

In contacts with the organic movement an urgent request is heard also to include research question no 2, concerning the quality concept, into this project. The aim to develop a thorough quality concept is ambitious and is best served by a good co-operation between (inter)-national experts on different parameters. So the Louis Bolk Institute invited several colleague-researchers, to participate in this project with their specific techniques and to participate in the discussion on understanding and integrating the different parameters in the quality concept.

### Participants:

- Louis Bolk Institute at Driebergen (NL), J. Bloksma (M.Sc. biology), M.A.S. Huber (M.D. physician), Dr. M. Northolt (food science), P.J. Jansonius (M.Sc. agriculture), M. Matze (M.Sc. nutrition), H.J.T.M. Hospers (M.Sc. biology) and A.M. de Weerd: co-ordination, quality concept, supporting the apple growing, description of orchard and crop, leaf series, external quality, Brix analysis, vitamin C, dry matter, disintegration test, sensory test, copper chloride crystallisations, capillary pictures.
- Meluna Bio-photon Research at Geldermalsen (NL), Dr. R. van Wijk: measurements of bio-photons i.c.w. A. Popp and quality concept.
- Kwalis Qualitätsforschung Fulda GmbH (D), Dr. J. Strube en Dr. P. Stolz: measurements of bio-photons with coloured light (modification of Popp's method), amino-acid analyses and quality concept.
- Arbeitskreis Qualität der BTQ (Gesellschaft für Boden, Technik Qualität) Kirchberg (D), Dipl.Ing.agr. H. Heilmann: electrochemical measurements and quality concept.
- University of Copenhagen/Hertha (DK), J.O. Andersen: quality concept.
- Forschungs Institut für Biologisch Landbau at Frick (CH) Dr. F. Weibel: quality concept and experienced in alternative quality research in apple.
- Inst. for biodynamic Research Darmstadt (D), I. Hagel: quality concept.

### Further collaboration with:

- Orchard Ter Linde at Oost Kapelle (NL): growing and storing the apples.
- Laboratory of Research Station for Fruit Growing (PPO) at Randwijk (NL): measurements of colour, firmness, Brix, starch, acid.
- Laboratory for Soil, Crop and Environment, Zeeuws Vlaanderen (NL): analyses of dry matter and minerals.
- TU-München, Fachgebiet Obstbau (D): Prof. Dr. D. Treutter: analyses of phenolic compounds.
- Ruth Manderla (expert capillary pictures).
- Statistics: M. Engelmoer (M.Sc. biology)
- Text-work: I. Schwagerman.
- English language: P. Doesburg (LBI) and German language: Maike Damhuis.

## 1.8 Funding

This project is financed by

- the Louis Bolk Institute (NL),
- Meluna Bio-photons (NL)
- Kwalis (D),
- Stichting Triodos Fonds (NL),
- Stichting Klaverblad (NL),
- the Software Stiftung (D)
- the Zukunftsstiftung Landwirtschaft (D).

We thank them all.

## 2 Introduction on apple growing

*This chapter is meant for those who are not familiar with the growth and ripening processes in the apple.*

### 2.1 Growth and ripening processes in apple

Chapter 2.1 is intended for people who are not familiar with the crop apple. Some basic processes in growth, ripening and the influence of picking date, crop load, light exposure and maturing on fruit quality are summarised from literature and practise.

#### 2.1.1 Processes of mass formation or 'growth'

Growth processes are associated with the growing volume of mass; the tree is spreading more and more into the surrounding area. In varying periods of the season apple trees show:

- length and succulent growth of roots (Feb.-May)
- length growth of twigs and spreading of leaves (May-Aug)
- succulent growth of the tree trunk (May-Aug)
- unfolding clusters and flowers on last year's reserves (End of April)
- fruit setting (May)
- development of fruit and fruit size (first by cell division; later cell enlargement) (May-Sept)
- filling of flower buds, bark and wood with reserves (aminoacids, sugars, starch) for next year (Aug-Nov)

The growth processes are guided by plant hormones like auxin, gibberellin and cytokinin and are favoured by water and nutrients taken up from the earth. The fruit grower stimulates these processes by watering, fertilisation, and growth stimulating pruning or reduces these processes by root pruning, controlled water stress etc.

#### 2.1.2 Processes of differentiating or ripening

Differentiation processes are associated with a complex morphology and physiology, fruit and seed formation, ripening and syntheses of specific compounds. In varying periods of the season apple trees show:

- pollination (April-May)
- flower bud initiation (May-June for flowering next year)
- ceasing shoot growth and lignification (=forming wood) (June-Sept)
- ripening of fruit: getting colour (red blush and ground colour from green to yellow), forming flavour (aroma, sugars) (Aug-Oct)
- developing seeds (May-Oct)
- ripening of shoots and leaves: colouring leaves and withdrawing compounds from leaves into bark, buds and wood for next year reserve.
- more intensive respiration in the fruit around ripeness (the moment of 'climacterium').
- syntheses of secondary metabolites like wax on the skin, phenolic compounds, vitamins, etc. (June-Oct)

The differentiating processes are guided by plant hormones as abscisic acid and ethylene and are favoured by warmth and light. The fruit grower stimulates these processes by optimal light entrance into the tree, summer pruning etc.

To get an optimal differentiating process first some mass must be formed. If not you will see for example 'emergency flowers', no successful pollination or fruit without aroma because of lack of assimilates.

#### 2.1.3 Vital quality as harmonious integration of growth and ripening

##### Apple as a bi-annual crop

During summer time the apple tree is working at three stages:

1. growth of roots, shoots and leaves and production of assimilates for this year;

2. growth and ripening of the apples for harvest this year;
3. initiation and filling of buds for next year with reserves.

An apple tree is botanically seen as a perennial crop. But you also can look at an apple twig as a biannual crop: bud formation in the first season (comparable with released seed in the soil) with a pause in winter. In the second season: flowering, fruiting, shooting and bud formation (comparable with an annual crop up to seed formation). This second view makes it easier to understand the various simultaneous processes and how to support the crop with cultural measurements. It is like singing a canon with three voices.

## Alternate bearing

Typical for some apple varieties (e.g. Elstar, Boskoop) is the habit of alternate bearing. In a year with high bearing, only few new buds for next year are formed and in a year with low bearing (by spring frost e.g.) too many buds are formed and the tree 'over-flowers' itself and loses energy for growing. So without fruit growers measures the tree jumps from one extreme into the opposite extreme next year. For a wild apple tree this habit is not a problem. Seen over many years the tree has enough chances to grow and spread seeds into the world and maintain its existence.

But for an agricultural crop with an aim at high food quality and sufficient production this is not enough. This alternation from one extreme into the other extreme brings less fruit quality and less mean production over the years. So the aim for fruit growers is to support and balance those processes by cultural measures like grafting on low or high growing rootstocks, pruning, root-pruning, thinning, training, fertilising, watering, trunk notching, etc. Fruit growers are used to speak about 'growth and ripening', 'growth and production' or 'vegetative and generative' referring to this polarity.

## Integration for fruit quality

In fruit growing literature many examples are known about the benefits of the balance between growth and yield. A harmonious grown tree is more capable to regulate itself. An irregularly grown tree reacts more to water stress. And an irregularly grown apple tree produces apples that store shorter. Table 1 shows some quality aspects in connection with bearing, summarised from literature.

**Table 1: Characteristics of under-bearing, optimal bearing and over-bearing.**

low bearing	optimal bearing	high bearing
many leaves per fruit	35-50 leaves per fruit	few leaves per fruit
low photosynthesis activity per leaf	Moderate photosynthesis activity per leaf	high photosynthesis activity per leaf
high sugar and acid thus much taste	fresh taste	low sugar, low acid, thus less taste
big sized fruit	Medium sized fruit	small sized fruit
early ripening	Ripening in time	later ripening
Red blush, yellow ground colour	Moderate colour	less blush, green ground colour
Firm	firm	less firm
Physiological storage diseases: Bitter pit, internal brown, rot	Maximal storage quality	physiological storage diseases

## Optimum between quantity and quality

Fruit growers have to choose the optimal position between yield and fruit quality. Some decades ago in conventional apple growing we saw quantity was more important than quality. On average high yielding varieties with less aroma like Golden and Red Delicious were grown and bought. High yield was achieved by optimal growing conditions and chemical help (drip irrigation with dissolved nutrients, artificial hormones for growth and yield regulation, pesticides) at the cost of fruit taste. From experiments with increasing levels of nitrogen it is known that the optimum for different properties is found at different levels of nitrogen. Increasing nitrogen will first result in an optimum for skin colour, then for taste and storage potential, then for texture and finally for yield (Stoll 1997).

But nowadays in an overproducing market for apples, the call for high quality, particularly taste and colour is heard. The high producing varieties are making place for varieties with good taste and colour like Elstar and the Jonagold mutant with improved colour. For Elstar the advised target yield has gone down from 60 tons/ha of

bulk fruit to 40 tons/ha of quality fruit For the organic market with the high consumers' prices it is of major importance to choose for good taste.

## 2.2 What is optimal ripeness?

Table 2 contains a number of characteristics of ripening stages in the period from some weeks before to some weeks after the picking time. The choice of picking date depends on the season and the expected storage period. The longer the expected storage time the earlier the picking. The later the picking date the longer the natural ripening process at the tree is going on and so more aroma is formed. The tastiest apple is ripened at the tree until fully ripe and consumed immediately, see table 2.

**Table 2: Characteristics of ripening stages from experience and literature**

	Not yet ripe	picking time for long storage	Consumers-ripe	Over-ripe
size	small	some bigger	big	Big
form	cone	rounder	round	Round
ground colour	green	yellow-green	green-yellow	Yellow
red blush	some	some more	more	Most
wax	thin	moderate	thick	Thickest
firmness	hard	moderate hard	moderate hard	Softer
Starch	high	moderate	low	Low
Sugar	low	moderate	high	high or some lower
Acid	high	moderate	low	Low
pH	ca. 3.5	ca. 3.7	ca. 3.8	ca. 4.0
Ethylene	Very low	suddenly raising	maximum	decreasing
Taste	too acid, no aroma, dry, hard	too acid, little aroma, juicy, crisp	optimal sweet/acid, much aroma, juicy, crisp	too sweet, little aroma, dry, soft, mealy

## 2.3 Texture of apple flesh

In apple fruit you will find watery parenchyma tissue with large polyedric cells drenched with vasculars. The cell walls contain (hemi-) cellulose stuck together with calciumpectin. Main properties of the texture for the consumer are firmness, crispness, juiciness and mealiness. A strong and elastic cell wall and a cell liquid with high viscosity (high sugar) enable a high juice pressure and firmness (Stoll 1997).

## 2.4 Influence of light

The Netherlands lay on the North border of the apple growing area. In our climate, light is a limiting factor for production. This is illustrated by the minimal amount of leaves to feed fruit: 20-30 in North of Italy and 30-40 in the Netherlands. To optimise catching light the fruit grower uses special systems of spacing trees, row direction, training young trees and pruning older trees to 'open trees'. In bio-dynamic fruit growing it is believed that the field-preparations support the use of light.

Leaves in the sun have a thicker assimilation tissue than leaves in the shadow and produce more assimilates. Fruit in the sun has a thicker wax layer on the skinskin ('cuticula') to compensate for the higher evaporation rate, the skin is more red coloured, the fruit is bigger sized and stores longer.

## 2.5 Alteration and senescence

Maturation is the natural process after ripening. The fruit has to disintegrate to release its seeds. The membrane structure of the cells loses its cohesion, tissues get soft and turn brown, cells slide from each other and the consumer says the apple is getting 'mealy'. The electric conductance of the tissue increases. The process of maturing is slowed down by the modern storage techniques but continues during shelf life (Stoll 1997).

## 2.6 Polarity between seeds and fruit flesh

After being young, small and extremely firm the seeds and flesh are developing into opposite characteristics: The flesh gets softer, juicy, aromatic, white, round, open to the surrounding, produces ethylene, easy to disintegrate and suitable for human nutrition. The seeds, on the other hand, get hard, dry, brown, pointed, sustainable, closed, produce gibberellins and are intended for reproduction. Table 3 shows differences in mineral content between flesh and seeds, seed data is from former research on another apple variety.

**Table 3: Nutrients in flesh and seeds in the apple variety Red Boskoop (LBI 1999)**

	% dry matter	N	P	K	Mg	Ca	emphasis on
		in mg/100 gram fresh weigh					
flesh	16	45	12	125	6	5	carbohydrates
seeds	80	3000	415	600	210	16	nitrogen

# 3 The reference series and experimental design

*This chapter describes the circumstances of our experimental results.*

## 3.1 Circumstances in the orchard

### Soil and climate

Orchard Ter Linde is situated in the south-western part of the Netherlands, in a sunny mild sea climate with a slight salty spray by sea wind. The soil is limy humus sea loam on limy sand with trickle irrigation. This soil has been bio-dynamically cultivated for more than seventy years.

### The orchard

The orchard is 10 years old, on dwarf rootstock M9, 2460 trees/ha with mowed grass in the tramline and most of the year a clean cultivated tree strip. The grower is able to prune to open trees with a quiet top. Sulphur and a small amount of lime sulphur are applied for scab control and some microbiological treatments against insects. Composted cow manure with Bd-compost-preparations is used every three years. In the other years, as this year, dry chicken manure pellets are used for additional fertilisation. For this experiment we used some rows without fertilisation. The differences in % nitrogen in leaves of fertilised and none-fertilised were not noticeable: and averaged 2,0% N in August.

This orchard is known for low nitrogen levels in soil and leaves and good fruit quality. The bearing differs from year to year depending on flowering conditions from 15 (spring frost) to 150 (too little fruit thinning) fruit per tree.

### The variety Elstar

The apple variety is Elstar, strain Elshof, pollinated by Alkmene. Elstar is one of the best tasting varieties in Holland; also under sub-optimal conditions you will have good fruit quality, especially colour and taste wise. The fruit is round, size small to moderate. The skin is green-yellow with a red shade and quit red, which is typical for the Elshof strain. Exposure to light is of great importance to colour the skinskin. For this variety exposure to light is of great importance to colour the blush. Among apples the apple variety Elstar has the habit of alternate bearing and of unequal ripening. Fruit growers usually pick Elstar in two or three rounds.

## 3.2 The season 2000

In the former year (1999) the roots were pruned after a frost year with low bearing and too much growth. In the season 2000 the balance between growth and bearing returned. The pollination conditions were good, the fruit set was good and needed moderate hand thinning. The optimal yield for this orchard is estimated at a 100 fruit per tree for a regular bearing every year. Rain fell nicely spread throughout this summer season, so soil mineralisation was good and twig and fruit grew continuously. In general, this leads to good storage quality and high bearing capacity. Also the heavy bearing trees, that have no twig growth in most other years, now have a moderate growth. This year the opposition between growth and yield is not as pronounced as we are used to. This year had an average number of sun hours and was very late with cold nights which colour the fruit red. The autumn was relatively warm.

There was only a small amount of fruit with insect damage this year. For measuring the quality we only used sound fruit.

## 3.3 Description of the reference series

The aim was to create series with only one varying factor increasing in small steps and standardised for all other influences. Due to our knowledge about apple quality and how it depends on the place in the tree, exposure, size, fertilisation, etc, we knew what had to be standardised. Because of adding series C later, we had to use more fertilised rows for these series. In table 4 we summarised the main factors and how we succeeded in standardising.

**Table 4: Overview of the varying and constant factors in the series:**

Series	Nr. of treatments	fertilising	exposure to the sun	Bd-field preparations	yield of fruit per tree	growth	picking date	Days of cold (4°C) Storage	fruit size of sample in mm
A ripening	5	low	Full	yes	130	moderate	1, 12, 21, 29 Sep; 9 Oct	20(A1, A2, A3), 12, 0(A5).	70-75 to 75-85
B bearing	5	low	Full	yes	35(B1); 75; 100; 125; 140(B5)	strong to weak	22 Sep	4	75-85 (1=80-90)
C light and preparations	3	medium	no(1); half; full (3)	yes	130	moderate	18 Sep	1	75-85
	3	medium	no (4); half; full (6)	no	120	slightly more than moderate	18 Sep	1	75-85
D shelf life	1 + 4	low	Full	yes	130	moderate	29 Sep	12(D1), 101(D2), 98(D3), 94(D4), 90(D5),	75-85

We checked the series with a couple of conventional parameters (Streif-index, sugar, colour, firmness) that are described well in literature for ripening, bearing, light and storage life. Although we tried, it is impossible to vary only one factor. Several factors are connected to each other in apple growth, for example low bearing is correlated with big fruit. When we tried to standardise the other factors we realised we weren't taking a representative sample of the whole tree which had consequences for the interpretations. Below you will find the description and the choices of the four series from this point of view.

### 3.3.1 Series A: ripening

To get an idea of the characteristics of ripening expressed in different parameters, we made a series with different picking dates from unripe to over-ripe, with about a one-week interval. We chose date 2 as optimal for long storage and date 3 as optimal for short storage. We had a row of 10 trees in the orchard from which we picked every week a sample of 120 apples from the sun side and with a 75-85 mm size.

#### Early picked means stored longer

Next we had the methodological choice between measuring every week one fresh sample or to store the samples until the last one was picked and measure five samples at the same time and conditions. For practical reasons as many laboratories are involved and to have a better opportunity to compare, we chose for the second option. That means we have a ripening series with different picking dates and different storage periods together. So early picked means stored longer and the last picking is not stored at all. This combination might have important consequences.

After picking the first unripe sample, we found the quality of very unripe: small size, very firm, hardly any red skin, low in starch and an astringent taste. During the five weeks of storage the ripening process continues partially. Some ripening processes go on during storage: converting starch into sugar. Other ripening processes only occur on the tree: increase in fruit size, increase in red skin, forming skin wax, increase in aroma. After waiting until the five samples were picked, we only saw a slight difference in starch between the five ripeness samples and also in taste we found hardly any differences in astringency. Accidentally no. 1 and 2 were not cooled until no. 3 was picked. In the usual ripening process, firmness decreases. Also in storage the firmness will decrease. In series A the overall effect of a later picking date and shorter storage period on firmness is more or less constant.



## Other varying parameters

In series A, you will also find an increasing fruit size. In the last five weeks of the ripening process between the first picking and the last picking the apples grew about 15 mm in diameter. We tried to standardise fruit size, but for the first and the last picking date this was not possible and the compromise is an average increase of 8 mm between the smallest and the biggest standardised sample.

We expected date 5 to be over-ripe, but in the sensory test those apples were still good. See series D for real over-ripe apples. The LBI-samples are checked for ripeness with the Lugol test and the extremes within one sample were deleted. Series A was not as extreme as we hoped for.

### 3.3.2 Series B: bearing

The trees for these series were fruit thinned in two steps in June and July. Those trees were part of a larger experiment with many independent repetitions in this orchard looking for the optimal bearing capacity. In fruit growing it is known that high bearing trees in a particular year initiate less flower buds for the next year and the other way around. For the fruit grower an equal bearing from year to year will give more production in the long term, less work and better fruit quality. The aim is to find out the maximal bearing for a regular every year yield of good quality and where the optimum in quality lays.

#### 2000 was a year with high bearing capacity and good fruit quality

We consider bearing level 3 as the best in this bio-dynamic orchard with skilled labourers and bearing 4 as more usual for a conventional orchard. Around these yields we chose high and low extremes. We planned an extreme of 175 fruit/tree but by natural dropping the highest bearing we could obtain was 140 fruit/tree. The lowest bearing was 35 fruit/tree by severe thinning. Finally we used the bearings 35, 75, 100, 125, 140 fruit/tree which corresponds with a yield of 14, 30, 40, 50, 56 ton/ha. This year had extremely good production conditions. Therefore, the extremes in bearing did not show the known effects in fruit quality very clearly. The good conditions also lead to less severe fruit growth retardation than usually. The expected strong polarity between growth and yield is not reached in these series. See table 5 for a summary of varying parameters.

The good year 2000 results in enough flower bud initiation for the next year 2001 except in trees with the highest bearing. After spring frost in 2001 the flowering was overall too low, but anyway it was clear that an increased bearing of fruit in 2000 showed a decrease in flower-index in 2001: 3,7; 1,9; 1,6; 1,0; 0,5.

*The flower-index has a range from 1-10; from no flower up to every cluster with flowers.*

## Other varying parameters

This increase in fruit per tree corresponds with a decrease of numbers of leaves per fruit that assimilate and evaporate from roughly 130, 55, 42, 35 to 30. We had 10 trees of each bearing level in the orchard.

In series B with the increased bearing and a decrease in fruit size we tried to standardise fruit size. This was possible for bearing level 2, 3, 4, 5 but at the extreme low bearing level 1, the fruit size was too big for storage quality.

In these series we counted seeds. The amount of seed per apple varied per fruit from 1 to 8 with an average of 3,7 and no significant difference by bearing. During picking the ripeness is very nicely standardised and uniform, see results of Streif-index.

**Table 5: bearing series**

code	fruit/tree 2000	yield in tons/ha 2000	twig growth 2000	leaves/fruit 2000	flower-index 2001
B1	35	14	moderate-severe	130	3,7
B2	75	30	moderate-severe	55	1,9
B3	100	40	moderate	42	1,6
B4	125	50	weak-moderate	35	1,0
B5	140	56	weak-moderate	30	0,5

### 3.3.3 Series C: light and bio-dynamic field preparations

For these series we had to use rows in the orchard that were fertilised a slightly more than rows in the other series, but no significant higher % nitrogen appeared in leaves or fruit. The reason for using a fertilised row is a practical reason because we needed more trees than planned to obtain enough fruit growing in the shadow.

#### Bio-dynamic field preparations

This year the bio-dynamic field preparations were used only in two thirds of the 3 ha parcel: horn manure preparation on the 14th and 26th of April and horn silica preparation on the 30th of June and the 11th of August. Although we looked carefully for comparable trees in the orchard, we noticed during the year some differences between the part of the orchard with, and the part without preparations. This is probably caused by a difference in thinning. The trees without preparations had slightly less fruit per tree, more growth and slightly bigger fruit with less blush. The leaf analyses showed slightly less nitrogen (1,9% and 2,0%, but not significant). So the trees without preparations seem a little more vegetative. This might also be due to tree variation. In the open trees with preparations there was not enough fruit growing in the shadow. So, some fruit without the standardised quality was added to the sample.

*We subjectively checked in the field if and where the vitality of the soil (with the Bovis intuitive method, see §17) increased. At three different places this was found 2 rows wider than the border treated with the preparations and parallel to the border. This indicates that the preparations have some effect. The distance between application border and effect border of about 6 meters is more often found in experiments with preparations.*

No conclusions about the preparations could be drawn due to lack of independent repetitions in the field. We hope to obtain the independent repetitions in successive years.

#### Sun-shadow

For these series we had two rows with 15 trees each of moderate size that were thinned for this experiment in a special way to get more shaded grown fruit than normally. The picker has standardised for size very well (see fruit size). We see a correlation between less sun and later or less ripeness (more starch and less sugar, so a higher Streif index).

Realise, in all trees, also where shaded grown fruit is picked, the outer side of the tree is exposed to the sun and the leaves have been assimilating in the sun. So, only the position of the fruit in the tree is in full sun, half sun or in the shadow.

### 3.3.4 Series D: shelf-life

Fruit for this series is collected from series A (picking date 4) and is stored in mechanical storage for three months at 4°C and high humidity. At the end of December every 4 days part of this fruit is taken out of the storage and kept at room conditions (16°C and varying humidity). So at the second week of January we collected a series of apples with different shelf lives. D1=A4 is used as short stored reference and is already measured in October. D2, D3, D4, D5 are all stored for about 3 months and have an increased shelf-life. See table 4 for the summary. To get a series with very altered fruit, we chose a very late picking time for mechanical storage purpose. Despite this we got no bad quality like mealy tasting, soft and rot. A compliment for the orchard, but a pity for this project. After storage the fruit, with little rot spots was not tested.

# 4 Method

*This chapter describes the working sequence, the methodological steps, experimental design, sampling method, statistics and the structure of the report.*

## 4.1 Working sequence

In §1.1 already the risk is mentioned of reasoning in circles. We are searching for a new concept with new parameters. We cannot avoid this risk but when describing our line of thought as clear as possible we can be addressed for thinking faults and wrong assumptions. This is the only way to introduce new concepts and new parameters. So we cannot speak about a traditional proof but only check for consistency and suitability of a hypothesis in different situations and products. Here we try to do the first check on experimentally grown series of apples and refer to the methodical steps as described in Bouter/van Dongen (1995) and in Houten/Diegem (1990).

### **Our method and working order was:**

1. We had a preliminary hypothesis about a new quality concept being more suitable for organic products, introduced in §1.1 in terms of what we consider as the basic life processes in organisms: 'growth', 'differentiation' and 'integration'.
2. We chose 5 experimental series (ripening, bearing, light exposure, bio-dynamic preparations, ageing) because in every series we expected one or more aspects of the life processes will vary.. This is our 'golden standard' for the criterion-validation.
3. To make this quality hypothesis operational we selected conventional and experimental parameters that may be useful to the concept. In each chapter (§5-§17) concerning a single parameter this assumption is explained in the subject heading 'background', the method is described and the results of our measurements are first discussed as facts and if relevant, compared with earlier experience in literature. After considering the correlations in §18, the headlines in the series in §19 and the life processes in §20, we discussed each parameter in relation to the series and the hypothetical concept and added this to each parameter chapter.
4. We checked the sub-sample similarity for some parameters in more than one sub-sample (for practical reasons we had to work with different sub-samples in the different laboratories) in §18.2 and drew conclusions about the similarity.
5. We compared the parameters with each other in §18.3 for relevant correlations and costs. This helped to classify parameters in §20 and check the several domains on internal consistency. After a number of similar projects we can choose the most promising and least costly parameters for quality control.
6. We summarised the results of the parameters in each series separately as facts and next arose a whole image around the changing factor(s) per series in §19. First we compared this with our expectation based on the results of the conventional parameters and next checked the consistency and expectation of the results of the experimental parameters.
7. We did this the other way around in §20 and summarised the three life processes in relation to the different parameters to double check on consistency of the traditional parameters and to find the relationship between the experimental parameters based on correlations in §18 (face-validity).
8. We checked the suitability of the concept in discussions with fruit growers and consumers and this led to the double and connected lines in the vital quality concept (first steps according to content-validity). One aspect of suitability is the communication with growers in terms of processes they are used to managing in their fruit growing practice (growth, differentiation and integration). The other aspect of suitability is the communication with the consumer about properties of the product, making clear that a vital quality apple differs from a 'bag of water with nutrients and flavours'. We found the quality concept sufficient suitable to present in this report, but are still searching to find a better proof for the process of integration. More details are discussed in the following chapters.

## 4.2 Experimental design, sampling and analyses

We had no independent repetitions of treatments in the orchard. We collected one sample of 120 apples from 10 to 20 trees per treatment and divided it into equal sub-samples judged by eye. To reduce natural variation we standardised as much as possible (fruit size, bearing, exposure to the sun, medium or high position in the tree). Because we work with series we get certainty from a consequent line in the successive treatments instead of the independent repetitions.

A project with many partners at different places in the Netherlands and Germany has the disadvantage of working with different sub-samples on different dates and long transport. The costs of analyses do not permit as many repetitions as we wished.

The apples mature slowly in the fridge (4-6° C) and faster outside. When we compare the samples of different analyses we must realise this influence.

See table 5 for which parameters share the same sub-sample and for the out-of-the-fridge-period of the samples.

All measurements and judgements are done with blind samples. This is especially important for the parameters where a subjective part is included as in crystallisation and Bovis.

## 4.3 Statistical analyses

The statistical analyses were performed with the statistical package SPSS version 8 (Norušis 1998).

### Analysis of variance

Analysis of variance was used to test the differences between the various treatments, since nearly always normally distributed data was collected. At first it was tested whether the variances from the different samples could be considered as homogeneous by using the Levene test. When such was the case, test statistics were based upon a t-test calculating the square root of the smallest variation between a sample pair. When the variances had to be considered as unequal, the pair wise comparisons were based upon the calculation of Tamhane's T2-statistic, which is a conservative test based upon a t-test.

Differences between means were made visible with 'a', 'b', etc in the tables. When no differences at the 95% level were found, the same letter was used for all means of one parameter in all series together. In the figures of parameters with independent repetitions we used error-bars for the standard error of the mean (sem).

### Correlations

The mean values of the different variables per parameter were also linear related to each other. Pearson's  $r^2$  was calculated in annex 14.2 for correlation with the confidence at  $p < 0,05$  and  $p < 0,01$ . In annex 14.3 some meaningful correlations are shown in several figures.

It was not possible to perform a cluster analysis or an analysis of covariance, since there were hardly any parameters which were measured in all series. Some parameters were not determined when biologically not of interest.

**Table 6: Parameters, institutes, sub-samples and different period out of the fridge**

Parameter	Institute	Independent samples	fruit per sample	Duplicates per analysis	date of analysis				hours outside the fridge between orchard-storage and analysing			
					A	B	C	D	A	B	C	D
Leaf series	LBI	4	-	-	-	21Sep	-	-	-	-	-	-
Exterior	LBI	1	>100	-	10 Oct	26 Sep	19 Sep	9 Jan	8	10	8	10
Brix, acid	PPO	1	25	2	13 Oct	29 Sep	22 Sep	12 Jan	36	36	48	36
Firmness, colour, starch	PPO	25	1	1	13 Oct	29 Sep	22 Sep	12 Jan	36	36	48	36
Minerals	ZVI	1	25	2	--	5 Oct	5 Oct	-	48	48	60	-
Vitamin C	LBI	1	8	2	11 Oct	2 Nov	-	10 Jan	24	24	-	24
Brix, pH	LBI	1	8	1	11 Oct	27 Sep	20 Sep	10 Jan	24	24	24	24
Self-disintegration	LBI	8	1	1	11 Oct	27 Sep	20 Sep	10 Jan	24	24	24	24
Dry matter	LBI	8	1	1	11 Oct	27 Sep	20 Sep	10 Jan	24	24	24	24
Sensory test	LBI	1	8	7	11 Oct	27 Sep	20 Sep	10 Jan	24	24	24	24
Crystallisation	LBI	1	5-8	4	11 Oct	28 Sep	20 Sep	10 Jan	24	12	24	24
Capillary pictures	LBI	1	5-8	1	11 Oct	28 Sep	20 Sep	10 Jan	24	12	24	24
Delayed luminescence	Meluna	10	1	2	11,12, 13 Oct	27,28, 29 Sep	20,21, 22 Sep	10,11, 12 Jan	24	24	24	24
Spectral range luminescence	Kwalis	12	1	4	12-14 Oct		14-19 Oct		60		60	
Amino-acids	Kwalis	1	5	4	17 Oct		19 Oct		60		60	
Phenolic compounds	München	1	10	1	dried later	-	dried later	-	60		100?	
Electrochemical	Heilmann	1	10	1	14 Oct	29 Sep	23 Sep	13 Jan	48	48	60?	75?
Bovis	LBI	1	10-25	4	10 Oct	26 Sep	19 Sep	9 Jan	8	10	8	10

*Parameters for the same sub-samples share a double lined compartment*

# 5 Leaf series and twig growth

*Making leaf series is a method by LBI to visualise growth and regularity in vegetative development of the apple twig.*

## Background

Making leaf series as an image of shoot growth is a method developed at Louis Bolk Institute (Bloksma and Jansonius, 2000). The successive leaves of one shoot are viewed as a biography of the growing season. Shoot length is smaller when a lot of fruit competes for growth. Leaf size and internodes are smaller when growth is temporarily limited by, for example, drought.

## Method

We labelled 4 standardised shoots (1 fruit/shoot, 45° up from side branch) per treatment and wrote down some shoot top dates in the growing season. We did not choose an average but a characteristic shoot for making a drawing. This year was very unusual in the way that the growth was less dependant from bearing than it mostly is. So we exaggerated a little this year to show this rule in fruit growing.

## Results

### B: Bearing

We only picked twigs with leaves in series B because bearing usually has a large influence on growth. In annex 1 you see the drawn leaf series and symbols for bearing and the proportion leaf to fruit as is presented in table 5.

In the season 2000 the growing conditions were so well that also the heavy bearing trees produced some twig growth. We chose twigs characterising something between the main rule and this years conditions. More fruit per tree leads to smaller leaves and earlier stop of growth and less flower bud initiation for the next season.

## Discussion

### Relations between firmness and other parameters

The main consequence of the ratio leaf/fruit, as is indicated in the figure, is the supply of assimilates for the fruit. You will find this consequence in the decreasing sugar content (annex 3.3).

### Relations with growth, differentiation and integration

Growth is reflected in leaf size and irregular leaf shape, in twig length and in internode length. Differentiation is found in regular shape and in details as fine leaf serrations. Integration is found in regularity in the leaf form sequence (Bloksma and Jansonius 2000). Because of the polarity between twig growth and fruit growth in the tree it is not necessarily that growth full leaves reflect a growth full quality in the fruit.

## 6 Exterior of the fruit

*Exterior quality, as size, grading, colour, smooth and blemish, is important for the traders. In this research we only used sound fruit and standardised for size and sun exposure, so these measurements have the role to check the standardisation and only partially for information about characteristics of the series.*

### 6.1 Fruit size

#### Background

Fruit size has a major influence on several quality parameters, so we tried to standardise for size as is shown in table 4. Measuring size, in table 6 and annex 3, is a check for this standardisation and not a parameter influenced by the treatments. From the commercial point of view the best size for an Elstar apple is 65-80 mm.

There is no fixed relationship between size and weight, because the specific density varies in the season, from season to season and between varieties of apples.

As a rule of thumb we use the following relationships (M. Kers, DLV 2000)

mm size	60	65	70	75	80	85
gram	92	107	128	158	193	235

#### Method

Fruit size is measured in horizontal diameter in mm or in weight in gram. PPO-laboratories measured size in mm on 25 individual apples.

### 6.2 Skin colour

#### Background

Skin colour plays an important role in the preference of the consumer and has a relation with the ripening process.

#### Method

With a chromameter (Minolta CR-300) 3 values of 25 individual apples are measured at the ground colour side: L-value for intensity of reflection (higher value means more reflection); A-value for green ground colour (higher value means less green); B-value for yellow ground colour (higher value means yellower). Besides that % red blush is measured by a prototype of a sorting camera (AWETA), see table 6 for results.

### 6.3 Shape, blush colour and shine

#### Background

Exterior plays an important role in trading and ripeness indication.

#### Method en results

LBI described visually shape, colour, shine and amount of wax in a box with 100 apples. See annex 2.1 for photographs of apples in boxes and description in annex 2.2.

#### Discussion

As expected there is a large difference between series A and C. Ripening on the tree produces big irregular apples

with purple red, fat and glossy skin and ripening in the store does not change size and blush, only ground colour from yellow to deep yellow. Bearing or preparations do not influence the exterior and could be nicely standardised.

After storage a thin layer of white moulds is found on the skin. The apples with the longest shelf life (D3, D4) had some small rotten spots and were deleted from the series.

### **Relations between colour and shine and other parameters**

The percentage blushed skin is correlated with many unripeness parameters: firmness ( $r^2=0,26$ ), starch ( $r^2=0,61$ ), acid ( $r^2=0,48$ ), Streif index ( $r^2=0,74$ ), N, P, K, Mg ( $r^2=0,63-0,81$ ), vitamin C ( $r^2=0,48$ ), both protein and free amino acids (both  $r^2=0,84$ ), raw flesh ( $r^2=0,28$ ), astringent skin ( $r^2=0,52$ ) and emission at  $t=3$  in delayed luminescence ( $r^2=0,40$ ). Blush is positively correlated with Brix-1 ( $r^2=0,22$ ), protein ratio ( $r^2=0,77$ ), yellow/blue ratio in spectral range luminescence ( $r^2=0,75$ ), electrical resistance ( $r^2=0,60$ ), P-value ( $r^2=0,23$ ) and differentiation in crystallisation ( $r^2=0,27$ ).

Ground colour and shine are not examined in the correlation table.

### **Relations with growth, differentiation and integration**

In blush we easily recognise the ripening process (differentiation) as well as in the series (A and C) and in the correlations. Moreover we find a slight correlation between blush and integration parameters.

As discussed in the background we expect ripening as the main factor for colour (green ground turns into yellow; red blush turns into purple) and for wax. We expect sun exposure as main factor for percentage of blush. Both parameters acting at the level of differentiation.



# 7 Basic parameters: firmness, starch, sugar, acid and calculated indexes

*Parameters, as firmness, starch, sugar and acid form our basic to check the quality of the experimental series and to communicate with common quality researchers. These parameters are cheap and also known in combinations in several calculated indexes.*

## 7.1 Firmness

### Background

Firmness is a quality measure that is correlated to sensory tests. Firmness is appreciated especially by young people. For traders this is the main quality parameter because a firm apple would not be damaged so much under transport conditions. To get very firm apples traders want apples to be picked unripe.

Consumers experience the firmness of Elstar as crisp (>6), as firm (4,5-6), as soft (4-4,5), as too soft (3,5-4) and as mealy (<3,5) (v.Diepen 1990). Firmness is the result of the combination of cell turgor (water, sugar) and elasticity of cell walls.

### Method

Firmness in kg/cm<sup>2</sup> is measured with a penetrometer with the 11 mm plunger at 8 mm depth on 25 individual apples at the ground colour side without skin.

### Results and discussion

The means of firmness and figures in kg/cm<sup>2</sup> are shown in annex 3.

#### A: ripening

The expected decrease in firmness during ripening in series A is not found. The explanation is already mentioned in §2.3.1: firmness decreases both during ripening and storage.

#### B: bearing

The slow decrease of firmness with increased bearing is in harmony with the expectation, with the crispness in the sensory test and with literature (Wagenmakers 1999, Awad 2001).

#### C: light

There is no significant difference as a result of growth in different light intensities.

#### C: preparations

There is no difference when preparations are used.

#### D: shelf life

As expected, firmness strongly decreased after storage due to water loss and decreased slightly with prolonged shelf-life.

## Discussion

### Relations between firmness and other parameters

Firmness is correlated with many other parameters that are linked to ripeness or ageing, however most of these parameters are highly correlated with firmness because of the presence of two separated groups: series A+B+C versus series D, see for example general appreciation in annex 14.3.6.

### **Relations with growth, differentiation and integration**

Firmness is the result of turgor and cell elasticity. Turgor (after taking up water) can be seen as a growth aspect whereas cell wall elasticity and the capacity of not losing its water is more an integrational aspect.

## **7.2 Starch**

### **Background**

The Lugol-test is a rough measure for ripeness for the fruit grower and is easy to perform in the orchard. During ripening starch levels are decreasing, which is reflected in an increase on the scale of the EU-standardised colour chart for decreased starch levels. For Elstar the optimal picking time is between scale 2 and 3 on the colour chart for long storage and between 5 and 8 for direct consumption.

### **Method**

Lugol-iodine-test (=3 gram I<sub>2</sub> and 10 gram KI per litre) on starch is performed on 25 individual half apples. Scale numbers 1 to 10 are used of the EU-standardised colour chart. A higher number means a lower starch level.

### **Results and discussion**

In annex 3 the means and figures of 'decreased starch levels' are denoted. The lugol test on starch shows a large variation between individual fruit samples. However, the means are in a consequent line.

#### **A: ripening**

When picked later we see a decrease in starch content.

#### **B: bearing**

With more fruit per tree the starch content decreases slightly as is understandable when assimilates have to be shared by more fruit.

#### **C: light**

The Lugol test shows fewer differences with light or preparations which fits with the constant sugar levels. In the sun there is slightly less starch which is in accordance with earlier ripening.

#### **D: shelf life**

After picking in a fairly ripe condition the lugol test varied from 5 to 9, with an average of 7,4. After storage, starch in all apples was hardly gone and not measured in detail any more.

### **Discussion**

#### **Relations between starch and other parameters**

Starch is correlated with many other parameters that have to do with ripeness and sun exposure. Notable is the negative correlation between starch and the yellow/blue ratio in spectral luminescence (see annex 14.3.29 with  $r^2=0,95$  based on series A+C).

#### **Relations with growth, differentiation and integration**

We recognise in starch as basic potential building material a clear growth aspect.

## **7.3 Sugar in Brix**

### **Background**

A high amount of both sugar and acid improve the taste, the balance between both is important.

For good taste fruit growers strive for a good proportion between sugar and acid: Brix >12 and acid: 8-10 g/l. Sugar is not spread homogeneously in the fruit: more near the skin than near the core and more near the bottom than near the top (Stoll 1997).

## Method

Brix is measured twice in juice by PPO and by LBI. At PPO-laboratories juice is made of a mixed sample of 25 half apples, including skin and core. At LBI juice is made without core and filtered by cloth, see §13 about crystallisations. Brix is measured with an optical Brix-meter and indicates total soluble solids, mainly sugars (glucose, saccharose, fructose, and sorbitol).

## Results

Sugar is measured in 2 sub-samples in Brix (sample PPO in annex 3 and LBI in annex 4) and is tasted as sweetness (annex 9). These three examinations show a good similarity (see annex 14 for correlation). This points out the similarity of the sub-samples. Further all sugar results are in a consequent line so there is no indication for an unknown factor, which could interfere when independent replicates are not included.

### A: ripening

Sugar content increases slightly. It does not vary as much as we expected from literature and experience. The explanation is already given (§2.3.1) in the fact that the early picked apples are stored relatively long. The process of transforming starch into sugar is going on, also in a cooled storage.

### B: bearing

Sugar content shows a decrease with an increasing yield. This is also found in other experiments (Wagenmakers 1999, Awad 2001, etc). This is easy to understand, because fruit from high bearing trees have less leaves per fruit to deliver assimilates.

### C: light

Both in annex 3 and in annex 4 we see a more or less constant level of sugar in the apple series with preparations. It is known that a tree distributes its assimilates through the phloem equally over all fruit, grown either sunny or shady.

### C: preparations

The slightly lower amount of sugar in the sun-grown apples without preparations is not clearly understood, and discussed further in §19.

### D: shelf life

After storage the sugar content was decreased, but during the shelf life period it didn't change. It is known that the sugar content decreases by dissimilation in storage. It is also known that during the shelf life period, breakdown of sugar is preceded by breakdown of other compounds, so finally sugar levels will decrease. This last phase is not reached here.

## Discussion

### Relations between Brix and other parameters

Brix is correlated with many other parameters that have to do with bearing and thus distributing assimilates. We found positive correlations between Brix and dry matter (see annex 14.3.4;  $r^2=0,73$ ), Brix and protein ratio (see annex 14.3.5;  $r^2=0,69$ ) and Brix with the yellow/blue ratio in spectral range luminescence (see annex 14.3.30;  $r^2=0,69$ ). We only found a weak positive correlation between Brix and sweet tasting (see annex 14.3.10;  $r^2=0,26$ ). Negatively correlated were Brix and calcium content (see annex 14.3.3;  $r^2=0,81$ ), and Brix with both protein and free amino acids ( $r^2=0,65$ ;  $r^2=0,68$ ).

### **Relations with growth, differentiation and integration**

For sugar we have the same argument as for starch to see this as a growth parameter. The ratio sugar/starch can be seen as a ripening indication and sugar/acid as a balance for taste and an indication for the integration process.

## **7.4 Malic acid**

### **Background**

A high amount of both sugar and acid improves the taste, the balance between both is important. For good taste fruit growers strive for a good proportion between sugar and acid: Brix >12 and acid: 8-10 g/l.

### **Method**

Malic acid is determined in duplo by endpoint-titration (pH=8,1) in juice, total acid is measured, of which more than 95 % is malic acid, it is assumed that acid =malic acid in gram/litre.

### **Results and discussion**

In annex 3 the results are shown. The decrease in acid levels in the ripening series, the decrease in the bearing series, the constant level in the light series and the decrease in the shelf life are all according to our experience and literature (Awad 2001). The same tendencies are found for sourness in the sensory test.

#### **Relations between malic acid and other parameters**

Acid is correlated with many other parameters that have to do with ripening and bearing and thus the distribution of assimilates. Acid is rather well positively correlated with firmness ( $r^2=0,87$ ), starch ( $r^2=0,73$ ), Streif index ( $r^2=0,71$ ), both phosphates and potassium ( $r^2=0,53$ ;  $r^2=0,42$ ), vitamin C ( $r^2=0,68$ ), both protein and free amino acids ( $r^2=0,72$ ;  $r^2=0,71$ ). Acid is also positively correlated, but weaker, with dry matter ( $r^2=0,40$ ), crispness ( $r^2=0,53$ ), and juiciness ( $r^2=0,49$ ), rawness ( $r^2=0,63$ ), general appreciation (see annex 14.3.7;  $r^2=0,47$ ), delayed luminescence at 3 sec. ( $r^2=0,48$ ) and Bovis-value ( $r^2=0,47$ ). Acid and sourness are positively correlated (see annex 14.3.11;  $r^2=0,72$ ).

Acid is rather well negatively correlated with protein ratio ( $r^2=0,77$ ), the yellow/blue ratio in spectral range luminescence (see annex 14.3.31;  $r^2=0,89$ ) and electrical resistance ( $r^2=0,74$ ). Acid is also negatively correlated, but weaker, with blush ( $r^2=0,48$ ) and disintegration ( $r^2=0,31$ ). Do not confuse acid with acidity (pH). These are not the same properties as is illustrated in the only moderate negative correlation ( $r^2=0,53$ ).

#### **Relations with growth, differentiation and integration**

For acid we have the same argument as for sugar and starch to see this as a growth parameter. The ratio sugar/acid as a balance for taste can be seen as an indication for the integration process.

## **7.5 Streif-index**

### **Background**

For fruit growers the Streif-index is a measure for maturity and is calculated from parameters that can be measured relative easy at the orchard: firmness, Brix and starch in the units as mentioned before (Streif 1996). A lower value indicates a riper product. For Elstar the optimal picking time for long storage is at Streif-index 0,3 and for short storage at 0,2 and optimal for taste for direct consumption is a Streif-index below 0,15.

### **Method**

Streif-index = firmness / (Brix x starch).

## Results and discussion

See for data annex 3.

### A: ripeness

The Streif-index shows a moderate decrease, during ripening but not as strong as expected. This methodological point is mentioned earlier (§2.3.1) due to the fact that the early picked apples are stored relatively long. The processes of transforming starch into sugar and the decrease in firmness are going on, also in a cooled storage.

### B: bearing

We expected from starch and firmness levels slightly more ripeness in the high bearing trees, but the low sugar levels conceal this in the overall Streif-index.

### C: light

We see slightly less or later ripening in the shaded grown fruit than we expected.

### D: shelf life

In series D no starch is measured because none was expected, so the Streif-index is also not meaningful.

## Discussion

### Relations between Streif maturity index and other parameters

The Streif index is correlated to many other parameters that have to do with ripeness and these correlations are very similar to those discussed in §7.4 for starch.

### Relations with growth, differentiation and integration

In accordance with its name the Streif-maturity-index looks like a ripening (=differentiation) parameter. In connection with our quality concept it is worth mentioning that this index is built up by a formula consisting of only growth parameters of which the mutual proportions change as an indication of maturing. Maturing in this way can also take place in the store and is not necessarily restricted to the tree. In series A the difference between apples matured in store versus ripened on the tree in the sun is clearly demonstrated (see §19.1). For our quality concept the differentiating process in the sun is obliged to reach high vital quality.

## 7.6 Technical quality index (TQ-index)

### Background

Many authors tried to find a cheap measure for eating quality to replace the expensive sensory test. Here we checked the conceptual FIBL-technical-quality-index as described for Golden Delicious (Weibel et al, 2000). The index is calculated from 3 parameters and on average corresponds rather well with results obtained from sensory tests with Golden Delicious. A higher value is predicted as a better eating quality. First we will check this formula and then look for another formula that possibly will fit better for Elstar.

### Method

$TQ\text{-index} = 1x \text{ Brix} + 2x \text{ firmness (kg/cm}^2\text{)} + 3x \text{ acid (g/l)}$ .

### Results

The results are shown in annex 3.

## Discussion

### Relations between technical quality index and sensory properties

The TQ-index for Golden Delicious based on Brix, firmness and acid is rather well correlated with the general appreciation in the sensory test (see annex 14.3.8,  $r^2=0,55$  when linear  $r^2$  increases with a more complex line). As discussed in §18 this good correlation is due to the presence of two separated groups: series A+B+C versus series D. We searched for a better predicting TQ-index based on these three technical measurements for our Elstar series, however we didn't find a higher correlation than the one with only firmness (see annex 14.3.6;  $r^2=0,68$ ). This means that firmness is the best technical parameter for the prediction of taste in these data.

# 8 Mineral content (N, P, K, Mg, Ca) and dry matter

*Mineral content and dry matter also are basic parameters.*

## Background

Minerals are of importance during the storage period. Misbalances in minerals are correlated with physiological storage problems as 'internal breakdown'. For good storage quality the amount of nitrogen should be low, phosphate and calcium high and potassium low in relation to calcium. A little nitrogen is necessary because nitrogen is present in chlorophyll and flavour compounds. Potassium favours size, colour and aroma. Dry matter content is not easy to interpret. It is known to be related to cell size (smaller cells have higher dry matter) and to sugar content.

## Method lab Zeeuws Vlaanderen

The mineral content is measured by the laboratory of Zeeuws Vlaanderen. We sent them one mixed sample of 25 half apples with skin and core but without seeds from the PPO-laboratories. They were immediately dried at 70°C for at least 48 hours. The tissues were destructed by sulphuric acid, copper sulphate and hydrogen peroxide in 2 hours at 330°C. Both treatments were performed in duplo. Minerals were analysed by:

- spectrophotometry: Nitrogen at 660 nm (NEN 7434) and Phosphorus at 880 nm (NEN 7435).
- flame-photometry: Potassium at 769,9 nm (NEN 7436); Magnesium at 285,2 nm and Calcium at 422,7 nm (NEN 6450).

Mineral content is expressed both as % of dry matter and in mg/100gram fresh flesh.

## Method LBI

For the self-disintegration test LBI measured the dry matter. Because only a very small dry room at 70°C was available, LBI dried at 30°C for one week and corrected the outcome with a factor to compensate for the difference between 30° and 70°C.

## Results and discussion of the dry matter

Dry matter is measured once in series A en D (LBI in annex 4) and twice in series B and C (IZVI in annex 3 and LBI in annex 4).

### A: ripening

We found a slight increase in dry matter in the 2 ripest samples.

### B: bearing

Both measurements equal well and have a more or less consequent decreasing line with increasing bearing.

### C: light

Both measurements equal quite well and show an increasing dry matter with more light (more evaporation in the sun) and a higher dry matter without preparations (no explanation).

### C: preparations

In apples with preparations we find a lower dry matter. We expected juicier apples or apples with a lower sugar content, but do not clearly find this.

### D: shelf life

After storage dry matter drops down due to dissimilating sugar in storage. During shelf life we see little effect, although we expected an increase due to evaporation.

## Discussion about dry matter

### Relations between dry matter and other parameters:

Dry matter is measured in two laboratories: dry matter 1 (PPO) in series B and C and dry matter 2 (LBI) in all series. The correlation between these both is only moderate (annex 14.3.2;  $r^2=0,58$ ), which is explained by the less precise method at LBI. The correlations between the dm-1 samples or between the dm-2 are not equal either. A clear positive correlation is found thrice with Brix (see annex 14.3.4;  $r^2=0,77$ ; 0,38; 0,93). A negative correlation is found with calcium ( $r^2=0,71$ ) and with delayed luminescence ( $t=3$   $r^2=0,66$  and lag time  $r^2=0,68$ , see annex 14.3.25).

Weaker positive correlations are found with firmness ( $r^2=0,38$ ; 0,36), vitamin C ( $r^2=0,57$ ), many sensory parameters ( $r^2=0,20-0,34$ ), electrical resistance ( $r^2=0,25$ ) and Bovis-value ( $r^2=0,30$ ).

### Relations with growth, differentiation and integration

Dry matter is a complex parameter to place in the quality concept. Dry matter is the result of water uptake (growth), sugar content (growth), cell-compactness (inverse growth) and maybe more.

Up to now we place dry matter with a question mark at the growth-level.

## Results and discussion about minerals: N, P, K, Mg, Ca

The results are shown in annex 3. Minerals are only measured in series B and C and are shown both in milligrams per 100 gram dry matter and per fresh matter. Magnesium is shown only in the table, not in a figure.

### A: ripening

In these series we have no own measurements because we expected no difference. From experience it is known that mineral content doesn't change so much during the ripening process. Only by increasing fruit size mineral contents are diluted slightly, but here we standardised for size. Rain during the period fruit hang on the tree often induces a severe increase in potassium. This is no ripening effect but an uptake effect (De Jager, pers. comm.).

### B: bearing

With an increase in bearing we see a decrease in N, P, K and an increase of Ca both in fresh and in dry weight. The decrease is explained by dilution due to enhanced fruit growth. The higher Ca can be caused by less competition from fewer growing twigs in high bearing trees. The proportion K/Ca, as indication for storage potential, is twice as good in the high bearing trees because K decreases and Ca increases.

Wagenmakers (PPO unpubl.) found in a bearing series of Elstar (1998: 60, 100, 140 fruit/ tree) an equal N, Mg, Ca and a decrease in K and P. In the later in summer defruited trees Ca was higher.

### C: light

Both with and without preparations we see a decrease in N, P, K and an equal Ca which increases in light, so the K/Ca ratio decreases in more sun. This induces an overall increase in storage quality for sun-exposed fruit. This effect has been found earlier in comparing inner and outer fruit in trees, but an explanation failed (de Jager, 1995). We see corresponding high amounts of proteins and amino acids in shaded-grown apples (see annex 6).

### C: preparations

No difference was found in N, P, K per dry matter between apples which were or weren't treated with preparations. Ca is slightly higher in apples with preparations (possibly related to less twig growth). Because of the lower dry matter with preparations the mineral content in fresh weight is higher

### D: shelf life

Is not measured, because of the expectation that it would not differ.

## Discussion about the minerals

### Relations between minerals and other parameters:

Minerals are only measured in series B and C, and the correlations are based on only 11 measurements. We found



quit often a high correlation between the N, P, K, Mg contents and the Ca content.

High N, P, K, Mg- contents are found in unripe (Streif) and shaded-grown apples. So it wasn't surprising to find nitrogen positively correlated with starch ( $r^2=0,60$ ) and negatively with blush ( $r^2=0,80$ ) and the yellow/blue ratio in spectral range luminescence (see annex 14.3.32;  $r^2=0,86$  based on only 6 measurements in series C).

As expected, nitrogen is positively correlated with protein ( $r^2=0,74$ ) and free amino acids ( $r^2=0,80$ ), but negatively with protein ratio ( $r^2=0,77$ ). It is interesting to find a high positive correlation between nitrogen and disintegration (see annex 14.3.15;  $r^2=0,72$ ) which fits with the experience of fruit growers that apples with high nitrogen will rot soon. There is a weak positive correlation with the P-value ( $r^2=0,39$ ).

The P, K and Mg correlations mainly follow the line of N. Specific for phosphate is the positive correlation with Brix ( $r^2=0,86$ ) and the negative correlation with electrical resistance ( $r^2=0,65$ ) and integration in crystallisation ( $r^2=0,63$ ).

Specific for potassium is a positive correlation with acid ( $r^2=0,42$ ) and Brix ( $r^2=0,87$ ), which is known in fruit science, see subject heading background, but which is not found as a positive correlation with taste in our series. We found a positive relation with electrical resistance ( $r^2=0,67$ ) and a negative correlation with the yellow/blue ratio in spectral range luminescence (see annex 14.3.39;  $r^2=0,90$  based on only 6 measurements in series C).

Calcium is moderate positively correlated with self-maintenance (see annex 14.3.20;  $r^2=0,59$ ), lag time in delayed luminescence (see annex 14.3.26;  $r^2=0,51$ ) and acidity ( $r^2=0,48$ ).

Calcium is strong negatively correlated with both Brix-measurements (see annex 14.3.3;  $r^2=0,81$  and  $r^2=0,99$ ).

We found negative correlations between calcium and firmness ( $r^2=0,76$ ), crispness (see annex 14.3.13;  $r^2=0,71$ ), dry matter ( $r^2=0,71$ ) and weaker with sourness ( $r^2=0,49$ ), aroma ( $r^2=0,42$ ) and general appreciation ( $r^2=0,47$ ).

### **Relations with growth, differentiation and integration**

Looking at the concept of growth (uptake from earth, §20) we expect all minerals to be growth parameters. N, P, K and Mg are diluted in series B due to increased fruit bearing and are higher in shaded-grown apples. This is indeed found in the correlations with the experimental data.

Calcium levels increase in series B and are not influenced by sun in series C. Also in many other correlations Ca levels are the inverse of the other mineral levels. The uptake of calcium is also known as an exception in a physiological way: it is sucked up by the hormone gibberellin formed in growing tissues (especially by seeds in the fruit) and once in the tissue it is not easy to reallocate. Calcium (and especially the Ca/K ratio) makes an apple more resistant against ageing or disintegration. This is why we consider placing calcium at the level of structure, although calcium is not correlated with many ripening parameters.

# 9 Protein and free amino acids

*Protein and amino acid are usually not measured while this content is very low in apple. We add this parameter to learn about processes. This chapter is written in co-operation with Peter Stolz.*

## Background

Various viewpoints can be chosen in examining amino acids in plants and vegetables.

One viewpoint is based on the evaluation of the nutritional-physiological quality of the plant material from the point of human need for amino acids. This view is also generally held in the determination of amino acids after hydrolysis of the protein, which simulates human digestion. The criterion here is to meet the human need for amino acids especially the essential amino acids.

The second viewpoint is that the amino acids and proteins in the plant are related to the physiological state of the plant. Here the criterion is to what extent amino acids and proteins reflect the physiological state of the plant. Apples contain a low amount of protein (0,2-0,5g/100 gram fresh weight; Souci 1994) Therefore, protein and free amino acids should be considered from the second point of view.

In the past, Schuphan (1976) evaluated agricultural crops on the basis of the amount of free amino acids (i.e. all non-protein nitrogen compounds) in relation to total nitrogen. This assessment is based on the following consideration: The plant – depending on its physiological state– takes in nitrogen as nitrate or ammonium and makes amino acids and proteins out of it. This process will go on only if all growth factors are optimal. The supply of water from the earth to the plant, light intensity and, to a lesser extent, temperature, are considered as the decisive growth factors. For this view we use 'amino acid ratio'.

Here, the hypothesis is put forward that the plant shows a higher quality when it has formed its metabolic processes in a manner appropriate to its species. That is after it has converted nitrate or ammonium into amino acids and further into proteins.

When the concentration of protein is high in relation to total protein + amino acids, a plant has to a large extent formed its metabolic processes. For this view we use 'protein ratio'.

To test the total amount of protein, one has to determine what protein contents of the plant parts used as food are typical for the plant's species. Plant seeds like peas, beans and grains, but also apple pips are high in protein (see table 3). In these generative plant parts, protein appears to be necessary for its function or is specific for the natural expression.

On the other hand, plant parts like apple flesh, contain relatively low protein contents of 0,2-0,5 g protein per 100 gram fresh weight. The nature of the apple fruit appears to lie more in its sugar and acid content. The protein appears to be needed only for the metabolic system. Therefore low protein content is to be expected for a properly grown apple.

## Method

### Crude-protein determination according to Kjehldal

Method: VDLUFA Methods Volume III Animal-Feed Testing: Determination of crude protein, by method 4.1.1: crushing; decomposition using sulphuric acid/catalyst; alkalisation; steam distillation; titrimetric quantification.

### Determination of free amino-acids

Crushing; protein precipitation with trichloroacetic acid; centrifuge/filtering; determination of free amino acids with amino acid analyser (ion-exchange chromatography), detection using post-column derivatives with ninhydrin.

### Calculated 'free amino acid ratio' and 'protein ratio'

Free amino acid ratio =  $\text{free amino acids} / (\text{protein} + \text{free amino acids}) \times 100\%$

Protein ratio =  $\text{protein} / (\text{protein} + \text{free amino acids}) \times 100\%$

Both ratios are each other opposite.

## Results and discussion

The results are shown in annex 6.

### A: ripening

During ripening we found hardly any differences in the amounts of protein and amino acids.

The contents of free amino acids in the ripening series range from 38 to 52 mg/100g, and protein contents from 175 to 194 mg/100g (= 0,18 – 0,19 %) which is relatively low within the range expected for apples with a low amount of fertiliser added. The protein ratio is high (79-89%) and lies within the range of weakly fertilised apples. Based on this available data it is not possible to make a meaningful differentiation of the state of maturity or ripeness.

### C: increased fertilisation

The content of free amino acids in the light series ranges from 72 – 243 mg/100g and is significantly above the values for the ripening series A (at 38 – 52 mg/100g) which was weakly fertilised. Even the protein content which ranges from 213–419 mg/100g for the light series, compared to 175-194 mg/100g for the weakly fertilised ripening series, points out the effect of fertilisation with nitrogen. In the light series however, the protein ratio is much lower than in the unfertilised series (63-75%) and indicates that the plant was less able to convert the amino acids into protein compared to its unfertilised counterpart in series A.

### C: light

With increasing light both protein and free amino acid contents decrease, but the protein ratio increases. Clearly beaten are the shaded-grown samples with values of 237 and 243 mg/100g for the total sum of free amino acids, 413 and 419 mg/100g for protein and values of 63% for protein ratio. This shows that the shaded-grown apples were insufficiently able to convert the amino acids into proteins.

### C: preparations

The use of preparations shows a decrease in both protein and in free amino acids but also an increase in protein ratio. The effect of preparations is the strongest in the sun-grown apples (38% less protein, 56% less free amino acids, 10% higher protein ratio), slightly less in half shadow (respectively 17% less protein, 29% less free amino acids, 6% higher protein ratio) and is completely absent in the shadow. The shaded-grown apples are similar with and without preparations. The apples grown in half shadow with preparations are similar to the apples in full sun without preparations. Here we see an influence of the preparations in the same degree as more sun. Oddly enough the preparations do not have any influence on the shaded-grown apples.

The apples with preparations in the sun have a protein ratio almost as high (75%) as the unfertilised series A in the sun (79-84%).

## Discussion

### Relations between protein and free amino acids and other parameters

The correlations for protein and for free amino acids are very similar. As expected protein is positively correlated with total nitrogen compounds ( $r^2=0,74$ ), free amino acids ( $r^2=0,99$ ), protein ratio ( $r^2=0,94$ ), and negative with Brix/N ( $r^2=0,88$ ).

Protein is positively correlated with many parameters for ripeness or shaded-growth like firmness ( $r^2=0,53$ ), starch ( $r^2=0,58$ ), Streif-index ( $r^2=0,75$ ), acid ( $r^2=0,72$ ), blush (inverse  $r^2=0,84$ ), Brix (inverse see annex 14.3.5;  $r^2=0,69$ ) and rawness ( $r^2=0,46$ ). Remarkable is the strong positive correlation with magnesium ( $r^2=0,94$ ).

Memorable are the negative correlations with electrical resistance ( $r^2=0,84$ ), the yellow/blue ratio in spectral range luminescence (see annex 14.3.34 with  $r^2=0,67$ ) and phenolic compounds ( $r^2=0,58$ ).

Surprisingly, and only found for free amino acids is the strong positive correlation with potassium ( $r^2=0,87$ ).

### Relations between the protein ratio and other parameters

For the protein ratio, also expressed as % incorporated amino acids, positive correlations are found with many parameters indicative of ripening like blush ( $r^2=0,77$ ), Brix ( $r^2=0,68$ ), phenolic compounds (see annex 14.3.21 with  $r^2=0,58$ ), not raw ( $r^2=0,46$ ), firmness (inverse  $r^2=0,71$ ), starch (inverse  $r^2=0,59$ ), Streif-index (inverse  $r^2=0,79$ ), acid (inverse  $r^2=0,77$ ),

We also found positive correlations with yellow/blue ratio in spectral range luminescence (see annex 14.3.40 with  $r^2=0,64$ ), electrical resistance ( $r^2=0,85$ ) and unexpectedly a weak negatively correlation with self-maintenance ( $r^2=0,38$ ).

### **Relations with growth, differentiation and integration**

Free amino acids are easy to recognise as building stones, so 'amino acids' is a clear growth parameter. Protein is already synthesised a little further, but still can be considered as a growth parameter. The identical behaviour of amino acids and protein in the table of correlations support this choice. The many negative correlations between amino acids and integration parameters suggest an arrest of differentiation/integration by high concentrations of amino acids.

The protein ratio (protein/protein+amino acid) as a measure for incorporated building stones can be seen as an integration parameter. (Or when expressed as 'amino acid ratio' we must speak about a inverse integration parameter). This is supported by the high protein ratio in apples in the sun and with preparations in series C and also in many correlations with integration parameters as yellow/blue ratio in spectral range luminescence and electrical resistance.

# 10 'Health' compounds: vitamins and phenolic compounds

*With regard to human health, vitamin C and phenolic compounds are important as anti-oxidants. For these components we do not assume more is better or healthier. When eating apples, these compounds might contribute to human health. Here we try to explain these 'health' components or secondary metabolites as a result of growth and ripening processes.*

## 10.1 Vitamin C

### Background

Elstar apples contain 10-20 mg vitamin C/100 gram fresh weigh (Stoll 1997, PPO pers.comm.).

### Method

The vitamin C-content (=ascorbic + dehydroascorbic acid) in mg/100 gram fresh-weight was analysed by LBI in apple juice from a mixed sample of 8 apples with skin per treatment. The analysis followed the titration procedure described by Gersons (1972) with a fresh-made 20% 2-5-dichloorfenol-indofenol (Na-salt) as reagent in a 2%-solution of metaphosphoros acid to prevent natural oxidation processes.

### Results and discussion

The results are shown in annex 4. All values are lower than expected (<10 mg/100 gram fresh-weight) and near the detection limit of this method. The values in series A and B vary so much, there is no strait line, so we expect measure faults. The low values in series D and even lower after shelf life are as expected.

#### Relations between vitamin C and other parameters

The correlations between vitamin C and other parameters are mainly determined by the difference between series B (fresh apples) and series D (older and less fresh apples) and are expected.

#### Relations with growth, differentiation and integration

From the concept we thought vitamin C, as a secondary metabolite, would be a differentiation parameter. In the series and in the correlations we see vitamin C also behaves like a growth parameter. So we put a question –mark at both levels. The measurements are not precise enough to distinguish between growth and differentiation with our experimental data.

## 10.2 Phenolic compounds

### Background

In flesh and skin of apple many phenolic compounds are present. The colour of the skin is caused by anthocianin pigment (mainly the phenolic compound cyanidin 3-galactoside). Chlorogenic acid, catechins, procyanidins and quercetin-glycosides are potent antioxidants with potential to prevent oxidation processes such as building up of superficial scald (a storage disorder). Furthermore, they are known to inhibit galactosidases of apple thus having the potential to prevent fruit softening. A high level of these compounds, particularly in the fruit skin may increase the storage potential and, thus, fruit quality (~Barden and Bramlage 1994). They may also have a function in the resistance of apples to diseases in the orchard (Mayer at all 1995, Michalek Diss. TUM). As anti-oxidant they also have a meaning for human health (Cornell University in Nature 22-6-2000).

In general, the biosynthesis of phenylpropanoids depends on the supply of carbohydrates. Studies by Treutter and Awad (2001) revealed that a high C/N-ratio is necessary for high amounts of phenolic compounds.

In organic grown apples a higher amount of phenolic compounds are found than in conventionally grown apples (Weibel et all. 2000; Søggaard pers. comm. 2001). Until now we haven't the knowledge to interpret the various

phenolic groups separately, so we only present here the total amount in figures.

## Method

Apples from different treatments were longitudinally cut into eight slices, which were frozen at -20°C and lyophilised. From the dry pieces the brown and oxidised surface was removed and representative parts were cut from the top, the middle and the bottom of the fruit slices. These pieces (skin, core, inner, outer flesh in a weight proportion as in the apple) were combined and ground in a mixer while methanol was added. After further extraction in an ultrasound water bath for 30 min, the samples were centrifuged and the supernatant concentrated before analysis by HPLC. The analysis was performed as described by Treutter et al. (1994) and Mayr et al. (1995). Flavanols (13 components), cinnamonic acids (3 components), phloretin-glycosides (2 components) and quercetin-glycosides (5 components) were determined.

These results cannot be compared with those of Awad and de Jager (1999, 2000), as they used another method.

## Results and discussion

The results are shown in annex 7.

### A: ripening

We find a decrease of total phenolic compounds during ripening.

A general decrease of phenolic content during fruit development and ripening can be expected but is not well established and not intensively studied (Mayr et al. 1995). Awad (2001) found in Elstar apple skin during maturation a decrease in concentration but an increase content per fruit. This can be explained by dilution due to fruit growth and continuing syntheses.

### B: bearing

Awad and the Jager (2001) found no significant differences in the phenolic compounds in apples with respect to bearing. Therefore we didn't analyse series B.

### C: light

Besides the effect of preparations we found the highest levels of phenolic compounds in the most exposed apples. Thus, more shadow during growth results in lower phenolic levels. Because of the higher sugar content and the lower nitrogen content the C/N-ratio of the sunny-grown apples is twice as high. A high C/N-ratio is of importance for the synthesis of phenols (pers.com. D. Treutter). So this difference is in line with the physiology. The minor compounds (phloridzin, quercetin) also show exceptions here, without consequences for the overall tendency.

Awad (2001) showed an increase of cyanidins (the main anthocyanin pigment in the skin) from August in sun-exposed fruit and hardly in shaded-grown fruit.

### C: preparations

Independent of light exposure we see thrice that apples with preparations have a  $\pm$  20% higher level of overall phenolic compounds. As far as known, this is the first time to find such an effect.

### D: shelf life

Awad and de Jager (2000) found no significant differences in the phenolic compounds measured in apples with respect to shelf-life. Therefore we did not analyse series D.

## Discussion

### Relations between phenolic compounds and other parameters

Astringent taste is caused by phenolic compounds so we expected a correlation and found a strong one (see annex 14.3.14;  $r^2=0,94$ ), but this is only based on 5 measurements of series A.

We found a positive correlation between phenolic compounds and protein ratio (see annex 14.3.21;  $r^2=0,58$ ) and with electrical resistance ( $r^2=0,38$ ). Negative correlations are found with proteins and free amino acids ( $r^2=0,58$ ;  $r^2=0,37$ ).

### **Relations with growth, differentiation and integration**

Phenolic compounds are secondary metabolites and that is why we place this parameter in the level of differentiation. Also the higher concentration of phenols in series C in sun-grown fruit and in fruit treated with preparations support this idea. The decrease in concentration of phenols with riper fruit in series A may look like the contrary, but remember, a decrease in concentration can also mean an increase in mg per fruit. Phenols are correlated with some integration parameters (protein ratio and electrical resistance), so maybe there is also an integrational aspect?

# 11 Self-disintegration (SD)

## Background

This test is developed to measure 'self-maintenance'. Therefore a slice is taken out of fresh fruit and exposed to air and incubated in a petri-disk unto disintegration by autolysis and opportunistic micro-organisms. See for further background annex 19. LBI uses this test in many crops, but with apples the results were poor, see under method improvement and annex 19. In this project we gave this test on apple another chance.

## Method

This test was carried out by LBI with 8 apples per treatment.

From each apple two slices (ca. 0,50-0,75 cm thick) were cut trough the middle of the apple. The remaining upper and lower parts of the apples were used for the other tests (taste, vit.C, crystallisation, and capillary picture).

Both slices of each apple were weighed. Then one was dried in a stove for initial dry weight and one was used for self-disintegration and dried for dry weight after the test. From this data the dry matter loss of every individual apple was calculated.

The slice in the petri-dish for disintegration was placed one hour in open air in the laboratory for natural infection by micro-organisms and then covered with a lid with ridges to allow gas exchange. The petri-dish was placed in a dark incubator of 20°C and a relative humidity of circa 100%.

After 7 days the apple slices were judged as to the following parameters:

### Visual judging, qualitative:

1. Presence of black-sporulating fungi. These fungi have proved to be very aggressive, and when they are present the percentage white surface is not a good parameter any more. So, these slices were excluded and in some cases the amount of observations without black mould became very small.
2. Percentage of the slice-surface (without black mould) that was still white as an indicator for 'self-maintenance'.

### Dry weight judging, quantitative:

3. Loss of dry matter after self-disintegration.

$$\text{Degree of disintegration} = \Delta d.s. = (d.s._{\text{before}} - d.s._{\text{after}}) / d.s._{\text{before}} * 100\%.$$

## Results

This parameter is shown in annex 4. We presented 2 values: '%dry matter loss' in one week and '%area white' after a week of 'self-maintenance'. The first parameter is calculated from all slices, the second without slices with black fungi. The variation between the 8 apple slices in one sample is so large that no significant (95%) differences between the means appeared. Nevertheless we discuss the tendencies.

### A: Ripeness

Later picking shows a tendency to faster disintegration especially of the ripest samples, and an equal self-maintenance. With riper samples we expected an increase in disintegration and a decrease in self-maintenance. These results give the impression that none of these apples are so ripe that they loose their form and only the ripest ones disintegrate a little more than average.

### B: Bearing

Series B has one lost sample, the remaining values show a constant 'disintegration' and a decreasing tendency in 'self-maintenance' with increased bearing.

### C: Light

Series C shows only a small difference between 'self-disintegration' and 'self-maintenance'. More sun exposure leads to less disintegration and equal self-maintenance.



## C: Preparations

There is a slight but consequent difference for apples with preparations in a way we did not expect, they seem to disintegrate and loose their form a little easier.

## D: Shelf-life

Series D shows a consistent increase in disintegration and a decrease in self-maintenance as expected.

## Discussion

### Relations between disintegration and other parameters

Disintegration is positively correlated with nitrogen content (see annex 14.3.15 with  $r^2=0,72$ ) and with phosphate, potassium and magnesium. The nitrogen content fits with the experience that apples with a high nitrogen content store shorter than apples with a lower nitrogen content.

Disintegration is negatively correlated with vitamin C (see annex 14.3.17 with  $r^2=0,59$ ). Vitamin C is known as an antioxidant and from the figure we derived that a minimum of 4 mg/100gram protects against a disintegration of more than 7% dry matter loss in a week.

Disintegration is also negatively correlated with firmness (see annex 14.3.16 with  $r^2=0,60$ ), many sensory properties (crisp, juicy, sour, not raw, aromatic, general), and Bovis-value (see annex 14.3.18 with  $r^2=0,57$ ). These correlations are again mainly due to the presence of two groups of apples: series A, B, C on the one side and the older, softer and easy to disintegrate apples from series D on the other side. The negative correlation with acidity (see annex 14.3.19 with  $r^2=0,54$ ) is caused by the group of apples from series B with an unexplained low pH. It is also a reasonable assumption that a low pH prevents disintegration.

We expected a negative correlation between disintegration and self-maintenance, a negative correlation between disintegration and dry matter (as found earlier however in this experiment only with  $r^2=0,39$ ) and a positive correlation between disintegration and Brix (sugar as an easy to assimilate substrate), but none of these correlations were very profound.

### Relations between self-maintenance and other parameters

Self-maintenance is positively correlated with calcium-content (see annex 14.3.20 with  $r^2=0,59$ ). Apples with high calcium-content are known to be well structured and long storable and self-maintenance fits with these properties. There is a weak positive correlation with Brix (PPO,  $r^2=0,33$ ) and some weak negative correlations with protein ratio ( $r^2=0,38$ ) and crispness ( $r^2=0,43$ ).

We expected a correlation between self-maintenance and disintegration, vitamin C or phenolic compounds, but none of these correlations were very profound.

### Relations with growth, differentiation and integration

From the concept, both resistance to disintegration and self-maintenance are parameters involved with keeping your own form. Which fits with the level of integration. Looking at the series we expected a decrease in self-disintegration during ripening and ageing and an increase with sun exposure, preparations and moderate bearing, of the above only ageing and light exposure were found.

Looking at the correlations we found only a few correlations with other integration parameters. As mentioned earlier we are not satisfied with the precision of this method, maybe this is the reason we didn't find the experimental evidence, maybe our way of hypothesising is not correct?

## Method improvement

To improve this test we need to measure dry matter more precisely and use more sub-samples to compensate for the loss of slices due to the aggressive black fungi. See annex 19 for experiences and questions with self-disintegration test on apples.

# 12 Sensory properties

*Taste is one of the most important parameters for apple quality in consumer's eyes. This chapter is written in co-operation with Mirjam Matze.*

## Background

Sensory panels are mostly used for judgements between varieties, but LBI has the experience that a trained panel is also able to judge between various treatments within one apple variety.

## Method: expert panel and preparation of the tasters

At LBI we determine the taste as objective as possible by controlling the taste circumstances and dividing them into taste-characterisation and judgement, see form in annex 15. LBI chose to use a trained panel of 7 to 8 people. The tasters are trained to judge apples and are checked for individual consistency of the results.

Eight people have been selected who: do not smoke, are familiar with eating apples and like them and are familiar with eating organically grown products. In the week before each sensory session these tasters received a 'trial-bag' with about 6 – 8 apples of different varieties, ripeness, aroma and production systems (bio-dynamic, organic or conventional). With the aid of these trial apples the tasters were asked to train themselves at home by tasting the apples to get a good feeling about how much an apple can vary in different aspects. In this way they calibrated themselves from the range of 1 (=very little) to 10 (=extreme) in the varying aspects and get used to the question list.

## Method

### Preparations of the apple segments

At LBI the same apples were used for many parameters. The apples are cut horizontally to obtain 2 slices for the self-disintegration test. Both remaining halves have been divided with an apple divider on a glass plate into 2x10 segments; the core is eliminated. Half of these segments have been used for the sensory test; the other halves are used to make juice to test the other parameters. The segments of 8 half apples per treatment are combined in a glass bowl, mixed well and covered under plastic for the rest of the time (about 5 to 15 minutes) before tasting. Each taster received about 8 segments per treatment at random in a coded tasteless paper dish. In one session 5 or 6 treatments were compared. Every taster had another code sequence, because of the well know preference for the first sample.

Series A, B, D are served with skin. The light series C, with clearly visual skin differences, was served peeled to avoid psychological influence.

### Answer form with objective characterisation and personal judgement

The taster starts with one segment in the mouth. There are three categories of questions in a fixed sequence: concerning biting the skin (thick, astringent), biting the flesh (crispy, juicy, mealy), and tasting the flesh (raw, sweet, acid, aroma). In every category the different aspects have to be characterised objectively between 1 and 10 and next to be judged personally between 1 and 10. These two exercises are important to correct for personal preference. A very sweet apple for example: one person who likes sweet apples marks a high score for sweetness for characterisation and for judgement. Another person who finds this apple too sweet marks a high score for characterisation but a lower for judgement. The personal preference is seen in the judgement.

At every form some space is left for other remarks. After comparing other samples the taster can repeat with the left segments as much as he/she likes for more certainty.

After incorporating the data we correlated all scores of one person's characterisations with their judgements to get a 'personal taste profile' for the different aspects and to see how consistent a person judges the different series and samples. A consistent person shows a narrow line (straight or bend down). We used only the data of consistent tasters (7 of the 8).

## Results and discussion

The results are shown in annex 8.

## A: Ripeness

Riper apples gave a slight (not significant) improvement of total judgement. Probably this has to do with increased sweetness (especially treatment 5), less sour, less astringent skin and less 'rawness' (but only small differences).

In some aspects we see an optimum in the intermediate treatments (which is also optimal from the point of view of the fruit grower): the thinnest skin (3), the highest juiciness (4) and the best results for aroma (3). These all confirm general accepted knowledge about taste and confirm the measured sugar and acid contents.

The juiciness in the sensory test and the measured %dry matter are positively correlated. Crispness increases until ripening treatment 3; beyond this treatment the crispness stays constant.

## B: Bearing

The bearing does not influence the general appreciation. Only the heaviest bearing shows a slight decrease. This is strange because some important sensory aspects decrease by bearing: crispness, sweetness (especially bearing 5), sourness and aroma.

In series B the skin is characterised as relatively thick compared to series A (session effect?) and is independent of bearing. The skin is relatively astringent, especially treatment 1.

## C: Light and preparations

For taste the differences due to light exposure or preparations are smaller than we saw with other parameters and aren't significant. The total judgement and aroma are independent of light exposure.

## D: Shelf-life

For general appreciation and most detailed aspects of taste we see a severe decrease after storage and a slight decrease during the period of shelf life. It is well known that apples lose their good taste in cooled storage and during shelf life.

## Discussion

### Relations between taste and other parameters

The **general appreciation** is positively correlated with firmness (see annex 14.3.6 with  $r^2=0,68$  although this is based on two separated groups; series A, B and C versus series D), with acid (see annex 14.3.7 with  $r^2=0,72$ ) and strongly with the Bovis-value (see annex 14.3.9 with  $r^2=0,87$ ). We found a weaker positive correlation with dry matter ( $r^2=0,20$ ), acidity ( $r^2=0,27$ ) and DL-emission at  $t=3$  in delayed luminescence test ( $r^2=0,31$ ). General appreciation is moderate negatively correlated with calcium ( $r^2=0,47$ ), dry matter ( $r^2=0,42$ ) and disintegration ( $r^2=0,40$ ). Remarkably there is no correlation with Brix.

Within the sensory parameters we see a group of crispness, juiciness and non-mealiness that is strong positively correlated with general appreciation ( $r^2=0,72-0,99$ ). Sourness ( $r^2=0,54$ ) and aroma ( $r^2=0,42$ ) are moderate positively correlated while sweetness is only weakly correlated ( $r^2=0,21$ ).

In §7.6 we discussed an attempt to find a formula that predicts taste based on some easy to measure technical parameters. Based on this small amount of data the best technical parameter seems to be firmness. Of all parameters the Bovis-value has the best prediction-value for taste.

Looking at the various aspects of taste we can mention some remarkable correlations.

With an **astringent skin taste** many parameters of unripe ness are correlated: starch ( $r^2=0,62$ ), Streif ( $r^2=0,71$ ), sweetness (inverse  $r^2=0,32$ ) and rawness ( $r^2=0,31$ ). With a non-astringent skin taste a number of parameters linked to sun-exposure are correlated, like blush ( $r^2=0,52$ ), magnesium ( $r^2=0,90$ ) and electrical resistance ( $r^2=0,33$ ). This is a new phenomenon for us, and is found in other series than the light series (series C is tasted without skin because of the psychological influence skin colour has on the taste perception).

As expected, an astringent skin is positively correlated with phenolic compounds (see annex 14.3.14 with  $r^2=0,94$ ) because the astringent components have a phenolic origin.

**Crispness** is correlated with many parameters. As expected a strong positive correlation is found with firmness (annex 14.3.12 with  $r^2=0,80$ ). Crispness is an important determinant for general appreciation ( $r^2=0,72$ ). Narrowly related with juiciness ( $r^2=0,85$ ), acid ( $r^2=0,53$ ), pH (inverse  $r^2=0,56$ ) and sourness ( $r^2=0,70$ ). Crispness is correlated with aromatic taste ( $r^2=0,60$ ), vitamin C ( $r^2=0,48$ ), dry matter ( $r^2=0,34$ ), Brix/N ( $r^2=0,37$ ), DL-emission at  $t=3$  in delayed luminescence test ( $r^2=0,27$ ), yellow/blue ratio in spectral range luminescence ( $r^2=0,59$ ) and Bovis-value

( $r^2=0,70$ ).

Remarkable is the strong negative correlation with calcium (see annex 14.3.13 with  $r^2=0,71$ ); here we recognise the polarity between storage potential (calcium) and taste (crisp).

**Juiciness** is strongly correlated with crispness ( $r^2=0,85$ ), so the majority of the correlations found for crispness are also found for juiciness. Remarkable is the negative and not expected correlation with disintegration ( $r^2=0,62$ ).

**Mealiness** is only measured in series D. The high correlations found in annex 14.1 are only based on 4 measurements and are left out of the discussion.

**Rawness** is an aspect of taste and as expected correlates with unripe-ness parameters: firmness ( $r^2=0,44$ ), crispness ( $r^2=0,20$ ), starch ( $r^2=0,42$ ), acid ( $r^2=0,63$ ), pH (inverse  $r^2=0,35$ ), sourness ( $r^2=0,30$ ), Streif-index ( $r^2=0,43$ ), dry matter ( $r^2=0,30$ ), blush (inverse  $r^2=0,28$ ), protein ( $r^2=0,46$ ), amino acids ( $r^2=0,45$ ), protein ratio (inverse  $r^2=0,46$ ), astringent skin ( $r^2=0,31$ ), vitamin C (inverse  $r^2=0,60$ ), DL-emission at t=3 in delayed luminescence test, yellow/blue ratio in spectral range luminescence (inverse  $r^2=0,59$ ) and electrical resistance (inverse  $r^2=0,57$ ).

**Sourness** in taste is as expected well correlated with acid (see annex 14.3.11 with  $r^2=0,57$ ) and acidity (inverse  $r^2=0,63$ ). Besides this we find correlations with freshness parameters like firmness ( $r^2=0,80$ ), crispness ( $r^2=0,70$ ), dry matter (inverse  $r^2=0,30$ ), vitamin C ( $r^2=0,43$ ), general appreciation ( $r^2=0,54$ ), DL-emission at t=3 in delayed luminescence test ( $r^2=0,54$ ) and Bovis-value ( $r^2=0,59$ ).

We found negative correlations with calcium ( $r^2=0,49$ ), disintegration ( $r^2=0,45$ ), and weaker negative correlations with electrical resistance ( $r^2=0,28$ ), differentiation and integration in crystallisation images ( $r^2=0,29$  and  $r^2=0,26$ ).

**Sweetness** is only correlated with a few parameters. We found a weak positive correlation with Brix (see annex 14.3.10 with  $r^2=0,26$ ) although we expected a stronger one. A weak positive correlation was found with general appreciation ( $r^2=0,21$ ), yellow/blue ratio in spectral range luminescence ( $r^2=0,38$ ) and a weak negative correlation with astringency ( $r^2=0,32$ ).

**Aromatic taste** showed moderate positive correlations with crispness ( $r^2=0,60$ ), juiciness ( $r^2=0,53$ ), sourness ( $r^2=0,48$ ), acidity (inverse pH  $r^2=0,21$ ), general appreciation ( $r^2=0,42$ ), Bovis-value ( $r^2=0,48$ ). Weak positive correlations with firmness ( $r^2=0,35$ ), Brix ( $r^2=0,21$ ), acid ( $r^2=0,20$ ), ripeness-index (inverse Streif  $r^2=0,26$ ), dry matter ( $r^2=0,23$  and  $0,40$ ). It seems the tasters judge the sweet/sour ratio often as 'aroma'. Negative correlations were found with calcium ( $r^2=0,42$ ) and disintegration ( $r^2=0,47$ ).

## Relations with growth, differentiation and integration

Appreciation is a complex parameter. We are glad we performed a very detailed sensory test. From the concept we recognise growth aspects in sweetness, sourness and juiciness. Crispness also has a growth aspect, but similar to what is said for firmness there is also an integrational aspect involved with sustaining the crispness. Mealiness can be seen as a loss of vitality (growth) and loss of coherence between cells (integration). Physiologically seen, a condition wherein aroma is gained requires a high level of basic substances (sugar, sour, etc) together with a differentiation process, so we place gaining aroma at the integrational level with a question mark for differentiation. Loss of rawness or astringent skin can be seen as a result of ripening. Overall taste depends on certain proportions, as is seen in the sweet/sour ratio.

Because of the small differences and the large variation we have only little experimental evidence from the series to support this hypothesis. The tendencies we recognise in series D that support this theory are the loss of vitality (sourness, crispness, juiciness) and integration (aroma, overall) with ageing. In series B we find that the reason for lower appreciation for apples from the highest bearing trees is too little sweetness (insufficient sugar for all fruit), and like wise insufficient growth.

Looking at the correlations it is remarkable to see that overall appreciation is mainly correlated with growth parameters, so juiciness, crispness, sweet/sour ratio are important. The correlations with differentiation/integration parameters are illustrated by rawness of flesh, astringent skin and sweet/sour ratio in the ripening process.

## Method improvement

It has hardly ever been done; comparing the taste between apples of only one variety grown in the same orchard, and moreover when there is no bad tasting sample present. So the tasters were provoked to sense very small differences, smaller than we had anticipated. With this in mind it is understandable to find a variation which is too large around the means in the experimental data. An improvement can be a correction factor or standardisation to decrease this variation. Another aspect might be a better training to improve sensing the difference between sweet/sour and aroma.

A well known phenomenon, and also found here, is the high appreciation of the first tasted apple. That is why the various samples are presented in a random sequence to the members of the taste panel. An improvement might be to correct for this phenomenon. When analysing the variation in judgement we see an increase in variation from the sixth apple onwards, this means the tasters are getting tired and lose their refined tasting ability, so we conclude to present a maximum of 5 samples per taster in future.

# 13 Copper chloride crystallisation (=CC)

*This is one of the experimental parameters to learn about life processes.*

## Background

The copper chloride crystallisation method (CC) has been developed from a viewpoint that living organisms do not just exist of substances, but have directing and organising 'living, architectural, structuring forces'. These structuring forces direct form and function of the organism. To demonstrate the existence of this structuring force the CC was developed around 1930 by Ehrenfried Pfeiffer in Switzerland and since then the CC is gradually being further developed to a method with which experts can distinguish different types of structuring forces (Engquist, 1970). The method can be applied with juice from a living organism. The juice is mixed with a solution of copper chloride which is incubated in a climate chamber, where the water evaporates and the solids crystallise after several hours. The pattern of the crystals is then examined. It is thought that the type of crystal patterns indicates the living, architectural and structuring forces, here called the life processes in the growing product. The forces that are supposedly reflected in these patterns, play an important role in the provisional quality concept. The method is already longer in use with blood from human beings, as an additional method for medical diagnostics. In the Louis Bolk Institute and some other European institutes this method is now in development for food quality. It is 'used' in this project in an exploratory way to gain experience and reference material.

## Method

### Preparing juice

In §4 the way of sampling is described. The apple segments of 5 apples were grated manually to a mixed sample. This grated apple was then squeezed through a cloth with a 110  $\mu$  diameter. The resulting juice is left for 30 minutes to allow the debris to settle. One ml of apple juice is combined with 33 ml aquabidest and 10 ml of copper chloride 20% solution.

### Crystallisation process

4.5 ml of the mixture is pipetted into a well-cleaned glass plate with a width of 9.5 cm and glued to a glass rim with a mixture of paraffin and bee-wax. Four replicate glass plates are prepared from one sample of juice. The crystallisation takes place in a climate chamber of 31 C° and 60% RH. The copper chloride starts to crystallise after 8 - 11 hours. The next day the rim is removed and the plate is dried further with filtration paper and stored as a glass plate in a plastic bag.

The CC is a very sensitive method and the current climate chambers at LBI do not have enough constant conditions. In expectation of the improved chambers, we did the crystallisation twice, on two separate days with two separate sub samples. The 'best day' - judged by clear differences between variants and few differences between the four replicates- was chosen to be described.

### Observation and judgement

The series are placed on a light-board to be judged. Judging starts with an empathic first impression of the gestures and qualities in the patterns, expressed in terms of coherence, vitality, differentiation; terms that emerge in this method as self-evident, natural concepts. Then the more objective facts are scored in terms of measurable properties, like length of crystallisation needles, angles between branches and side needles, etc. Finally a free description is given. This process of judgement was first done blindly and was repeated after revealing the sequence of samples in the series.

One representative image per variant is digitally scanned and presented in annex 9. Choosing one image of the four is a subjective process of characterisation, which is based on the impression of the essential changes in the sequential series. The described observations are based on 4 images per sample. So the presented image may not contain all aspects described in the text. In annex 9 the different categories, classified in the observation and judgement process, are explained.

## Results

The results are shown in annex 9.1 (details method); annex 9.2 (representative pictures) and 9.3 (figures per series).

### A: ripening

In these series we needed to reveal the sample codes before we could rank the images. Then a development started to appear. From a very abundant dividing and subdividing of small needles in the multi-centered images A1 and A2, both with a very dense and thick needle-structure, the series developed from image A3 onwards to more one-centeredness. The head branches make bigger gestures, the structure of the side needles is less abundantly divided, more air appears between the needles and the structures open more to the periphery. The images become more coherent towards A5. It also seems that in the images the centre of attention is gradually drawn more towards the periphery. The ripening series are in accordance with our expectations about differentiation and coherence.

### B: bearing

In these series it was possible to blindly find the right sequence. The series starts in B1 with a very thin, long and slack image filling needle structure as one extreme. The 'middle images' in B3 have a well filled ground structure with short and bristle-like needles, and an apple-typical structure. The other extreme, B5, shows images with a quite empty surface of the plate and faintly apple-typical characteristics. Gradually the needles become somewhat sharper. The extreme under bearing situation seems to show a 'luxurious but inharmonious growth'. The extreme overbearing (B5) shows 'poorness' and the crystal structure does not reach the border. The lowest bearing, expected to be very vital, was surprisingly weak but yet coherent. For both vitality and for differentiation B3 had the highest scores and for coherence B4 scored a little higher. From the crystallisations point of view this series had the expected optimum in the middle.

### C: light

Although the series with and without preparations are very different, in both, with the increasing amount of light, an increase can be seen in one-centeredness and in coherence of the images. Also an increase in the refinement of form of the needles is seen, as well as in the transparency of the images.

### C: preparations

In the coded samples a clear difference can be seen between two groups of three images. One group (C1, C2, C3, later revealed as the group with preparations) shows slender and quite straight needles which have relatively few side needles; these images appear very 'transparent' but also somewhat 'thin'. The other group (C4, C5, C6, later appearing to be the group without preparations) has a much fuller needle-structure and more bunches of side needles. The needles of the first group are distinctly longer than in the second group. It was a surprise to find that the 'thinner, more transparent group' with a slightly lower vitality- and differentiation-score, was the one treated with preparations. The coherence was, as expected, a little better in the apples with preparations.

### D: shelf life

The just-out-of-the-storage-apples (D2) show a rather inharmonious, kind of 'shocked' image. Apples-4-days-on the shelf (D3) are regarded as the most harmonious ones. In the next two images the attention is drawn increasingly towards the outer, peripheral zone. Parallel to this the structure changes in the sense of much more dividing into long thin side needles that appear as very un-apple-like. A loss of coherence, which might have been expected, was not seen, moreover an increase was found! Apples until 12 days on the shelf, stay vital and coherent. No ageing signs, as we expected, appeared.

## Discussion

The crystallisations form a holistic image, and aid to the interpretation of the conventional parameters for growth, differentiation and integration

The images have brought information of a totally different quality than the other methods. Observing these images brings the experience of the reality of life processes closer. It changes the way of thinking and makes it easier to integrate other data, including the conventional ones, into a more holistic overview about what is

converting in the various series, see §19 en annex 9.3. The results brought some confirmation in series A, B and C for light and also some surprises in series C for preparations and in series D.

Crystallisations are especially valuable for the vital quality concept because crystallisations can be judged on all three aspects of the concept. Vitality is recognised as smooth, quite dense needle structures filling the whole plate. Structure is recognised as , well ordered, one-centred, regular pattern of sharp side-needles with a wide angle. Coherence is recognised as a whole where the parts are interconnected, without isolated structures and in a way typical for apple.

### Relations between crystallisations and other parameters

In the table of correlations (annex 14.1) only the empathic judgements are included, the impressions of vitality, structure and integration. We found no correlations between what is experienced in these images as **vitality** and any other parameter. A handicap in the search for correlations is the rough score-scale used, which seldom will give significant correlations. In this report we only evaluated the empathetical observations of the first impression. The other scores will be kept until we have collected more data from other projects.

With the impression of **differentiation** we found some moderate correlations: negative correlations with unripeness parameters like firmness ( $r^2=0,20$ ), malic acid ( $r^2=0,29$ ) and sourness ( $r^2=0,29$ ) as we expected for ripening is an aspect of differentiation. With the delayed luminescence test we found a negative correlation with the DL-emission at  $t=3$  ( $r^2=0,30$ ; annex 14.3.41 and not expected) and with lag time ( $r^2=0,30$ ; annex 14.3.42). The last correlation is as expected because lag time is known to be an inverse differentiation parameter.

We found a negative correlation with redoxpotential (Eh:  $r^2=0,22$ ) and P-value ( $r^2=0,33$ ; annex 14.3.43). This is as expected because P-value is known to be an inverse ordering parameter.

We found a moderate positive correlation with the impression of integration in the same images ( $r^2=0,35$ ; annex 14.3.44) as fits with the hypothesis that to a certain amount differentiation is a prerequisite for integration.

With the impression of **integration** we found a negative correlation with aromatic taste ( $r^2=0,34$ ; annex 14.3.45) which we expected to be positive because we assume aroma as an integration-parameter. With sourness ( $r^2=0,26$ ) and DL-emission at  $t=3$  in the delayed luminescence test a moderate negative correlation is found. This is understandable as both parameters are thought to belong to the growth process and when growth is too severe the integration is inhibited. We found a negative correlation with redoxpotential (Eh:  $r^2=0,31$ ) and P-value ( $r^2=0,33$ ; annex 14.3.47). This is as expected because P-value is known to be an inverse ordering parameter. And we repeat the moderate positive correlation between the impression of differentiation and of integration in the same images ( $r^2=0,35$ ; annex 14.3.44 as expected). No correlation with Bovis-value is found (see annex 14.3.48) unless we had expect.

### New steps in the method during this project

During this project a new method of judging CC on a quantitative scale was introduced. This facilitates comparing within series and with other parameters. Quantification was performed with the empathically judged aspects of vitality, differentiation and coherence, which arose from the images. There were only a few moderate correlations with other parameters, which are thought to indicate aspects of growth, differentiation and integration. This brings into question whether we have enough consensus about the aspects of vitality, differentiation and coherence and about the concept of vital quality. Also the selected items described in the quantitative scale can be improved.

A new insight from the correlations is that 'transparency' of images is a measure for differentiation.

### Method improvement for the future

Our aim is to develop the CC to a reliable scientific method giving information about living, architectural and structuring forces and thus about the life processes in food products, with repeatable judgements , if the same technical and judgmental conditions are used. To reach this goal we need some improvements.

First the technical requirement to make reproducible images. As the CC is a very sensitive method it requires good technology, and also 'green fingers' for the method. We mentioned previously that the current climate chambers at the LBI do not have optimal stable conditions. To circumvent this, two separate crystallisation series were made on different days, and the technically 'best day' was chosen. Improvement of the chambers is necessary to obtain better control. But nevertheless with a single repeat we obtained results that could be interpreted.



Next, is the requirement for capable judging of the images. One way is to analyse the objective facts about the needle structure. A computer could do that and discriminate between the physically recognisable properties between different images on the level of growth and differentiation. But the empathic judgements on the level of integration still ask for human observations.

This is the way of the personal judgement by trained observers. Research in the past showed that a group of skilled observers can reach acceptable intra- and inter-observer repeatability with respect to objectively as well as emphatically judged aspects of images. (Amons, van der Bie, Huber, Koopmans, 1992, 1999).

Last step is the interpretation of the images: what do the observed facts and characteristics mean? To make interpretations, references have to be available to correlate to and also a conceptual network has to be present. Studies like this apple study are suitable to obtain references. In this CC method expert-validity will be the first and most feasible to obtain. A co-operation and result-comparison with a colleague-laboratory would be most useful and will take place in the future.

# 14 Capillary rising Picture method (CP)

*This is one of the experimental parameters to learn about processes.*

## Background

The capillary rising picture method (CP) was developed with the same purpose as the copper chloride crystallisation (CC) to make aspects of life processes in living organisms visible. The method was developed from ascending chromatography (Kolisko 1939) and is also called capillary dynamolysis (A.Tingstad 2001).

In the CP a different method and medium is used than in the CC, so other aspects of life processes might appear than in the CC. In the Louis Bolk Institute this method has only recently been introduced. The aim was to obtain reference material to see whether it is worthwhile developing this method further at LBI.

## Method

### Preparing juice and rising process

The same juice as for the CC is used. Two concentrations are made, 100% and 50%, for which either 0.6 ml apple juice or 0.3 ml juice with 0.3 ml aqua bidest is allowed to rise in chromatographic paper (Whatmann no. 1) and dry during 3 hours in a room of 20 C° and 60% RH. Next the filter is placed in a solution of 0.7 ml silver nitrate 0.25 % which is allowed to migrate vertically up the filter paper up to 1 mm over the juice-line, under a glass bowl during 30 min. After drying, 2 ml of iron sulphate 0.25 % is added to rise nearly to the top of the paper under a glass bowl during 0.5 to 2 hours. Then the paper is dried for 3 hours. The last two stages take place in a dark room.

## Observations

For training in technique and judgement of the images the help of an expert, Mrs. Ruth Mandera from Germany, was asked. She considered the technical quality of the images as good. She first described the CP's blindly, then with known series sequences, but she could not interpret the images because she had not worked with apples before. From her descriptions of the two concentrations of the four series, we chose to present here the ones that show the most obvious signs. Which can be of either concentration.

## Results

The technical terms are indicated in annex 10.1 and some photos with description in annex 10.2.

### A: ripening

CP A1 and A5 are very different in the way that in A1 the middlezone lies like a barrier between the lower part and the upper part of the images. Whereas in A5 the middlezone connects in a lively way the lower and the upper part and the images seem more like a whole. In A1 the bowls of the middlezone are round, whereas in A5 the bowls have opened into vertical lines. The content of the bowls in A1 is a solid grey, whereas in A5 this area is filled with vertical lines. Also more flags appear. In images A2-A4 a transition is recognised. Summarising, the ripening series showed a continuous process from closed horizontal gestures into open vertical gestures.

### B: bearing

Again the middlezone draws most attention. In B1 it is rather pale, with a pale silverline under the bowls. In B5 the bowls in the middlezone are strongly formed and are contoured by a strong brown lower silverline. Also the forms of the bowls seem to change: in B1 the tendency is towards an irregular form whereas in B5 the tendency is towards a rounder and regular form. B2-B4 shows a gradual transition from pale forms to stronger and clearer forms, but not so obvious that it was found blindly.

### C: light

The full sunlight series show the roundest forms and contours in the bowls of the middlezone and few, swinging flags. There is openness between the flags. The shaded grown series show less round forms and a paler middlezone and have many more flags, which are stiff. The half sunlight grown series are somewhere in between. So increasing sunlight showed a gradual change from stiff to more bowl-like and open forms.

### **C: preparations**

No differentiating criteria could be found. The only impression is that the forms have more contrast.

### **D: shelf life**

After storage the images showed great differences with the original; in annex 10.2 all the five are shown. The juice did not rise well and produced increasingly broadened bowl forms in the middlezone. This might be due to an increased 'stickyness' of the juice.

## **Discussion**

The images are only described, not explained. It is a first step to find which phenomena in plant development relate with the phenomena in the capillary pictures. In future research the images will gradually get their meaning in the context of the other parameters.

# 15 Delayed luminescence

*This is one of the experimental parameters to learn about processes.*

## Introduction to ‘Bio-photons’

Plants in darkness emit a permanent and extremely weak radiation of light [Ruth 1976, Popp 1979, Colli, 1954, Günther 1983, Neurohr 1992]. This emission becomes stronger when preceded by an illumination phase (induced emission). The intensity of the light emission decreases following the illumination phase and, after some time, equals the weak permanent emission. This transition time depends on the sample material and varies from a few seconds to some hours.

Popp designated bio-photon analysis as the examination of the weak light emission by biological samples, though no clear differentiation was made between induced and permanent emission.

Meluna Bio-photon Research co-operates with Popp in measuring delayed luminescence and Kwalis developed an own method with spectral range luminescence. Both methods are used in this project and described in more detail in the next two chapters. Differences in the methods are summarised below in figure 7:

**Figure 7: Differences between the two methods of delayed luminescence.**

	Meluna: delayed luminescence	Kwalis: spectral range luminescence or fluorescence-excitation-spectroscopy
measured part of the apple	2 disks from the inner apple from both the blushed and the shaded side (2x)	whole fruit in the skin at 4 sides (4x)
repetitions	10 apples	12 apples
colour of excited light	white (in 2 intensities)	8 colours from the light spectrum
duration of measuring luminescence	3 to 200 sec	1 to 50 sec.
presented values	<ul style="list-style-type: none"> <li>emission at <math>t=3</math> sec (for metabolic energy)</li> <li>lag-time, figure determining the form of the hyperbolic curve</li> </ul>	<ul style="list-style-type: none"> <li>% yellow/blue</li> <li>emission of all 8 colours/blue</li> </ul>

## 15.1 Delayed luminescence by Meluna

*written in co-operation with Roel van Wijk*

### Background

Meluna characterises the development of a plant or an organ as the co-operation between two forces: a force forming mass through metabolic activity and a differentiating force for compartmentalisation and order.

A compartment is then defined as a part of the whole with a specific (different from the whole) composition, structure and/or function. The increase in size or mass of a compartment during development might also be different from the increase of mass and size of the whole.

When the metabolic activity decreases, more energy is stored in the differentiated structure via an integration process. Fully ‘integrated’ is the situation with a maximum order together with the highest metabolic energy. Because of the similarity in thinking with the concept described in §1 and §20, we expect a good alignment in this work which appears in the discussion indeed.

Long-term delayed luminescence, which can be detected with sensitive light detecting devices, presents information about the energy-storage capacities of apple flesh. The characteristics of this energy-storage capacity are derived from the shape of the curve representing the delayed luminescence. A high start after 3 second ( $t=3$ -value) means a high metabolic activity. A real hyperbolic decrease in luminescence is found in a well differentiated situation and is expressed as a low ‘lag-time’ in the curve, see example in annex 18.

Input of energy is obtained by excitation with two different protocols, differing in intensity and duration of the excitation, high intensity and short: 'flash', and longer with a low intensity: 'LED'. The use of this variation in excitation conditions increases the probability to detect differences, but in this case the results were the same and only the results obtained with LED are shown in the figures.

## Method

Measuring 5 to 6 flesh repetitions/apple of 10 to 12 individual apples takes 3 days. On each day measurements were performed for maximally 12 hours, during which 3-4 apples per treatment were tested.

### Sample preparation

For the present studies, apples were kept in the dark at room temperature for at least 30 minutes. The subsequent sampling of the flesh of the apples was performed at a very low light intensity. The procedure for sample preparation was as follows.

Each apple was judged for its most red-coloured and most green-coloured side. These two areas were selected for sampling by cutting the selected convex disks (slices) at a diameter of approximately 5 cm (with a maximal thickness of 1.5 – 2 cm). The centre part of each disk was used to prepare a smaller (3 cm diameter) disk and the skin side of this disk was removed. This resulted in a draughts-like disk (3 cm diameter; 1 cm thick). This disk was placed in an aluminium foil-coated petri-dish of 3.3 cm diameter with the former skin side facing upwards. The sample contained neither skin nor core parts. Each disk was prepared in this manner immediately before measurement.

### Excitation protocol of delayed luminescence

In the first set-up the sample is illuminated with a very high intensity source (xenon flashlight X-3644; 25 Watt seconds) situated 43 cm above the sample, and parallel to the photomultiplier tube. In the second type of excitation the sample was illuminated at a moderate light intensity with a LED (white light LED type 5 mm; Conrad Electronic) for 1 minute. The LED illumination source is also situated 43 cm above the sample, and parallel to the photomultiplier tube. Immediately after illumination, the sample was transferred under the photomultiplier.

### Measurement of delayed luminescence

Delayed luminescence was measured in the period from 2 seconds to 200 seconds after illumination with a dwell time of 0.2 sec. The luminescence was measured with a photon counter, which was equipped with a Hamamatsu R550 photomultiplier tube (spectral response 280-850 nm, 1.5 kV) kept at  $-20^{\circ}\text{C}$ .

Standard high performance photon counting electronics consisting of a low-noise pre-amplifier, amplifier, discriminator and rate-meter were used. The petri-dishes containing the samples were situated in the measurement chamber, with the sample approximately 6 cm below the photomultiplier window.

### Analysis of luminescence decay

The variety of curves representing delayed luminescence suggests that the decay of luminescence cannot be sufficiently described by a simple exponential curve. Some curves can be described by a simple hyperbolic curve. However most curves are composed of two hyperbolic parts and a transition between these two parts.

Since each measurement consists of the counts in a fixed, 50 m sec. period, the log-log plotted scale of the graph causes the individual measurement points to deviate relatively more in the tail part of the curve than at the start of the measurement. In order to minimise the deviation in the data points, the total measurement time was divided in such time periods that the average value of the logarithm of the time points is equally distributed on the time axis, with the exception of the first measured values. This reduces the number of data points from 1000 to 68. The number of photons in these time intervals was averaged. In this way the relative standard error of the mean of the data points over the measured time range is almost equal. Examples of curves are presented in annex 18.

In order to describe two hyperbolic parts and the transition, the log-log plotted delayed luminescence curves obtained after excitation by 'LED' and 'flash' are characterised by six parameters.

1. **Initial slope** ('Slope A'): The initial slope of the log-plotted curve.
2. **Final slope** ('Slope B'): The final slope of the log-plotted curve.

3. **T=3 sec emission** ('t=3 sec'): emission measured at 3 sec after illumination. A higher emission indicates a higher metabolic energy.
4. **T=200 sec emission** ('t=200 sec'): emission measured at 200 sec after illumination.
5. **Transition** ('Apparent lag time'): time that extrapolation of the final slope reaches the 3 sec value. A lower lagtime is indicative of more differentiation.
6. **Hyperbolic character** ('Degree of hyperbolic decay'): The reverse of the calculated deviation from a hyperbolic curve. High hyperbolicity closely resembles the 'lower lag time' condition and indicate, at least for apples, a high energy's storage capacity.

Parameters 3 and 5 are thought to be the key parameters in the two life processes and are presented in figures in this report.

## Results Delayed luminescence

See annex 11 for results.

### A: Ripening

Increasing ripeness is reflected in the steadily decrease of the emission after 3 seconds and the steadily increase of the hyperbolicity of the curve (inverse lag time). It is interesting to note that all points given in the table and graphs are average values obtained from two sites of 10 apples. Particularly at the ripest stage, A5, many individual apples showed a real hyperbolic curve. Apples with the condition that luminescence is largely decreased and hyperbolicity is lost were not seen in this series, so we saw no overripe apples.

### B: Bearing

Increased bearing is reflected in the steadily increase of the emission after 3 seconds and the steadily decrease of the hyperbolicity of the curve.

### C: Light

The emission after 3 seconds decreases a little with more sun and the hyperbolicity of the curve (inverse lag time) is relatively high. However no consistent relation is observed between the hyperbolicity and light condition. The exception is the sample grown in the shadow with preparations (C3). We have to do with an unexplained effect.

The decrease in emission at 3 seconds is in line with the expected higher degree of ripeness in the sun. The decrease of hyperbolicity is contrary to what we expected for growth in the sun and for this we have no explanation.

### C: Preparations

The emission after 3 seconds increases a little with preparations and the hyperbolicity of the curve decreases more with sunny and half sunny-grown apples. Also here the exception is the sample grown in the shade with preparations (C3). The decrease of hyperbolicity is contrary to what we expected for preparation treatment and for this too, we have no explanation.

### D: Shelf life

A longer shelf life is reflected in the slight decrease of the emission after 3 seconds and a maximum hyperbolicity of the curve in the apples 4 days out of storage (D3).

## Discussion

### Relations between delayed luminescence and other parameters

Emission at 3 seconds is correlated with many parameters. First a positive correlation with the group of parameters related with unripeness like firmness ( $r^2=0,51$ ), starch ( $r^2=0,49$ ); Streif-index (see annex 14.3.22;  $r^2=0,73$ ); acid ( $r^2=0,48$ ); crispness ( $r^2=0,27$ ); non-mealiness ( $r^2=0,30$ ), rawness ( $r^2=0,27$ ), pH (inverse  $r^2=0,44$ ), blush (inverse  $r^2=0,40$ ) and dry matter (inverse  $r^2=0,66$ ).

We also found positive correlations with aspects we usually find in more ripened fruit like sweetness ( $r^2=0,47$ ), general appreciation ( $r^2=0,31$ ), P-value ( $r^2=0,28$ ) and Bovis ( $r^2=0,26$ ). Unexpectedly we found negative

correlations with electrical resistance ( $r^2=0,23$ ), Brix (Brix-2;  $r^2=0,55$ ), differentiation and integration in crystallisation (resp.  $r^2=0,30$  and  $r^2=0,28$ ).

Emission at 3 seconds is positively correlated with the other delayed luminescence parameters like hyperbolicity (see annex 14.3.23: 'inverse DL-lagtime'  $r^2=0,56$ ) and yellow/blue ratio in spectral range luminescence (see annex 14.3.12;  $r^2=0,68$ ).

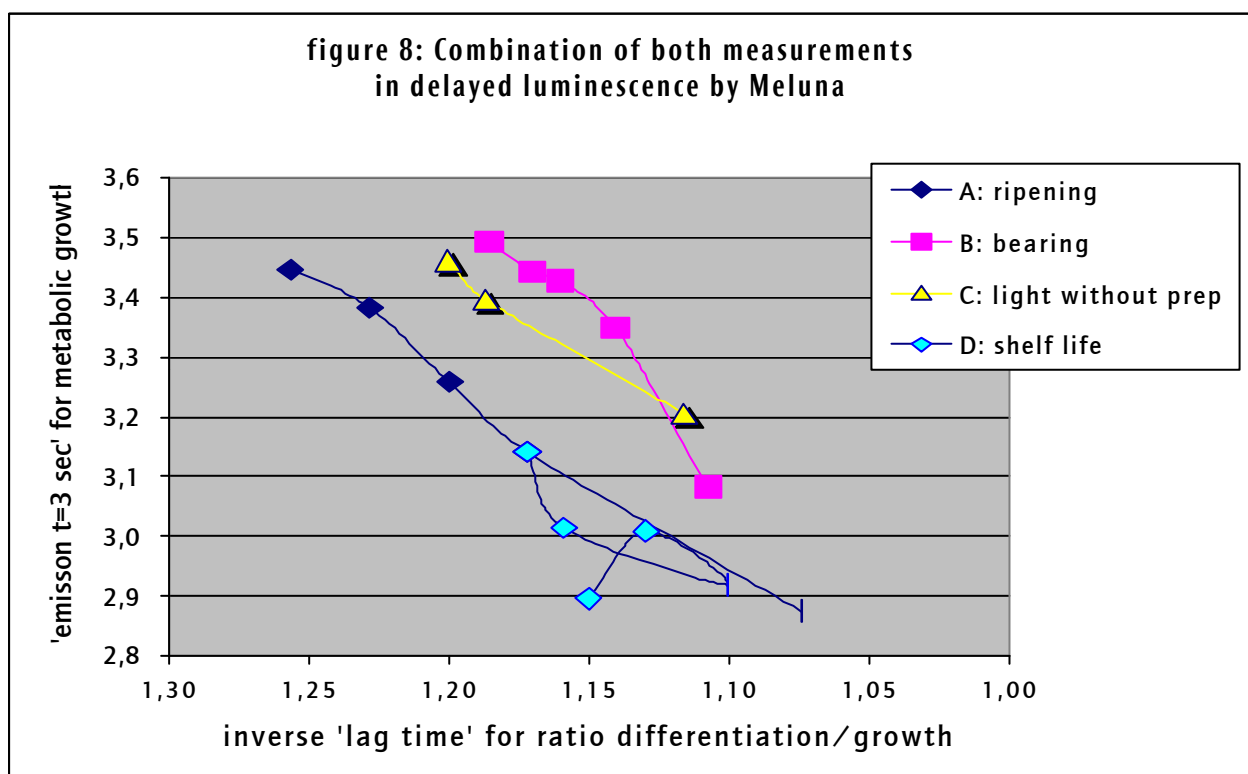
The hyperbolicity of the delayed luminescence (inverse DL-lagtime) is negatively correlated with only a few parameters: calcium (see annex 14.3.26;  $r^2=0,51$ ), redoxpotential (Eh:  $r^2=0,32$ ), P-value ( $r^2=0,28$ ) and emission at 3 seconds (DL  $t=3$ ,  $r^2=0,56$ ) and negatively correlated with dry matter (see annex 14.3.25;  $r^2=0,68$ ) and differentiation and integration in crystallisation (resp.  $r^2=0,30$  and  $r^2=0,45$ ).

### Relations with growth, differentiation and integration

Meluna assumes the 'emission of biophotons at 3 seconds' to be a measure for 'metabolic energy', the driving force for both growth processes and differentiation processes, a potential (or vitality) for both growth continuance and for differentiation, maintaining structure (or in other words preventing disintegration).

We recognise this assumption in these apple series in the phenomenon that correlations are found with both processes indeed. We see a decrease when this energy is used in the ripening process (found in the later picked in series A, the lowest bearing of series B and in the sun in series C) or in the ageing process (series D).

Meluna assumes the **hyperbolicity** to be a measure for the ratio between growth and differentiation processes in a way that a high hyperbolicity (=low lagtime) indicates more differentiation and a low hyperbolicity (=high lagtime) is indicative of more growth. Looking at the correlations we recognise this in correlations with inverse redoxpotential (P-value too) and differentiation in crystallisations (both assumed to be differentiation parameters) and dry matter (assumed to be a growth parameter). Regarding the series we found a switch from growth to differentiation in series A with increased ripening, in series B with lower bearing and in series C with more sun all as is expected.



### Proportion of metabolic energy versus the differentiation/growth ratio

The ripening process in **series A** is illustrated by the lower availability of metabolic energy and its use for differentiation, see figure 8. Increase in tree load in **series B** is illustrated by higher availability of metabolic energy (for growth) relative to its (lower) use for differentiation. At first the apples from low bearing trees were interpreted only as riper, but considering the decreased starch levels (annex 3.3) we only slightly can explain this

aspect. Apples from low bearing trees are full of sugar, so it might be suggested that under this condition a relatively high availability of metabolic energy is combined with a relatively high degree of differentiation. The more sun-exposed apples in **series C** are characterised as having less metabolic energy and more use for differentiation. Preparations induce the apple's vitality and delay the differentiation. In **series D** we found a nick in the figure, possibly explained by a loss of metabolic energy and a need for further ripening (differentiation) onto the fourth day (D3) and after that a loss of differentiation/structure as a start of disintegration (D4, D5).

Another remarkable thing is to find two main lines, one for series A and D and one line for series B and C. We can understand the lower metabolic energy in A+D from the fact that these apples are stored for a varied length of time or used their metabolic energy during the ripening process (series A and D are slightly riper than series B and C). Series C is overall high in metabolic energy and low in differentiation/growth. A possible explanation for this, is that series C is the only fertilised series.

Looking at the A- and D-lines, It is surprisingly how well the D1, D2, D3-samples of ripening in January after storage fit in the line between A1 to A5 of the ripening process in September. That means that a 3 months stored apple after 5 days of shelf life (D3) is quite similar to an apple freshly picked but one week over due in September (A5). Another interesting thing is that D1 and D2 differ in a 3 months storage period, while no difference in the ratio differentiation/growth is found. However there is some decrease in metabolic energy. After the maximum in differentiation is reached at D3 we see a turn with a slight revival in metabolic activity before breaking down as is found in many ageing products.

## 15.2 Spectral range luminescence in flesh and seeds by Kwalis

*written in co-operation with Jürgen Strube*

### Background

This method measures the delayed luminescence after excitation. Measurement is performed for 7 excitation colours plus white instead for white only. So the spectral range is used to excite samples and fluorescence-excitation-spectroscopy is an adequate description reflecting what is done.

Basis for the rating of samples is a proved theoretical concept [Strube 1998, Strube 1999, Strube 2000]. This concept relates spectral data to plant properties. The concept has two foundations, primarily measured phenomena and in addition Popp's theory of cell communication.

The basic phenomenon is shown by a green plant versus a chemical. A green plant shows a broad excitation spectrum, a chemical shows response only after blue excitation. This can be explained, as to photosynthesis of green plants contributes the whole spectrum of light. Chemicals show only the normal fluorescence excited by blue and uv-light. The excitation spectrum of dormant seeds is in between, a broad spectrum as green plants have, but with its maximum after excitation with blue light [Strube 1998]. So, in general samples can be classified as vegetative (when related with broad spectrum) or seed-like (when related with spectrum less broad or even concentrated on blue).

Popp's theory of cell communication assumes best conditions of life when the inner structure of living matter communicates equally on all frequencies, that means all modes of structural resonance are present. That means best differentiated structure is followed by broad resonance. And indeed fluorescence-excitation-spectroscopy shows a broad excitation range with maximum at red-green-yellow for ripest apples. Dormant seeds show restricted resonance as vegetative activity and cell communication is on minimum.

Basis for the evaluation are pilot studies with leaf crops, beans and other seeds. The method was mainly used to test seeds [Strube 1998, Strube 1999, Strube 2000]. Broad spectral range excitability correlates with a vegetative character, narrow spectral range excitability with a seed-like character.

Fruits and seeds of the same fruit were investigated at these apples the first time. The expectation was that best apple flesh will have the maximum red-yellow-green response related to blue and corresponding the apple seeds will behave most seed-like for ripest apples (reduced red-yellow-green values related to blue).

So this method is expected to give information about the most fruit-like character of apple-flesh and the seed-like character of apple seeds. The method is expected also to show ageing, but this was not applied during this study.



## Method

In this report a brief description is given as an easy overview. A more extended description is provided earlier [Strube 1999, Strube 1998, Strube 2000]. The excitation spectra used for the analysis are derived by exciting a sample using subsequently light of various wavelengths (colours) and measuring the total emission. The sample reacts to the various excitation colours with an afterglow of different intensity and duration (fluorescence excitation spectroscopy). The red-yellow-green range is compared to blue.

### Measuring equipment

The device used for fluorescence measurement basically consists of a chamber impervious to light in which the sample is placed. A shutter similar to those of a camera allow the sample to be illuminated for limited periods of time (5 s) by means of a 150 W tungsten lamp, a set of commercial colour filters (Schott) and collimating lenses (sample distance 25 cm). Excitation is followed by the subsequent (0,2 s dwell time) measurement of the induced light by two photomultipliers (EMI 9558QA cooled to  $-28^{\circ}\text{C}$ ) highly sensitive in the 190-850 nm range with a response according to S20 cathode (EMI). The measurement position is turned  $90^{\circ}$  from excitation direction. For the principal arrangement see figure in Annex 16. Standard single photon counting electronics is applied.

### Excitation and measurement protocol

The typical time course of measurements is depicted in the following chart (annex 17). First the sample is illuminated for 5 sec by coloured light. During the following measurement phase, the emission radiated by the sample becomes continuously weaker.

With apples, the time required for the emission to reach a constant level recedes at a significantly slower pace than illustrated in annex 17. As shown by preliminary analyses, an adequate measuring period was 50 seconds with 500 msec counting period time. For apples the mean of the luminescence from the 30th to the 50th second (40 count intervals of 500 msec) is designated as 'R40'.

Excitation and measurement is repeated for each colour 5 times. 7 colours and white are applied, so 40 sequential excitations and measurements are performed during one measurement sequence. The colour sequence is dark-red, red, light-red, yellow, green, blue, uv and white, using standard colour filters from the Schott Co.

### Measurements with apple fruit

Samples were stored in a refrigerator at  $5 (+/-1)^{\circ}\text{C}$ . They were removed from the refrigerator 12 hours prior to testing, stored at  $15 (+/-2)^{\circ}\text{C}$  and subsequently measured also at  $15^{\circ}\text{C}$ .

Each apple was tested as a whole from 4 different sides ( $90^{\circ}$  rotation). Since each sample consisted of 12 apples, there were 48 individual measurements of 8 colours conducted on each sample.

### Measurements with seeds

The drying process of the fresh seeds necessary prior to measurement took some 2-3 months. Drying was at temperature of  $15^{\circ}\text{C} (+/-2^{\circ})$  in the presence of silicagel. The seeds of the 12 apples of a sample were measured together. Each sample was measured 8 times at the same colour with different filling arrangement to involve most of the surface of the seeds. As for flesh 8 colours were measured.

### Collected data

After each excitation the complete response for 50 sec in 500 msec intervals was collected and stored.

### Calculated data

Response of same excitation colour was averaged point by point forming the averaged response of the 5 excitations. Parts of the response are designated as follows:

- Mw1 Mean of first reading following excitation (highest response value).
- R40 Mean of the readings from the 31st – 50th second (mean of 40 values).
- R80 Mean of the readings from the 11th – 50th second (mean of 80 values).

Above data are absolute values. In order to become independent of the sample's physical size (e.g. size of an apple), normalized data are calculated by dividing the response of each colour by response of white (Mw1%w, R40%w, R80%w) or by response of blue (R40%bl, R80%bl).

- Mw1%w Like Mw1, however a relative spectrum based on white light.

- R40%w Like R40, however relative spectrum based on white light.
- R80%w Like R80, however relative spectrum based on white light.
- R40%bl Like R40, however relative spectrum based on blue light.
- R80%bl Like R80, however relative spectrum based on blue light.

According to Popp the curvature of the response curve is of particular importance in evaluating coherent systems [Popp 1986, Popp 1993]. The curvature is evaluated by calculating the quadratic difference between response curve and the best possible hyperbola and between response curve and the best possible exponential reference curve. The quotient of the square differences is designated Chi E/H. Small values (below 1) correspond to an exponential course of the curve, values greater than 1 correspond to a more hyperbolic curvature.

- Chi E/H 25 Coefficient of the curve's course calculated over seconds 1 – 12,5.
- Chi E/H 50 Coefficient of the curve's course calculated over seconds 1 – 25.
- Chi E/H100 Coefficient of the curve's course calculated over seconds 1 – 50.

### Analysis of data

Spectra of R40%bl reflect all information needed to discriminate between fruit-like and seed-like as mentioned above (see 'background'). The very similar R80%bl spectra reflect same information but may be helpful for kinds of samples not measured before (in case of different response timing).

Spectra of Mw1 relate to ageing of apples. Ageing was not investigated during this study. In addition Mw1 has a weak correlation to seed-character.

In general Chi E/H 50 correlates weak to R40%bl. For flesh of apples also a strong correlation can occur.

In order to reflect the character of a sample in one figure, yellow/blue ratio of R40 may be used (that is 'yellow' bar out of spectrum R40%bl).

Results of measurements are presented as spectra for R40%bl and as data table. For ease of evaluation also graphs of yellow/blue ratio R40 are provided, see annex 12.

### Evaluation of samples

High values of yellow/blue R40 indicate most fruit-like apples. Low values of yellow/blue R40 indicate most seed-like kernels.

### Statistics

From the 8 x 48 individual measurements, the mean values and mean-value standard deviations were calculated and depicted in graphs for each of the respective testing parameters.

The differences between any two samples were tested for their significance using the two-sided t-test (using GraphPad).

For this study, all parameters were calculated possibly capable of providing statements. In order to maintain the simplicity of the overview, only a selection is illustrated. All data can be provided on request.

## Results for flesh and seeds

See annex 12 for results.

### A: Ripening

The increase of ripening correlates significantly with the decrease of the broadness of the excitation spectrum. This means that for the whole apple higher values at red, light-red and yellow in relation to blue were found. The broadness of the spectrum is expressed in the figures in annex 12.4 in the yellow/blue ratio, and gives an equal line for red, light-red and yellow. So, in the ripest apples the highest yellow/blue ratio is measured in the whole fruit and the lowest in the seeds.

### C: Light exposure

The light exposure of apples correlates also significantly with the broadness of the excitation spectrum. A high yellow/blue ratio is measured in the apples exposed to sunlight, lowest values are measured in apples grown in the shadow. This is also valid for apples with and without bd-field preparations.

Because of too little seeds in series C (only 20 from 10 apples), there is no data on the seeds.

## C: Bd-field preparations

The influence of Bd-field preparations is also reflected in the broadness of the excitation spectrum. A higher yellow/blue ratio is measured in the apples with preparations under sunlight and under half-shaded conditions. The inverse effect was found for the apples grown under shaded conditions.

This effect is also reflected by a lot of the other methods, as is seen in annex 15 and §19.4

## Discussion

### Relations between spectral range luminescence and other parameters:

The yellow/blue ratio in spectral range luminescence is clearly correlated with many ripeness and/or sun parameters: we found negative correlations with firmness (see annex 14.3.28;  $r^2=0,57$ ), starch (see annex 14.3.29;  $r^2=0,95$ ), Streif-index (see annex 14.3.27;  $r^2=0,89$ ), acid (see annex 14.3.31;  $r^2=0,89$ ), rawness ( $r^2=0,59$ ), minerals N,P,K and Mg ( $r^2=0,71-0,90$ ; see annex 14.3.32 and 14.3.39), proteins (see annex 14.3.34;  $r^2=0,67$ ), free amino acids (see annex 14.3.33;  $r^2=0,64$ ) and positive correlations with blush ( $r^2=0,75$ ), Brix (see annex 14.3.30;  $r^2=0,69$ ), sweetness ( $r^2=0,38$ ).

Positive correlations are found with Brix/N-ratio ( $r^2=0,86$ ), protein ratio (see annex 14.3.40;  $r^2=0,63$ ), crispness (see annex 14.3.35;  $r^2=0,59$ ), DL-emission at  $t=3$  (see annex 14.3.24;  $r^2=0,68$ ), electrical resistance (see annex 14.3.36;  $r^2=0,63$ ) and a negative correlation with disintegration ( $r^2=0,45$ ). Only a weak positive correlation is found with the Bovis-value (see annex 14.3.37;  $r^2=0,23$ ).

### Relation with growth, differentiation and integration

Kwalis assumed the yellow/blue ratio in spectral range luminescence to be a parameter for fruit-character and 'being alive'. In our quality concept this is at the level of integration. Thus, the clear correlations with protein ratio, electrical resistance, disintegration and Bovis are expected. As expected the ripening series and the sunnier grown fruit from the light-exposure series, have an increasing amount of ripe fruit (see annex 12.3).

### The concept 'fruit-like' and 'seed-like'.

This data fits perfectly in the context of the preliminary spectral range luminescence measurements of leaves, seeds and chemicals. Living plants are open to the full spectrum of sunlight. This openness is measured as light emission especially after excitation with the colours red, light-red, yellow and green to which dead chemicals react very weakly. This is valid only for plant leaves and fruit. For seeds the inverse is found. So a broad spectrum in red, light-red, yellow and green is called 'fruit-like' and a narrow spectrum around blue, 'uv' and white is called 'seed-like' (see annex 12.4).

In this context the flesh of riper apples is characterised to be more fruit-like than the unriper ones and corresponding the seeds from riper apples are more seed-like. This is reflecting clearly the inner differentiation which is part of the new quality concept.

In addition, apples grown in sunlight have to be characterised as more fruit-like than shadow grown apples. Data also show that Bd-field preparations result in apples which are more fruit-like. The influence of sunlight seems to be stronger than the Bd-field preparations. This is suggested by the fact that full sunlight results in apples that are more fruit-like than half-shadow grown apples with Bd-field preparations with respect to the measured data.

### Fruit and seed are polar

The same experiments showed that polar phenomena arise for seeds on the one hand and leaves on the other hand. The more plants are being investigated, the more it becomes a general phenomenon that seeds can be considered of a better quality if they emit little after red, yellow and green light excitation. From this point of view we tried successfully to interpret the results of the coded samples of apples (and samples of beans and Calendula in other studies). In §2.1.7 the polarity between seeds and fruit flesh has already been seen in other parameters.

This is important for methodological reasons but it also has practical implications. It can be interpreted that a very

ripe apple with the highest quality of flesh also contains the best seeds. So there is no contradiction between the vegetative and reproductive parts. A contradiction may arise, when fertilisation is achieved in a different way. Then the vegetative development may be stimulated solely. This is an intriguing research question that should be addressed. Application of Bd-field preparations has a harmonious development (in a vegetative and reproductive sense) of the plant as goal and in this case it was achieved.

## Supplementary data and method improvement

Just for completeness and not for regular evaluation  $\chi^2/h$ -values (degree of hyperbolic decay) were calculated in addition for all colours, see annex 12.2. This shows that mainly 'white' values correlate often with the R40%bl or R80%bl values. 'Blue' and 'uv' values have an even better correlation. The R40%blue figures give the same pattern as Meluna's lagtime.

The question arose whether only the red skin of the apples was measured, which correlates with ripeness. Perhaps this may be also the fact, however, the seeds show the same ripeness order and so do the slices measured by Meluna. Kwalis measurements performed on some peeled samples of apples showed the same spectral characteristics as whole apples. So there are some aspects strongly supporting the concept, that structural behaviour is measured.

Normally seeds are measured in an amount greater than 100 seeds per sample. The measuring-technique for seeds has been improved for samples with fewer seeds for this apple project. It was possible to obtain results with 36 seeds per sample from ripeness series with a special seed holder, but the only 23 seeds per sample from the light series was beyond reliable measurement levels.

# 16 Electro-chemical parameters

*This is an experimental parameter to learn about properties as vitality, structure and ageing.  
This chapter is written in co-operation with Hartmut Heilmann.*

## Background

Life processes in plants and animals can be described as chains of electro-chemical or redox reactions. Vincent (1955) developed a bio-electrical theory to derive an electrical energy value of food from measurements of pH, redox potential and electrical resistance of a watery solution of the food. He suggested that food with high reducing power, later expressed as low P-value (as a combined parameter of the three mentioned parameters above), was health promoting. Later, M. Hoffmann (1988) analysed different products and determined factors influencing the P-value. In studies with J. Streif, he found that a low P-value correlated with a high apple quality. Harvest time and storage had a profound influence on the P-value. The P-value and apple quality could be influenced by soil fertility and application of different types of fertilisers (H. Keppel, 1998).

### PH-Value (acidity)

The pH-Value is the best known electrochemical parameter, and is used to measure the acidity. The pH is measured by potentiometry using suitable electrodes. The measured mV value is logarithmically transformed to the pH-Value. The potentiometric equilibrium is at pH 7, and 0 mV. A difference in pH of 1 (at 25°) equals 59 mV. In plants proton activity reflected in pH has energetic aspects. Plant cells push protons into the cellular lumen and use the proton motive force to generate ATP in the cell membranes (Mengel 1991). So, the consumption of protons and increase of pH means a loss of vitality (Stoll 1997, Kinzel 1989).

### Redoxpotential

The redoxpotential is of central interest for electrochemical research because it reflects the gradient of electrons which life processes utilise for their cellular work (Kollath, 1978). The redoxpotential can be determined with suitable electrodes of gold or platinum; Heilmann's laboratory prefers gold. The redoxpotential represents the respiratory equilibrium between oxidising and reducing substances in a liquid milieu. When the energetic power is higher (the redoxpotential is low), plant cells can use more free enthalpy for their activity. Such plants convey more to the consumer's organism. As this is the aim, decrease of the energetic power is looked upon as denaturation (Kollath, 1987).

### Redoxpotential as Eh [in mV]

Traditionally the flow of electrons was considered to be the main respiratory energy transport in an organism. Oxygen is the terminal electron acceptor.

$$E_h = E_{h_0} + E_{ref}$$

$E_{h_0}$  is the read off value of the potentiometer.

$E_{ref}$  is the tension of the reference electrode.

$E_h'$  or rH is  $E_h$  with pH-correction to pH7.

$$rH = 2 E_h / E_{(Nernst)} + 2 \text{ pH}$$

$$E_h' = E_h + (\text{pH} - 7) \times E_{(Nernst)}$$

$$E_h' = (rH / 2 - 7) \times E_{(Nernst)}$$

$E_{(Nernst)}$  is the Nernst-factor of 59,1 mV (25°C)

### The Electrical Resistance R [in Ω]

High values of electrical resistance indicate that electrolytes and other cellular ions are more integrated in membranes and cell organelles. Low values indicate free-moving electrolytes, which might be a sign of deterioration of plant cells and tissues. The conductivity G [mS] is the inverse of R.

### P-value as measure of dissipation [in μW]

The P-value is used to determine the tendency of an organism to dissipate its energy. High values represent the most probable state of higher entropy, low values show low entropy. It takes into account the gradient of the

redoxpotential and the role of the electrical resistance.

$$P = Eh^2 / R$$

In the 8<sup>th</sup> Congress Electrochemical Quality Research, Vienna 2001, a modification for energy dissipation was suggested (Heilmann 2001).

$$DP = (Eh')^2 / R$$

In the DP, the pH value is taken into account. The data were presented also according to this aspect and will be discussed. In each case lower values of P or DP of food are considered as of higher value.

### **Aim of electrochemical research**

The pattern of energy regulation is based on experiences with food like water, vegetables, fruit, potatoes and grains. The thermodynamic question is: how does the organism deal with its energy flow? Which level of entropy can it maintain? The aim is to characterise the pattern of energy loss or maintenance of entropy level. Higher P and DP values can be interpreted as more openness, while lower values are a sign of ordering or coherence.

Healthy legumes, fruit and grains usually show low levels of entropy. In a comparative examination of seven pairs of organic and conventional products, the organic products had lower P-values than the conventional products (Heilmann & Hoffmann 1995). Products not yet ripe or infected with a parasites had higher P-values.

## **Method**

### **Preparing juice**

Each sample consists of 10 unwashed apples. In series D some apples were damaged by transport and were sorted out (maximum 3/sample were damaged). The apples are cut into quarters, the inner part is cut away, because this is not consumed. Juice is collected from 7-10 apples with a household device. The juice is left to settle for about 5 minutes until the foam floats on top and clear juice can be taken for analysis. The juice was not centrifuged because the juice separated nicely.

### **Preparation of electrodes**

The redox-electrodes are adapted to the specific range of work. For apples they are put into freshly prepared apple juice for some time. The pH-electrode is calibrated with pH 7 and pH 4,01 buffers. The electrode for electrical resistance is checked monthly.

### **Measurements**

The juice is made successively and measured immediately after preparation. Usually 3-5 measurements are performed, calculations are made and converted into graphs.

## **Results**

Results are presented in annex 13. Because the juice is obtained from a mixed sample of 7-10 apples, no statistical significance can be calculated.

### **A: ripening**

The pH stays constant and increases from the 4th picking date onwards. The redoxpotential decreases firmly to increase a little on the latest picking date. The resistance stays constant and decreases on the latest picking date. The P-value reflects mainly the trend of the redox. The trend of the P-value in the ripening series is similar to what is found in previous ripening series (Streif 1996, Hoffmann 1997).

### **B: bearing**

Bearing has no effect on the pH and is remarkably low compared to the other series (no explanation). The resistance increases in the first three bearings. The redoxpotential and P-values are constant except for the second bearing, which gives higher values. Transport damage might be an explanation for this, although the outcome of other parameters did not show such exception in series B.

## C: light

The pH is constant. The resistance generally increases with more light, and the shaded-grown apples without preparations have a somewhat higher resistance. The redox-potential and P-value clearly decrease with increasing light.

## C: preparations

The pH is constant. In apples with preparations, the resistance in (half) sun is slightly higher, this is also found for the redoxpotential and the P-value. This was not expected. Heilmann interpreted this as increased ripeness due to preparation usage. This, however is not supported by other parameters.

## D: shelf life

The pH shows a large increase which is a logical continuation of series A. The resistance shows a maximum after 4 days of shelf life and afterwards decreases a little. The redoxpotential and P-value also increase with a longer shelf life, as was expected from earlier experience with fruit.

## Discussion

### Acidity (pH)

#### Relations between acidity and other parameters

Acidity (pH) is correlated with many other parameters that have to do with ripening and ageing. In these processes the pH increases (=less acid). So as expected acidity is negatively correlated with firmness ( $r^2=0,68$ ), acid ( $r^2=0,53$ ), vitamin C ( $r^2=0,59$ ), sourness ( $r^2=0,63$ ), crispness ( $r^2=0,56$ ), and juiciness ( $r^2=0,48$ ), rawness ( $r^2=0,35$ ), DL-emission at  $t=3$  ( $r^2=0,44$ ). Acidity is weak negatively correlated with general appreciation ( $r^2=0,27$ ), aroma ( $r^2=0,21$ ) and Bovis-value ( $r^2=0,38$ ) and positively correlated with calcium ( $r^2=0,48$ ), nitrogen ( $r^2=0,77$ ), magnesium ( $r^2=0,75$ ), disintegration ( $r^2=0,54$ ), Brix-2 ( $r^2=0,29$ ) and electrical resistance ( $r^2=0,23$ ). We cannot explain the positive correlation with the minerals and resistance. The others fit into the quality concept, see below.

#### Relations with growth, differentiation and integration

For fruit like apples, acidity can be seen as a growth parameter, as a potential for further ripening, as is illustrated in the negative correlation with many ripeness parameters. In ageing in series D the fruit loses its vitality, the pH increases and so the basis for integration decreases, as is illustrated in negative correlations with taste, Bovis-value, and the positive correlation with disintegration.

### Redoxpotential (Eh)

#### Relations between redoxpotential and other parameters

Redoxpotential (Eh) is correlated with only a few parameters: weak positively with DL lagtime ( $r^2=0,32$ ) and weak negatively with differentiation and integration in crystallisations ( $r^2=0,22$  and  $0,31$ ). Because of the P-value formula a high correlation with the P-value is expected ( $r^2=0,92$ , see annex 14.3.43).

#### Relations with growth, differentiation and integration

In series A and D we saw the redoxpotential decreasing heavily during ripening and increasing a little during over-ripening and ageing. Heilmann's assumption of the redoxpotential being a parameter for order, together with the correlations with a number of differentiation parameters, suggest that the redoxpotential is a differentiation parameter. In these results we also see the ability to show the loss of structure in over-ripe fruit, which is an aspect of integration.

### Electrical resistance (R)

#### Relations between electrical resistance and other parameters:

Resistance (R) is negatively correlated with parameters like firmness ( $r^2=0,50$ ), starch ( $r^2=0,55$ ), acid ( $r^2=0,74$ ), Streif-index ( $r^2=0,69$ ), vitamin C ( $r^2=0,66$ ), sourness ( $r^2=0,28$ ), rawness ( $r^2=0,57$ ), astringent taste ( $r^2=0,33$ ), phosphate ( $r^2=0,65$ ), protein and free amino acids ( $r^2=0,84$  and  $0,85$ ), DL-emission at  $t=3$  ( $r^2=0,23$ ). Positively

correlated with blush ( $r^2=0,60$ ), potassium ( $r^2=0,67$ ), protein ratio ( $r^2=0,85$ ), yellow/blue ratio in spectral range luminescence ( $r^2=0,63$ ; see annex 14.3.36), and has a weak positive correlation with dry matter ( $r^2=0,25$ ), phenolic compounds ( $r^2=0,38$ ) and pH ( $r^2=0,23$ ).

### **Relations with growth, differentiation and integration**

In series A and D we saw the resistance slowly decreasing during the last phase of ripening and ageing. Sun and preparations in series C and the higher bearing trees in series B gave the highest resistance. Considering this, we expected electrical resistance to be an integration parameter. This is supported by good correlations with other integration parameters like the yellow/blue ratio in spectral range luminescence and protein ratio.

In series B and C the overall level is lower than in series A and D. The same difference in overall level we see in ripeness (A and D are riper) and in the DL-emission at  $t=3$  (A and D have less metabolic energy). So this suggests a certain amount of differentiation is obliged for this integration process, and so electrical resistance is a differentiation parameter too.

## **P-value**

### **Relations between P-value and other parameters**

The P-value is correlated very strongly with the redoxpotential (Eh:  $r^2=0,92$ ; see annex 14.3.43). This is what we expected as the redoxpotential plays a severe role in the P-value-formula.

The P-value is slightly positive correlated with blush ( $r^2=0,23$ ), nitrogen and phosphate ( $r^2=0,39$  and  $0,51$ ), DL-emission at  $t=3$  ( $r^2=0,28$ ), and DL-lagtime ( $r^2=0,28$ ). The P-value is slightly negative correlated with differentiation and integration in crystallisations ( $r^2=0,32$  and  $0,33$ ; see annex 14.3.43 and 14.3.47).

### **Relations with growth, differentiation and integration**

Because of the close relation with the redoxpotential we expect the P-value also to be a differentiation parameter. Beside that we recognise also growth aspects (pH) and differentiation (Eh) and integration aspects (R) the P-value-formula. From the concept we expect 'dissipation' as an inverse parameter for integration, but in the correlations we found aspects of all three levels. For our quality concept a complex parameter like the P-value for differentiation and integration will give additional information when we use the separate aspects (pH, Eh, R) apart from each other.



# 17 Bovis-value

*Bovis is an intuitive measurement-scale for vitality made by Mr Bovis. It is performed by trained people who regularly calibrate each other, making it inter-subjective. This method has its meaning only when you know the people who are doing it and their integrity, their skill to concentrate and attentively connect with the object. This is not a parameter we expect to make objective in the way of independence of the observer and it is added as an experimental parameter to get an idea of its worth .*

## Method

People concentrate on the energy flow coming up from the earth and the flow going down to the earth through the apples, and the amount of both flows is counted in Bovis. This can be a parameter for the 'integration' aspect of quality.

For food the interpretation is: <3000 causing illness; 3000-6500 unhealthy; 6500-7000 neutral; >7000 health supporting. Here we present in kilo-Bovis to avoid big numbers.

Joke Bloksma performed the Bovis blindly (i.e. without knowing the treatment) . This quick measurement was also used to check the similarity of the sub-samples before sending them to the various partners and was performed on at least 4 sub-samples.

## Results

The results are shown in annex 4 as kilo-Bovis.

### A: ripening

This series shows no clear difference in Bovis-value with increased ripeness. Picking date 4 has the only distinguishable higher value and the last picking date is again lower, possible due to over-ripeness.

### B: bearing

The different bearings have a rather constant Bovis-value, a decrease is found with the highest bearing (overbearing?).

### C: light

Increase in light-exposure shows a large and consequent increase in Bovis-value, both with and without preparations.

### C: preparations

Apples with preparations show a slight increase in Bovis-value, but only significantly higher in full sunlight.

### D: shelf life

In storage and shelf life period the Bovis-value decreases sharply. After 4 days of shelf life the Bovis-value drops below the value that is said to support human health.

## Discussion

Very important for the Bovis-value is the maturation in series D, but also optimal picking date, sun and preparations have effect.

## Relations between Bovis-values and other parameters

Bovis-value is strong positively correlated with general appreciation ( $r^2=0,88$ ; annex 14.3.9 and with many aspects of taste: crispness, juiciness, sourness and aroma) and with firmness ( $r^2=0,70$ ). Weaker positive correlations are found with acid ( $r^2=0,47$ ), pH (inverse 0,38), dry matter ( $r^2=0,30$ ), vitamin C ( $r^2=0,34$ ), emission at t=3 in delayed luminescence test ( $r^2=0,26$ ) and yellow/blue ratio in spectral range luminescence ( $r^2=0,23$ ; annex 14.3.37). Bovis is negatively correlated with disintegration ( $r^2=0,58$ ; annex 14.3.18). We found no correlation with crystallisations (see annex 14.3.48).

## Relations with growth, differentiation and integration

The big changes in Bovis-value are found in series D (a decrease during ageing) and in series C (higher in sun and with preparations). These series are thought to show integration/differentiation (C) or loss of integration (D and last picking date in A). From the assumption that Bovis is a measure for the connection between the up-going and down-going energy flows through a plant or through the fruit, Bovis is thought to be related to integration. Bovis-values positively correlate with integration parameters like taste, SRL-yellow/blue and inverse disintegration. We found weaker correlations with some growth-parameters like acid, pH, dry matter, DL-emission at t=3 and a differentiation parameter like vitamin C. This places Bovis-value as a preliminary integration parameter.

# 18 Comparing the parameters

## 18.1 Costs of parameters

In this phase of developing a new quality concept we permit ourselves to work with as many parameters as possible to learn from each an aspect of quality. For the routine phase later, we have to choose which quality measurements are sufficient for routine usage and are a compromise between much information and little costs. See table 10 to get an idea what the parameters will cost.

**Table 10: Global costs (year 2000, excl. tax) in Euro of 1 sample when measured in a series of about 5 samples**

Parameter	laboratory	remarks	Euro
Leaf series	LBI	picking in the field, drying, drawing	100,-
Exterior	LBI	global description	16,-
Starch by lugol	LBI	12 individual apples	34,-
Making juice	LBI	1 mixed sample of 12 apples	52,-
Brix	LBI	in juice (in duplo)	26,-
Vitamin C: meth.Sprenger	LBI	in juice (in duplo)	30,-
Disintegration incl. dry matter	LBI	8 individual apples, incl. photo	75,-
Sensory test	LBI	trained panel of 8 apple experts	110,-
Crystallisation	LBI	4 images per mixed juice sample, 2 concentrations, 1 repetition	410,-
Capillary picture	LBI	1 image per mixed juice sample, 2 concentrations, 1 repetition	300,-
Brix and acid	PPO	duplo on mixed sample	15,-
Starch, firmness, skin colour	PPO	25 individual apples	18,-
Minerals, incl. dry matter	LZVI	duplo destruction, mixed sample	57,-
Delayed luminescence	Meluna	10 individual apples 2 types of excitation; 2-200 sec.	900,-
Delayed luminescence in fluorescence excitation spectra	Kwalis	10 individual apples complete spectra with 8 types of excitation	950,-
Amino acids, protein	Kwalis	1 mixed sample of 10 apples	250,-
Phenols	TU-München	1 mixed sample of 10 apples	300,-
Electrochemical measurements pH, R, Eh and calculated P-value	Heilmann	1 mixed sample of 10 apples 4x measured in proceeding time	130,-
Bovis	LBI	4 independent repetitions	18,-

## 18.2 Similarity between sub-samples

For practical reasons we had to work with different sub-samples for different laboratories. Because of this we risked obtaining non-comparable results from the different laboratories. In table 5 we present an overview of sub-samples and dates of measurements in the different laboratories. We worked with highly standardised fruit, see table 4, which minimises the variation during sub-sampling. Besides this we checked for similarity in a number of ways:

1. Bovis-value of 4 sub-samples before sending to different laboratories. The difference between mixed sub-samples was below 100 Bovis and that means the sub-samples were very similar.
2. Brix is measured in 2 sub-samples: PPO (1) and LBI (2). In annex 14.3.1 the correlation is shown which is rather good ( $r^2=0,76$ ). It would be better if every laboratory had measured Brix (as an easy measurement) to check similarity of sub-sampling and possible transport-delay.
3. Delay or damage during transport or faults during sub-sampling can cause unexpected deviations in results. This can be the case with the electro-chemical parameters in B2.

## 18.3 Relations between parameters

To get an idea of the relations between parameters we made a correlation table for (only!) linear correlations with a certain amount of confidence and significance (selected for  $p<0,05$  and  $r>0,5$ ); see annex 14.1 and 14.2. We

looked for narrow correlations with a methodological or biological meaning and presented these figures in annex 14.3. The conclusions drawn for the individual parameters are discussed in the various parameters' chapters and are collected in this chapter again. The next list is only for people with interest in correlations. Others better continue at § 19.

### **Relations between colour and shine and other parameters**

The percentage blushed skin is correlated with many unripe-ness parameters: firmness ( $r^2=0,26$ ), starch ( $r^2=0,61$ ), acid ( $r^2=0,48$ ), Streif index ( $r^2=0,74$ ), N, P, K, Mg ( $r^2=0,63-0,81$ ), vitamin C ( $r^2=0,48$ ), both protein and free amino acids (both  $r^2=0,84$ ), raw flesh ( $r^2=0,28$ ), astringent skin ( $r^2=0,52$ ) and emission at  $t=3$  in delayed luminescence ( $r^2=0,40$ ). Blush is positively correlated with Brix-1 ( $r^2=0,22$ ), protein ratio ( $r^2=0,77$ ), yellow/blue ratio in spectral range luminescence ( $r^2=0,75$ ), electrical resistance ( $r^2=0,60$ ), P-value ( $r^2=0,23$ ) and differentiation in crystallisation ( $r^2=0,27$ ).

The correlation between ground colour and shine is not examined in the correlation table.

### **Relations between firmness and other parameters**

Firmness is correlated with many parameters that are indicative of ripeness or ageing, but most of them are highly correlated with firmness due to the presence of two separated groups: series A+B+C versus series D, see for example general appreciation in annex 14.3.6.

### **Relations between starch and other parameters**

Starch is correlated with many parameters that are indicative of ripeness and sun exposure. Notable is the negative correlation between starch and the yellow/blue ratio in spectral range luminescence (see annex 14.3.29 with  $r^2=0,95$  based on series A+C).

### **Relations between Brix and other parameters**

Brix is correlated with many parameters that are indicative of bearing and thus the distribution of assimilates. We found positive correlations between Brix and dry matter (see annex 14.3.4;  $r^2=0,73$ ), Brix and protein ratio (see annex 14.3.5;  $r^2=0,69$ ) and Brix with the yellow/blue ratio in spectral range luminescence (see annex 14.3.30;  $r^2=0,69$ ). We only found a weak positive correlation between Brix and sweet taste (see annex 14.3.10;  $r^2=0,26$ ). Negatively correlated were Brix and calcium content (see annex 14.3.3;  $r^2=0,81$ ), and Brix with both protein and free amino acids ( $r^2=0,65$ ;  $r^2=0,68$ ).

### **Relations between malic acid and other parameters**

Acid is correlated with many parameters that are indicative of ripening and bearing and thus the distribution of assimilates. Acid has a rather well positive correlation with firmness ( $r^2=0,87$ ), starch ( $r^2=0,73$ ), Streif index ( $r^2=0,71$ ), both phosphates and potassium ( $r^2=0,53$ ;  $r^2=0,42$ ), vitamin C ( $r^2=0,68$ ), both protein and free amino acids ( $r^2=0,72$ ;  $r^2=0,71$ ). Acid also has a positive correlation (albeit weaker) with dry matter ( $r^2=0,40$ ), crispness ( $r^2=0,53$ ), and juiciness ( $r^2=0,49$ ), rawness ( $r^2=0,63$ ), general appreciation (see annex 14.3.7;  $r^2=0,47$ ), delayed luminescence at 3 sec. ( $r^2=0,48$ ) and Bovis-value ( $r^2=0,47$ ). Acid and sourness are rather well positively correlated (see annex 14.3.11;  $r^2=0,72$ ).

Acid is rather well negatively correlated with protein ratio ( $r^2=0,77$ ), the yellow/blue ratio in spectral range luminescence (see annex 14.3.31;  $r^2=0,89$ ) and electrical resistance ( $r^2=0,74$ ). Acid is also negatively correlated (albeit weaker) with blush ( $r^2=0,48$ ) and disintegration ( $r^2=0,31$ ). Do not confuse acid with acidity (pH). These are not the same properties as is illustrated in the only moderate negative correlation ( $r^2=0,53$ ).

### **Relations between Streif maturity index and other parameters**

Streif index is correlated with many parameters that are indicative of ripeness, these correlations are very similar to those discussed in §7.4 for starch.

### **Relations between technical quality index and sensory properties**

The TQ index for Golden Delicious based on Brix, firmness and acid is rather well correlated with the general appreciation in the sensory test (see annex 14.3.8,  $r^2=0,55$  when linear;  $r^2$  increases with a more complex line). As discussed in §18 this good correlation is due to the presence of two separated groups: series A+B+C versus series D. In an attempt to find a better predicting index for the general appreciation based on Brix, firmness and acid measurements in our Elstar series, firmness was found to have the highest correlation (see annex 14.3.6;

$r^2=0,68$ ). Conclusively, firmness is the best technical parameter for the prediction of taste.

### **Relations between dry matter and other parameters:**

Dry matter is measured in two laboratories: dry matter 1 (PPO) in series B and C and dry matter 2 (LBI) in all series. The correlation between both measurements is only moderate (annex 14.3.2;  $r^2=0,58$ ), which is explained by the less precise method at LBI. The correlations between the dm-1's or the dm-2's are not equal either. A clear positive correlation is found thrice with Brix (see annex 14.3.4;  $r^2=0,77$ ; 0,38; 0,93). A negative correlation is found with calcium ( $r^2=0,71$ ) and with delayed luminescence ( $t=3$   $r^2=0,66$  and lagtime  $r^2=0,68$ , see annex 14.3.25).

Weaker positive correlations are found with firmness ( $r^2=0,38$ ; 0,36), vitamin C ( $r^2=0,57$ ), many sensory parameters ( $r^2=0,20-0,34$ ), electrical resistance ( $r^2=0,25$ ) and Bovis-value ( $r^2=0,30$ ).

### **Relations between minerals and other parameters:**

Minerals are only measured in series B and C, and the correlations are based on only 11 readings. We found many times a high correlation between N, P, K, Mg in one group and Ca separately.

High N, P, K, Mg- contents are found in unripe (Streif) and shaded-grown apples. So it wasn't surprising to find that nitrogen is positively correlated with starch ( $r^2=0,60$ ) and negatively with blush ( $r^2=0,80$ ) and the yellow/blue ratio in spectral range luminescence (see annex 14.3.32;  $r^2=0,86$  based on only 6 readings in series C).

As expected, nitrogen is positively correlated with protein ( $r^2=0,74$ ) and free amino acids ( $r^2=0,80$ ), but negatively with protein ratio ( $r^2=0,77$ ). It is interesting to find a high positive correlation between nitrogen and disintegration (see annex 14.3.15;  $r^2=0,72$ ) as fits with the experience of fruit growers that apples with high nitrogen levels rot quickly. A weak positive correlation was found with the P-value ( $r^2=0,39$ ).

The P, K and Mg correlations mostly follow the line of the N correlations. Specific for phosphate is the positive correlation with Brix ( $r^2=0,86$ ) and a negative correlation with electrical resistance ( $r^2=0,65$ ) and integration in crystallisation ( $r^2=0,63$ ).

Specific for potassium is a positive correlation with acid ( $r^2=0,42$ ) and Brix ( $r^2=0,87$ ), which is known in fruit science, see above in the subject heading background, but which is not found as a positive correlation with taste in our series. We found a positive correlation with electrical resistance ( $r^2=0,67$ ) and a negative correlation with the yellow/blue ratio in spectral range luminescence (see annex 14.3.39;  $r^2=0,90$  based on only 6 readings in series C).

Calcium is moderate positively correlated with self-maintenance (see annex 14.3.20;  $r^2=0,59$ ), lagtime in delayed luminescence (see annex 14.3.26;  $r^2=0,51$ ) and acidity ( $r^2=0,48$ ).

Calcium is strong negatively correlated with the two independent Brix-measurements (see annex 14.3.3;  $r^2=0,81$  and  $r^2=0,99$ ).

We found negative correlations for the relations between calcium and firmness ( $r^2=0,76$ ), crispness (see annex 14.3.13;  $r^2=0,71$ ) and dry matter ( $r^2=0,71$ ) and slightly less negative correlations with sourness ( $r^2=0,49$ ), aroma ( $r^2=0,42$ ) and general appreciation ( $r^2=0,47$ ).

### **Relations between protein and free amino acids and other parameters**

The correlations for protein and for free amino acids are very similar. As expected protein is positively correlated with total nitrogen compounds ( $r^2=0,74$ ), free amino acids ( $r^2=0,99$ ), protein ratio ( $r^2=0,94$ ), and negative with Brix/N ( $r^2=0,88$ ).

Protein is positively correlated with a lot of parameters indicative of ripeness or shaded-growth like firmness ( $r^2=0,53$ ), starch ( $r^2=0,58$ ), Streif-index ( $r^2=0,75$ ), acid ( $r^2=0,72$ ), blush (inverse  $r^2=0,84$ ), Brix (inverse see annex 14.3.5;  $r^2=0,69$ ) and rawness ( $r^2=0,46$ ). Remarkable is the strong positive correlation with magnesium ( $r^2=0,94$ ). Memorable are the negative correlations with electrical resistance ( $r^2=0,84$ ), the yellow/blue ratio in spectral range luminescence (see annex 14.3.34 with  $r^2=0,67$ ) and phenolic compounds ( $r^2=0,58$ ).

Surprisingly, and only found for free amino acids is the strong positive correlation with potassium ( $r^2=0,87$ ).

### **Relations between the protein ratio and other parameters**

For the protein ratio, also expressed as % incorporated amino acids, positive correlations are found with many parameters indicative of ripening like blush ( $r^2=0,77$ ), Brix ( $r^2=0,68$ ), phenolic compounds (see annex 14.3.21 with

$r^2=0,58$ ), non-rawness ( $r^2=0,46$ ), firmness (inverse  $r^2=0,71$ ), starch (inverse  $r^2=0,59$ ), Streif-index (inverse  $r^2=0,79$ ), acid (inverse  $r^2=0,77$ ).

We also found positive correlations with the yellow/blue ratio in spectral range luminescence (see annex 14.3.40 with  $r^2=0,64$ ), electrical resistance ( $r^2=0,85$ ) and unexpectedly a weak negative correlation with self-maintenance ( $r^2=0,38$ ).

### Relations between vitamin C and other parameters

The correlations between vitamin C and other parameters are mainly determined by the difference between series B (fresh apples) and series D (older and less fresh apples) and are as expected.

### Relations between phenolic compounds and other parameters

Because astringent taste is caused by phenolic compounds we expected, and found a strong correlation between these two parameters (see annex 14.3.14;  $r^2=0,94$ ), although this is only based on 5 measurements of series A. We found positive correlations between phenolic compounds and protein ratio (see annex 14.3.21;  $r^2=0,58$ ) and with electrical resistance ( $r^2=0,38$ ). Negative correlations are found with proteins and free amino acids ( $r^2=0,58$ ;  $r^2=0,37$ ).

### Relations between disintegration and other parameters

Disintegration is positively correlated with nitrogen content (see annex 14.3.15 with  $r^2=0,72$ ) the same stands for phosphate, potassium and magnesium. The nitrogen content fits with the experience that apples with high nitrogen store shorter than with a lower nitrogen content.

Disintegration is negatively correlated with vitamin C (see annex 14.3.17 with  $r^2=0,59$ ). Vitamin C is known as an antioxidant and the figure denotes a minimum of 4 mg/100gram of vitamin C to protect against a disintegration of more than 7% dry matter loss in a week.

Disintegration is also negatively correlated with firmness (see annex 14.3.16 with  $r^2=0,60$ ), many sensory properties (crisp, juicy, sour, not raw, aromatic, general), and Bovis-value (see annex 14.3.18 with  $r^2=0,57$ ). These correlations are mainly due to the presence of two separated groups: series A+B+C on one side and the older, softer and easier to disintegrate apples from series D on the other side. The negative correlation with acidity (see annex 14.3.19 with  $r^2=0,54$ ) is caused by the group of apples from series B with an unexplained low pH. It is also imaginable that a low pH prevents disintegration.

We expected a negative correlation between disintegration and self-maintenance, a negative correlation between disintegration and dry matter (as earlier found and here only with  $r^2=0,39$ ) and a positive correlation between disintegration and Brix (sugar as an easy to assimilate substrate), but none of these correlations were found very clearly.

### Relations between self-maintenance and other parameters

Self-maintenance is positively correlated with calcium-content (see annex 14.3.20 with  $r^2=0,59$ ). Apples with high calcium-content are known to be well structured and long storable and self-maintenance fits with these properties. There is a weak positive correlation with Brix (PPO,  $r^2=0,33$ ) and some weak negative correlations with protein ratio ( $r^2=0,38$ ) and crispness ( $r^2=0,43$ ).

We expected a correlation between self-maintenance and disintegration, vitamin C or phenolic compounds, but none of these correlations were found very clearly.

### Relations between sensory properties and other parameters

The **general appreciation** has a positive correlation with firmness (see annex 14.3.6 with  $r^2=0,68$  although again based on the presence of two separated groups: series A+B+C versus series D), with acid (see annex 14.3.7 with  $r^2=0,72$ ) and a strong positive correlation with Bovis-value (see annex 14.3.9 with  $r^2=0,87$ ). We found a weaker positive correlation with dry matter ( $r^2=0,20$ ), acidity ( $r^2=0,27$ ) and DL-emission at  $t=3$  in delayed luminescence test ( $r^2=0,31$ ). General appreciation has a moderate negative correlation with calcium ( $r^2=0,47$ ), dry matter ( $r^2=0,42$ ) and disintegration ( $r^2=0,40$ ). Remarkable is that no correlation was found with Brix.

Within the mutual sensory parameter we see a group consisting of crispness, juiciness and non-mealiness that has a strong positive correlation with general appreciation ( $r^2=0,72-0,99$ ). Sourness ( $r^2=0,54$ ) and aroma ( $r^2=0,42$ ) are only moderate positively correlated whereas sweetness is weakly correlated ( $r^2=0,21$ ).

In §7.6 we discussed an attempt to establish a formula that predicts taste based on some easy to measure technical parameters. Based on this small amount of data the best technical parameter is firmness. Of all

parameters Bovis-value has de best prediction-value for taste.

Looking at the various aspects of taste we can mention some remarkable correlations.

With an **astringent skin taste** many parameters of unripe-ness are correlated: starch ( $r^2=0,62$ ), Streif ( $r^2=0,71$ ), sweetness (inverse  $r^2=0,32$ ) and rawness ( $r^2=0,31$ ). With a non-astringent skin taste we find correlations with parameters related to sun-exposure like blush ( $r^2=0,52$ ), magnesium ( $r^2=0,90$ ) and electrical resistance ( $r^2=0,33$ ). This is a new phenomenon for us which is found in series other than the light series (series C is tasted without skin because of the appreciation the skin colour would on the tasters).

As expected, astringent skin is positively correlated with phenolic compounds (see annex 14.3.14 with  $r^2=0,94$ ) as the astringent components have a phenolic origin.

**Crispness** is correlated with many parameters. As expected a strong positive correlation with firmness (annex 14.3.12 with  $r^2=0,80$ ) was found. Crispness is an important addition for general appreciation ( $r^2=0,72$ ). Narrowly related with juiciness ( $r^2=0,85$ ), acid ( $r^2=0,53$ ), pH (inverse  $r^2=0,56$ ) and sourness ( $r^2=0,70$ ). Crispness is correlated with aromatic taste ( $r^2=0,60$ ), vitamin C ( $r^2=0,48$ ), dry matter ( $r^2=0,34$ ), Brix/N ( $r^2=0,37$ ), DL-emission at t=3 in delayed luminescence test ( $r^2=0,27$ ), yellow/blue ratio in spectral range luminescence ( $r^2=0,59$ ) and Bovis-value ( $r^2=0,70$ ).

Remarkable is the strong negative correlation with calcium (see annex 14.3.13 with  $r^2=0,71$ ); here we recognise the polarity between storage potential (calcium) and taste (crisp).

**Juiciness** is strongly correlated with crispness ( $r^2=0,85$ ), so many correlations discussed for crispness are also found for juiciness. Remarkable is the negative and unexpected correlation with disintegration ( $r^2=0,62$ ).

**Mealiness** is only measured in series D. The high correlations found in annex 14.1 are only based on 4 measurements and are left out in the discussion.

**Rawness** is an aspect of taste and as expected correlated with unripe-ness parameters: firmness ( $r^2=0,44$ ), crispness ( $r^2=0,20$ ), starch ( $r^2=0,42$ ), acid ( $r^2=0,63$ ), pH (inverse  $r^2=0,35$ ), sourness ( $r^2=0,30$ ), Streif-index ( $r^2=0,43$ ), dry matter ( $r^2=0,30$ ), blush (inverse  $r^2=0,28$ ), protein ( $r^2=0,46$ ), amino acids ( $r^2=0,45$ ), protein ratio (inverse  $r^2=0,46$ ), astringent skin ( $r^2=0,31$ ), vitamin C (inverse  $r^2=0,60$ ), DL-emission at t=3 in delayed luminescence test, yellow/blue ratio in spectral range luminescence (inverse  $r^2=0,59$ ) and electrical resistance (inverse  $r^2=0,57$ ).

**Sourness** in taste is well correlated with acid (see annex 14.3.11 with  $r^2=0,57$ ) and acidity (inverse  $r^2=0,63$ ) as we expected. Additionally, positive correlations were found with many freshness parameters like firmness ( $r^2=0,80$ ), crispness ( $r^2=0,70$ ), dry matter (inverse  $r^2=0,30$ ), vitamin C ( $r^2=0,43$ ), general appreciation ( $r^2=0,54$ ), DL-emission at t=3 in delayed luminescence test ( $r^2=0,54$ ) and Bovis-value ( $r^2=0,59$ ).

We found negative correlations with calcium ( $r^2=0,49$ ), disintegration ( $r^2=0,45$ ), weaker negative correlations with electrical resistance ( $r^2=0,28$ ), differentiation and integration in crystallisation images ( $r^2=0,29$  and  $r^2=0,26$ ).

**Sweetness** is only correlated with a few other parameters. We found a weak positive correlation with Brix (see annex 14.3.10 with  $r^2=0,26$ ) although we expected this to be stronger. A weak positive correlation was found with general appreciation ( $r^2=0,21$ ), yellow/blue ratio in spectral range luminescence ( $r^2=0,38$ ) and weakly negative with astringent ( $r^2=0,32$ ).

**Aromatic taste** showed moderate positive correlations with crispness ( $r^2=0,60$ ), juiciness ( $r^2=0,53$ ), sourness ( $r^2=0,48$ ), general appreciation ( $r^2=0,42$ ), Bovis-value ( $r^2=0,48$ ) and weak positive correlations with firmness ( $r^2=0,35$ ), acidity (inverse pH  $r^2=0,21$ ), Brix ( $r^2=0,21$ ), acid ( $r^2=0,20$ ), ripeness-index (inverse Streif  $r^2=0,26$ ), dry matter ( $r^2=0,23$  and  $0,40$ ). It seems the tasters judge the sweet/sour ratio often as 'aroma'. Negative correlations were found with calcium ( $r^2=0,42$ ) and disintegration ( $r^2=0,47$ ).

### Relations between crystallisations and other parameters

In the table of correlations (annex 14.1) only the empathic judgements are included, the impression of vitality, structure and integration. We found no correlations between what is experienced in these images as **vitality** and any other parameter.

With the impression of **differentiation** we found moderate correlations: negative correlations with unripe-ness

parameters like firmness ( $r^2=0,20$ ), malic acid ( $r^2=0,29$ ) and sourness ( $r^2=0,29$ ) as we expected. With the delayed luminescence test we found a negative correlation with the DL-emission at  $t=3$  ( $r^2=0,30$ ; annex 14.3.41 which we hadn't expected) and with the lagtime ( $r^2=0,30$ ; annex 14.3.42). The last correlation is as expected as lagtime is said to be an inverse differentiation parameter.

We found a negative correlation with redoxpotential (Eh:  $r^2=0,22$ ) and P-value ( $r^2=0,33$ ; annex 14.3.43). This is as expected as the P-value is said to be an inverse ordering parameter.

We found a moderate positive correlation with the impression of integration in the same images ( $r^2=0,35$ ; annex 14.3.44) which fits with the hypothesis that a certain amount of differentiation is a prerequisite of integration.

With the impression of **integration** we found a negative correlation with aromatic taste ( $r^2=0,34$ ; annex 14.3.45) which we expected to be positive because we assume aroma to be an integration-parameter. With sourness ( $r^2=0,26$ ) and DL-emission at  $t=3$  in the delayed luminescence test a moderate negative correlation was found. This can be explained by the fact that both parameters are thought to belong to the growth processes and when growth is too severe it inhibits the integration. We found a negative correlation with redoxpotential (Eh:  $r^2=0,31$ ) and P-value ( $r^2=0,33$ ; annex 14.3.47). This is as expected as the P-value is said to be an inverse ordering parameter. And we repeat the moderate positive correlation between the impression of differentiation and integration in the same images ( $r^2=0,35$ ; annex 14.3.44 as expected). Unexpectedly no correlation with the Bovis-value was found (see annex 14.3.48).

### **Relations between delayed luminescence and other parameters**

Emission at 3 seconds is correlated with many parameters. First a positive correlation with the group of parameters indicative of unripe-ness was found: firmness ( $r^2=0,51$ ), starch ( $r^2=0,49$ ); Streif-index (see annex 14.3.22;  $r^2=0,73$ ); acid ( $r^2=0,48$ ); crispness ( $r^2=0,27$ ); non-mealiness ( $r^2=0,30$ ), rawness ( $r^2=0,27$ ), pH (inverse  $r^2=0,44$ ), blush (inverse  $r^2=0,40$ ) and dry matter (inverse  $r^2=0,66$ ).

Positive correlations were found with aspects we usually find in more ripened fruit like sweetness ( $r^2=0,47$ ), general appreciation ( $r^2=0,31$ ), P-value ( $r^2=0,28$ ) and Bovis ( $r^2=0,26$ ). We found unexpected negative correlations with electrical resistance ( $r^2=0,23$ ), Brix (Brix-2;  $r^2=0,55$ ), differentiation and integration in crystallisation (resp.  $r^2=0,30$  and  $r^2=0,28$ ).

Emission at 3 seconds is positively correlated with the other delayed luminescence parameters like hyperbolicity (see annex 14.3.23: 'inverse DL-lagtime'  $r^2=0,56$ ) and yellow/blue ratio in spectral range luminescence (see annex 14.3.12;  $r^2=0,68$ ).

The hyperbolicity of the delayed luminescence (inverse DL-lagtime) is negatively correlated with only a few parameters: calcium (see annex 14.3.26;  $r^2=0,51$ ), redoxpotential (Eh:  $r^2=0,32$ ), P-value ( $r^2=0,28$ ), emission at 3 seconds (DL  $t=3$ ,  $r^2=0,56$ ), dry matter (see annex 14.3.25;  $r^2=0,68$ ) and differentiation and integration in crystallisation (resp.  $r^2=0,30$  and  $r^2=0,45$ ).

### **Relations between spectral range luminescence and other parameters:**

The yellow/blue ratio in spectral range luminescence is clearly correlated with many ripeness and/or sun parameters: we found negative correlations with firmness (see annex 14.3.28;  $r^2=0,57$ ), starch (see annex 14.3.29;  $r^2=0,95$ ), Streif-index (see annex 14.3.27;  $r^2=0,89$ ), acid (see annex 14.3.31;  $r^2=0,89$ ), rawness ( $r^2=0,59$ ), minerals N,P,K and Mg ( $r^2=0,71-0,90$ ; see annex 14.3.32 and 14.3.39), proteins (see annex 14.3.34;  $r^2=0,67$ ), free amino acids (see annex 14.3.33;  $r^2=0,64$ ) and positive correlations with blush ( $r^2=0,75$ ), Brix (see annex 14.3.30;  $r^2=0,69$ ), sweetness ( $r^2=0,38$ ).

Positive correlations are found with Brix/N-ratio ( $r^2=0,86$ ), protein ratio (see annex 14.3.40;  $r^2=0,63$ ), crispness (see annex 14.3.35;  $r^2=0,59$ ), DL-emission at  $t=3$  (see annex 14.3.24;  $r^2=0,68$ ), electrical resistance (see annex 14.3.36;  $r^2=0,63$ ) and a negative correlation with disintegration ( $r^2=0,45$ ). Only a weak positive correlation is found with Bovis-value (see annex 14.3.37;  $r^2=0,23$ ).

### **Relations between acidity (pH) and other parameters**

Acidity (pH) is correlated with many parameters that are indicative of with ripening and ageing. In these processes the pH increases (=less acid). So as expected acidity is negatively correlated with firmness ( $r^2=0,68$ ), acid ( $r^2=0,53$ ), vitamin C ( $r^2=0,59$ ), sourness ( $r^2=0,63$ ), crispness ( $r^2=0,56$ ), and juiciness ( $r^2=0,48$ ), rawness ( $r^2=0,35$ ), DL-emission at  $t=3$  ( $r^2=0,44$ ). Weak negative correlations were found with general appreciation ( $r^2=0,27$ ), aroma ( $r^2=0,21$ ) and Bovis-value ( $r^2=0,38$ ) and positive correlations with calcium ( $r^2=0,48$ ), nitrogen ( $r^2=0,77$ ), magnesium ( $r^2=0,75$ ), disintegration ( $r^2=0,54$ ), Brix-2 ( $r^2=0,29$ ) and electrical resistance ( $r^2=0,23$ ). We cannot



explain the positive correlation with the minerals and the electrical resistance. The others fit into the quality concept, see below.

#### **Relations between redoxpotential (Eh) and other parameters:**

The redoxpotential (Eh) is correlated with only a few parameters: weak positively with DL lagtime ( $r^2=0,32$ ) and weak negatively with differentiation and integration in crystallisations ( $r^2=0,22$  and  $0,31$ ). Because of the P-value formula a high correlation with the P-value is expected ( $r^2=0,92$ , see annex 14.3.43).

#### **Relations between electrical resistance (R) and other parameters:**

Resistance (R) is negatively correlated with parameters like firmness ( $r^2=0,50$ ), starch ( $r^2=0,55$ ), acid ( $r^2=0,74$ ), Streif-index ( $r^2=0,69$ ), vitamin C ( $r^2=0,66$ ), sourness ( $r^2=0,28$ ), rawness ( $r^2=0,57$ ), astringent taste ( $r^2=0,33$ ), phosphate ( $r^2=0,65$ ), protein and free amino acids ( $r^2=0,84$  and  $0,85$ ), DL-emission at t=3 ( $r^2=0,23$ ). Positively correlated with blush ( $r^2=0,60$ ), potassium ( $r^2=0,67$ ), protein ratio ( $r^2=0,85$ ), yellow/blue ratio in spectral range luminescence ( $r^2=0,63$ ; see annex 14.3.36), and weakly positive with dry matter ( $r^2=0,25$ ), phenolic compounds ( $r^2=0,38$ ), pH ( $r^2=0,23$ ).

#### **Relations between P-value and other parameters:**

The P-value is very strongly correlated with the redoxpotential (Eh:  $r^2=0,92$ ; see annex 14.3.43). This is what we expected because the redoxpotential plays a key role in the P-value-formula.

P-value is positively correlated with blush ( $r^2=0,23$ ), nitrogen and phosphate ( $r^2=0,39$  and  $0,51$ ), DL-emission at t=3 ( $r^2=0,28$ ), and DL-lagtime ( $r^2=0,28$ ). Surprisingly the P-value is negatively correlated with differentiation and integration in crystallisations ( $r^2=0,32$  and  $0,33$ ; see annex 14.3.43 and 14.3.47).

#### **Relations between Bovis-values and other parameters**

Bovis-value is strong positively correlated with general appreciation ( $r^2=0,88$ ; annex 14.3.9 and with many aspects of taste: crispness, juiciness, sourness and aroma) and with firmness ( $r^2=0,70$ ). Weaker positive correlations are found with acid ( $r^2=0,47$ ), pH (inverse  $0,38$ ), dry matter ( $r^2=0,30$ ), vitamin C ( $r^2=0,34$ ), emission at t=3 in delayed luminescence test ( $r^2=0,26$ ) and yellow/blue ratio in spectral range luminescence ( $r^2=0,23$ ; annex 14.3.37). Bovis is negatively correlated with disintegration ( $r^2=0,58$ ; annex 14.3.18). We found no correlation with crystallisations (see annex 14.3.48).

# 19 Characteristics of the varying factors

*When we consider the patterns in the various figures for each series we find clusters of parameters with a similar result. We could distinguish a steady increase, a decrease, constant values, or an optimum and come with a qualitative description, see annex 15. In this chapter we describe each series from this point of view and a \* means a significant relationship, inaccurate parameters are left out (SDT, vitamin C). In the discussion the concept of vital quality including vitality, structure and coherence is already used, see §1 and §20 for more detailed introduction.*

## 19.1 Ripeness: unripe, overripe

Later picking (and shorter storage) results in:

- a continuous increase of size\*, irregular shape\*, redness\*, fat and glossy skin, Brix-1 (slow), crispness, sweetness, ripe flavour, hyperbolic character of delayed luminescence\* (Meluna: inverse lagtime), one-centredness, openness to periphery and coherence in crystallisation pattern.
- at first constant and at the 4th and 5th picking date an increase of Brix-2, dry matter, pH and vitamin C.
- a continuous decrease of starch\*, Streif-index, malic acid, sourness, astringent skin taste, general appreciation, phenolic compounds, emission at 3 sec. of delayed luminescence\*, subdividing of needles and density in crystallisation-pattern.
- an optimum at the 3<sup>rd</sup> picking date in thin skin, aroma and TQ-index.
- an optimum at the fourth picking date in: juiciness, low P-value and low redox-potential and high Bovis-value\*.
- an optimum at the third and fourth picking date in free amino-acids and a minimum in protein ratio.
- a broader spectrum in whole fruit and a smaller spectrum for seeds in spectral range luminescence (Kwalis: %yellow/R40blue).
- no difference in firmness, protein, self-maintenance.
- In an earlier study with Jonagold apples, ripeness was related with contents of sugar, malic acid, taste, self-maintenance and Bovis-value (Bloksma, de Jonge and Brands 1996) and with phenolic compounds and basic parameters (Awad 2001).

## Discussion

### Ripening in the store versus ripening at the tree

We found no difference in firmness and only a slow conversion from starch into sugar, normally known as strong ripeness indicators. This is caused by compensation of a later picking by a longer storage of the earlier pickings. As we know in storage this conversion from starch into sugar goes on and firmness decreases. So this series is more or less indicative of ripened-in-the-store versus ripened-at-the-tree!

For growers who want to reach good quality, it is interesting to see which aspects of the ripening process will continue in storage and for which aspects ripening at the tree is a must. Here we see that the conversion from starch into sugar and the decrease of firmness continue perfectly well in storage. These two parameters are important aspects for the conventional Streif-index for ripeness (loss of vitality). But for quality parameters like skin colour, flavour, aroma, luminescence, P-value, crystallisations and Bovis (structure and coherence), the ripening process on the tree is important.

### Ripening series without the expected extremes

This series didn't reflect the broad range between unripe and overripe as was theoretical possible because unripe picked apples ripened during storage more than we expected and the last picking date was too early for real overripeness.

Because of this prolonged ripening and/or long storage the quality of series A is much lower and/or riper than in series B and C. This is illustrated in firmness, malic acid, Streif-index, delayed luminescence, electrical resistance and P-value.

### **Ripening can be seen as a process of transition from solid into soluble and volatile compounds**

Looking at the ripening series the ripening process is illustrated quite nicely. The ripening process can be seen as a successive transition of a firm, sour, astringent fruit into an optimal stage for human consumption in taste, before disintegrating and releasing seeds. The early ripening started with transition of solid substances like starch, acid and phenolic compounds into soluble and volatile substances like sugar, juice and aromatic-compounds. Around the third and fourth picking date some other compound levels started to decrease, such as protein, amino-acids and aromatic-compounds whereas the pH suddenly increased. The transition process can also be seen as the increasing 'openness towards the periphery' in the crystallisations, the weaker luminescence after 3 seconds, and the better hyperbolic and fruit-like character in luminescence. The fifth picking date is already over-ripe for human consumption, as is seen in a disbalance between sweetness and sourness (sweet without sour tastes dull), loss of aroma, less electrical resistance, loss of vitality in crystallisations and lower Bovis-values.

### **Ripening can be seen as a transition from vitality into structure and coherence**

The fruit grower expected the optimal ripeness for direct consumption around the third and fourth picking date. And indeed, around these picking dates we recognised some aspects of what is called the 'climacterium' in literature (the physiological stage in fruit around real ripeness with a short and high ethylene-ripening-hormone production and a high metabolic activity). So, useful parameters for the optimal ripeness (here optimal taste) seem to be: sweet/sour balanced taste, aromatic, high yellow/blue ratio in spectral range luminescence, open and periphery orientated crystallisation image, low P-value, high Bovis-value.

Parameters indicating vitality (high emission in DL at 3 sec.) decrease and parameters indicating structure and coherence (crystallisation, broad spectrum and hyperbolic character in delayed luminescence) increase without an optimum in the reach of this series. The transition from vitality into structure is clearly shown in annex 11.2 in the left-under figure.

## **19.2 Bearing: from underbearing to overbearing**

Increased bearing results in:

- a continuous increase of: calcium, delayed luminescence\* (Meluna: emission at 3 sec), electrical resistance and sharpness in needle-structure in crystallisations.
- a continuous decrease of: twig growth, Brix, sweetness, firmness, malic acid, sourness, TQ-index, dry matter\*, minerals N, P, K and hyperbolic character\* of luminescence (Meluna: inverse lag-time).
- some (not significant) decrease of: crispness, aroma, astringent skin and general appreciation.
- a constant value with a minimum quality at bearing 2 for: redoxpotential and P-value (this sub-sample might have been damaged).
- an optimum at bearing 3 for: apple typicality and balance between luxurious growth and emptiness in crystallisation.
- a slight optimum at bearing 4 for: sensory aspects (or maybe this sub-sample was riper?)
- no difference in: starch, ripeness (Streif-index), skin colour\*, thin skin, pH, Bovis-value\*.
- the standardisation for fruit size did not succeed completely; B1 was slightly bigger and B5 slightly smaller than the average of this series. The standardisation was good for ripeness and sun-exposed blush.

### **Discussion**

Amongst fruit growers it is known that fruit of high bearing trees will have a sub optimal taste but store better (here, we did not check the storage potential again). This is related to the decrease in the K/Ca-ratio, as is shown in this series. Maybe electrical resistance and sharpness of the needle structure indicate the same (this is to be proven in a later project)?

An increased bearing and a decreased number of leaves per tree means that each fruit has to be fed by fewer leaves (see annex 1). A partial compensation is found in the phenomenon that the photosynthetic activity per leaf increases with a higher demand (also see § 2.1.3). In this bearing series we see a decrease in all compounds related to the assimilation such as dry matter, sugar, acid and aroma and a decrease in minerals that have to be distributed to more fruit. These relations are commonly found in bearing research.

The underbearing trees have longer twigs on all sides, and gave the impression to be weaker, lazier, luxurious and showed a more vegetative growth. The same is seen for their fruit: a powerless slack crystallisation picture with a vegetative character and a weak luminescence. The luxurious aspect of these trees is expressed by the few but very nice sweet-sour-balanced and tasteful apples.

Fruit from overbearing trees showed poor crystallisation pictures that do not reach the periphery. This fruit is not very well filled with substances like sugar, acid, minerals and have a poor taste. But can we consider the higher metabolic energy, expressed as a higher delayed luminescence, to be the result of the increased photosynthetic activity of higher bearing trees?

In this orchard we expected bearings 4 and 5 to be overbearing with respect to flower bud formation for the next season. However, in 2001 it appeared that only the 5th bearing was actually overbearing, which is possibly due to the good season in 2000.

We also expected bearing 5 (and maybe also bearing 4) to be overbearing with respect to fruit quality. In this series we found only for the highest bearing a decrease in sweetness, crispness, aroma and astringent skin taste. However, we found hardly any decrease in the overall appreciation. This is hard to understand. In many parameters in B5 we recognised the preserving tendency as is described in §20.1 for fruit with growth shortage and severe differentiation.

In this series the expected optimum in fruit quality around bearing 4 is not demonstrated very clearly. Only in the crystallisation images we recognise an optimum between the two mentioned poles. The optimal bearing for the tree is not necessarily the optimal bearing for fruit taste, nor for optimal storage potential. This is seen in the fact that different parameters have their optimum at different fruit bearing levels. The season 2000 had such excellent weather (see §3.2) for apple growth that the highest bearing with 130 fruit per tree was not the extreme bearing that we had hoped for.

### **Increased bearing can be seen as a change in emphasis from vitality to structure**

In apples from the lowest bearing trees we see all vitality parameters at a high level. Twig vigour is high and so is the vitality of the fruit (sweet, juicy, crispy, minerals, dry matter, etc). In apples from the highest bearing trees we recognise more structure in: high calcium levels, high electrical resistance, sharp formed crystallisations, clear and contoured capillary pictures, increased hyperbolicity in delayed luminescence and the general knowledge that apples from high bearing trees will store longer. The only phenomenon we didn't find in line with an increased bearing, is the increase of the emission at  $t=3$  in delayed luminescence (thought to be a vitality parameter).

## **19.3 Light: exposure to sun, shadow**

With an increased exposure to sunlight we see:

- a slow decrease in starch\* and malic acid and an increase in sugar, so slightly less ripeness in the shadow.
- a continuous increase of red blush\*, yellow ground colour\*, dry matter\*, phenolic compounds, protein ratio, hyperbolicity of luminescence\* (inverse lagtime without preps), broad spectrum in spectral range luminescence\* for fruit, electrical resistance, Bovis\*-value and more transparency, coherence, and one-centredness in crystallisations.
- a continuous decrease of the minerals N, P, K, protein, amino-acid, emission at  $t=3$  in delayed luminescence\* (without preps), redoxpotential, P-value and dark vertical flags in the capillary pictures.
- an optimum at half shadow for the delayed luminescence (DL: both parameters with preps).
- no difference in firmness\*, general appreciation\*, sweetness\*, sourness\*, crispness\*, juiciness\*, aroma\*, calcium, pH.

### **Exposure to sun stimulates differentiation resulting in a more structured and coherent product**

In this series we must consider the influence of slightly earlier ripening of apples grown in the sun. This means that the shaded-grown apples can improve a little by picking them a couple of days later. But when we compare the figures of the ripening series, it is clear that a slightly later picking date will not compensate the big differences due to sun exposure.

As expected we found more red blush, more yellow ground, more phenolic compounds, more hyperbolicity in luminescence (DL inverse lagtime), all results of a stronger differentiation due to sun exposure.

Besides more differentiation, the sun-grown apples are also more integrated and more apple-like (broader

spectrum in luminescence, higher protein ratio, higher Bovis-value and more coherence in crystallisations). Surprisingly, we did not find a better taste and aroma in the sun-grown fruit. Remarkable is the enormous influence of shadow, which is expressed in high nitrogen, protein and free amino-acid levels. Whereas the low protein content in sunny apples is more incorporated (high protein ratio) into the apple. PPO (annual report 1998) reports a comparable phenomenon; higher nitrogen contents for the inner grown fruit compared to the outer grown fruit in the same tree, for this they have no physiological explanation. Similar to nitrogen, the potassium and phosphor contents are also much lower in the sun, whereas the calcium level remains constant. Consequently, the K/Ca-ratio of sun-exposed apples is much better for storage than the shadow-grown apples and also here sunny fruit can be seen as better structured. This is consistent with the experience of growers that well exposed apples will store better. Because of the easy transportable assimilates there is no big difference in sugar between sun and shaded-grown apples, so the C/N-ratio is much higher in the sun. The higher dry matter content and sugar levels are normal phenomena because of an increased evaporation in the sun. We see a similarity in properties of apples from both the shaded-grown and the more fertilised trees.

## 19.4 Bd-field preparations

Addition of preparations results in quite complicated and not easy to understand effects:

- higher for all light-exposure levels are: the levels of phenolic compounds, the transparency of the crystallisations and with more light the crystallisations have slender and quite straight needles with few side needles.
- higher, but only for the sun-exposed fruit are the emission at t-3 in delayed luminescence\*, Ca-content, protein ratio, acidity, Bovis-value\*.
- higher, but only for the shaded-grown fruit is the P-value.
- higher, for the sun-exposed fruit; lower for the shaded-grown fruit, are electrical resistance and broad spectrum in spectral range luminescence\*.
- lower at all light-levels is dry matter (1x).
- lower, only for the sun-exposed fruit are protein, amino acid, K/Ca and hyperbolicity of luminescence\* (DL inverse lagtime).
- lower, for the sun-exposed; higher for the shaded-grown fruit, but not very extreme are Brix (2x) and dry matter (1x).
- no difference in blush\*, firmness\*, starch, malic acid, ripeness (Streif-index), N-, P-, K-content, capillary pictures, general appreciation\* and all sensory parameters\*.

### Discussion

When interpreting these complex effects, we should keep in mind that this series has no repetitions in the field. The slightly increased growth and slightly lower bearing of the trees without preparations, as is described in §2.3, can be a result of not using preparations or simply be due to natural occurring variations. Nevertheless this series is well standardised for ripeness and size and thus can be compared with the other series.

While the preparations don't effect in most conventional parameters, including taste, it is remarkable to see some influence in the experimental parameters like delayed luminescence, protein ratio, phenolic compounds, crystallisations, P-value and Bovis-value.

### Preparations where expected to improve differentiation and integration processes and this is partly found

According to our idea and former experience we expected more differentiation and integration as a result of Bd-field preparation usage (Lammerts van Bueren & Beekman 1995; Bisterbosch 1994).

Some aspects of both processes can be recognised. From the integration-hypothesis we can easily imagine that a higher water tension (less dry matter), higher protein ratio, broader range in spectral luminescence and higher Bovis-value are integrational aspects.

Differentiation phenomena are found in higher phenolic compound and calcium levels, lower K/Ca ratio and lower free amino acids. All are known to improve the storage potential. Other aspects of differentiation, like taste, make no difference (or only a little, non-significant difference).

We also see some aspects that will have an inverse effect on differentiation and integration processes like a decreased hyperbolicity in delayed luminescence and a higher P-value.

### **Preparations where expected to level differences but this series does not support this idea**

According to our idea and former experience we expected that Bd-field preparations would bring some 'sunny'-aspects in shaded-grown fruit. That was the idea behind combining both factors in one series. We only find this levelling-hypothesis in Brix and ones in dry matter. Whereas in other parameters no levelling, or rather the inverse (K, N and protein) is found.

For protein, free amino acids and spectral range luminescence it seems the preparations mainly have an influence in the sun and not in the shadow, which is contrary to the hypothesis. Or maybe preparations need sun for some aspects of their action (protein-metabolism, spectral range luminescence) and not for other aspects (like forming phenols). All these 3 parameters are tested on one sub-sample in the Kwalis-laboratory. Maybe the C6-sample (shadow, no preps) was of relative good quality and not representative, or maybe the hypothesis is wrong.

## **19.5 Shelf life**

### **After 3 months of cold storage (D1 to D2) we see:**

- a sudden decrease in firmness\*, Brix, malic acid, dry matter, general appreciation\*, crispness\*, juiciness\*, sourness\*, aroma\*, Bovis\*.
- a sudden increase in disintegration and pH.
- a slow decrease (not significant) in vitamin C, delayed luminescence (Meluna t=3).
- no difference in sweetness\*, self-maintenance, P-value, redox-potential, electrical resistance.
- in crystallisations a shock-effect (D2)

### **With longer shelf life (D2→D5) we see:**

- a decrease in firmness\*, general appreciation\*, vitamin C, emission at t=3 in delayed luminescence\*, Bovis-value\*.
- a slow decrease (not significant) in malic acid, self-maintenance, crispness, juiciness, aroma.
- a slow increase in dry matter, P-value, pH, redox-potential.
- an optimum at D3 in pH, electrical resistance, crystallisation, hyperbolicity of luminescence\* (DL lagtime).
- no difference in Brix, sweetness, sourness.
- in crystallisation a recovery from the shock (D3) growing out to an openness and retreating towards the periphery with long thin needles.

## **Discussion**

### **Shelf life as a loss of vitality, structure and coherence**

The dates are conform what we expected in storage and shelf life by literature. From other quality research it is known that apples are able to remobilize sugar from tissue compounds to keep a steady sugar level for a very long period of time, also when firmness and sourness already decrease. This is also found here.

From experience it is known that apples need some days to revive from cold storage and to re-release aromatic flavour again. It is surprising to find the optimal revival time at D3, which is expressed very well in many experimental parameters like pH, electrical resistance, crystallisation and hyperbolicity of luminescence.

Because of the expected decrease of all three aspects of vital quality in this series we will not be able to distinguish vitality, structure or coherence parameters.

### **Shelf life until twelve days was not long enough to see real ageing and disintegration**

The overall good quality of the apples in this orchard prevented the real extremes (fresh to aged) to occur after 12 days of shelf life. Some vitality parameters remain at a good level (vitality in crystallisations) and some parameters indicate some ageing in this period (general appreciation, firmness, vitamin C, self-maintenance, capillary pictures, DL-emission at t=3, P-value, Bovis-value).

## 20 Reflection on research questions

*In §1.1 we introduced a hypothetical concept for vital quality and its grounds. After the experimental series we evaluated the various aspects of the quality concept with the measured parameters. In §20.1 we summarise the three aspects of the vital quality both as processes and as properties and in §20.2 we describe our first ideas about the relationship between parameters and vital quality aspects.*

### 20.1 A More detailed description of the three aspects of vital quality

Based on the outlines of the series in §19, we are now able to describe in more detail what we consider to be growth, differentiation and integration processes in apples and what the corresponding properties are. Dividing the reality into two basic life processes is only a mental tool to get a better grip on the meaning of the balance. When a living organism grows, it is always the result of the co-operation of both processes, sometimes with the emphasis on growth processes (vegetative phase) sometimes on differentiation processes (generative phase). Besides distinguishing the two basic life processes, as is performed in this first report, a more detailed qualification in specific processes is possible (Bockemühl 1972 and 1995, and many others).

#### 20.1.1 Growth processes

Growth is defined as the expanding process of filling space with basic unformed substance, e.g. cell division, twig growth, root growth and trunk growth, unfolding of leaves and flowers in a cluster and fruiting. The basic cell substances are formed by photosynthesis (the primary production system of sugar) and by biosynthesis of starch, malic acid, amino acids and the more complex cellulose and protein. Although the photosynthesis integrates the uptake of carbon dioxide, water and minerals (typical growth processes) using energy and warmth of sunlight (differentiation aspects) we find the emphasis lays on growth.

The phytohormones involved in growth are auxins, gibberellins and cytokinins and in human physiology these growth processes are related to anabolic processes.

Fruit growers stimulate growth processes, for example by watering, fertilising, defruiting and pruning in a growth-stimulating way. On the other side they know how to reduce growth by root pruning, pruning in a growth-reducing way, trunk notching and controlled water stress.

#### Vitality is a property resulting from growth

A vital crop has a lot of green mass and a good yield. Vital fruit is well-sized, is firm, crispy and juicy due to a high tension. Vital seeds are eager to germinate. The product has a high amount of starch, acid and sugar and the proportion depends on the ripening stage. The acidity (pH) of fruit is low. Fruit is amino acid and protein rich. A vital growing product is still metabolising, busy building and dissolving, many easy-transportable substances are found (sucrose, amino acids) and the delayed luminescence immediately after excitation is high. In the crystallisations we find a well-filled plate with dense needles.

#### 20.1.2 Differentiation processes

Differentiation is defined as the process of refinement or getting a specialised form and function like differentiation in cells, leaf serration, flower bud formation and pollination, forming secondary metabolites during the ripening process: colouring the fruit skin and autumn leaves, flavour and compounds for resistance. (We are not sure whether we consider the transport of calcium by hormonal processes to the fruit flesh as a differentiation process too).

The phytohormones involved in differentiation are ethylene and abscisic acid and in human physiology differentiation are related to catabolic processes.

Fruit growers stimulate differentiation processes, for example by choosing a light and warm habitat, pruning the tree to an open architecture to expose to light and sun as much as possible, reducing heavy growth, bringing in beehives for pollination and binding down young twigs.

## Structure is a property resulting from differentiation

The product has a detailed form and colour. Twigs have flower buds for the next year. Autumn leaves are coloured, fruit have a yellow ground colour and a blush and are aromatic with a waxy, glossy skin. Secondary metabolites for resistance like phenolic compounds and vitamins are found in the fruit, the tree bark contains tannin. The fruit contains a lot of seeds and has a high calcium/potassium-ratio, the tissue is ordered with optimal texture and has a high storage potential. After excitation in the delayed luminescence test, the tissue is capable to store light energy and release it slowly as biophotons, which produce a clear hyperbolic curve (small lagtime). The crystallisation-image shows one-centred, well formed and open angled side-branches.

### 20.1.3 Integration processes

Normally, growth processes should be at a moderate level to allow differentiation processes to occur in a proper way. Looking at plants with stress, emergency flowering can be seen as an example of differentiation without enough growth. Fruit growers know that twigs with canker in the wood, mildew or fruit with rosy apple aphid will bear a lot of poor quality fruit of poor quality. These are examples of differentiation without enough growth, resulting in a dry preserving process. Examples of the other extreme are the very growthfull twigs with pests and diseases like scab and green aphid.

These examples show different ratios between growth and differentiation. But, realise there is a difference between 'balance' and 'integration'. Balance is the ratio between growth and differentiation, both processes may occur alongside each other. Integration is the co-operation between growth and differentiation. When growth and differentiation processes intermingle and co-operate at the same time in rhythm we are truly talking about integration. We expect some balance in the ratio between growth and differentiation as a prerequisite for the integration process. This is no static balance, but a dynamic alternation of polar conditions.

The typical balance between the two processes depends on the context. Firstly, we distinguish a 'nature context', like species and variety choice and developing stage, and secondly a 'nurture context' like soil, climate and orchard management. The integration process helps a crop or a fruit to self-regulate after fruit growers' interference. The contrary is compartmentalisation.

Fruit growers exhibited the knowledge of the importance of balance between growth and differentiation for fruit quality. In practise they try to obtain this balance mainly by stimulating or slowing down the growth process. Bio-dynamic growers expect the bio-dynamic field preparations to play a role in the integration process, Although this has not been proven yet (Lammerts van Bueren & Beekman 1995; Bisterbosch 1994). Managing the integration process is still the most elusive process. Also during breeding and selection the germ is laid for the possibility of the integration process.

## Coherence is a property resulting from integration

The final product shows combinations of the polar properties. Apples have a good taste and a high storage potential. The product should be tasty, juicy, crispy, sweet/sour-balanced and aromatic for a long time and have a species and context typical form and taste. The product is ready for harvest, amino acids are incorporated into protein and the ratio protein/amino acids is high. Crops or seeds are capable of reproduction. The crop or fruit is resistant to disintegration, stress and diseases. The tissue is elastic and keeps turgor for a long time. The electric resistance and self-maintenance are high and the redox-potential is low.

The delayed luminescence tests shows a high start, a hyperbolic delay and a species-typical spectrum.

The crystallisation shows a coherent image, the parts are connected (no separate 'marbles') and a species-typical gesture is shown. The Bovis-value is high.

## Integration/coherence is a relational aspect of quality

In judging vitality or structural aspects, the observer may be objectively and non-involved. Also in the crystallisation method computers with image analysis are used to judge to a certain degree these two aspects (Anderson 2001).

But to judge the third aspect (integration or coherence) the observer must be involved and related, and so a subjective part appears. An involvement is desired to choose an aim as reference for quality. The fruit grower



brings in his direction. He chooses the optimal ratio between growth and differentiation to obtain the desired compromise between high yield and good taste or between a high storage potential and better tasting apples. An active personal involvement is also desired to perform some of the measurements, like the sensory tests, emphatic judgement in the crystallisation test and intuitive tests like Bovis. To develop these parameters in scientific manner requires a special interest in the method of inter-subjective judgement and cluster analysis. Development of tests to measure coherence is of great importance for the vital quality concept. In our opinion the aspect of coherence is the essence of quality.

## **20.2 Perspective for practical relevance of the vital quality concept**

The processes and properties described above for apple are generalised in a scheme in figure 11. We hope to investigate more series of all kinds of products to test and further develop this hypothesis as a general concept. Growers recognise (or can learn to recognise) both processes in their growing crop and should know how to bring both processes into balance.

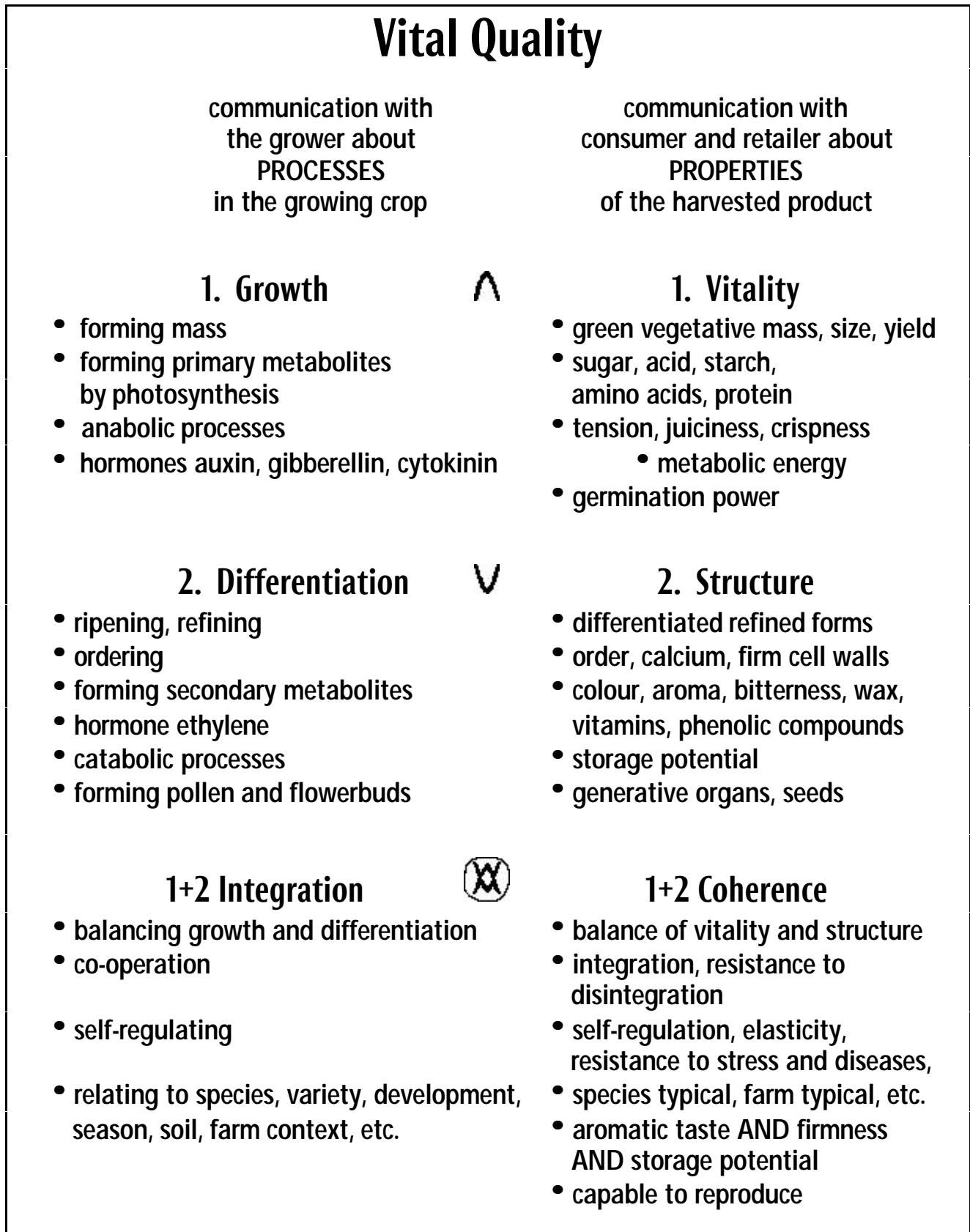
We assume that products with well-balanced growth and differentiation processes are of high vital quality and help humans create coherence in their own being. This should be proven by nutritional studies. Only then consumers will be provoked to purchase products with high vital quality.

Traders recognise both processes and their integration in the final product. And if there are some imperfections in quality the trader can communicate in terms growers can deal with.

The next step, the relevance for the consumers' health, has to be proven in a later stage.

For future enlargement of figure 11, we can image a third column at the right hand side about resonance phenomenon with the human health and a fourth column at the left hand side with the cultural practices for growers.

Figure 11: Generalised Vital Quality concept in keywords (in Dutch on page 14 and in German on page 22)



## 20.3 In which amount do the various parameters reflect vitality, structure and coherence?

In real life it is hard to distinguish between the two life processes, growth and differentiation. We cannot expect one measured parameter to represent only one aspect of vital quality. But for most parameters we can recognise emphases on one or more aspects of the vital quality concept. We made this preliminary classification both by thinking about the concept and by looking at the experimental results. Also the conventional parameters are interpreted as a result of these processes in a more holistic way than usual.

We realise that various parameters all concerning the same aspect of our quality concept can show different levels of the aspect. To belong to the same aspect of the quality concept does not automatically mean that their correlation (see annex 14.2) must be high. We still have to get a lot of more experience to validate the parameters' character. Here we present our first research on this topic, including the unanswered questions and realise that more experimental series will bring more and more certainty.

After this first project we cannot say which parameters are so similar that it makes the other redundant. Until now we learned something from every parameter to develop our quality concept. Most inspiring for the new quality concept were the crystallisations, the delayed luminescence and the Bovis-value.

In this chapter we collected remarks about the various parameters relevant to the processes growth, differentiation and integration. These remarks are presented as text in the various parameter chapters (§5-§17) before and also summarised in table 12 for an overview .

### Leaf series

Growth is reflected in leaf size and irregular leaf shape, in twig length and in internode length. Differentiation is found in regular shape and in details like fine leaf serrations. Integration is found in regularity in the leaf form sequence (Bloksma and Jansonius 2000). Because of the polarity between twig growth and fruit growth in the tree it is not necessarily that growth full leaves reflect a growth full quality in the fruit.

### Blush and ground colour and shine

In blush its easy to recognise the ripening process (differentiation) as well as in the series (A and C) and in the correlations. Moreover correlations are found between blush and integration parameters.

In our experience we expect ripening as the main determinant for colour (green ground turns into yellow; red blush turns into purple) and also for wax formation. We expect sun exposure as main determinant for the percentage of blush. Both parameters acting at the level of differentiation.

### Firmness

Firmness is the result of turgor and cell elasticity. Turgor, after taking up water, can be seen as a growth aspect whereas cell wall elasticity and the capacity of not loosing this water is more an integration aspect.

### Starch

We recognise in starch as basic potential building material a clear growth aspect.

### Sugar and sugar ratios

For sugar we have the same argument as for starch to regard this as a growth parameter. The ratio sugar/starch can be seen as a ripening indicator and sugar/acid as a balance for taste and an indication for the integration process.

### Acid

For acid we have the same argument as for sugar and starch to regard this as a growth parameter. The ratio sugar/acid as a balance for taste can be seen as an indication for the integration process.

### Streif-index

In accordance with its name the Streif-maturity-index looks like a ripening (=differentiation) parameter. In

connection with our quality concept it is worth mentioning that this index is built up by a formula consisting of only growth parameters of which the mutual proportions change as an indication of maturing. Maturing in this way can also take place in the store and is not necessarily restricted to the tree. In series A the difference between apples matured in store versus ripened on the tree in the sun is clearly demonstrated (see §19.1). For our quality concept the differentiating process in the sun is obliged to reach high vital quality.

### **Dry matter**

Dry matter is a complex parameter to place in the quality concept. Dry matter is the result of water uptake (growth), sugar content (growth), cell-compactness (inverse growth) and maybe more. Up to now we place dry matter with a question-mark at the growth-level.

### **Minerals**

Looking at the concept of growth (uptake from the earth, see §20.1) we expect all minerals to be growth parameters. N, P, K and Mg levels are diluted with an increased bearing in series B and are higher when grown in the shadow. This is also found in the correlations of the experimental data.

Calcium levels are the inverse of the N, P, K and Mg levels in series B and are not influenced by sun in series C. Also in many correlations Ca levels are the inverse of the other minerals' levels. The uptake of calcium is also known as an exception in a physiological way: it is sucked up by the hormone gibberellin formed in growing tissues (especially by seeds in the fruit) and once in the tissue it is not easy to reallocate. Calcium (and especially a high Ca/K ratio) makes an apple more resistant against ageing or disintegration. This is why we consider placing calcium at the level of structure, although calcium is not correlated with a lot of ripening parameters.

### **Free amino acids**

Free amino acids are easy to recognise as building stones, so 'amino acids' is a clear growth parameter. Protein is already synthesised a little further, but still can be considered as a growth parameter. The identical behaviour of amino acids and protein in the table of correlations support this choice. The many negative correlations between amino acids and integration parameters suggest an arrest of differentiation/integration by high concentrations of amino acids.

The protein ratio (protein/protein+amino acid) as a measure for incorporated building stones can be seen as an integration parameter. (Or when expressed as 'amino acid ratio' we must speak about a inverse integration parameter). This is supported by the high protein ratio in apples in the sun and with preparations in series C and also in many correlations with integration parameters as yellow/blue ratio in spectral range luminescence and electrical resistance.

### **Vitamin C**

With respect to the vital quality concept, we thought vitamin C, as a secondary metabolite, would be a differentiation parameter. In the series and in the correlations we see vitamin C also behaves like a growth parameter. So we put a question –mark at both levels. The measurements are not precisely enough to distinguish between growth and differentiation with our experimental data.

### **Phenolic compounds**

Phenolic compounds are secondary metabolites and that is why we place this parameter in the level of differentiation. Also the higher concentrations of phenols in series C in sun grown fruit and in fruit with preparations support this idea. The decrease in concentration of phenols with increased ripening in series A may seem the contrary, but remember, a decrease in concentration can also mean an increase in mg per fruit. Phenols are correlated with some integration parameters (protein ratio and electrical resistance), so maybe there is also an integration aspect?

### **Disintegration and self-maintenance**

With respect to the vital quality concept, both resistance to disintegration and self-maintenance are parameters related to keeping its own form. This implies that the parameters exert their action on the level of integration. Looking at the series we expected a decrease during ripening and ageing and an increase with sun exposure, preparation usage and moderate bearing, which is only found in ageing and light.

Looking at the correlations we found only a few correlations with other integration parameters. As mentioned previously, we are not satisfied about the precision of the method, maybe this is the reason we didn't find the

experimental evidence, or maybe our hypothesis is not correct?

### **Sensory properties**

Appreciation is a complex parameter. We are glad we performed such a detailed sensory test. With respect to the vital quality concept, we recognise the growth level in sweetness, sourness and juiciness. Crispness also has a growth aspect, but similar to what is said for firmness; remaining crisp can be seen as an integration aspect too. Mealiness can be seen as a loss of vitality (growth) and as a loss of coherence between cells (integration). Physiologically seen, gaining aroma requires a high level of basic substances (sugar, etc) together with a differentiation process, so we place gaining aroma at the integration level with a question-mark for differentiation. Loss of rawness or astringent skin can be seen as a result of ripening. Overall taste is dependent on proportions, as is seen in the sweet/sour ratio.

Because of the small differences and the large variation we have only little experimental evidence from the series for these hypotheses. The tendencies supporting these theories, recognised in series D, are the loss of vitality (sourness, crispness, juiciness) and integration (aroma, overall taste) during ageing. In series B the reason for a lower appreciation of apples from the highest bearing trees is too little sweetness (insufficient sugar for all fruit), and thus insufficient growth.

Looking at the correlations it is remarkable to see in these series that overall appreciation is mainly correlated with growth parameters, as juiciness, crispness and the sweet/sour ratio. The correlations with differentiation/integration parameters are illustrated by rawness of flesh, astringent skin and sweet/sour ratio in the ripening process.

### **Crystallisations**

Crystallisations are especially valuable for the vital quality concept because crystallisations can be judged on all three aspects of the concept. Vitality is recognised as smooth, quite dense needle structures filling the whole plate. Structure is recognised as , well ordered, one-centred, regular pattern of sharp side-needles with a wide angle. Coherence is recognised as a whole where the parts are interconnected, without isolated structures and in a way typical for apple.

### **Capillary pictures**

We do not have enough experience to classify the capillary pictures.

### **Delayed luminescence**

Both methods of delayed luminescence were important for the vital quality concept.

For the delayed luminescence by Meluna the emission at  $t=3$  is recognised as the amount of available energy for the development of either growth or differentiation. The hyperbolicity of the curve is indicative for a highly differentiated state. A combination of a highly differentiated state and a relatively high availability of energy allows the organism to have a high degree of coherence. So every aspect of the vital quality is represented. In the spectral range luminescence Kwalis assumed the yellow/blue ratio to be a parameter for fruit-character and 'being alive'. In our quality concept this is at the level of integration.

### **Acidity**

For apples, acidity can be seen as a growth parameter, as a potential for further ripening, as is illustrated in the negative correlation with many ripeness parameters. During ageing in series D the fruit loses its vitality, the pH increases and so the basis for integration decreases, as is illustrated in negative correlations with taste, Bovis-value, and the positive correlation with disintegration.

### **Resistance**

In series A and D we saw the resistance slowly decreasing during the last phase of ripening and ageing. In series C sun and preparations and in series B the higher bearing trees had the highest resistance. These phenomena prompted us to regard electrical resistance as an integration parameter. This is supported by good correlations with other integration parameters like the yellow/blue ratio in spectral range luminescence and protein ratio. In series B and C the overall resistance level is lower than in series A and D. The same difference in overall level we see in ripeness (A and D are riper) and in the DL-emission at  $t=3$  (A and D have less metabolic energy). So this suggests a certain amount of differentiating is a prerequisite for integration and therefore electrical resistance is a differentiation parameter too.

### **Redox-potential**

In series A and D we saw the redoxpotential decreasing heavily during ripening and increasing a little during over-ripening and ageing. Heilmann's assumption of the redoxpotential being a parameter for order, together with the correlations with a number of differentiation parameters, suggest that the redoxpotential is a differentiation parameter. In these results we also see the ability to show the loss of structure in over-ripe fruit, which is an aspect of integration.

### **P-value**

Because of the close relation with the redoxpotential we expect the P-value also to be a differentiation parameter. Beside that we recognise also growth aspects (pH) and differentiation (Eh) and integration aspects (R) the P-value-formula. From the concept we expect 'dissipation' as an inverse parameter for integration, but in the correlations we found aspects of all three levels. For our quality concept a complex parameter like the P-value for differentiation and integration will give additional information when we use the separate aspects (pH, Eh, R) apart from each other.

### **Bovis-value**

The big changes in Bovis-value are found in series D (a decrease during ageing) and in series C (higher for sun-exposed fruit and with preparations). These series are thought to show integration/differentiation (C) or loss of integration (D and last picking date in A). From the assumption that Bovis is a measure for the connection between the up-going and down-going energy flow through a plant or through the fruit, Bovis is thought to be linked to integration. For the Bovis-value is positively correlated with integration parameters like taste, SRL-yellow/blue and inverse disintegration. We found weaker correlations with some growth-parameters like acid, pH, dry matter, DL-emission at t=3 and the differentiation parameter vitamin C. This makes Bovis-value a preliminary integration parameter.

Figure 12: Assumed relationships between parameters and aspects of vital quality

	growth vitality	differentiation structure	integration coherence	remarks
leaf series	++	+	+	info about twigs and leaves, not necessarily about fruit.
glossy and waxy skin		++	+	
skin ground colour yellow		++		
skin blush colour red		++	+	
firmness	++		+	as result of turgor (growth) and cell elasticity (integration)
sugar	++			
malic acid	++			
starch	++			
sugar/starch		+		
sugar/malic acid		+	+	
protein	+	+?		
amino acids	++			
protein/amino acids+protein			++	
nitrogen (N)	+			
phosphate (P)	+			
potassium (K)	+			
calcium (Ca)		+?		
K/Ca		+?	+	
vitamin C	+?	++?		
phenolic compounds		++	+?	
general appreciation	+		++	
sweet	++			
sour	++			
aroma		+?	++	partly judged as sweet/sour?
mealy	+		+	as a loss of vitality and coherence.
crispy	+		+	
juicy	++			
sweet/sour		+	+	
raw, astringent		+?		
dry matter	+?			as a contrary result of water uptake, sugar content and cell compactness.
disintegration			+?	insufficient experimental evidence.
self-maintenance			+?	insufficient experimental evidence.
crystallisations	+	+	+	
capillary picture	+?	+?	+?	still too little experience in judging.
delayed luminescence: DL emission t=3	+			as metabolic energy for all processes.
DL lag time		+	+	as the growth/differentiation ratio.
DL emis. t=3/lag time			+	as maturity of a typical organ.
SRL yellow/blue	+?	+?	++	
P-value		+?	+?	P-value too complex; separate components are more useful.
pH	+			
Eh		+	+?	
R		+?	++	
Bovis			++	well correlated with appreciation

## 20.4 Recommendations for further research

This is only the first project in our ambitious FQH-program. We feel it as the first step.

We hope to continue with the next recommendations for further research to concept- and method-development:

- comparable experimental design with apples in a series of increasing nitrogen fertilisation, and anew series with Bd-preparations, see discussion in § 19.3.
- comparable experimental design with other products, notably carrot, red beet, potato, tomato and milk and determine whether the three aspects of vital-quality can also be found in these products.
- methodical improvement of the parameters: taste, self-disintegration, crystallisation and Bovis-value.
- further development and interpretation of biophotons and copperchloride crystallisations.
- which cultivation circumstances improve the integration process?
- nutritional research to determine the significance of vitality, structure and coherence for human health.

With this project we hope to have sown a seed to inspire all who are working on a better suiting quality concept for organic production. We are open to hear all improvements and hope to find colleagues to collaborate further in the context of the Food, Quality and Health programme.



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