Dienst Landbouwkundig Onderzoek

Instituut voor Agrotechnologisch Onderzoek

2[ND] ANNUAL PROGRESS REPORT

Mealiness in fruits
Consumer perception and means for detection

FAIR CT95-302

Participants: KUL, ATO-DLO, UPM, IFR,IATA, BTL
Report period : 01.01.97 - 31.12.97

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Elvis Biekman (Daily Coordination)
Carmen Boeriu (NIRS)
Truke Ebbenhorst-Seller (HPLC)
Yvonne Gerritsen (GC)
Sorel Muresan (HPLC)
Alex van Schaik (Storage and Handling)
Eugene Schijvens (Texture Measurement)
Jannemieke Termeer (Sensory Work)
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ato-dlo

January 1998

ATO-DLO

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Summary

Mealiness in fruits: consumers perception and means for detection

Objectives
Within this project measurement techniques for quantification of 'mealiness' investigated by ATO-DLO are: sensory analysis, gaschromatography of aroma compounds, HPLC determinations of sugars and acids, near infrared spectrocopy (NIRS) and impedance measurement. Except from impedance measurement, in the previous period the measurement methods were optimised c.q. further developed for measurement of apples with different degrees of mealiness. Impedance measurement were investigated to some extend by KUL. In addition ATO-DLO investigated the use of (enzymatic) maceration/ cell sloughing to investigate the change of the 'state' of the middle lammela during development of mealiness.

In this period two main experiments were performed.

Experiment 1: Aim of this was to investigate the relation between mealiness and NIRS-, HPLC- and GC-data. In this experiment a sample of 10 apples was taken as representative for the batch of apples. The sensory mealiness in this experiment was assessed by the trained analytical panel.

Experiment 2: Aim of this experiment was to investigate the relation between mealiness and some instrumental measurement of individual apples. As all the measurements were performed on the same apple the number of panellist assessing the apple was limited to four. Therefore in this experiment use was made of an expert panel trained to assess mealiness. In addition to the methods mentioned above, also texture measurements were performed. One of the methods was based on the methode applied by UPM.

In a seperate investigation an experiment was performed, to investigate the chewing behaviour of the panelist of the analytical panel.

Discussion
Cox and Jonagold were investigated in experiment 1, while in experiment 2 Elstar was used. The experiments with Cox and Jonagold were performed in February and March and the experiments with Elstar in July. The sensory assesment of the Cox and Jonagold showed that: 1) Cox's Orange Pippin and Jonagold apples behave similar in their proces of becoming mealy. 2) When Cox's Orange Pippin or Jonagold apples age, the firmness, crispness, moistness and sourness decrease while the mealiness, graininess and dryness increase. The sweetness and total aroma intensity differ between different apples (within product variation). In both experiments the results with NIRS showed a low R-squared of Cross validation.

Attention was paid to the extraction procedure for determination of tast compounds, in order to find a method which mimics the liberation of these compounds during chewing. In all cases the same results were obtained for pieces of apples and juice suggesting that both sampling techniques are suitable for analysis of non-volatile constituents of apples. The total sugar content of the Cox and Jonagold were similar and consistent with the fact that no significantly differences in sweetness were perceived by the sensory panel. A relation between the malic acid content and mealiness scores assessed by the sensory panel can be observed.

GC-analysis revealed that the majority of the 15 identified compounds were present in both cultivars. However a different behaviour is observed in development of flavour compounds during storage in the plastic bags. There was no clear relation between the level of mealiness and the GC-profile. With respect to texture measurements, in experiment 2, the best relation is observed between mealiness and juice area (the amount of juice pressed out during compressive test).

The enzymatic maceration showed a relation with the degree of mealiness. This is attributed to a change in the state of the pectin in the middle lammella, making it more susceptible to endo-PG hydrolysis.

Conclusion

Sensory Measurements

From the sensory measurements in experiment 1 it can be concluded that during storage in plastic bags at 20°C:

- Cox's Orange Pippin and Jonagold apples behave similar, in that more or less the same changes occur with respect to the texture properties. However, the timescale for the changes to occur is shorter for Cox.
- the firmness, crispness, moistness and sourness decrease while the mealiness, graininess and dryness increase. The sweetness and total aroma intensity differ between different apples (within product variation).

The Chewing experiments revealed that:

- The assessors of the analytical sensory panel agree on the attributes juicy and mealy.
- For the attribute crispy and firm the assessors don't behave in the same way.
- The maximum scores given for the texture attributes don't differ that much, only the moment at which the maximum score is reached differs.
- The apple pieces were not homogeneous enough to create good profiles for sweetness and sourness.

In experiment 2 an increase of mealiness, assessed by an expert panel, was observed for Elstar during storage in plastic bags 20°C. The largest decrease was observed in the first week of storage in the bags.

NIRS

In experiment 1 the best models were obtained in calibration of the NIRS spectra with firmness, mealiness and sourness. R-squared for validation RSQC, using 3 factors, were all above 0.9 while R-squared for cross validation RSQV were 0.62, 0.69 and 0.78 respectively. The lower R-squared for cross validation is attributed to: large variation within the batches, the high standard deviation in the sensory data and the relative small data set used.

In order to improve the results experiment 2 was performed: in this experiment the variation within a batch was omitted by using individual apples and the data set was larger. This approach had the draw back that the analytical sensory panel could not be used. The R-squared of calibration, RSQC= 0.93, was approximately the same as that for the experiment 1, while the R-squared for cross vallidation was somewhat lower, RSQV=0.64. Prediction of malic acid content by NIRS showed the same prediction potential as for mealiness.

The results thus far clearly show the possibility of to predict sensorial attributes by NIRS spectral data. However, further development is required.

Gas chromatographic Analysis
Most determined compounds are present in both Cox and Jonagold, but the relative intensity and behaviour in time are different. The increase of the peaks is larger in Jonagold than in Cox. Aroma determined by sensory panel decreases during storage while 'total aroma' determined by GC increases. This is in agreement with the hypothesis that mealiness retards the liberation of compounds located insight the cells during chewing.

HPLC Analysis of Taste Compounds
Comparison of extraction of taste compounds from homogenised apple and from apple juice showed little or no difference. So, it was concluded that both procedures are suitable for analysis of non-volatile compounds in apples gave the same results Also a real 'storage' will be followed.

Cox and Jonagold had similar total sugar content, which is consistent with the observation that no significant differences in sweetness were perceived by the sensory panel.
For Cox and Jonagold in experiment 1 as well as for Elstar in experiment 2 a relation between the malic acid content and mealiness scores assessed by the sensory panel was observed.

For Elstar, in experiment were a individual apple approach was followed, a linear relation with r^2=0.65 between malic acid content and mealiness scores was obtained.

Mechanical Measurement
By means of the applied method, linking the sensory and instrumental measurement to one and the same apple, it appeared to be possible to find relatively good relationships: the liquid separation and the total area (parameters of the ATO-method) showed good relationship with the sensory mealiness. With this experiments it is shown that liquid separation and total area are potential predictors of the sensory mealiness. To confirm the predictive capacity of these two parameters, these experiments have to be repeated in a larger scale with more apple varieties.

Enzymatic Maceration/Cell Sloughing
It can be concluded that this technique might be used for investigation of the decrease in middle lamella strength. In addition the individual cells obtained during the maceration experiment can be used for measurement of the cell wall strength. By this approach it might be possible to relate the change of the strength of the middle lamella and cell wall to development of mealiness or more general to texture changes.

Tomatoes

The mealiness levels of the tomatoes were very low and that and the differences if any were small. This means that there was no range for training a panel or performing a measurement session.

Conclusion
The results show that the mealiness levels were very low and that differences were very small. So there was no range for training a panel or performing a measurement session. Without mealy tomatoes it is difficult to continue this part of the project. However, by redefinition of the problem (softness instead of mealiness) we might continue.

Redefinition of the problem
An important textural problem of tomatoes is getting soft. It is generally accepted that softness in tomatoes is related to the activity of endo-polygalacturonase. This enzyme degrades the pectin of e.g. the middle lamella causing i.a. cell separation, eventually resulting in softness.

Because the development of mealiness in apples is also attributed to the degradation of the middle lamella the hypothesis can be postulated that the phenomena leading to softness in apples are the same as those which lead to mealiness in apple and that the difference in sensorial perception is caused by a different level of the cell wall strength of the individual cells: In tomatoes it is low so that cells are broken very easy through the cell walls; in apples it is high so that cells are more resistant to mechanical stress. Using this approach the central theme of the project 'degradation of the middle lamella' is kept.

1. Research performed in the reporting period

1.1 Deliverables covered by this report

Subtask 2.1.2.2
Report describing the experimental procedures to be followed for both the physical and the chemical instrumental techniques for *apples*

Subtasks 2.1.2.2
Reports in which the measurements results of the physical and the chemical properties of *apples* with varying degree of mealiness are described

Subtasks 5.1
Reports on statistical models of the relationship between chemical and the physical properties and the sensory description of mealiness of *apples*

Within these tasks the *impedance measurement* has not been performed by ATO. It was decided that the feasibilty of this measurement technique will be investigated by KUL. Instead of this measurement technique ATO has performed mechanical tests and has investigate cell sloughing as a means for characterization of mealiness of apples.

As there was no range of mealiness in the investigated *tomatoes,* the work involved within this tasks was limited.

Other deliverables

Subtasks 3.1. and 3.2
The report covering the deliverables of these subtasks for apples is send as a separate report

Subtask 5.2
For this task no report is available yet. It is expected that based on the results of UPM and IATA and the experiments being performed by KUL and ATO a dynamic model can be made for *apples.*

Task 6.
Within the framework of this task an article has been submitted to the Dutch Journal 'Fruitteelt'

1.2 Apples

In the Progress Report of 1996 it was mentioned that the problems encountered especially with NIRS, may be due to the large variations within a batch as was visualized via difference in size, blush, green-yellow ratio. In addition the batches of the different harvesting periods turned mealy in an different order than was expected. Because of this it was suggested that these batches may also have a different origin. With respect to the sensorial measurements panellists were trained on the basic mouth feel descriptors for one apple variety (Cox). The range of samples available appeared to be unsuitable to investigate flavour differences of this variety.

In this year the research performed on apples was directed towards further development c.q. improvement of the measurement techniques and the training of the panel for assessing the different sensory attributes. Two main experiments were performed:

Experiment 1
Aim of this experiment was to investigate the relation between mealiness and NIRS, HPLC- and GC-data. In this experiment a sample of 10 apples was taken as representative for the batch of apples. The sensory mealiness in this experiment was assessed by the trained analytical panel.

Experiment 2
Aim of this experiment was to investigate the relation between mealiness of individual apples and some instrumental measurement. As all the measurements were performed on the same apple the number of panellists assessing the apple was limited to four. Therefore in this experiment use was made of an expert panel trained to assess mealiness.

Collaborative Test
A first collaborative test was performed at IFR (Reading, England) from 26th to 30th of November 1996. Results of this experiment were presented at the progress meeting held at KU Leuven 18-19 september 1997. Because of practical problems, not all measurement techniques were used, this second collaborative measurement, to be held at ATO-DLO, was agreed upon.

It was also agreed to use the experimental design which was used at the first collaborative test.

In this second test efforts have to be made to use all relevant techniques which are investigated in this project. In the reporting period preparations were made this test (see appendix).

1.3 Tomatoes

At the last meeting of the mealiness project, held at the IFR in Reading, the question was put forward how to obtain mealy tomatoes, in order to conduct a sensory panel training.
In the past two months experts from ATO-DLO evaluated several batches of tomatoes with respect to mealiness.

Evaluations were made on tomatoes obtained from two different sources.
1. Dutch tomatoes obtained via the 'market'
2 Belgian tomatoes obtained via KU-Leuven/VBT

2. Materials and methods

2.1 Experimental set up, apple variety and storage conditions

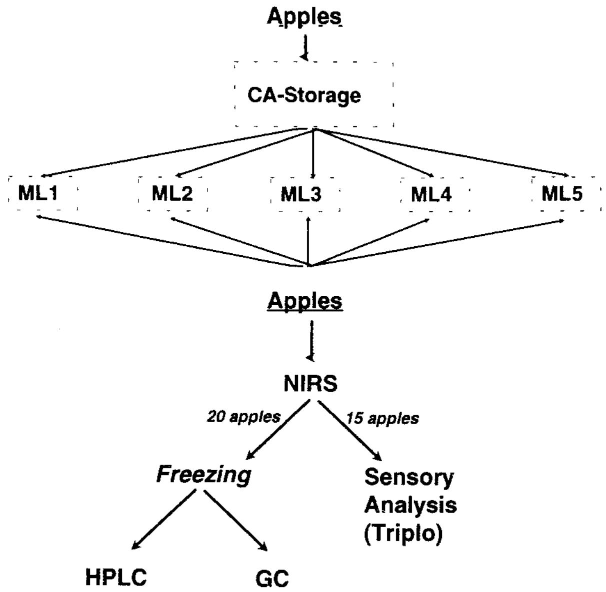


Figure 1: Setup of Experiment 1

Experiment 1
The Cox and Jonagold apples used in this experiments were purchased from a commercial storage facility in The Netherlands. On arrival at ATO-DLO they were immediately stored under CA-conditions: Jonagold 1°C, CO_2=4.5%, O_2=1.2%; Cox: 4°C, CO_2=0.7%, O_2=1.3%. These conditions were the same as of commercial storage.

In this experiment a batch approach was followed. This means that from the apples of each mealiness level a sample 10 apples for sensory analysis and 20 apples for HPLC and GC was regarded as representative for the batch.

Each of the apple for sensory analysis and for the HPLC and GC determination were first measured by NIRS. The sensory assessment was performed by the trained analytical panel.

Experiment 2
The Elstar apples used in experiment 2 were purchased from a commercial storage facility in The Netherlands (in May 1997). Upon arrival at ATO-DLO they were stored at ATO-DLO under CA-conditions: T= 4°C, CO_2=0.7%, O_2=1.3%.
An individual approach was followed in this experiment. This means that each of 20 apples was measured by 1) NIRS, the sensory panel and HPLC or 2) NIRS, the sensory panel and mechanically (Instron). Because of this approach, the part of an apple available for the sensory panel was limited, the trained analytical panel could not be used. Therefore an expert panel (four persons), trained to assess mealiness, was used instead. The expert panel scored the appels from 1 to 5. As the amount of apples to be judged was very high the experiment was performed during four successive days. The experimental scheme is given in table 1.

Table 1: Experimental set up for experiment 2.

Method	Days	ML's	Apples	Total nr. of Appels/Day	Total nr. of Appels
HPLC Set					
NIRS ↕ Sensory ↕ HPLC	4	4	5	20	80
Mechanical Set					
NIRS ↕ Sensory ↕ Text. Anal	4	4	5	20	80
Maceration Set					
Maceration	2	4	5	5	10

In both experiments different levels of mealiness were developed by storage of the entire batch of apples at T=20°C in perforated plastic bags according to the scheme given in table 2. Each bag contained 10 kg of apples. Every 5 days for Cox, and 7 days for Jonagold and Elstar a fraction of the batch was transferred from CA-conditions into plastic bags, so that after 20, 28 and 21 days for Cox, Jonagold and Elstar respectively the different levels of mealiness were obtained.

Table 2: Scheme for development of mealiness in the apples used in experiment 1 and 2.

Exp. nr. (Period)	Variety	Mealiness Level				
		1	2	3	4	5
		Days Stored in Plastic Bags				
1	Cox	0	5	10	15	20
1	Jonagold	0	7	14	21	28
2	Elstar	0	7	14	21	---

2.2 Sensory analysis

2.2.1 Experiment 1

1. Product profiling

Cox's Orange Pippin and Jonagold apples were stored at 20°C in perforated plastic bags in order to develop five different levels of mealiness. Each apple was divided into six pieces from stem-end to calyx-end. Cores of apple pieces were removed and all the pieces were peeled. Samples were presented in four-digit coded plastic containers.

The sensory analytical apple-panel consists of 18 assessors aged between 25 and 35 years. The assessors are trained in evaluating apples for mouthfeel, taste and aroma. The used attributes are: mealiness, firmness, crispiness, moist, grainy, dry, sweet, sour and total aroma intensity. Profiling of the apples is performed by the use of 'Quantitative Descriptive Analysis' (QDA^R, Stone & Sidel, 1993). For this test a line scale from 5 to 95 was used. Each session 5 products were evaluated by the assessors. The analyses were performed in triplicate. Data were collected with the PSA-SYSTEM (v.1.64, OP&P, The Netherlands). The tests were performed in separated booths, using red light and a little over pressure.

2. Chewing experiment
Jonagold apples of three mealiness levels were used. For the first bite the assessors used 1/4 of an apple, for the next chewing steps the assessors were provided with standardized apple pieces. The apples were cut and peeled and the pieces were minimized to a weight of 10 ± 2 grams.

For a chewing experiment 7 assessors out of the analytical panel were selected. The seven assessors were trained in evaluating apples for mouthfeel and taste aspects during chewing. The attributes used are: mealy, firm, juicy, crispy, sweet and sour. For the test a line scale (0-80) was used and the data were collected on paper. With round-the-table sessions the assessors developed a method to analyse the apples. For mouthfeel they analysed the apples after the first bite and after 1,2,3,4,6,8,10,15 and 20 times chewing. For taste the assessors analysed the apples after the first bite and after 2,4,6,8,10,15 and 20 times chewing. Two experiments were performed, during the first experiment the assessors scored all the mouthfeel attributes after one chewing step and the setup for taste was the same. During the second experiment the assessors scored the mouthfeel attributes separately after every chewing step. The final tests were performed in separated booths, using red light and a little over pressure.

Data-analysis
Principal Component Analysis (PCA) and analysis of variance (ANOVA, with a confidence level of 95%) were used to examine differences between the different products. In addition, the statistical packages UNSCRAMBLER (v.6.1, CAMO, Norway) and SPSS (v.6.1.4, SPSS, The Netherlands) were used to analyse the data. The statistical package SENSTOOLS (v2.2, OP&P/Talcott, The Netherlands) was used to study the assessors behaviour and the correlations between individual scores and mean scores for each descriptor.

2.2.2 Experiment 2

The sensorial measurements were performed by an expert panel of four persons (two male, two female). This panel scored mealiness on a scale of 1 - 5, were 1 is very mealy and 5 is not mealy. Every day five apples of each mealiness was measured for the 'HPLC-set' and for the 'Mechanical-set'. So, every day the panel assessed 40 apples, 20 in the morning and 20 in the afternoon.

The apples for maceration were not measured by NIR nor were they assessed by the panel. So, in this case the batch approach was used for relating cell sloughing to mealiness.

2.3 NIR and mealiness of apples.

2.3.1 Experiment 1

Aim of the study was to investigate the relationship between NIR spectral properties and sensory properties of apples (sensory panel). Apples with 5 mealiness (cv. Jonagold) stages were first measured by NIR and were then subjected to sensory analysis.

NIR measurement
NIR analysis was done with an InfraAlyzer IA500 spectrometer (Bran& Luebbe), with a PbS detector and a fiber optic accessory. The spectra were recorded for the wavelength range: 1100-2500 nm, with 4 nm increment. For measurements, the fruits were laid down on a static ring and a cover maintained the sample in darkness during measurements. No preparation was required and the whole apples were measured without any destruction. The reflection spectra were taken along the equator of the apple. The mean spectrum for 10 apples were used in calibrations.

Data analysis
Software package used: Unscrambler 5.5 and 6.1 (Camo, Norway). Regression technique: Partial Least Squares (PLS) - multi linear regression data pretreatment: different pretreatment of spectral data, such as MSC (scattering correction), or derivation (calculation of first and second derivative of the spectra); different calibrations were conducted: (i) the full range of wavelength (1100 - 2500 nm) and (ii) reduced range of wavelength (1100 - 1900 nm).

2.3.2 Experiment 2

Aim of this experiment was to investigate relationships between NIR spectral data and sensory mealiness for individual apples.

Experimental Set-up
Apples of 4 mealiness stages (cv. Elstar) were first measured by NIR and then were subjected to chemical and sensory analysis.

NIR measurement
NIR analysis was done with an Infra Alyzer IA500 spectrometer (Bran& Luebbe), with a PbS detector and a fiber optic accessory. The spectra were recorded for the wavelength range: 1100-2500 nm, with 4 nm increment. For measurements, the fruits were laid down on a static ring and a cover maintained the sample in darkness during measurements. No preparation was required and the whole apples were measured without any destruction. The reflection spectra were taken along the equator of the apple, on four equidistant positions from the central axis. The mean spectrum of four measurements were used in calibrations, for every fruit.

Chemical and Sensory analysis
The fruits were cut in 10 equal pieces; Pieces: garlic press; in the juice: malic acid analysis the pulp: rehydration Half of the pieces- analysed by a expert panel to determine the mealiness scores

Data analysis
Software package used: Unscrambler 5.5 and 6.1 (Camo, Norway). Regression technique: Partial Least Squares (PLS) - multi linear regression data pretreatment: different pretreatment of spectral data, such as MSC (scattering correction), or derivation (calculation of first and second derivative of the spectra); different calibrations were conducted: (I) the full range of wavelength (1100 - 2500 nm) and (ii) reduced range of wavelength (1100 - 1900 nm).

2.4 Gaschromatographic analysis of flavour compounds

Experiment 1
In the past period the composition of volatile compounds of two cultivars of apple, Cox and Jonagold, of five levels of mealiness was investigated. The applied techniques were static head space isolation, gas chromatography and mass spectrometry.

After the storage in plastic bags (to develop mealiness) apples were peeled, cut in pieces immediately frozen in liquid nitrogen, and stored at -80°C until analysis.

Frozen apple pieces, 45 grams, were homogenised under liquid nitrogen with a blender for 10 minutes. After 5 minutes 45 grams of saturated $CaCl_2$ solution (125 gram $CaCl_2$ in 100 ml milliQ) was added.

Three replicates of 20 grams of homogenate were weight in 500-ml glass bottles which were then sealed with a septum lid, with an opening in which a glass tube with 80 mg Tenax TA adsorbent was fitted. The samples were stirred for 2 hours at 35°C, to allow the volatiles to evaporate and adsorb on the Tenax. Experiments were performed in triplo.

Volatile compounds were desorbed from the Tenax tubes at 200°C for 5 minutes, and trapped on a cold trap at -100 °C (Chrompack TCT-2 injector). After desorption from the cold trap at 220°C for 5 minutes the volatiles were separated in a GC-system (Carlo Erba, HRGC 5300 mega series) with a DBWax column (50 m, 0.32 mm ID, 1.2 µm film thickness), and a FID-detector. Chromatographic conditions were 40°C for 10 min, 3°C.min^{-1} until 190°C, 10°C.min^{-1} until 250°C, 250°C for 5 min. Data acquisition and manipulation were performed using Chromcard software.

Volatile compounds were identified by using a GC-MS (Carlo Erba, Mega 3600, QMD 1000) which was equipped with a thermal desorption unit (Carlo Erba, Tekmar 5010). Tenax tubes were desorbed for 10 minutes at 220°C. The GC column and the column conditions were identical to those for gas chromatography except that the colomn temperature was kept for 1 minute at 250°C instead of 5 minutes. Positive ion electron impact mass spectral analysis was carried out at 70 eV and a source temperature of 200°C. The calculated Kovats indices (KI) and MS fragmentation patterns of each component were compared to those of the authentic compound as reported in the literature.

2.5 Sugars and acids

In order to monitor the changes in taste components in relation to the development of mealiness HPLC analysis aiming the quantification of taste components for apples at different mealiness stages was performed. To mimic the teeth action and to find chemical background for sweetness/sourness in relation to mealiness, a juice/press experiment was designed.

HPLC analysis was performed to determine the ascorbic acid, sugar, malic acid and citric acid contents for apples at different mealiness stages.

Preparing fresh samples
Fresh samples were peeled and cut into slices with an apple slicer to obtain 10 equal pieces. From the middle part of one red piece and one green piece cubes of approx. 1 cm^3 were taken.

These pieces were immediately put into liquid nitrogen to avoid enzymatic activity. Until extraction for analyses, the samples were stored in a freezer at - 25^{0}C. Before extracting with a suitable solvent the frozen samples were mixed with a Moulinex household mixer to obtain a homogeneous sample.

For the juice/press experiment the apple pieces were pressed using a garlic press. Only the resulted juice was used for analysis.

All the experiments were performed in duplicate.

Dry matter content
Weigh 5 g (±0.01 g) of fresh sample into a alumina plate. Predry during 16 hours at 70°C. Dry 3 hours at 105°C, cool in a desiccator at room temperature and weigh again. Calculate % dry matter (dry weight in g/fresh weight in g X 100 %).

Total ascorbic acid content (ascorbic acid + dehydro-ascorbic acid = TAA)
Ascorbic and dehydro-ascorbic acid is extracted from the sample with 9.5 % oxalic acid. After filtration of the slurry the pH is adjusted to 7 and dehydroascorbicacid is reduced into ascorbic acid with homocysteine. If necessary, interfering components are discarded with a Sep-Pak cartridge (C18 or Florisil dependant of the interfering components).An ion pair reversed phase system is used to separate ascorbic acid from other components. Results are monitored with a variable UV detector at 251 nm (isosbestic point of ascorbic acid).

Sugar content (sucrose, glucose, fructose, sorbitol)
Boiling Milli Q water is first added to a known amount of the fresh sample and boiled for 5 minutes to inactivate the enzymes. After cooling the sugars are extracted with a Ultra Turrax mixer. Samples are deproteinised with Carrez, made up to volume and filtrated. The clear filtrate is treated with a Sep-Pak C18 cartridge. Sugars are separated on a Sugar pak column, a calcium loaded anion exchange column. Separation of the sugars is a mixture of mechanisms, as hydrophilic, hydrophobic adsorption and separations based on molecular size.

Organic acid content (citric acid, malic acid)
The acids are extracted from the samples with Milli Q water at 4^0C and kept at that temperature until dilution with phosphoric acid to avoid enzyme reactions. After mixing with a Ultra Turrax mixer samples were filtrated through a Whatman GFC glass fibber filter. Dilution with 0.02 M phosphoric acid until a suitable concentration, clean up with a Sep-Pak C18 cartridge and the sample is ready for injection in the HPLC. Organic acids are separated on two in series connected H^+ loaded cation exchange columns (Shodex KC811 - Waters B.V.). Chromatograms are monitored with a UV detector set at 210 nm.

2.6 Maceration/Cel sloughing of apples

Experiment 2

Principle
Apple cells are linked together by the middle lamella which is composed mainly out of pectine. Endo polygalacturonase (PG) decomposes the pectin molecules of the middle lamella. Shaking apple pieces with endo PG results into sloughing of the pieces to give a suspension of loose cells and/or clusters of cells. The fraction of the pieces which is transformed into loose cells, as measured by sieving, may be a measure of the state of the pectin (and thus the middle lamella).

Apples (5) were peeled, cored and cut into cubes (a=0.95 cm). A sample of 50 grams (10 grams of each apple) was vacuum infiltrated with 50 mM NaCitrate buffer, containing 1.6 ml endo-PG (Rohament PL, Röhm GmbH, Darmstadt, Germany). Apple cubes infiltrated entirely, were selected and incubated at 40°C in the 50 mM NaCitrate buffer, during 90 minutes in a rotating water bath. A second sample of 50 grams was incubated, without being vacuum infiltrated. In this case the enzyme was added at the start of the incubation. Finally a thirth sample of 50 grams was incubated without being vacuum infiltrated and without enzyme.

At the end of the incubation, the enzymatic reaction was stopped by cooling the incubation mixture in a ice bath and adjusting the pH to 2. The incubation mixture was filtered with a kitchen sieve to seperate the larger apple pieces from the macerate and both the fractions were weighed. Subsequently the fraction containg the macerate was screened, using three sieves with mesh size 2.8, 0.25 and 0.063 mm. Each fraction was subsequently washed over a Buchner funnel and weighed.

2.7 Texture Measurements

Sample preparation
Elstar apples of five mealiness levels (generated by storage time intervals of seven days) have been used for the combined sensory and texture measurements. The separation for the sensory and texture measurements have been performed as illustrated in figure 2. From each slice of 17 mm thickness, five cylinders with a diameter of 17 mm were cut. From two cylinders the length was reduced to 8 mm for the 'ATO-method' measurements. The other three cylinders with a height of 17 mm were used for the the measurement with the texture analyser. The method was based on the method of UPM, except that the sample was not confined in a tube.

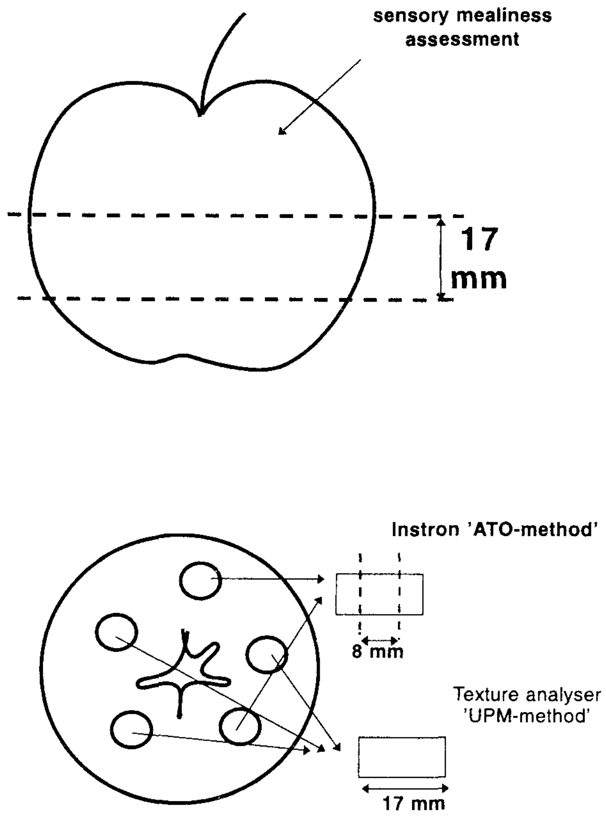


Figure 2: Sample preparation for the sensory and texture measurements

ATO-method
In this method an Instron 4301 instrument, equipped with a 50 mm diameter plate has been used. This plate was moved downwards with a speed of 10 mm/min, starting from 12 mm up to 2 mm above the load cell, where the plate returns to the start position. The bottom and

top side of the apple cylinder is covered with a stainless steel sieve with 0.063 mm diameter holes and filter paper to absorb the drained liquid (figure 3). From the weight of the filter paper and the registrated force data the following parameters were determined for each apple cylinder:

- liquid separation (g)
- failure stress (Mpa)
- area to failure (mJ)
- slope (N/mm)
- modulus (Mpa)
- total area (mJ)

Modified UPM-method

These measurements were performed with a SMS TA XT2i Texture analyser on apple cylinders of 17 mm diameter and 17 mm height. The plate was moved with a speed of 20 mm/min, first downwards up to a deformation of the apple cylinder of 2.5 mm and than upwards (figure 3). From the data the following parameters were determined for each apple cylinder (figure 3):

- Fmax (N)
- plastic deformation (mm)
- degree of elasticity (mm)

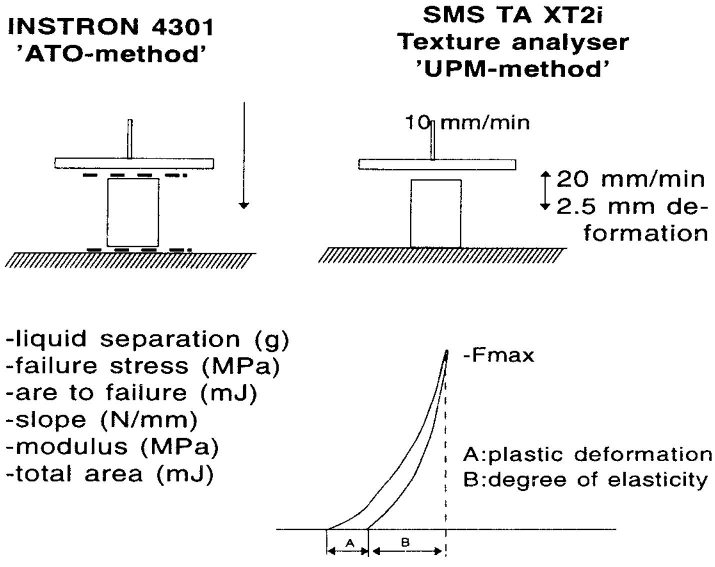


Figure 3: Texture measurement methods

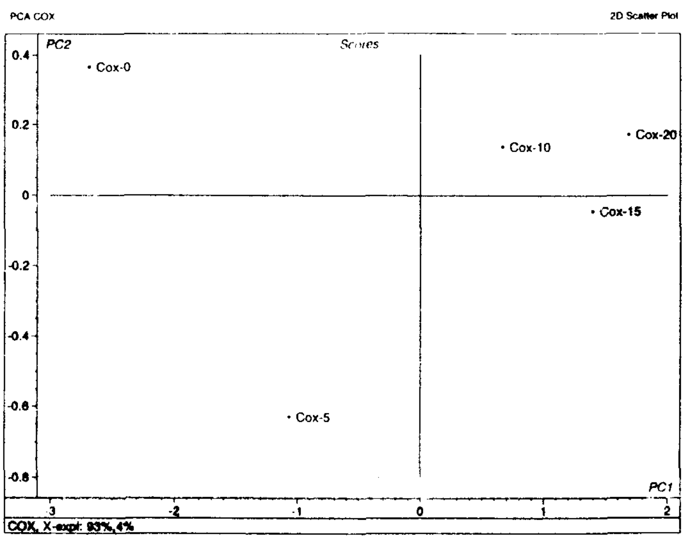
3. Result and discussion

3.1 Sensory analysis

Experiment 1

Product profiling
Five samples of Jonagold apples and five samples of Cox's Orange Pippin apples have been evaluated by the sensory analytical panel. After calculating standardized scores (z-scores) the data were analysed. The PCA-plot in figure 4 shows the distribution of the Cox's Orange Pippin samples and the attributes on the first and second principal component.The explained variance with these two components is 97%.

Figure 4 shows that non-mealy apples (cox-0) are firm, moist, crispy, sour and have a high total aroma intensity. They are located at the top of figure 4. On the bottom of figure 4 the most mealy apples are located with the characteristics mealy, grainy and dry. The three apple samples on the positive side of the first component are grouped (cox-10, cox-15, cox-20). Within this group the only significant differences are between cox-10 and cox-20, for sourness and mealiness. The other attributes do not differ significantly between these three products. Table 3 shows the significant differences between the different samples. From these data one can conclude that the Cox's Orange Pippin apples reached their most mealiest level after a storage of 10 days at 20°C.



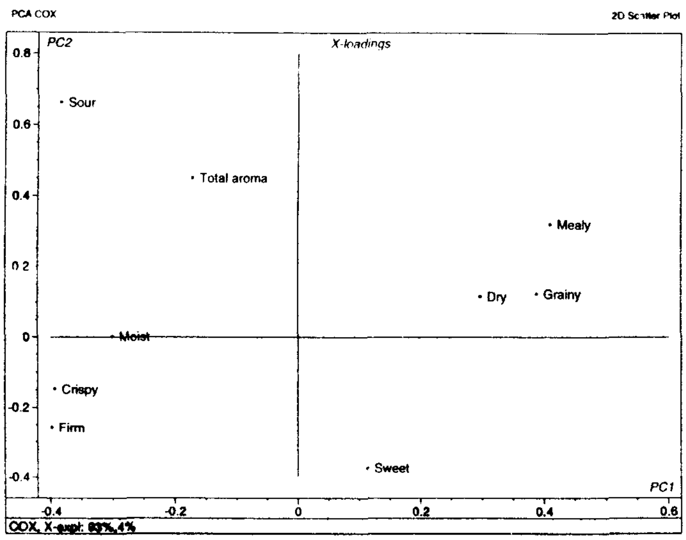


Figure 4: *Principal Component Analysis for Cox's Orange Pippin apples, for mouthfeel, taste and aroma attributes. The distribution of the products (scores) and the distrubtion of the attributes (loadings) on the first and second principal component (PC1 and PC2: respectively x-axes and y-axis) is shown. The apples are coded 0 for non mealy apples to 20 for the most mealy apples.*

Table 3: Significant differences ($p \leq 0.05$) between Cox's Orange Pippin samples. The apples are coded from not-mealy (cox-0) to most mealy (cox-20).

The apples become significantly more...	**The apples become significantly less...**
From cox-0 to cox-5	
grainy	crispy sour aroma
From cox-5 to cox-10	
dry grainy mealy	crispy firm moist
From cox-0 to cox-20	
dry grainy mealy	crispy firm moist sour aromatic

For the Jonagold apples the results are shown in figure 5. The PCA plot shows the distribution of the samples and the attributes over the first and second principal component. Together PC1 and PC2 explain 95% of the variance.

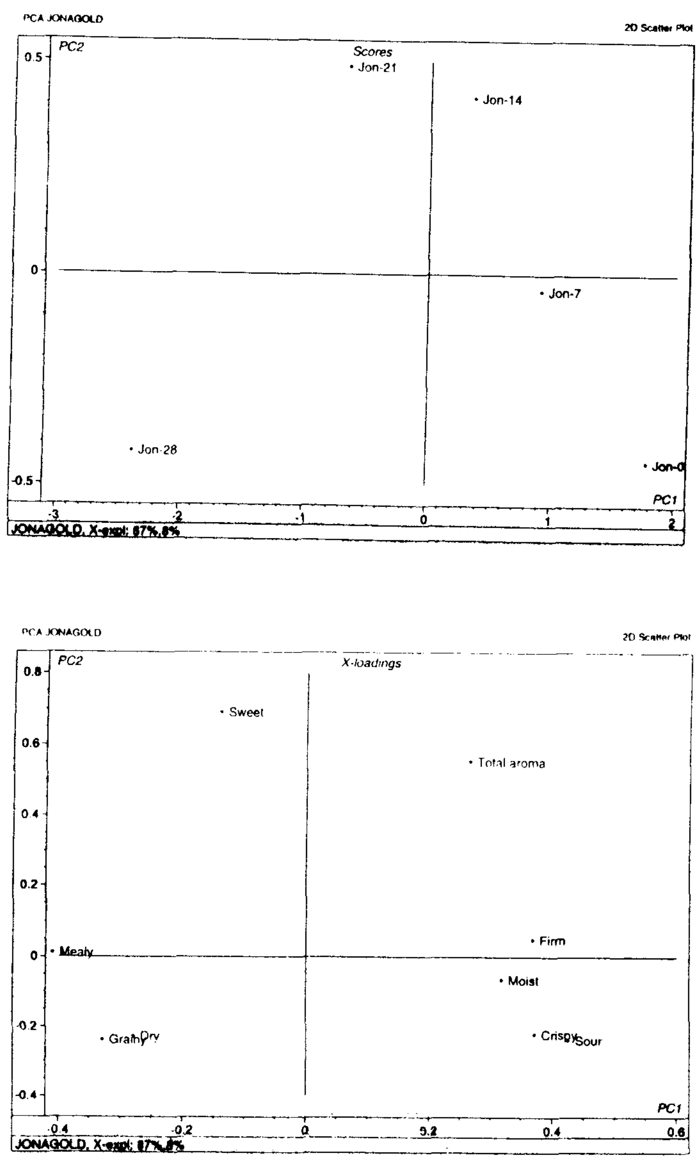


Figure 5: *Principal Component Analysis for Jonagold apples, for mouthfeel, taste and aroma attributes. The distribution of the products (scores) and the distrubtion of the attributes (loadings) on the first and second principal component (PC1 and PC2: respectively x-axes and y-axis) is shown. The apples are coded 0 for non mealy apples to 20 for the most mealy apples.*

Figure 5 shows that non-mealy Jonagold apples (jon-0) are firm, moist, crispy and sour. They are located at top of figure 5. On the bottom of figure 5 the most mealy Jonagold apples are located with the characteristics mealy, grainy and dry. From jon-7 to jon-21 the sweetness and total aroma intensity are increasing. The results for the Jonagold apples are different from the Cox's apples the Cox's apples reach their mealiest level after 10 days at 20°C. While for the Jonagold apples the mealiest level measured was after 28 days at 20°C according to figure 5. Most of the differences between Jonagold applesthat have been stored for 7, 14 and 21 days are not significant. Significant differences are observed between jon-0 and jon-7 and between jon-21 and jon-28. Table 4 shows these differences and also the significant differences between jon-0 and jon-28 are included. During storage at 20°C the Jonagold apples become more mealy, dry and grainy and the crispiness, moistness, firmness, sourness and total aroma intensity decrease. This result is the same as for the Cox's Orange Pippin apples.

Table 4: Significant differences between the Jonagold samples.

The apples become significantly more....	**The apples become significantly less...**
From jon-0 to jon-7	
	firm sour
From jon-14 to jon-21	
	sour
From jon-21 to jon-28	
grainy mealy dry	firm aromatic
From jon-0 to jon-28	
grainy mealy dry	crispy moist firm sour aromatic

Chewing experiments

For these experiments Jonagold apples of three mealiness levels were used. During the first session the assessors discussed about the method to evaluate the apple samples. During the next sessions the assessor analyzed the apples with the method they agreed on. For interpretation of the data so-called 'profiles'of the different assessors were created.

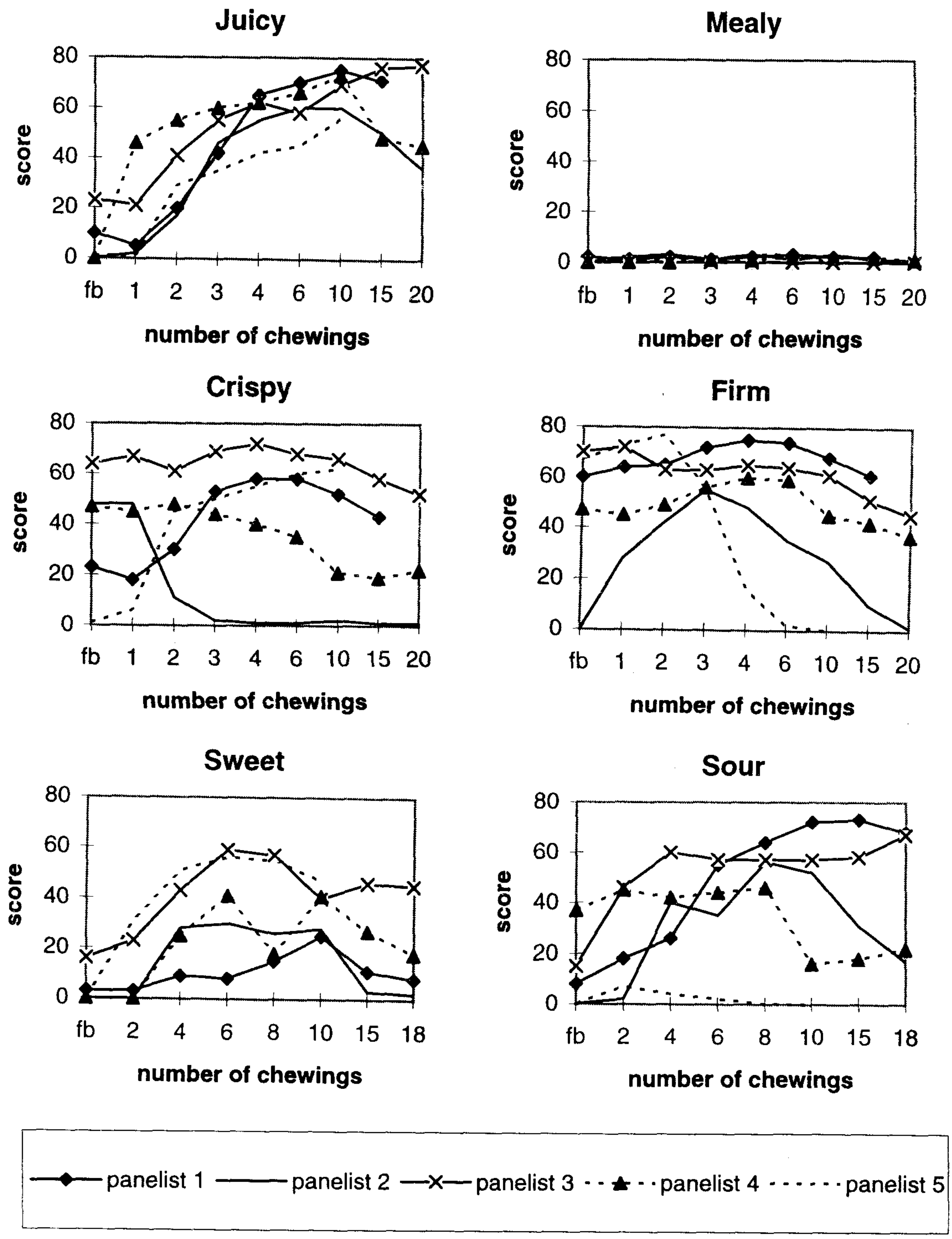


Figure 6: Profiles of six attributes for five assessors for non mealy Jonagold apples.

In figure 6 the profiles per attribute of five different assessors are shown. These data have been collected on not mealy Jonagold apples. From figure 6 it becomes clear that for juiciness all the assessors behave in the same way, they start low and give higher scores when they are chewing longer. The profile of mealiness shows that the apples were not mealy and that the assessors agree on that. For the attribute crispy the assessors don't behave in the same way. Some assessors give for crispiness a high score after the first bite and some give a low score. For firmness it is clear that three assessors have the same idea, but two assessors do something completely different. Assessor 5 starts high, but ends low and assessor 2 starts low, goes up and ends low. The other three assessors (1,3,4) start high and stay on the same level during the chewingsteps. From these data it becomes clear that the maximum values do not differ that much, whereas the moment at which the maximum score is reached differs considerably.

For sweetness and sourness the profiles are less consistent. The assessors (having pieces from their own apple) were confronted with the differences between pieces from the red and green side. After the test the assessors complained about the differences between the apple pieces, they expected them to be equal. The less consitstent results can be a result from these differences.

Conclusion

Profiling of Cox's Orange Pippin and Jonagold apples:

- Cox's Orange Pippin and Jonagold apples behave similar in their proces of becoming mealy.
- When Cox's Orange Pippin or Jonagold apples age the firmness, crispness, moistness and sourness decrease while the mealiness, graininess and dryness increase. The sweetness and total aroma intensity differ between different apples (within product variation).

Chewing experiments:

- The assessors agree on the attributes juicy and mealy.
- For the attribute crispy and firm the assessors don't behave in the same way.
- The maximum scores given for the texture attributes don't differ that much, only the moment at which the maximum score is reached differs.
- The apple pieces were not homogeneous enough to create good profiles for sweetness and sourness.

Experiment 2

The mealiness scores for the apples of different storage regimes were in the range of 5 (not mealy) to 1. The agreement between the different experts was good. The scores for the apples of the same storage regime showed a variation which increased as the time of storage became longer. In figure 7 it can be seen that the averaged scores decreased during storage in the plastic bags; which means that mealiness increased. The trend is same for all days, when the apples of the different levels were measured. The largest increase of mealiness (decrease of scores) is observed in the first week. In the weeks thereafter the decrease is lower but constant.

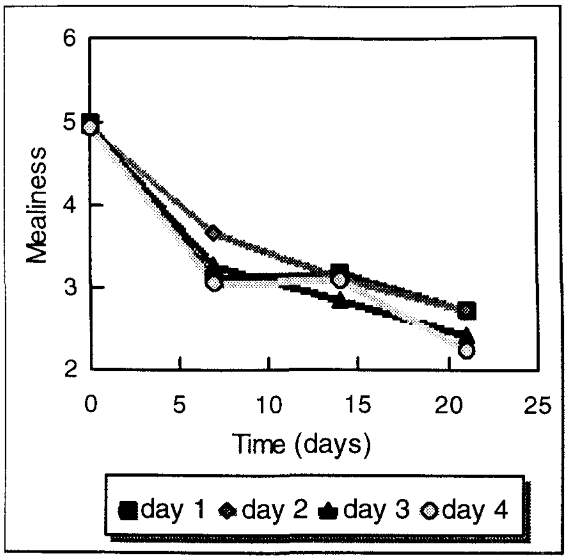


Figure 7: *Decrease of mealiness scores during storage of Elstar apples in plastic bags at 20°C. Each datapoint is the average of 20 apples.*

3.2 Relationships between NIR spectral properties and sensory and chemical characteristics of stored apples

Experiment 1

Correlation between NIR spectra and sensory properties of apples (batches)
Experiments were performed in order to develop a non-destructive method to assess and predict the sensory perceived properties of batches of apples from their Near infrared spectral properties. Measurements were performed for apples of varieties Cox and Jonagold, following the experimental set up described in Chapter 2.1. NIR-spectra of each apple from the sample batch (n=10) were recorded before the fruits were evaluated by the sensory panel, using the method described in Chapter 2.3. The mean spectrum was used for data analysis.

Sensorial Data
The sensory profile of the samples of apples of varieties Cox and Jonagold was discussed in detail in chapter 3.1. Here we address the texture data determined by the sensory panel as the reference data set for the NIR measurements. To avoid repetition, we exemplify the results for variety Jonagold. The mean, the minimum and maximum levels of texture properties and the correlation between mealiness and other sensory parameters are presented in table 5. The following general trends are observed:

- the texture characteristics are highly correlated
- mealiness is negatively correlated with texture attributes firmness and crispiness and positively correlated with grainy
- mealiness is negatively correlated with juiciness
- mealiness and sourness are negatively correlated
- there is no relation between mealiness and sweet.

In other words, an increase in mealiness will be accompanied by increase of graininess and decrease of firmness, crispiness, juiciness and sourness.

Table 5: Summary of sensory results with range, mean and correlation between mealiness and other sensory parameters.

	Range	**Mean**	**Correlation**
mealiness	16 - 47	23.5	
firmness	30 - 70	52.3	-0.86
Juicyness	34 - 67	56.3	-0.8
crispy	19 - 66	42.5	-0.89
dry	10 - 51	26.8	0.86
grainy	15 - 58	29.0	0.92
sweet	20 - 43	37.8	0.20
sour	20 - 65	40.8	-0.72

PLS calibrations

Before the calibration, the spectral variation of the data base has been analysed by principal component analysis (PCA), (data not shown); however, the very different samples (outliers) were not removed from the data set, since the number of samples was very small (n= 20). Different mathematical pre-treatments of the spectra were tested, and it was found that scatter correction (MSC) improved the calibration results. However, a first derivative with a smooth segment of 5 data points and a gap segment of 10 points gave the best results.

The essential relationship between NIR-absorbance spectra and the sensory properties of apple were analysed by PLS regression. PLS calibrations were conducted (X matrix: spectral data; Y matrix: reduced data set containing texture data), using cross-validation as model validation technique. Separate models were developed for every cultivar. Several kinds of calibrations were conducted: (I) full range of wavelengths (1100-2500 nm), and (ii) reduced range of wavelengths (1100-1700 nm). Best results were obtained when performing the calibration in the lower NIR range (1100-1700). In the models thus obtained, 3 factors (PC) explained approximately 70 % of the variance in the data set. This result is in agreement with data presented in literature for similar type of products. The prediction by PLS for the reduced range of spectra gave good results for the following sensory attributes: mealiness, firmness, sourness, since all the RSQ were superior to 0.9. However, in all cases, the correlation coefficient of the cross validation was lower ($0.6 \leq RSQV \leq 0.8$), indicating that some of the cross-validation samples were not representative for the data set. This effect can also be produced by the high correlation in the reference data set (sensory data).

In addition, we can assume that the following factors can affect the results:

- the difficulty in assessing the texture parameters by the sensory panel
- the high standard deviation in the sensory data
- experimental error for NIR measurements
- high variation in the batch
- small data set (for reliable models, a larger data set, spanning the whole range of variation for sensory data, is necessary)
- outliers in the data set
- possible non-linearity of the relationship between NIR spectral data and sensory data.

The results of the PLS calibrations developed for apples of varieties Cox and Jonagold are reported in table 6.

Figure 8 shows an example for the calibration curve for mealiness.

Table 6: Calibration and validation statistical results for the determination of texture parameters (PLS model with 1st derivative of NIR spectra; spectral range: 1100-1700 nm)

	SEC	RSQ	SECV	RSQV	PLST
Jonagold					
Mealiness	4.23	0.92	9.60	0.69	3
Firmness	4.32	0.94	9.88	0.62	3
Sour	5.47	0.89	7.74	0.78	3
Cox					
Mealiness	3.28	0.94	6.28	0.76	3
Firmness	4.56	0.93	7.92	0.70	3
Sour	4.68	0.90	7.74	0.80	3

SEC - residual standard deviation of calibration; RSQ - correlation coefficient of calibration; SECV- standard error of cross validation; RSQV- correlation coefficient of cross validation; PLST- number of principal components

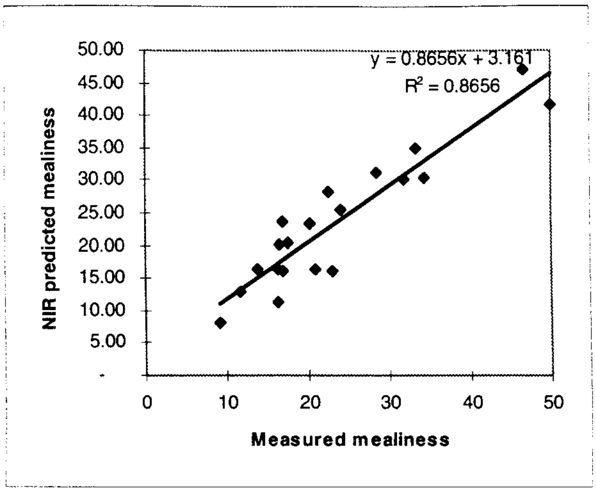


Figure 8: *NIR predicted vs. Sensory measured mealiness*

Conclusion

The results show that apples can be measured by NIR with good accuracy for the principal texture parameters, and particularly mealiness, which are taking in consideration for apple quality.

Experiment 2

Prediction of mealiness and malic acid content of apples (individual) from NIR spectral data

In this experiment, apples of variety Elstar, at four mealiness levels, were measured first by NIR and then subjected to chemical and sensory analysis (expert panel), using the methods described in chapter 2. Every apple was treated as a separate sample. Partial Least Squares analysis (PLS) was used to develop models for predicting sensorial perceived mealiness and malic acid content based on spectral data. Malic acid was considered an important chemical parameter indicative for mealiness, since there is a high negative correlation between malic acid content and mealiness of the fruit (data not shown).

PLS calibrations were conducted (X matrix: spectral data; Y matrix: malic acid concentration and mealiness scores), using cross-validation as model validation technique. Best results were obtained using the first derivative of the spectra with a smooth segment of 5 data points and a gap segment of 10 data points, and a spectral range of 1100-1700 nm, in the X matrix.

Table 7 shows the calibration and validation statistical results for mealiness scores and malic acid content. Also in this case it is seen that, for cross-validation, the standard error increases and the correlation coefficient decreases, as compared with the calibration. The same hypothesis as presented for Experiment 1 can be considered valid for this case also.

Table 7: Calibration and validation statistical results for the determination of sensory attributes. PLS model with 1st derivative of NIR spectra; spectral range: 1100-1700 nm

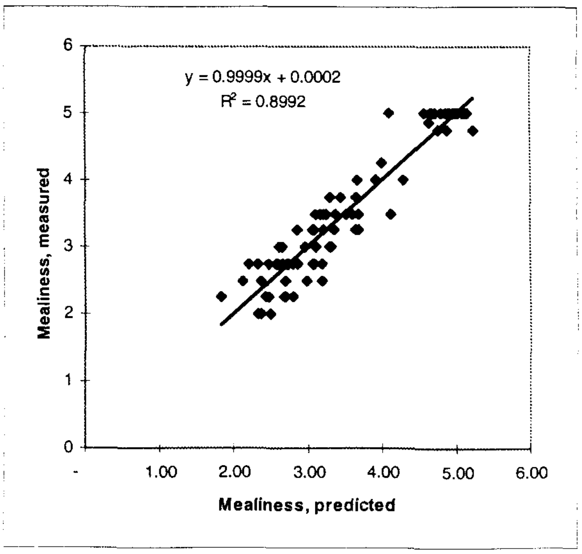
	Mealiness	**Malic Acid (mg/ 100 gr)**
Range	2-5	250-600
Std. Err Calibration	0.25	29
R-Squared	0.93	0.91
Std. Err. Cross-Validation	0.72	57
R-Squared Cross-Validation	0.64	0.66
Factors	2	2

Figure 9 shows the calibration lines for (A) mealiness and (B) malic acid.

Conclusion

Mealiness (SEC = 0.25, $r^2 = 0.93$ for calibration, and SEV = 0.72, $r^2 = 0.64$) and malic acid content (SEC = 29, $r^2 = 0.91$ for calibration, and SEV = 57, $r^2 = 0.66$) of apples can be predicted to a certain extent by the NIR spectral data. The low statistical parameters for the validation procedure can be explained, among others (see above) by the large variation in the biological material.

NIRS as a rapid, non-destructive method is a promising technique for instrumental prediction of mealiness of apples.



A.

B.

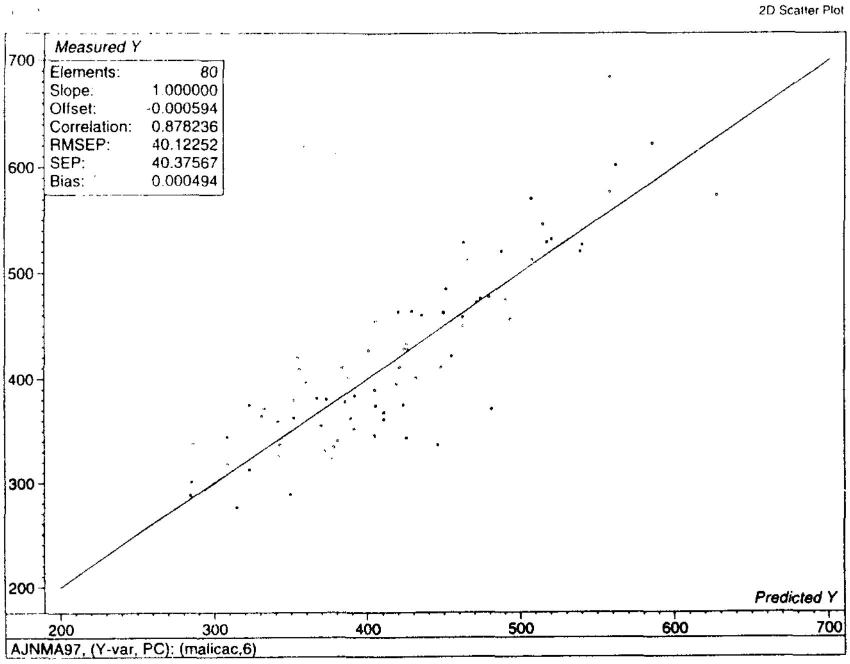


Figure 9: *NIR predicted vs. Measured (A) mealiness and (B) malic acid content of apples.*

3.3 Gaschromatographic analysis of aroma compounds

Experiment 1

GC-analysis was performed only in experiment 1. In figure 10 gas chromatograms are shown of Cox apples which have been stored for 0 (control), 5, 9, 15 or 21 days at 20 °C in air (see page 6 for a detailed description of the storage conditions). Figure 11 represents gas chromatograms of Jonagold apples which have been stored for 0 (control), 7, 14, 21 or 28 days at 20 °C in air. In both cultivars 27 peaks were selected on the basis of a minimum peak area of 10 mVs. Of these peaks 14 compounds were identified with GCMS (figure 12 and 13 and table 8). Numbers correspond with the numbers in the chromatograms. Peak 11 consisted of two compounds and the position of the double band of hexenylacetate of peak 25 could not be identified. In order to quantify the changes during storage the areas of the identified peaks were used. The standard deviation of some of the mean peak areas is high because of a big difference of the peak area of one of the peaks. The relative changes during storage were plotted in figure 14 and 15.

Influence of cultivar

It can be said that most of the identified compounds are present in Jonagold as well as in Cox (table 8). However the compounds differ in intensity. These results are in agreement with the results of Paillard (1967). He showed that in nine apple cultivars most identified components were always present, but in variable proportions, and that no identified component seemed to be characteristic of a single variety. In Cox the summarized peak areas of the esters are approximately five times higher than the summarized peak areas of the alcohols, in Jonagold approximately three times higher. Drawert (1968) classified apple cultivars by their volatile composition into two types: the ester type and the alcohol type. He classified Cox, Jonathan and Golden Delicious as ester type apples, since the ester production of these cultivars was approximately ten times higher than the alcohol production. Since Jonagold is a cross-breeding of Jonathan and Golden Delicious (Pijpers, 1992), it can be expected that Jonagold is an ester type cultivar. As mentioned above, the results presented in this report show only a three (for Jonagold), respectively five (for Cox) times higher ester production than alcohol production, so Cox and Jonagold might be ester type apple cultivars. The total area of the peaks of CA stored Cox is higher than the total area of the corresponding chromatograms of Jonagold (not shown). This difference is mainly caused by the high peak area of ethyl acetate in Cox (fig. 12 and 13). Figure 12 and 13 further show that the amounts of butyl acetate, butanol and hexyl acetate were high in both cultivars (CA storage). CA stored Cox contained relatively more ethyl acetate, ethanol, butyl acetate, hexanal/2-methyl propanol, butanol and less 2-methyl butyl acetate, 2-methyl butanol and hexanol than Jonagold. Propyl acetate and propanol were identified in Jonagold, but not in Cox. Girard (1995) also found propanol and Propyl acetate in Jonagold. Knee (1981) showed that if propanol was supplied as vapour to cortex and peel tissue of Cox apple the tissue was capable of acetylating propanol to Propyl acetate. This result implies the presence of a source of acetyl units and a esterifying system, but a lack of propanol supply in Cox. In Jonagold methyl acetate and ?-hexenylacetate were not detected. This is in agreement with Girard (1995) who also didn't find methyl acetate in Jonagold.

Influence of storage at 20°C

Storage in air, at 20°C, after CA storage resulted in an increase of almost all peaks for both cultivars (fig. 12 and 13). Particularly butyl acetate, butanol and hexyl acetate increased substantially in both cultivars. In Jonagold also 2-methyl butyl acetate, 2-methyl butanol and Propyl acetate showed a high increase during storage. Further, in Jonagold propanol and Propyl acetate showed a relatively high increase (fig. 15). In Cox butyl acetate, butanol and hexyl acetate relatively increase the most (fig. 14). In Cox methyl acetate, ethyl acetate and ethanol decreased strongly after a short storage in air, as well absolutely as relatively, while in Jonagold these compounds changed hardly. We found the same results in the former experiments with Cox (mealiness progress report December 1996). An explanation is that the O_2 concentration (1.4 % O_2) during the CA storage has been low enough so that the Cox apples produced ethanol by anaerobic respiration. Subsequently ethyl acetate could have been formed out of ethanol. In both cultivars 2-methyl propanol/hexanal, pentyl acetate and hexanol increased slightly. The identified peaks, which have not been discussed in this section, changed hardly during storage. The differences between the peak areas of the Cox samples increased as the storage times were longer, except for pentyl acetate which changed hardly after five days storage. The increases of the peaks during the storage of Jonagold were more gradually, and after 21 days in Jonagold most peaks decreased again. These results are in accordance with Brown (1965) who showed that the production of apple volatiles declined shortly after the peak of ethylene production. Ethylene initiates the ripening process. So Jonagold seems physiologically older than Cox.

Relation between GC-profile and sensory mealiness

The GC chromatograms were related to the sensory observed texture of the apples by statistical analysis. Ethanol was omitted from the analysis because the large changes were a result of anaerobic respiration during CA storage, and not of ripening of the apples themselves. The analysis was performed with 27 relative peak areas and with the total peak area of the 27 peaks. "Relative" means that the area of each peak of the chromatogram was divided by the total peak area of the 27 peaks. The average relative peak area of three chromatograms was used in the analysis. Principal Component Analysis (PCA) was applied to relate the GC-profiles of the two cultivars to each other and multivariate analysis (using PLS) and linear regression were used to relate the GC-chromatograms to mealiness. The results show a clear difference between the cultivars (PCA-plots in figure 16, PLS and linear regression results (not shown). For this reason the relation between mealiness and cultivar had to be determined for each cultivar seperately. This decreased the amount of objects from 10 to 5, so that the results are merely indicative. Results are summarized in table 8. The table shows that the relation between mealiness and GC-profiles is probably not the same for Cox as for Jonagold. Using PLS more peaks show a relation with mealiness than using linear regression, in Cox as well as in Jonagold. This indicates that the development of mealiness is probably connected to a complex shift of several peaks.

Table 8: *Identified volatile compounds isolated by static headspace analysis of Cox (C) and Jonagold (J) apples. Relation between relative GC peak areas and sensory observed mealiness (+ significant positive relation ; - significant negative relation ; (+) weak significant positive relation ; (-) weak significant negative relation).*

		Cox		Jonagold	
Peaknr.	**Compound**	**LR**	**PLS**	**LR**	**PLS**
1					
2	methyl				
3	ethyl acetate				
4	ethanol (C,J)				
5	propyl acetate		-	+	+
6	2-methyl				-
7			(-)		-
8				+	+
9	propanol (J)		-	+	+
10	butyl acetate		+	(+)	+
11	methyl		-		-
12					-
13	2-methyl		(-)	-	-
14			-		-
15					+
16				(+)	
17	butanol (C,J)		+	+	+
18	pentyl acetate				
19	2-methyl		-		
20			+		
21					
22	hexyl acetate				-
23					
24			-		
25	?-hexenyl		-		
26	hexanol (C,J)		(-)		-
27			-		-
Total Area			+	(+)	+

Conclusions

Most determined compounds are present in both Cox and Jonagold, but the relative intensity and behaviour in time are different.

The increase of the peaks is larger in Jonagold than in Cox. Aroma determined by sensory panel decreases during storage while 'total aroma' determined by GC increases.

In the coming period attention on the extraction procedure, to mimic chewing, will be continued. Also a real 'storage' will be followed.

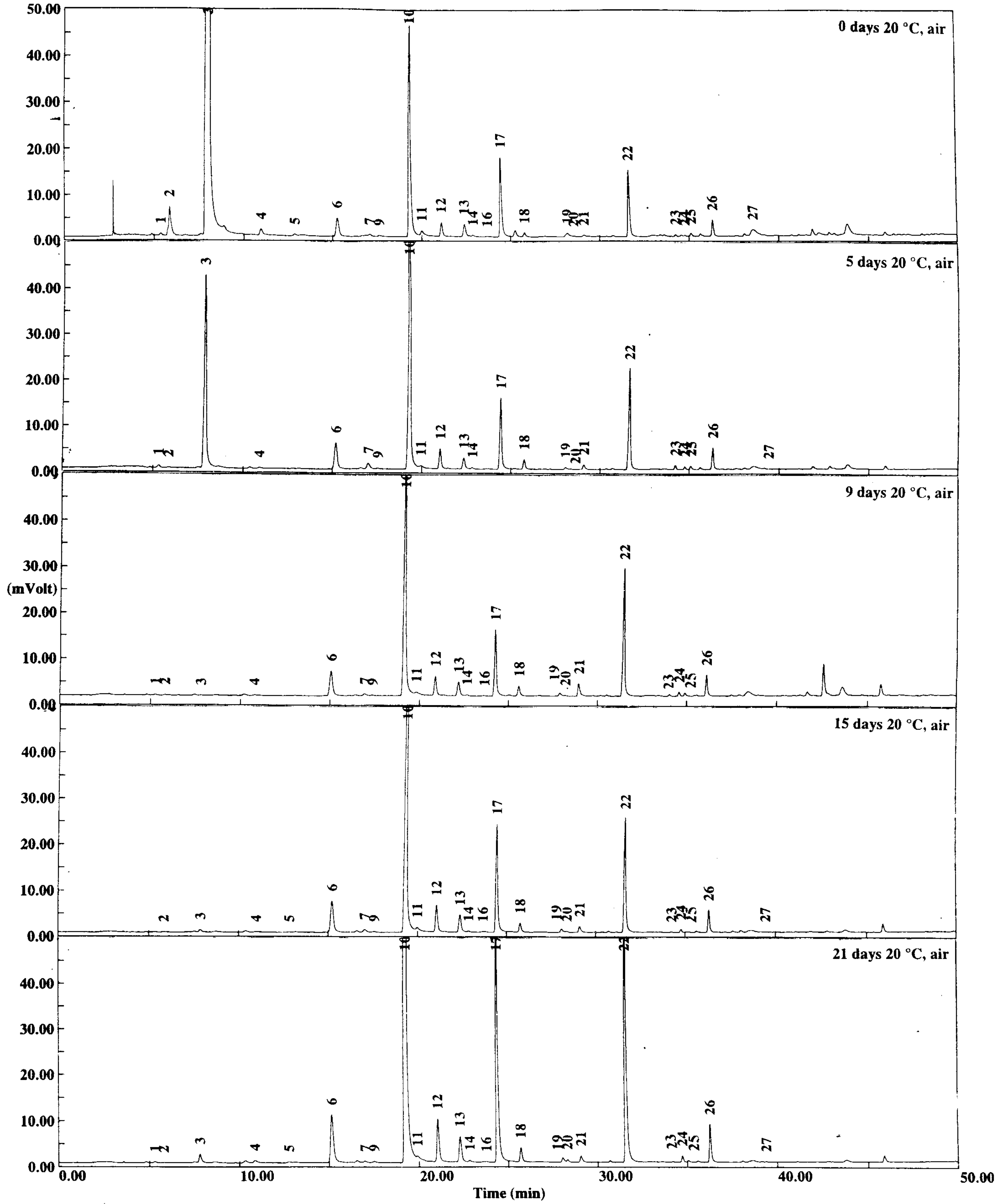


Figure 10: *Static headspace gas chromatograms of volatiles of Cox apple stored for 0 (control), 5, 9, 15 resp. 21 days at 20°C in air. Peak numbers correspond with compounds in fig. 12.*

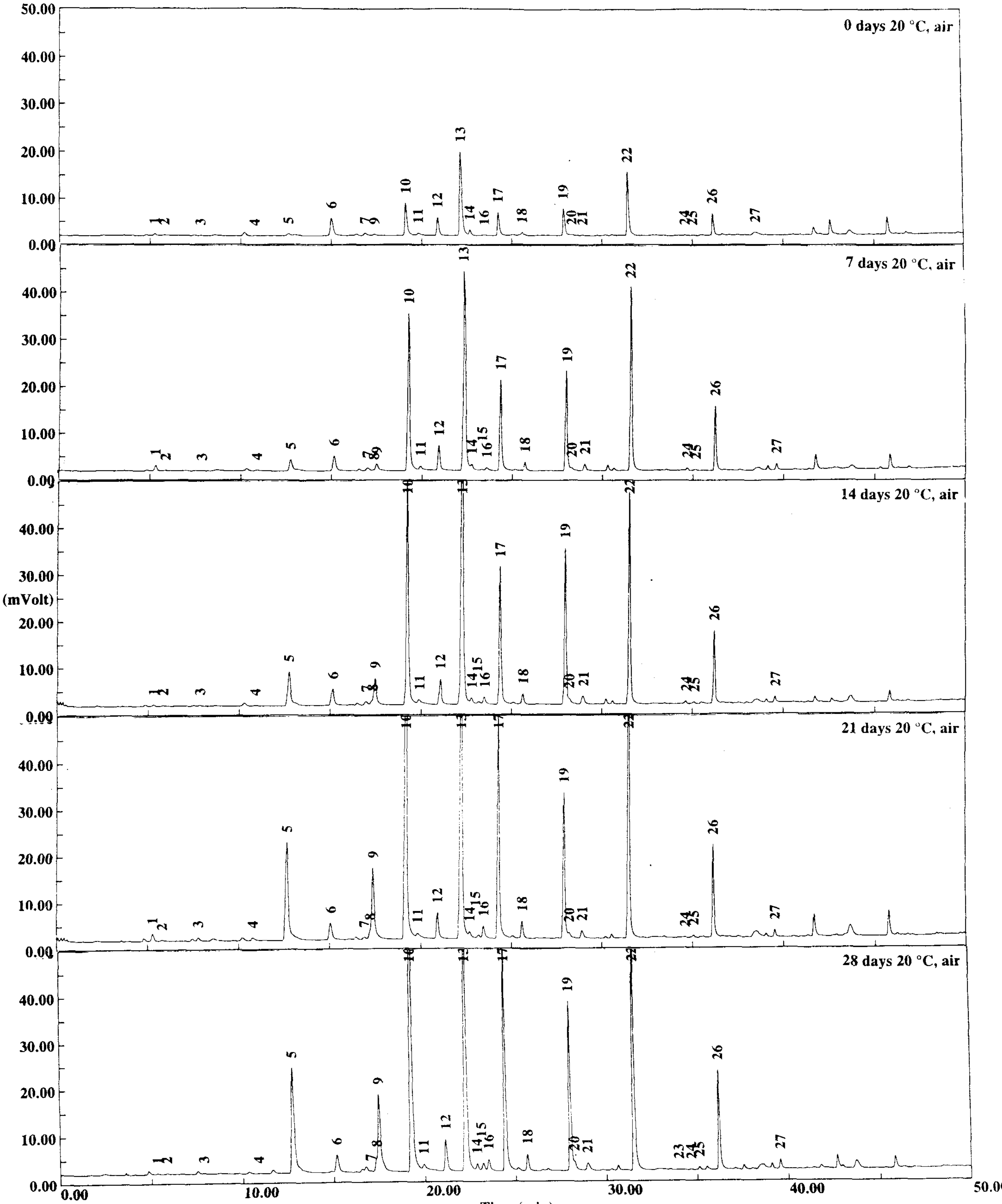


Figure 11: *Static headspace gas chromatograms of volatiles of Jonagold apple stored for 0 (control), 7, 14, 21 resp. 28 days at 20 °C in air. Peak numbers correspond with compounds in fig. 11.*

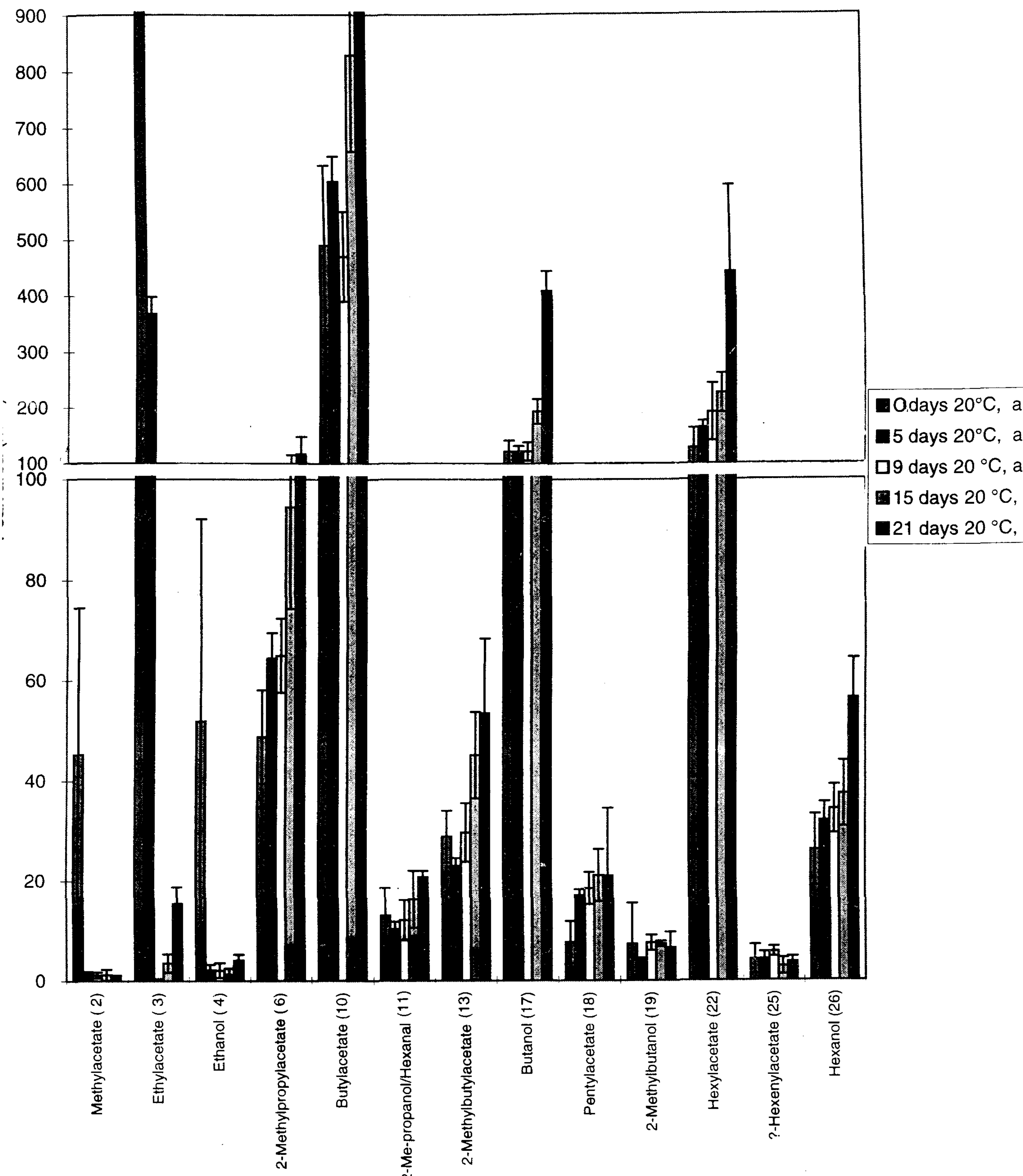


Figure 12: *Peak areas of 14 identified compounds of the gas chromatograms of Cox apple (fig. 8), stored for 0 (control), 5, 9, 15 resp. 21 days at 20°C in air.*

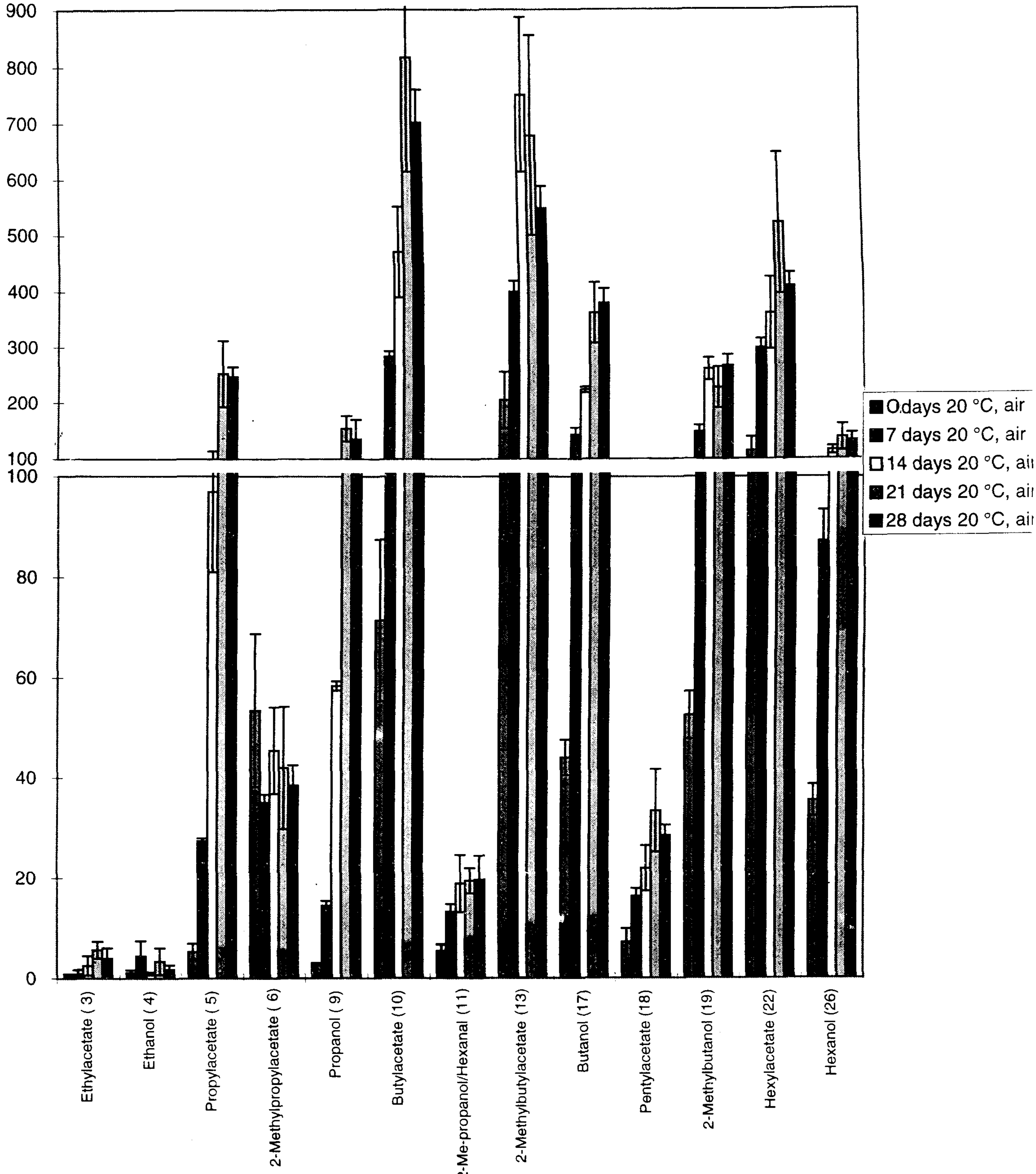


Figure 13: *Peak areas of 14 identified compounds of the gas chromatograms of Jonagold apple (fig. 9), stored for 0 (control), 7, 14, 21 resp. 28 days at 20°C in air.*

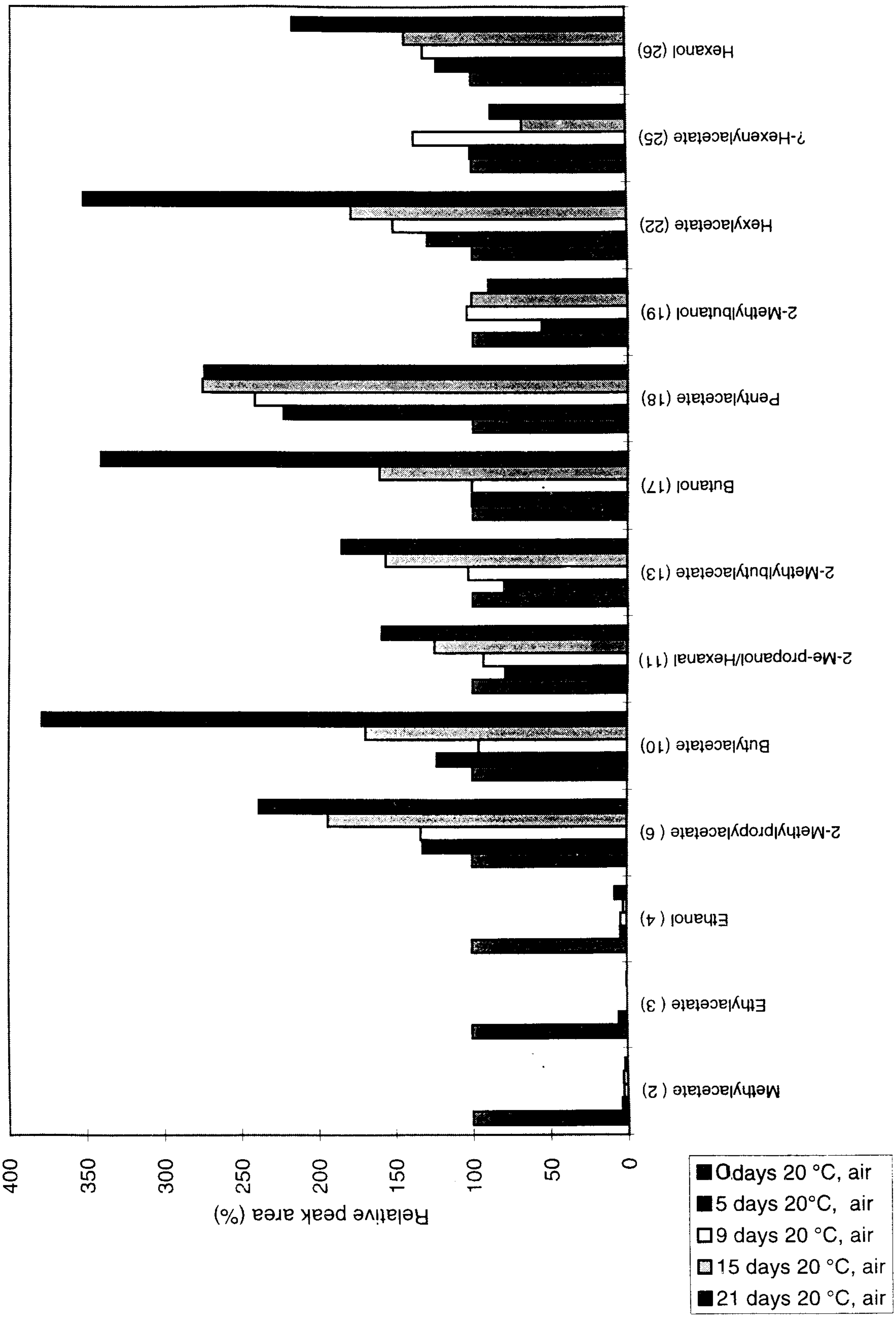


Figure 14: *Relative peak areas of 14 identified compounds of the gas chromatograms of Cox apple (fig. 8), stored for 0 (control), 5, 9, 15 resp. 21 days at 20°C in air.*

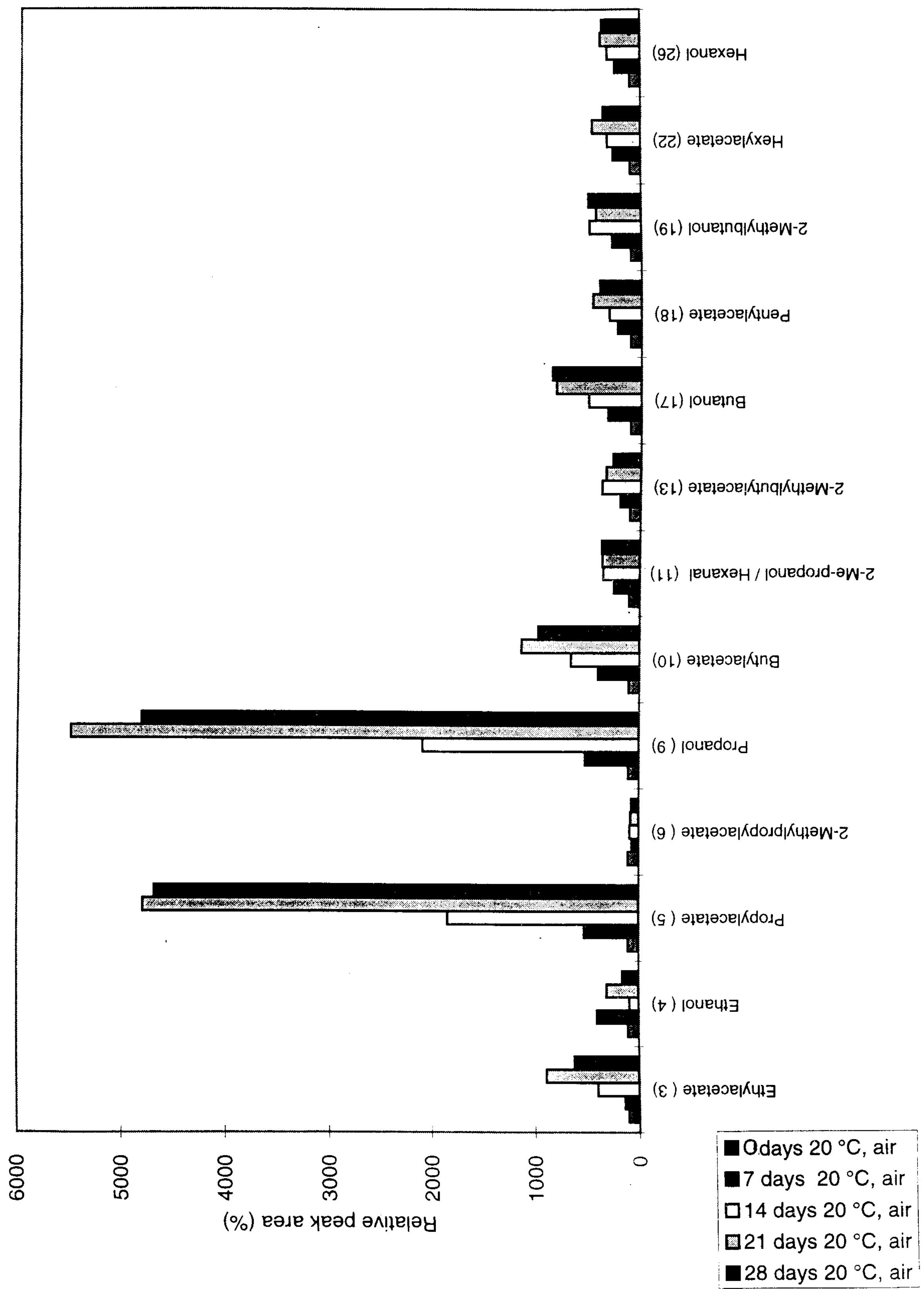
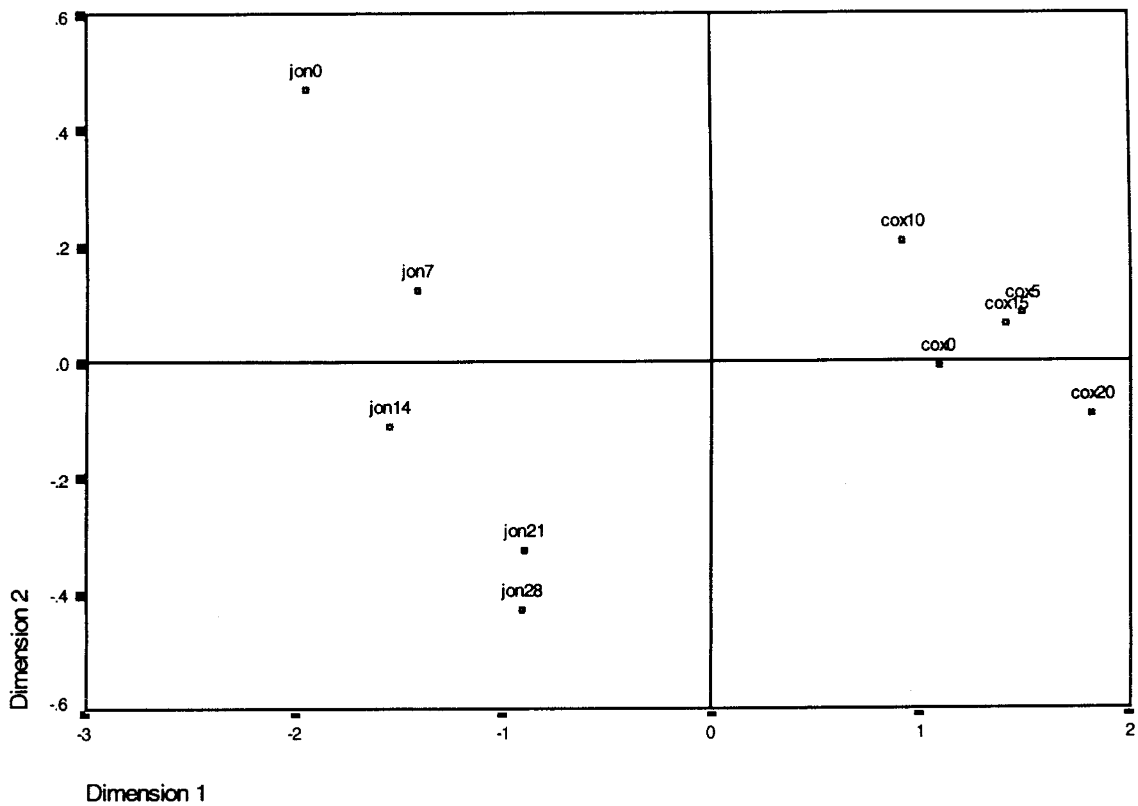
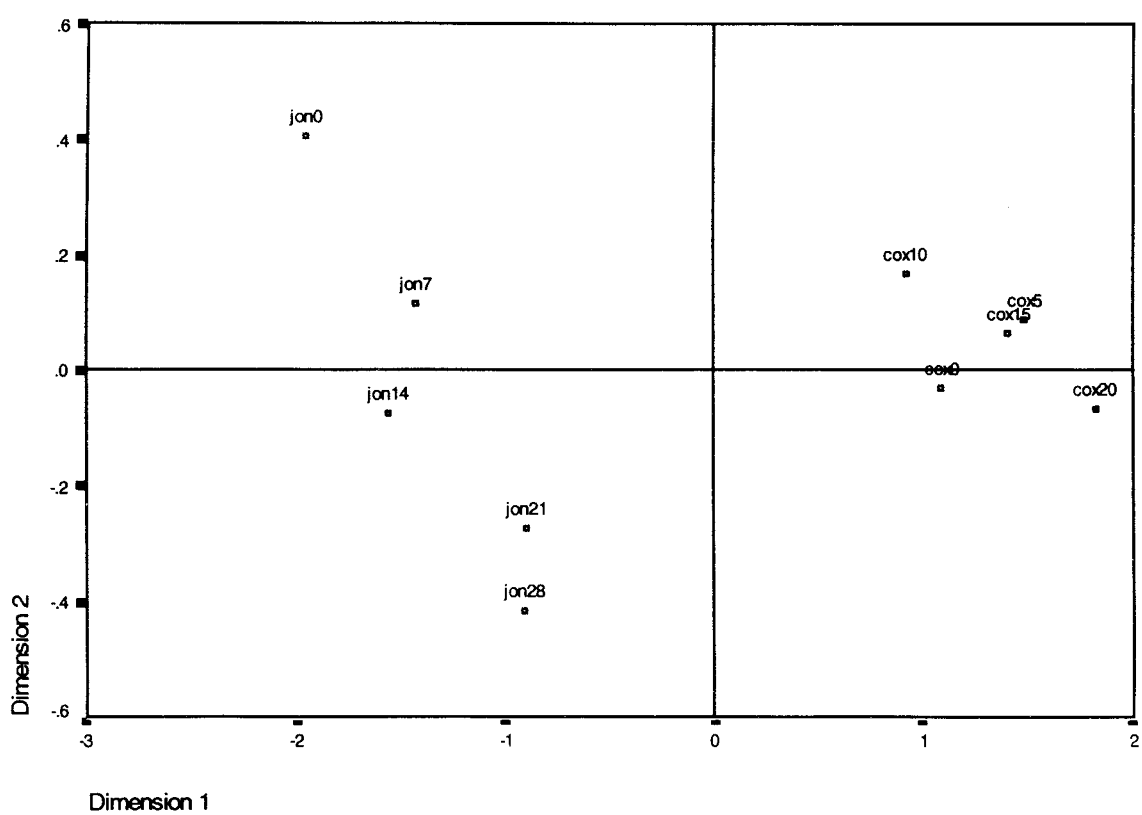


Figure 15: *Relative peak areas of 14 identified compounds of the gas chromatograms of Jonagold apple (fig. 9), stored for 0 (control), 7, 14, 21 resp. 28 days at 20°C in air.*

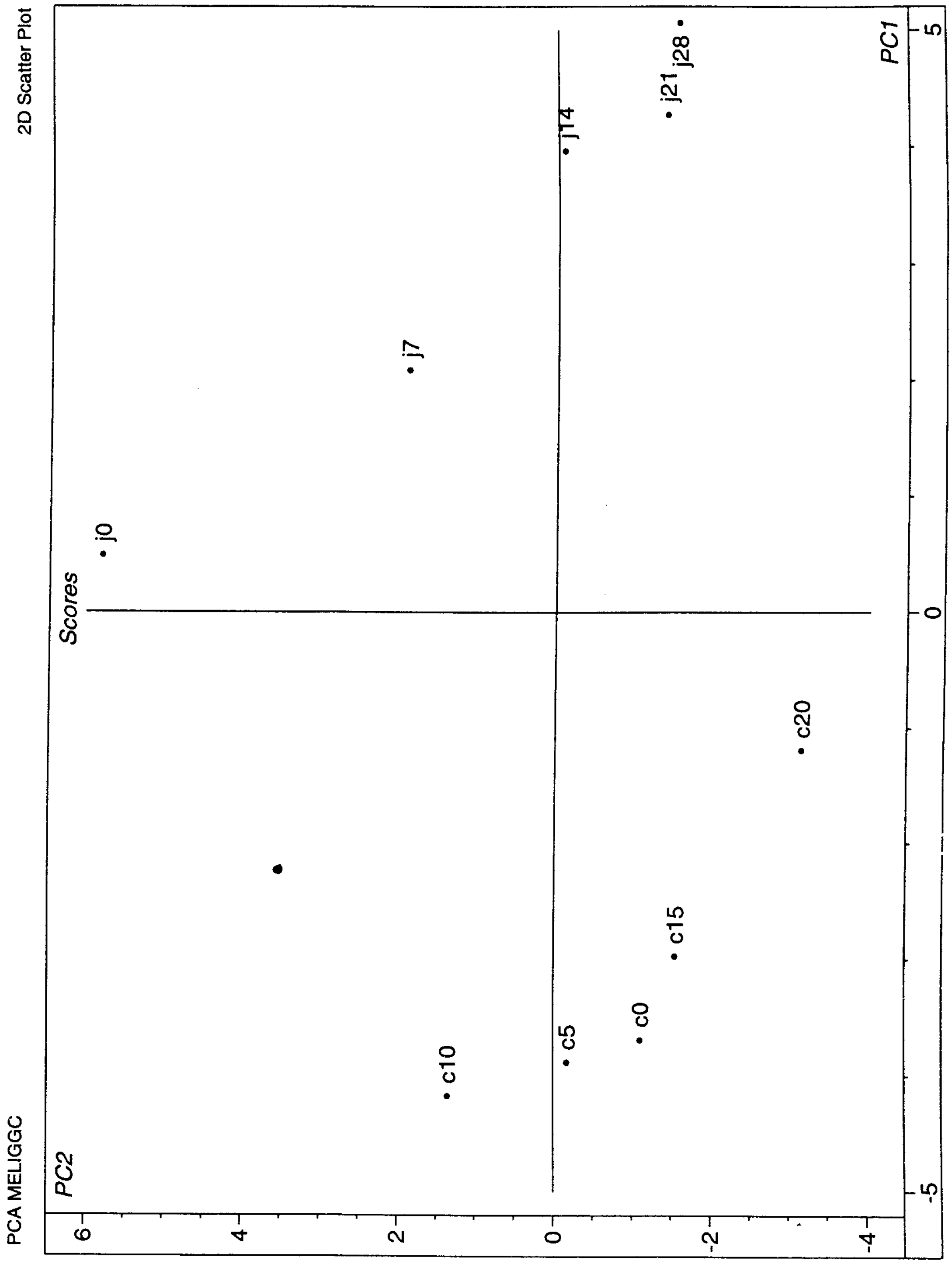
Derived Stimulus Configuration

Euclidean distance model





Principal Component Analysis of GC-data of COX and JONAGOLD



3.3 Taste compounds measured by HPLC

Experiment 1

Dry matter content

The dry matter content of Cox and Jonagold apples and for juice obtained from Jonagold apples is presented in Figure 16.

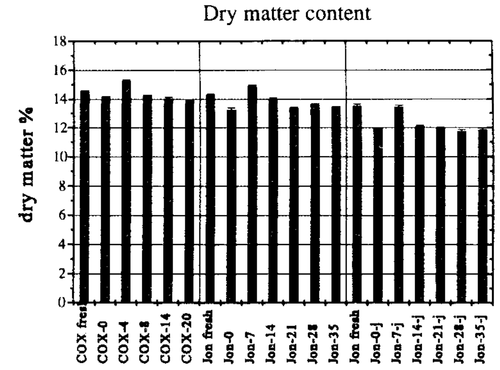
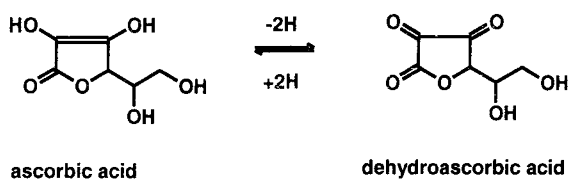


Figure 16: Dry matter content



The results indicate approx. 14% dry matter content for fresh and stored apples (for both Cox and Jonagold) and approx. 12% dry matter content for juice (consistent with the fact that sugars are the main components).

Total ascorbic acid content

The total ascorbic acid content (ascorbic acid + dehydroascorbic acid=TAA) is presented in Figure 17.

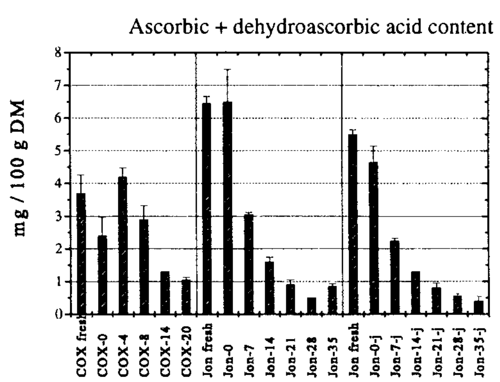


Figure 17: Total ascorbic acid content.

Total sugar content

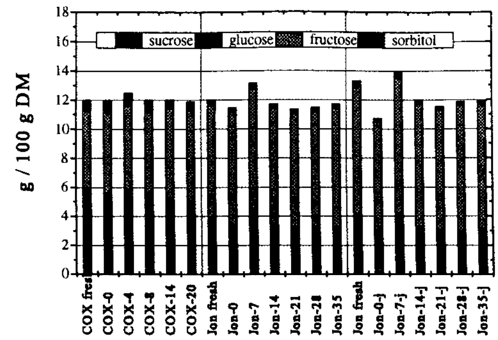


Figure 18: Total sugar content

A higher content of TAA in Jonagold as compared to Cox apples can be observed. There is a general trend of decreasing of TAA during storage due to oxidative processes.

c) Total sugar content

The total sugar content of Cox and Jonagold apples is presented in Figure 18. Approx. 12% total sugar content for both apple cultivar as well as for Jonagold-juice is obtained. Relating these results to the dry matter content it can be readily seen that sugars are the main non-volatile constituent of the apples.

Although the total amount of sugar is similar for Cox and Jonagold cultivars there are differences in composition:

Cox	fructose (5.3%) > sucrose (4.5%) > glucose (1.4%) > sorbitol (0.8%)
Jonagold	fructose (7.7%) > glucose (2.5%) > sucrose (1.4%) > sorbitol (0.4%)

Using the sweetness potency of the analysed sugars relative to sucrose as reference (Montcrieff): it is possible to calculate a theoretical sweetness index.

Table 9: Sweetness potency of some sugars

Sucrose	Glucose	Fructose	Sorbitol
100	74	174	54

The values obtained: 1519 (fresh Cox), 1707 (mealy Cox), 1682 (fresh Jonagold), 1965 (mealy Jonagold) are not significantly different. Also, no changes in total sugar content during storage were observed. These results are in agreement to the sensory results: the panel perceived no differences in sweetness between the 2 cultivars and for the same cultivar at different mealiness stages.

Organic acid content

Malic acid (HOOC-CH_2-CH(OH)-COOH) is the main organic acid constituent of apples and is related to the freshness/sourness of fruit, figure 18. There is a general trend of decreasing of malic acid content during storage for both cultivars. For Cox apples a level of about 325 mg / 100g DM was reached after 20 days storage at 20°C while for Jonagold apples a level of about 425 mg / 100g DM was reached after 21 days storage at 20°C. No significant differences in the citric acid content between different mealiness stages were observed.

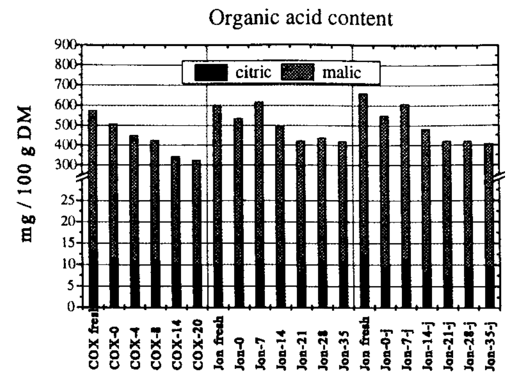


Figure 19: Organic acid content

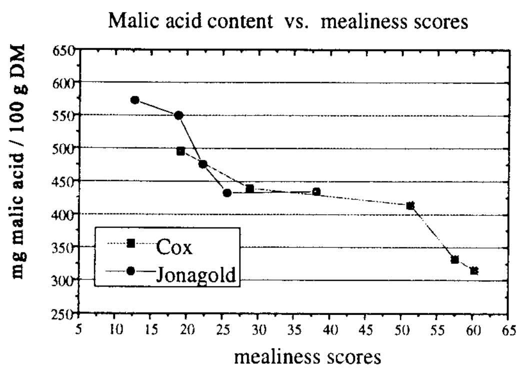


Figure 20: Relation between malic acid content and mealiness scores for COX and Jonagold apples.

Correlation between malic acid content and mealiness

An attempt was made to correlate the malic acid content with the mealiness scores as assessed by the sensory panel. The results are presented in Figure 20.

There is a general trend of decreasing of malic acid content with mealiness for both cultivars. The malic acid content for Jonagold is higher than for Cox.

Conclusions

a) For all experiments same results were obtained for pieces of apples and juice suggesting that both sampling techniques are suitable for analysis of non-volatile constituents of apples.

b) The total sugar content of the two cultivars analysed are similar consistent with the fact that no significantly differences in sweetness were perceived by the sensory panel.

c) A relation between the malic acid content and mealiness scores assessed by the sensory panel can be observed.

Experiment 2

HPLC analysis for malic acid content of juice obtained from Elstar cultivar was performed. The apples were sensory assessed by an expert panel.
Malic acid content of Elstar apples at different mealiness stages is presented in Figure 21. Large variation in experimental data are obtained due to the non-homogeneity of the biological material. During storage the level of malic acid decreased.

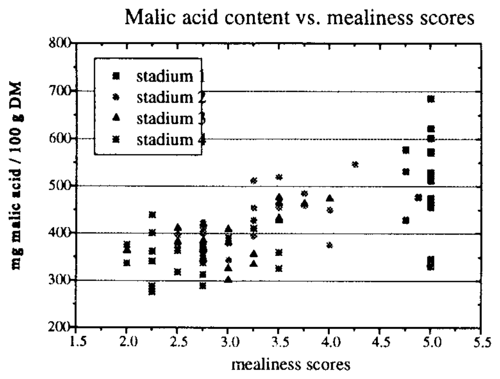


Figure 21: Malic acid content of ELSTAR apples at different mealiness levels

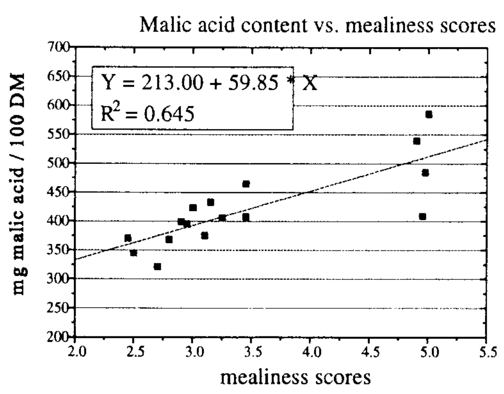


Figure 22: Correlation between malic acid content and mealiness for ELSTAR

A linear model was used in order to correlate the malic acid content and mealiness. The model obtained explained 65% of data variation.

Conclusion
A linear model with r^2=0.645 between malic acid content and mealiness scores was obtained. Mealiness can be described as a "multidimensional attribute" being usually related to a lack of crispiness and juiciness. In is possible that the use of a single variable is not enough to describe this complex attribute. Better linear/non-linear models might be obtained using a set of variables which encompass the changes in apples.

3.4 Maceration/Cell Sloughing

In figure 23 the weight percentage of the unmacerated tissue is plotted against expert panel mealiness scores. All datapoints are below the 100% value which means that in all cases there is a loss of tissue weight. As was observed visually the main contribution to this loss is caused by cell sloughing. Furthermore it can be seen that application of endo-PG decreases the amount of the unmacerated tissue, which correspond to a higher amount of cell sloughing. Evacuation of endo-PG into the tissue resulted in the highest amount of cell sloughing.

In all cases celll sloughing increased with increasing mealiness. In the first week, when the largest change in mealiness is observed, the increase is significantly larger in case that the enzyme is infiltrated into the tissue. In the following weeks the increase of cell sloughing with increase of mealiness is virtually constant.

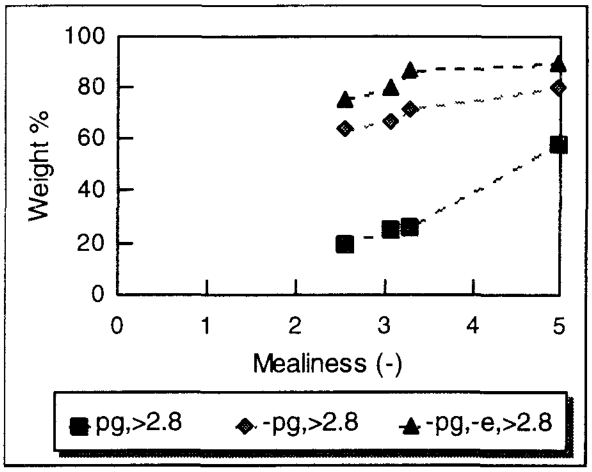


Fig.23: Relation between cell sloughing and sensory mealiness for Elstar apples.pg= incubated with pg, -pg=no pg was used; -e=no evacuation was applied.

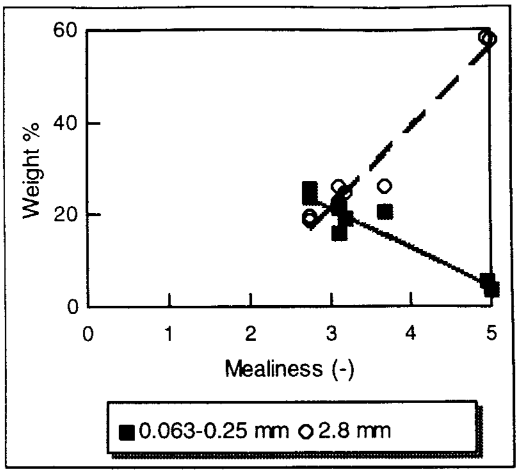


Figure24: Relation between cell sloughing (○), amount of individual loose cells (■) and sensory mealiness of Elstar apples.

In figure 24 it can be seen that the weight% remaning on the 0.063 mm sieve, consisting almost entirely of individual cells, is the mirror image of the unmacerated tissue weight%.

These observation are in agreement with the hypothesis that mealiness is accompanied by a change in the state of pectic fraction of the apple: the fact that endo-PG, an enzyme acting specifically on pectin, enhances cell sloughing as mealiness increases shows to an increasing susceptibility of the pectin (e.g. as a result of demethylation) to endo-PG action.

Conclusion
It can be concluded that this technique might be used for investigation of the decrease in middle lammella strength. In addition the individual cells obtained during the maceration experiment can be used for measurement of the cell wall strength. By this approach it might be possible to relate the change of the strength of the middle lammella and cell wall to development of mealiness or more general to texture changes.

3.5 Texture measurements

The relation of the liquid separation and the sensory mealiness is shown in figure 25. Because there was some variation in the weights of the cutted cylinders the liquid separation is corrected for the weight of the cylinder (liquid separation as % of weight of cylinder). Because the highest score (5) means not mealy at all and the lowest score (1) means very mealy, in all the figures the y axis is called “non mealiness”. It can be seen that there is a good relationship between mealiness and liquid seperation (R^2=0.72 and std. err.=0.56), with little liquid separation (20%) for mealy scored apples and more liquid separation (40%) for not mealy scored apples. This relation is in accordance with the expectations. After all the mealiness perception is strongly related with a dry feeling in the mouth and the lack of juiciness.

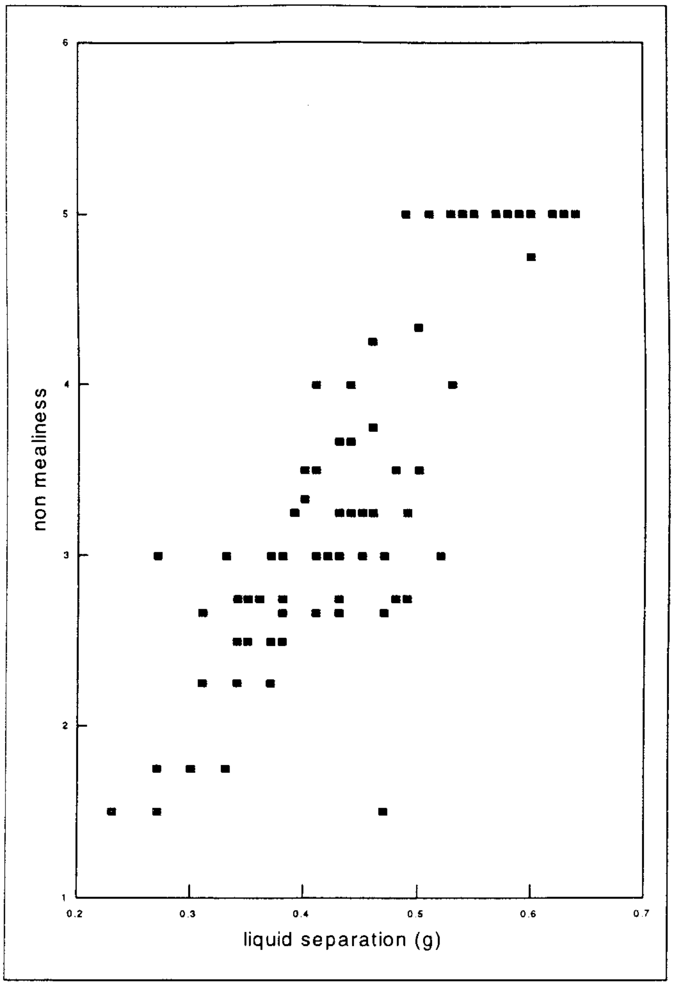


Figure 25: Sensory mealiness in relation to the liquid separation at the 'ATO-method' texture measurement

In figure 26 the relation between the sensory mealiness and the failure stress is shown. The relation between these two parameters (R^2=0.70 and std. Err=0.58) is almost the same as between liquid separation and sensory mealiness. The relation of the area to failure, slope and modulus with sensory mealiness are less good with R^2 of respectively 0.46, 0.65 and 0,55. However the relation of total area with the sensory mealiness is better than for the liquid separation with R^2=0.80 and a std. Err=0.48 and as can be seen in figure. 27. From this figure it can be concluded that it takes less energy to destruct mealy apples than not mealy apples.

The relations of the modified UPM-method with the sensory mealiness are lower than for the 'ATO-method' results. The R^2 values of Fmax, the plastic deformation and the degree of elasticity with the sensory mealiness are respectively 0.42, 0.0003 and 0.0003. However there was enough variation in these parameters (Fmax 20 to 80 N, plastic deformation of 0-0.5 and degree of elasticity of 0.5 to 1), no relation with sensory mealiness can be established.

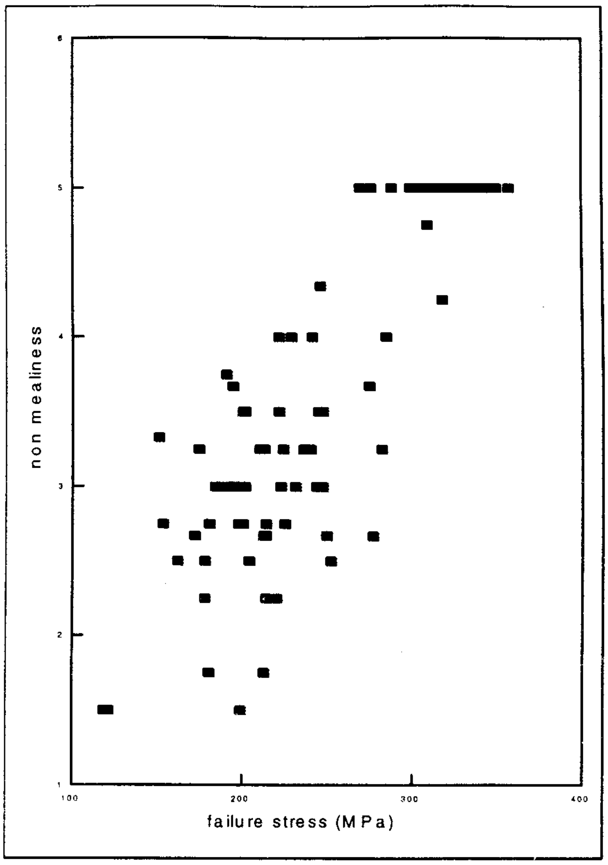


Figure 26: *Sensory mealiness in relation to the failure stress ('ATO-method')*

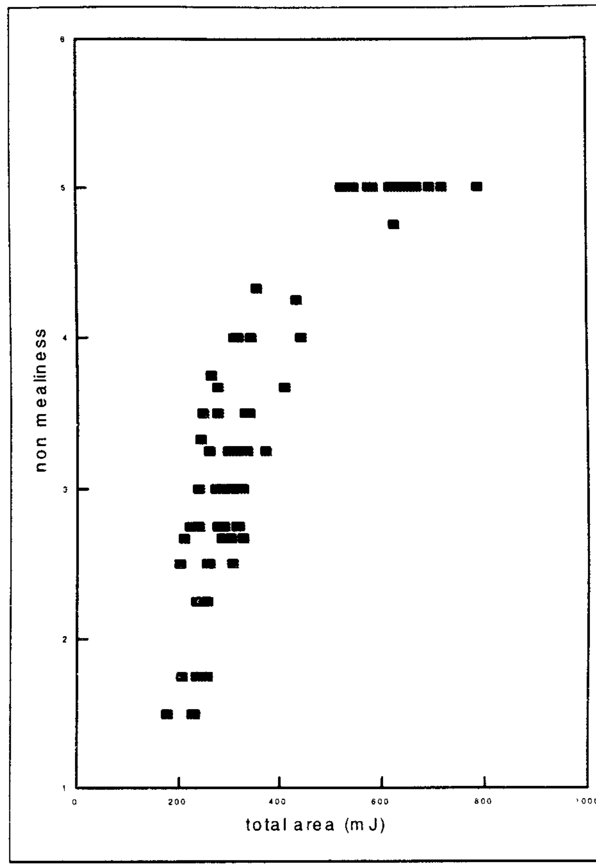


Figure 27: *sensory mealiness in relation to the total area ('ATO-method')*

Conclusions

By means of the applied method, linking the sensory and instrumental measurement to one and the same apple, it appeared to be possible to find relatively good relationships: the liquid separation and the total area (parameters of the ATO-method) showed good relationship with the sensory mealiness. With this experiments it is shown that liquid separation and total area are potential predictors of the sensory mealiness. To confirm the predictive capacity of these two parameters, these experiments have to be repeated in a larger scale with more apple varieties.

The linkage of the instrumental and sensory measurement to one and the same apple has also the advantage that each apple is a sample. When it is not possible to measure on the same apple, a lot of repetitions of the measurements have to be performed to obtain a good average of the batch and to be able to make comparisons between measuring methods. So, 10 to 20 apples have to be measured for each batch representing one sample.

4. Tomatoes

1. Dutch tomatoes obtained via the market

From approximately 80 batches of tomatoes traced at the market, a selection was made of tomatoes which might show mealiness. Criteria were the size (large tomatoes) and the type (beef tomatoes, multi segmented tomatoes). These tomatoes were subsequently assessed by a panel of three ATO-DLO-experts on texture, especially mealiness.
Based on the expert panel judgement of mealiness 7 batches were selected, showing some level of mealiness (see table 10).

The origin of four batches was traced via The Greenery and the growers were asked to deliver some samples for a second assessment of the texture.
The tomatoes were picked at the 'Auction-ripe' state and were delivered at ATO-DLO one day after harvest. Upon arrival at ATO-DLO they were immediately stored at 20°C to ripen. Three and six days later the multi segmented/round tomatoes and the beef tomatoes respectively were assessed by the expert panel on texture.

Table 10: Texture assessment of the 'Dutch' tomato

nr.	Tomato Type	Texture Description in the first assessment	Mealiness level 0------------------------------100
1	Multi segmented	mealy	Foreign tomato
2	Beef tomato	mealy	Foreign tomato
3	Tomato, large, multi segmented	not mealy, soft	Foreign tomato
4	Tomato, multi segmented	mealy, juicy, sweet	0------------------100 X
5	Beef tomato, 72-82 mm	mealy, sour, juicy	0------------------100 X
6	Round tomato, 57-67 mm	slightly mealy and sweet, high in aroma	0------------------100 X
7	Beef tomato, 72-82 mm	slightly mealy, firm, slightly sour, aroma less	0------------------100 X

The result is given in table 10. It can be seen that these tomatoes almost show no mealiness.

2. Tomatoes obtained via KU-Leuven/VBT

The tomatoes picked at the 'Auction-ripe' state were picked on Wednesday in Leuven and delivered at ATO-DLO the next day by express mail. Upon arrival at ATO-DLO they were immediately stored at 20°C to ripen.
Three days later the tomatoes were assessed by the expert panel on texture.

The result is given in table 11. It can be seen that these tomatoes also showed a minor level of mealiness and that there was little difference in it.

Table 11: Texture assessment of the 'Belgian' tomato

nr.	**Tomato Cultivar**	**Texture Description in the first assessment**	**Mealiness level** 0------------------100
1	Adelaide	Dry, not firm	0------------------100 20
2	Grace	Tough peel, mashy	0------------------100 15
3	Blitz	Tough peel, mashy	0------------------100 15-20
4	DRW3450	mashy, juicy	0------------------100 30
5	Tradiro	Pericarp is a bit mealy and a bit dry	0------------------100 30

Conclusion
The results show that:
- the mealiness levels are very low and that
- the differences are very small.

This means that a) there is no range for training a panel or performing a measurement session.

Without mealy tomatoes it is difficult to continue this part of the project. However, by redefinition of the problem (softness instead of mealiness) we might continue.

Redefinition of the problem
An important textural problem of tomatoes is getting soft. It is generally accepted that softness in tomatoes is related to the activity of endo-polygalacturonase. This enzyme degrades the pectin of e.g. the middle lamella causing i.a. cell separation, eventually resulting in softness.

Because the development of mealiness in apples is also attributed to the degradation of the middle lamella the hypothesis can be postulated that the phenomena leading to softness in apples are the same as those which lead to mealiness in apple and that the difference in sensorial perception is caused by a different level of the cell wall strength of the individual cells: in tomatoes it is low so that cells are broken very easily through the cell walls; in apples it is high so that cells are more resistant to mechanical stress.

Using this approach the central theme of the project 'degradation of the middle lammella' is kept.

In this alternative the question of the economic importance of texture problems in tomatoes remains. Another point to consider is that there already a tremendous amount of literature about the softness of tomatoes. A directed literature search is required.

4. Conclusion

Sensory Measurements

From the sensory measurements in experiment 1 it can be concluded that during storage in plastic bags at 20°C:

- Cox's Orange Pippin and Jonagold apples behave similar, in that more or less the same changes occur with respect to the texture properties. However, the timescale for the changes to occur is shorter for Cox.
- the firmness, crispness, moistness and sourness decrease while the mealiness, graininess and dryness increase. The sweetness and total aroma intensity differ between different apples (within product variation).

The Chewing experiments revealed that:

- The assessors of the analytical sensory panel agree on the attributes juicy and mealy.
- For the attribute crispy and firm the assessors don't behave in the same way.
- The maximum scores given for the texture attributes don't differ that much, only the moment at which the maximum score is reached differs.
- The apple pieces were not homogeneous enough to create good profiles for sweetness and sourness.

In experiment 2 an increase of mealiness, assessed by an expert panel, was observed for Elstar during storage in plastic bags 20°C. The largest decrease was observed in the first week of storage in the bags.

NIRS

In experiment 1 the best models were obtained in calibration of the NIRS spectra with firmness, mealiness and sourness. R-squared for validation RSQC, using 3 factors, were all above 0.9 while R-squared for cross validation RSQV were 0.62, 0.69 and 0.78 respectively. The lower R-squared for cross validation is attributed to: large variation within the batches, the high standard deviation in the sensory data and the relative small data set used.

In order to improve the results experiment 2 was performed: in this experiment the variation within a batch was omitted by using individual apples and the data set was larger. This approach had the draw back that the analytical sensory panel could not be used. The R-squared of calibration, RSQC= 0.93, was approximately the same as that for the experiment 1, while the R-squared for cross vallidation was somewhat lower, RSQV=0.64. Prediction of malic acid content by NIRS showed the same prediction potential as for mealiness.

The results thus far clearly show the possibility of to predict sensorial attributes by NIRS spectral data. However, further development is required.

Gas chromatographic Analysis

Most determined compounds are present in both Cox and Jonagold, but the relative intensity and behaviour in time are different. The increase of the peaks is larger in Jonagold than in Cox. Aroma determined by sensory panel decreases during storage while 'total aroma' determined by GC increases. This is in agreement with the hypothesis that mealiness retards the liberation of compounds located insight the cells during chewing.

HPLC Analysis of Taste Compounds
Comparison of extraction of taste compounds from homogenised apple and from apple juice showed little or no difference. So, it was concluded that both procedures are suitable for analysis of non-volatile compounds in apples gave the same results Also a real 'storage' will be followed.

Cox and Jonagold had similar total sugar content, which is consistent with the observation that no significant differences in sweetness were perceived by the sensory panel.
For Cox and Jonagold in experiment 1 as well as for Elstar in experiment 2 a relation between the malic acid content and mealiness scores assessed by the sensory panel was observed.

For Elstar, in experiment were a individual apple approach was followed, a linear relation with r^2=0.65 between malic acid content and mealiness scores was obtained.

Mechanical Measurement
By means of the applied method, linking the sensory and instrumental measurement to one and the same apple, it appeared to be possible to find relatively good relationships: the liquid separation and the total area (parameters of the ATO-method) showed good relationship with the sensory mealiness. With this experiments it is shown that liquid separation and total area are potential predictors of the sensory mealiness. To confirm the predictive capacity of these two parameters, these experiments have to be repeated in a larger scale with more apple varieties.

Enzymatic Maceration/Cell Sloughing
It can be concluded that this technique might be used for investigation of the decrease in middle lamella strength. In addition the individual cells obtained during the maceration experiment can be used for measurement of the cell wall strength. By this approach it might be possible to relate the change of the strength of the middle lamella and cell wall to development of mealiness or more general to texture changes.

Tomatoes

Conclusion
The results show that the mealiness levels were very low and that differences were very small.
So there was no range for training a panel or performing a measurement session. Without mealy tomatoes it is difficult to continue this part of the project. However, by redefinition of the problem (softness instead of mealiness) we might continue.

Redefinition of the problem
An important textural problem of tomatoes is getting soft. It is generally accepted that softness in tomatoes is related to the activity of endo-polygalacturonase. This enzyme degrades the pectin of e.g. the middle lamella causing i.a. cell separation, eventually resulting in softness.

Because the development of mealiness in apples is also attributed to the degradation of the middle lamella the hypothesis can be postulated that the phenomena leading to softness in apples are the same as those which lead to mealiness in apple and that the difference in sensorial perception is caused by a different level of the cell wall strength of the individual cells: In tomatoes it is low so that cells are broken very easy through the cell walls; in apples it is high so that cells are more resistant to mechanical stress. Using this approach the central theme of the project 'degradation of the middle lamella' is kept.

5. Dissemination

1 Muresan. S. et. al. Mealiness in Fruits: Consumer Perception and means for Detection: Instrumental assessment of mealiness. Lecture given at the COST96-Conference 'Interaction of Food Matrix with small ligands', 9-11 November 1997, Garching, Germany.

2 Muresan. S. et. al. Mealiness in Fruits: Consumer Perception and means for Detection: Instrumental assessment of mealiness. In: Proceedings of COST96-Conference 'Interaction of Food Matrix with small ligands', 9-11 November 1997, Garching, Germany. (To be published)

3 Biekman E., A. Van Schaik, J. Termeer, C. Boeriu, L. Tijskens en C. Van Dijk. Meligheid in appelen: Waarneming door de consument en methoden voor detectie. (Submitted to Fruitteelt).

6. Literature references

Bartley, I.M. and Knee, K. The chemistry of textural changes in fruit during storage. Food Chemistry, 1982, 9, 47-58.

Brown, D.S. Volatiles from apple fruits as related to variety, maturity and ripeness. Proc.Am.Soc.Hortic.Sci. 1966, 88, 98-104.

Drawert, F. Über die Biogenese von Aromastoffen bei Pflanzen und Fruchten. Phytochemistry, 1968, 7, 65-88.

Girard, B. and Lau, O.L. Effect of maturity and storage on quality and volatile production of 'Jonagold apples'. Food Research International, 1995, 28(5), 465-471.

Knee, M. The metabolism of alcohols by apple fruit tissue. J.Sci.Food Agric. 1981, 32, 593-600.

Paillard, N. Analyse des produits organiques volatils émis par quelques variétés de pommes. Fruits, 1967, 22, 141-151.

Pijpers, D. Fruit uit alle windstreken. Het Spectrum, Utrecht, 1992. ISBN 90 274 2992 8.
Fukushima, T. Chilling-injury in cucumber fruits. VI. The mechanism of pectin demethylation. Scientia Horticulturae, 1978, 9, 215-226.

Stone, H., Sidel, J.L., Sensory Evaluation Practices, Inc. London, 1993.

Appendix 1

2nd Collaborative Testing on Mealiness Assesment in Apple Comparison between Sensorial and Instrumental Measurements.
13-15 Januari 1998
ATO-DLO, Wageningen, The Netherlands

Objective:
Comparison between sensory perceived mealiness and instrumental measurement of mealiness.

Introduction
A first collaborative test was performed at IFR (Reading, England) from 26th to 30th of November 1996. Results of this experiment were presented at the progress meeting held at KU Leuven 18-19 september 1997.

Because of practical problems, not all measurement techniques were used, this second collaborative measurement, to be held at ATO-DLO, was agreed upon.
It was also agreed to use the experimental design which was used at the first collaborative test.

In this second test efforts have to be made to use all relevant techniques which are investigated in this project.

Measurement Techniques

Sensory Analysis	ATO	Jannemiek Termeer
Non-Destructive Measurements		
NIRS	B+L	Heinrich Prüfer
Acoustic Impulse Response	KUL	Veerle De Smedt
Ultrasonic Wave Propagation	KUL	Veerle De Smedt
Destructive Measurements		
Confined Compression	UPM	Pilar Barreiro
GC	ATO	E. Biekman/Y. Gerritsen
HPLC	ATO	Truke Ebbenhorst
Microscopy	KUL	Veerle De Smedt

Experimental Design
Three apple varieties are selected:COX, STARKING and JONAGOLD. These apples will be subjected to mealiness treatments, by VCBT, resulting in three levels of mealiness for every variety. The apples will be divided into three sets:

~~Set 1, Mechanical Set. Techniques Applied~~	~~NIRS, Mechanical resonance, confined compresion, ultrasound and microscopy~~ This set will not be necessary
Set 2, Sensorial Set. Techniques applied:	Sensory analysis, NIRS, Mechanical resonance, confined compresion, ultrasound and microscopy

Set 3, Chemical Set. Techniques applied HPLC and GC

As ATO uses a larger panel sensorial panel 12 apples/sample will be used

Table 1: Experimental Design

		~~ML1~~	~~ML2~~	~~ML3~~	~~Amount of appless~~
~~**Set 1**~~ ~~**Mechanical Set**~~	~~COX~~	~~C1..xx~~	~~C2..xx~~	~~C3..xx~~	~~18~~
		~~C1..xx~~	~~C2..xx~~	~~C3..xx~~	~~18~~
		~~C1..xx~~	~~C2..xx~~	~~C3..xx~~	~~18~~
	~~Starking~~	~~S1..xx~~	~~S2..xx~~	~~S3..xx~~	~~18~~
		~~S1..xx~~	~~S2..xx~~	~~S3..xx~~	~~18~~
		~~S1..xx~~	~~S2..xx~~	~~S3..xx~~	~~18~~
	~~Top Red~~	~~J1..xx~~	~~J2..xx~~	~~J3..xx~~	~~18~~
		~~J1..xx~~	~~J2..xx~~	~~J3..xx~~	~~18~~
		~~J1..xx~~	~~J2..xx~~	~~J3..xx~~	~~18~~
	~~**Total Amount of Apples Set 1/Cultivar**~~				~~**54**~~
Set 2 **Sensory Set**	COX	C1..xx	C2..xx	C3..xx	36
		C1..xx	C2..xx	C3..xx	36
		C1..xx	C2..xx	C3..xx	36
	Starking	S1..xx	S2..xx	S3..xx	36
		S1..xx	S2..xx	S3..xx	36
		S1..xx	S2..xx	S3..xx	36
	Jonagold	J1..xx	J2..xx	J3..xx	36
		J1..xx	J2..xx	J3..xx	36
		J1..xx	J2..xx	J3..xx	36
	Total Amount of Apples Set 2/Cultivar				**108**
Set 3 **Chemical Set**	COX	C1..xx	C2..xx	C3..xx	60
	Starking	S1..xx	S2..xx	S3..xx	60
	Jonagold	J1..xx	J2..xx	J3..xx	60
	Total Amount of Apples Set 3/Cultivar				**60**
Total Amoount of Apples for the whole experiment*/Cultivar					**212 (=50 kg)**

For each cultivar ca. 50 kg. are required in the experiments. Most probably a selection of 'good' apples (disgarding rot apples or apples with the wrong size) have to made made from a larger batch.

QUESTION: With which percentage we have to increase this amount??

TIMETABLE

Monday

Morning	- Labeling of apples/Installation of equipment
Afternoon	- labeling of apples/Installation of equipment
Evening	- Apples are taken out of cold storage

Tuesday Morning:First experiment; First Sensory Session; 36 apples

8.00 - 10.30	Measurement NIRS and Mechanical Resonance for the first sensory panel session Max. 4 minutes/apple
10.30 - 11.00	preparation of samples for sensory, TA and ultrasound
11.00 - 12.30	Measurement:sensory, TA and ultrasound

Tuesday Afternoon:First experiment; Second Sensory Session; 72 apples

10.30 - 13.30	Measurement NIRS and Mechanical Resonance for the second sensory panel session. Max. 2.5 minutes/apple
13.30 - 14.00	Preparation of samples for sensory, TA and ultrasound
14.00 - 15.30	Measurement:sensory, TA and ultrasound
15.30 - 17.00	Missings/Evaluation/Data analysis
Evening	- Apples are taken out of cold storage

Wednessday Morning:Duplo expeiment; First Sensory Session; 36 apples

8.00 - 10.30	Measurement NIRS and Mechanical Resonance for the first sensory panel session Max. 4 minutes/apple
10.30 - 11.00	Preparation of samples for sensory, TA and ultrasound
11.00 - 12.30	Measurement:sensory, TA and ultrasound

Wednessday Afternoon: Duplo experiment; Second Sensory Session: 72 apples

10.30 - 13.30	Measurement NIRS and Mechanical Resonance for the second sensory panel session. Max. 2.5 minutes/apple
13.30 - 14.00	Preparation of samples for sensory, TA and ultrasound
14.00 - 15.30	Measurement:sensory, TA and ultrasound
15.30 - 17.00	Missings/Evaluation/Data analysis
Evening	- Apples are taken out of cold storage

Thursday Morning: Triplicate experiment; First Sensory Session; 36 apples

8.00 - 10.30	Measurement NIRS and Mechanical Resonance for the first sensory panel session Max. 2.5 minutes/apple
10.30 - 11.00	Preparation of samples for sensory, TA and ultrasound
11.00 - 12.30	Measurement:sensory, TA and ultrasound

Thursday Morning: Triplicate experiment; Second Sensory Session: 72 apples

10.30 - 13.30	Measurement NIRS and Mechanical Resonance for the second sensory panel session. Max. 2.5 minutes/apple
13.30 - 14.00	Preparation of samples for sensory, TA and ultrasound
14.00 - 15.30	Measurement:sensory, TA and ultrasound
15.30 - 17.00	Missings/Evaluation/Data analysis

Measurement Scheme for the different Sets:

~~***Set 1,Mechanical Set.*** ***Required Space*** ***Measurement Time*** ***Sample Size***~~
~~1) NIRS ? ? Whole~~
~~2) Mechanical Resonance ? ? Whole~~
~~3a) Ultrasound ? ? 1/4~~
~~3b) Confined Compresion ? ? 1/4~~
~~(4a microscopy) ? ? ?~~

Set 2, Sensorial Set.

1) NIRS	?	?	Whole
2) Mechanical Resonance	?	?	Whole
3a) Sensory Analysis	?	?	2/4
3b) Ultrasound	?	?	1/4
3c) Confined Compression	?	?	1/4
3d) Microscopy	?	?	?

Set 3, Chemical Set

1a)HPLC	?	?	Whole
1b) GC	?	?	Whole

Partner information

Partner	Person	Arrival	Departure	Labspace (cm*cm)	Power Points	Measuring time (min)
KUL	Veerle De Smedt + 1 person	12/01	16/01	De Instron	4	1-2
UPM	Pilar Barreiro + 1 person	12/01	16/01	120 * 60 cm	3	1-2
B+L	Christel Kurowski	12/01	16/01	2.5 m labt.	4	1.5-2.5