Dienst Landbouwkundig Onderzoek
Instituut voor Agrotechnologisch Onderzoek

Mealiness in Fruits
Consumer perception and means for detection

1st annual progress report

CONFIDENTIAL

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Geachte heer Nicolai,

Hierbij zenden wij u in tweevoud het "1st annual progress report Mealiness in Fruits - Consumer perception and means for detection".

Met vriendelijke groet,

p/a NB Hayn

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Contents

Summary

Within this project measurement techniques for quantification of 'mealiness' investigated by ATO-DLO are: sensory analysis, gas chromatography of aroma compounds, HPLC determinations of sugars and acids, near infrared spectroscopy (NIRS) and impedance measurement. In the previous period the measurement methods were optimised c.q. further developed for measurement of apples with different degrees of mealiness.

The main experiments described in this report are performed with **COX appels** received from VBT. After arrival the apples were characterised visually and by a one person expert sensory evaluation and stored under controlled atmosphere conditions.
With respect to the **sensory analysis** the first aim is to train a panel of products experts sensorily which is capable of distinguishing and quantifying in the first place the texture and secondly the taste and aroma attributes.
With respect to **GC** and **NIRS** the aim was to investigate wether the technique can discriminate between apples with different degrees of texture and aroma developed during storage.
HPLC experiments were conducted to investigate 1) the within batch and 2) the within apple variation. *(Results were not yet available, they will be reported in next period).*
Electrical Impedance measurements were not yet performed.

A overall visual characterisation of the apples, on arrival at the ATO, showed that the batches were very heterogenous with respect to size.
In order to develop different levels of mealiness the appels transferred from CA to plastic bags and stored during 5, 10, 15 and 20 days at 20°C.
With respect to sensory characterization the majority of the panellist were able to distinguish and to quantify the different texture attributes. In general a change in texture could be observed as a function of storage time. However, apples stored for 10 days behaved differently. With respect to taste and aroma additional extra training of the panel is needed.
Different methods for homogenization and sampling for GC-determination of aroma components were investigated. Static, head space sampling with TENAX of homogenate made by blending of frozen tissue proved to be best method. A large different was observed between the control and apples stored for 10 days. The different between 10 and 20 days storage was lower but distinct. Aroma profile changed in that a relative decrease in lower boiling components was observed, while compounds with higher boiling points increased.
With NIRS a large variations in the spectra of individual apples from the same batch was observed. This probably masks the changes introduced by storage under the investigated conditions.

1. Introduction

Within the project ATO investigates the relation between on the one hand the sensory perception of apples and tomatoes and on the other aroma components, sugars, acids. Near infrared spectroscopy was used as non-destructive measurement technique and near infrared characteristics.
In the past period research was performed on apples and directed towards development c.q. improving the measurement techniques and the training of the panel for assessing the different sensory attributes.

1.1 Modelling

One of the implicit assumptions underlying the aim of the project is that:
'Mealiness perceived by the consumers is some **continuos** function of the mechanical, taste and aroma properties (concentration and composition) of the apple'.

$$M = f(m, t, a) \quad (1)$$

In which: M=perceived mealiness, m=mechanical properties, t , a= concentrations of taste and aroma compounds respectively.
In figure 1 a schematic model relating the different aspects with respect to mealiness is given. In this scheme it is implicitly assumed that the ratio of the cell wall/middle lamella-strength determines the amount of cells, broken during chewing. The level of 'ruptured' cells in turn influences the fraction of taste/aroma compounds liberated during chewing. The absolute amount of taste/aroma compounds liberated, however, is determined by the total concentration of taste/aroma compounds in the apples as well as the amount of ruptured cells.. Eventually, the mouth feel and perceived taste/aroma result in the perceived mealiness.

To be able to find quantitative relations between:
1) the perceived mealiness (M) and the perceived aroma and the 'mouth feel
2) the perceived mealiness (M) and the instrumental measurements.

It is necessary to assess whether or not there is a mealiness scale on basis of which we can relate the measurements. So, the first question which has to be answered is:

"Is the perceived mealiness M, for apples of different variety, maturity, origin etc., related to the predictive measurements according to the same function?".

If the answer is 'no', a different experimental set-up has to be followed than when the answer is 'yes'.

Answer is **Yes:**

- The decomposition of the 'system' is sufficient.
- If experimental determination of the variable is correct, the measured variables will explain the mealiness to a large extend.
- One universal mealiness-scale can be used for all apple samples
- Different samples with respect to **variety, sugar/acid-ratio, maturity** etc. can be used in the same sensorial assessment
- In the experiment the samples have to cover the complete or a broad range of the mealiness scale.

Answer is **No:**

- Decomposition is insufficient; the problem is not fully understood. There are one or more characteristic features of the system not accounted for in the predictive function
- Several scales have to be developed for different (groups of) apple types (variety, maturity etc.)
- Samples following different mealiness scales can't be used in the same sensorial assessment
- In the experiments the samples, belonging to the same type, have to cover the complete or a broad range of the appropriate mealiness scale.

1.2 Sensory Attributes

With respect to literature survey of the sensory work performed on apples we show to the technical report of IFR and IATA.

Table 1. Descriptors used by ATO in sensory evaluation of apples

Mouth feel	**Flavour**
- ***MEALINESS***	- **Sweetness**
- **Firmness**	- **Sourness**
- **Crispness**	- **Tartness**
- **Juiciness**	- **Total Aroma Intensity**
- **Dryness**	* **green aroma**
- **Moistness**	* **apple aroma**
- **graininess**	* **fruity aroma**

The scale of each individual attribute can be normalized by taking not mealy as 0 and maximum mealiness as 1. For 'calibration' of the sensory panel a range of apple samples with mealiness values distributed within this range is required. It is assumed that five mealiness levels (e.g.: 0, 0.25, 0.5, 0.75, 1) are sufficient to establish the scale. Figures 2 shows three types of relations between the perceived mealiness and the predictive function f, which type 3 is the most probable relation.

Type 1 is a linear scale over the whole range. It is very unlikely that human sensitivity shows this kind of behaviour. Type 2 is a step function: either the samples are not mealy at all or completely mealy. This will be the case all the cells have the same firmness properties. This is not to be expected. Type 3 is a sigmoidal curve.

Further more it will be assumed that:
- that the major factors influencing mealiness are in the first place the 'state' of the cell wall/middle lamella complex and secondly the (absolute) concentration of sugar and acids.
- the whole range of possible cell wall/middle lamella 'states' can be obtained by appropriate storage regimes of mature apples.

1.3 Gas chromatographic analysis of Apples

Introduction
Literature research revealed that over 300 components that contribute to apple flavour and aroma have been identified from many different cultivars (Dimick, 1983). Apple fruits without peel (cv. Cox's Orange Pippin) contains head space volatiles butyl acetate, hexyl acetate, butanol, 2 or 3-methyl-butylacetate and butyl butyrate (Knee, 1976). Mattheis (1991) and Brackmann (1993) analysed head space volatiles of apple fruits with skin. Mattheis mainly found aldehydes, alcohols and esters, including nonanal, ethanol, ethyl acetate, 2-methylbutyl acetate and hexyl 2-methylbutyrate in Bisbee Delicious apples. Brackmann detected mainly esters, including butyl acetate, hexyl acetate and hexylbutyrate in Golden Delicious apples.

GC-analysis of aroma compounds from fruits and vegetables involves three specific steps (see fig. 3): I) homogenisation , ii) extraction/adsorption of the compounds and iii) GC-determination. In general the extraction/adsorption of volatiles is performed via two methods: dynamic head space sampling on TENAX TA and Solid Phase Micro Extraction (SPME). Solid Phase Micro extraction (SPME) is a new sample preparation technique for GC. According to the manufacturer (Supelco) the advantages of SPME are that it is simple, fast, cheap and that no organic solvents are needed. Figure 4 shows the SPME device. The device consists of a holder and a replaceable fibre, coated with an appropriate stationary phase. By passing the needle with the retracted fibre through the sample vial septum and depressing the plunger, the fibre with stationary phase can be immersed in an aqueous sample or placed in the head space above a sample. Different types of fibres are available. The 100 µm polydimethylsiloxane (PDMS) coated fibre is non polar and suitable for low molecular weight, non polar compounds. Larger molecular weight compounds are more effectively extracted and released with a 7 µm PDMS fibre. To extract very polar analyses from polar samples the polar 85 µm polyacrylate coated fibre is suitable. A disadvantage of this fibre is that large, strong polar compounds bind too tightly and desorb slowly (Supelco Chromatography products 1996 catalog).

2. Materials and methods

2.1 Apple variety and storage conditions

Apples JONAGOLD, BOSKOOP, COX (see table) were received on 31st of October at the ATO and were immediately stored under CA-conditions:
Jonagold 1°C, CO_2=4.5%, O_2=1.2%, Boskoop: 4°C, CO_2=0.7%, O_2=1.2%; Cox: 4°C, CO_2=0.7%, O_2=1.3%.
Every variety was subdivide in three harvesting dates.

Table 2. Apples delivered by VBT

Variety	Picking date	Number of boxes	Amount in kg
COX	23.09 30.09 04.10	5 5 5	±50 ±50 ±50
JONAGOLD	02.20 15.10 24.10	5 5 5	±50 ±50 ±50
BOSKOOP	25.09 30.09 11.10	5 5 5	±50 ±50 ±50

In order to develop different mealiness levels COX-apples were stored at a T=20°C in perforated plastic bags according to the scheme given in table 3. Each bag contained 10 kg of apples. This means that every 5 days a fraction of a specific sub-batch was transferred from CA-conditions into plastic bags, so that after 20 days we had 5 samples with different levels of mealiness.

Table 3. COX apples

Picking date	Kg	Storage time in plastic bags (days)	*WEEK*			Totaal
			48	49	50	
			number (kg)			
23.09	50	0	45(9)	60(12)	35(7)	140(28)
		5	45(9)	60(12)	35(7)	140(28)
30.09	50	10	45(9)	60(12)	35(7)	140(28)
		15	45(9)	60(12)	35(7)	140(28)
04.10	50	20	45(9)	60(12)	35(7)	140(28)
		Total (number)	**225**	**300**	**175**	**700**
		Total (kg)	**45**	**60**	**35**	**140**

Apples stored in week 48 were used for training of sensory attributes of texture, GC-determination of aroma and NIRS-measurement. Apples from week 49 were used for training of the panel on taste and aroma attributes.

2.2 Sensory Assessment

Based on the time of the year, apple-experts expected that development of mealiness in Jonagold and Boskoop would be difficult if not impossible.
Therefore, training experiments on texture and aroma characteristics of apples were performed with COX apples which can easily be made mealy by changing the storage temperature and storage time. Other two varieties will be evaluated in February and March 1997.
Panellists were trained to assess and quantify the textural and flavour characteristics of apples. After subjected to the protocol for development of mealiness (5 levels, see table 3), the apples were presented to the panellists one by one.

Material
Apple variety (Cox Orange Pippin) was the subject for the training during this period. Samples (5) varying in mealiness were evaluated (see table 2).

Panel
The panel consisted of 20 men and women aged between 25-35 years.

Preparation of the samples
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 and data were collected by the help of a computerized data collection system (PSA-SYSTEM v.1.64, OP & P, Utrecht, The Netherlands).

Assessment of texture
Panel training was performed by two sensory methods: ranking and descriptive test.
Ranking helped panellists to understand the range of samples for individual texture descriptors. Panellists ranked five samples (differing in mealiness) for the attributes mealy, firm, moist, crisp, dry and grainy. For each descriptor, one piece of fruit from every samples was tasted by the panellists.
The aim of the descriptive test was to train the panellists on:
I) the use of the scale for the texture descriptors and
ii) investigating their reproducibility on the bases of scale use and
iii) differentiating the samples.

Descriptive Test was applied on a line scale anchored at both ends from "*present slightly* " to "*present strongly*". Samples were randomized for presentation and test was repeated three times.

Assessment of taste and aroma
Pre-screening of the samples revealed that the differences between samples were not that much pronounced for the individual aroma descriptors in this range. Only two basic taste descriptors (sweetness and sourness) and overall aroma intensity were chosen for the evaluation. The directional paired comparison and a descriptive test were performed by panellists. Directional paired comparison was used instead of ranking because it is more precise for the ranged. For a paired comparison 3 samples were chosen: 0 day stored, 10 days stored and 20 days stored. Each panellist evaluated three pairs. These were a) 0 day vs. 10 days stored, b) 0 day vs. 20 days stored and c)10 days stored vs. 20 days stored.The two permutations of the pairs were randomized over the panel and the order of pairs was randomized per panellist. The questions asked were:
- Which sample is more sweet?
- Which sample is more sour?
- Wich sample has more aroma?

In one session only one question was answered.
The panellists also rated these attributes on a line scale anchored both ends from "present slightly " to "present strongly". The samples were randomized per panellist, and were presented three times.

2.3 Gas chromatographic analysis of flavour compounds

During the past period we investigated: I) the homogenisation method and ii) the extraction/adsorption system. With respect to the extraction system the SPME and TENAX-technique were investigated. For the SPME system static head space and liquid sampling, whereas for TENAX static and dynamic head space were investigated.

Homogenisation
The following three methods were compared using COX apples (unknown origin) . They were purchased from a local store in Wageningen.

Method 1. Peeled and sliced apple tissue (45 grams) was homogenised with 45 grams of milli Q water with a blender for 10 seconds at low speed, at 20 °C.

Method 2. The apple was blended in the same way as for method 1, but the blended apple was further homogenised with an ultraturrax (speed 20000 min_{-1}) for 30 seconds at 20 °C.

Method 3. Frozen peeled apples slices (45 grams) were homogenised under liquid nitrogen with a blender at low speed for 5 minutes.

The homogenates were examined with a light microscope (Olympus BH-2).

SPME-technique and Gas chromatography of extracted samples
The SPME technique was investigated with a homogenate prepared from the COX apples of unknown origin. Homogenisation method 3 was used. The extraction system consisted of a100 µm PDMS fibre. This fibre was chosen because the volatile compounds of apple are semipolar and of low molecular weight (esters, alcohols and aldehydes). Two SPME-sampling methods were investigated: 1) liquid and 2) head space sampling.
For head space sampling the 100 µm PDMS fibre was incubated in 25 ml vials containing 15 grams homogenate at several time/temperature combinations, with extremes of 1 hour at 60 °C under stirring, and of 16 hours at 35°C. Yang (1994) found that because salt decreases the solubility of hydrophobic compounds in water, it increased the evaporation and adsorption of fruit juice compounds on SPME fibre, for both head space as well as liquid sampling. For this reason a head space sample, from 15 grams of homogenate in which 2.25 grams of NaCl was dissolved, was also taken during 1 hour at 60 °C under stirring.
For liquid sampling the fibre was incubated in the sample at several time/temperature combinations, with extremes of 1 minute at 20 °C, and 30 minutes at 35 °C under stirring. To determine wether or not non volatiles also adsorb on the fibre, the fibre was incubated in 5.9 % (w/v) fructose (from Merck and Serva), 2.6 % (w/v) sucrose and 0.55 % (w/v) malic acid, during 30 minutes at 20 °C under stirring.
The adsorbed compounds were analysed with a GC-system (Carlo Erba, HRGC 5300 mega series) with a DBWax column (30 m, 0.25 mm ID, 0.25 µm film thickness), and a FID-detector. Chromatographic conditions were as follows: the initial oven temperature of 80 °C was increased to 250 °C at 20 °C/min, and held for 5 min at 250 °C. Linear velocity of the carrier gas (Helium) was 28 cm/sec at 80°C. Data acquisition and analysis was performed using Chromcard software.

Dynamic and static head space sampling on Tenax TA and GC analysis
For homogenate preparation method 3 was modified by blending for 10 minutes instead of 5 and by adding after 5 minutes of blending 45 grams of saturated $CaCl_2$ solution (125 gram $CaCl_2$ in 100 ml milliQ) in order to inhibit enzymatic reactions (Buttery, 1987).

Static head space isolation: homogenate (20 grams) of COX apples received from VBT was weighed in 500-ml glass bottles which were subsequently sealed with a septum lid. The lid had 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. The experiments were performed in triplo.
Dynamic head space isolation: homogenate (20 grams) of COX apples received from unknown origin, was weighed in 500 ml glass bottles which were then sealed with a lid. The lid had an inlet and an outlet port. Glass tubes with 80 mg Tenax TA were connected to the outlet port. The samples were flushed with 30 $ml.min^{-1}$ purified nitrogen, supplied by the inlet port, for 2 hours at 35 °C under stirring.
Volatile compounds were desorbed from the tenax tubes for 5 minutes at 200 °C and trapped on a cold trap at -100 °C (Chrompack TCT-2 injector). After desorption from the cold trap for 5 minutes at 220 °C the volatiles were separated and detected 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. Linear velocity of the carrier gas (Helium) was 31 cm/sec at 80°C. Data acquisition and analysis were performed using Chromcard software.

2.4 NIR and mealiness of apples.

In the preceding research period two investigations were made with COX apples.

Experiment 1
Apples from the 6th and 7th harvest, stored either at 0°C or under controlled atmosphere conditions at 8° were used for the experiments. The samples from apples were treated with liquid nitrogen and stored at -20°C before they were analysed by Near Infrared Reflectance Spectroscopy. Prior to measurements, the samples were defrozen and the excess of water on the apple slices was removed with a tissue. The samples were homogenised (ultracentrifuge mill ZM 1000, Retsch) for about 60 seconds prior to NIR analysis.

Experiment 2
Apples (variety Cox) stored under controlled conditions for different periods of times (see table 3) and characterised by different degrees of mealiness, were used for the experiments. To have a representative sample for each batch of apples (one storage time) and to minimise the variability induced by the variation in the batch, minimum 10 apples were selected and analysed for each batch.

Instrumentation and acquisition of spectra
The NIR analysis was performed using a Near Infrared Spectrophotometer Infra Analyser 500 (Bran & Luebbe), using IDAS software. Reflectance measurements were obtained scanning in the range 1100-2500 nm, at 4 nm interval. Samples were measured at a temperature of about 20°C. The detected diffuse reflectances (R) were transformed to apparent absorbencies (log 1/R). Data collection was performed in two different ways: (I) Homogenised apples (set a) were packed into standard black cups closed

with a quartz window, and scanned. Two replicates were measured for each sample; (ii) the NIR spectra of whole apples and/or slices of apples were measured with an optic fibre. Five replicates were measured for each apple. The mean spectrum of two and respectively five replicates for each apple was used for data analysis.

Data analysis

The NIR spectrum contains information on the chemical composition of a sample, which determines the amount of absorbed near-infrared light. The information in a NIR spectrum is however non-selective because of interference of strongly overlapping constituents and because of light scatter variations. The spectral data for each batch were subjected to multiplicative scatter correction to reduce non-linear scatter effects due to specular reflection and structure of sample. Because of the multi-dimensional character of the data a dimension-reducing analysis technique was needed to analyse the data set. Accordingly, Principal Component Analysis (PCA) was used.

The spectral transformations and data analysis were executed with the software Unscrambler version 5.5 (CAMO A/S, Trondheim, Norway).

3. Result and discussion

3.1 Characterisation of Apples

An overall visual characterisation of the apples, on arrival at the ATO, showed that the batches were very heterogenous with respect to size. De maximum size difference measured was 20 mm. Also colour difference, as observed by blush and green-yellow ratio was large.
In addition an expert 'sensory evaluation' of the COX-apples revealed that there was already a start of mealiness and decrease of firmness. Because of these findings we expected a large variability within a batch.

3.2 Sensory Investigation

Ranking of Texture Attributes
The ranking data were evaluated according to the 'Expanded tables in the analysis of ranked data' (Newell 1987). Statistically significant rank are shown in table 4. For every descriptors, there were three different groups. The differences between 0 day and 20 days stored samples were clear for all the descriptors. Moderate group had more than one sample. The reason could be the forced storage conditions to make the samples mealy or large within sample variations especially 10 days stored samples.

Table 4. Statistically significant group of samples for texture descriptors ($p<0.05$)

Attribute	Less...	Moderate...	Very...
Mealy	0 5	15	10 20
Firm	20	15 10 5	0
Moist	20	15 10 5	0
Crispy	20 10	15 5	0
Dry	0	5 10 15	20
Grainy	0	5 15	10 20

Texture assessment by Descriptive Test
For each descriptors the mean of the panel was used to analyse the sensory texture data. Principal Component Analysis (PCA) was applied on the mean scores to reveal important descriptors or group of descriptors and the distribution of the samples between important descriptors. Also spider web diagrams were drawn to visualize the differences between samples.
Figures 5 and 6 show the loadings and samples scores on two principal components (PC). The First PC explained 95 % of the variation in the texture data. High negative correlations between dry and moist and grainy\mealy and crisp\firm are observed. The samples (each had three repeats) were lined from firm\crisp\moist to grainy\mealy\dry according to their storage days (0 day to 20 days). Again the place of mid-range (10

days and 15 days storage) was not clear on these distribution. All the repeats were close to each other for every sample except 5 days storage. High explained variation on the first PC also showed high agreement between panellists. Panellist agreement with group mean (correlations) was carried out by Senstools (OP & P, Utrecht, The Netherlands). The results have left only two panellists as outliers. Probably, these panellists need more training.

Figure 7 shows the distribution of samples on a spider web diagram. The centre of the diagram is 0. The distances from 0 on the branches (individual descriptors) shows the distribution of the samples according to theirs storage days. For example 20 days stored apples have higher values for mealy, grainy and dry and lower values for firm moist and crisp than the other apples. This diagram was drawn by using the average of three measurements.

Directional paired comparison test for taste and aroma
Significant differences ($p<0.05$) were found between the control and 20 days stored apples and between the control and 10 days stored apples for sweetness and sourness. Aroma differed significantly only between the control and 10 days stored apples. There was no differences between 10 days and 20 days stored apples and between control and 20 days stored apples.

Descriptive test (taste and aroma):
Panel agreement on taste and aroma descriptors was low. This range (depending on the forced mealiness conditions of apples) did not give a pronounced differences for the taste and aroma descriptors.

3.3 Gaschromatography of aroma compouds

Homogenisation
Microscopic investigation showed that apple homogenate prepared by homogenisation with a blender (method 1) contained mostly lumps of cells. The homogenate of method 2 (homogenisation with a blender and an ultraturrax) contained mainly single, intact cells. The homogenate obtained by grinding the frozen apple in liquid nitrogen (method 3) showed cell contents, broken cell walls and a few intact cells.
For the analysis of the total profile of volatile compounds of apple a homogenisation method is required which breaks the cells completely, so that volatile compounds can evaporate or/and adsorb directly. The (modified) method 3, homogenisation under frozen conditions, met these requirements best. With method 1 and 2 , homogenisation at room temperature the cells remained intact. An explanation is that the strength of the cell walls is stronger than the strength between the cell walls (the middle lamella) of adjacent cells. So at room temperature the cells are more easily separated via the middle lamella. Probably the friction of shearing forces introduced by the blender and the ultraturrax are not high enough to break through the cells. In contrast in frozen tissue the difference in mechanical properties of the tissue (including middle lamella and cell wall) is determined by the ice crystals. Obviously, the friction/shearing forces introduced in method 3 are high enough to reduce the size of the apple pieces to an extend lower than the size.

Solid Phase Micro extraction

GC-analysis showed that, for all time/temperature combinations including the extreme combinations of 1 hour at 60 °C, and 16 hours at 35 °C headspace sampling yielded only a few peaks (fig. 8). Addition of salt to increase the evaporation of volatiles didn't increase the amount of peaks.

Liquid sampling resulted in a chromatogram with approximately 40 peaks (fig. 9), for all time/temperature combinations including the extreme combinations of 1 minute at 20 °C, and 30 minutes at 35 °C. To examine if non volatiles also adsorb on the fiber, the fiber was incubated in solutions of some major compounds of apple: fructose, sucrose and malic acid, with the same concentrations as in apple (Souci, 1986). Fructose as well from Merck as from Serva yielded 6 peaks that seemed to corresponded to 6 peaks of an apple chromatogram (not shown). One peak of the sucrose chromatogram corresponded with a peak of the apple chromatogram, malic acid gave no peaks (not shown).

The conclusion is that headspace sampling with SPME is not a suitable method (with an FID-detector) because it resulted in only a few measurable peaks. Liquid sampling resulted in approximately 40 peaks. Some peaks were however descended from non volatile components of apple, including fructose. An explanation is that when the fiber is retracted from the solution a film of solution remains. Another possibility is that fructose adsorbs on the fiber. In the injection port fructose probably decomposes by pyrolysis into volatile compounds. This makes liquid sampling an unsuitable method.

Static and dynamic headspace isolation

Figure 10 represents a dynamic headspace chromatogram of Cox apple. The number of peaks and the area of the peaks is high. Because of the large number of peaks and the high areas not many peaks are separated, which makes correct integration and identification of these peaks difficult. Also some peaks can't be integrated correctly because they exceed the detection limit.

Static headspace sampling was applied on the same homogenate. Results showed lower peaks with base-line separation of at least 18 peaks. For this reason in the actual measurement the static headspace sampling method with TENAX was applied.

Changes in volatiles as a result of storage of COX-apples.

In figure 11 static headspace chromatograms are shown of Cox apples, which have been stored at 4 °C in CA (control), 10 days at 20 °C in air, or 21 days at 20 °C in air. Comparison of a static headspace chromatogram with a dynamic headspace chromatogram shows that static headspace sampling yields less and lower peaks, with base-line separation, so consequently can be integrated correctly. Another advantage of static headspace sampling is that this method approximates 'a person eating an apple', better than dynamic headspace sampling. During sampling the composition of the headspace will change from a mixture of lower boiling components to a mixture of higher boiling components in time. Consequently a static headspace sample will contain relatively more lower boiling components. Probably the amount of volatiles that is released during the eating of an apple will be very low so the volatiles will also have low boiling points.

Figure 11 further shows that storage of apples at 20 °C changed the volatile composition. In order to quantify the differences 18 peaks were selected. The selection went as follows: the average area of each of the 18 peaks was compared for the three storage conditions; if the average area of at least one storage condition was higher than 90 kVolt.sec then the peak was selected. Their areas are represented in fig.6. In order to show the reproducibility error bars were plotted. It is shown that most of the standard deviations are low. The standard deviation of control peak 3 is high. This is because one of the peaks of the triplo was two times higher than the other two. Figure 11 further shows that peaks 1, 2 and 3 decreased, that peak 4 changed hardly, and that the other peaks increased during storage. The largest change was observed for peaks 2,6,10 and 14. The differences between CA storage and 10 days 20 °C were larger than between 10 and 21 days °C. In order to quantify the relative changes in the 18 components relative changes in peak areas were plotted (figure 13). The figure shows that the largest decrease was observed for peak 1, followed by peak 2 and 3. With respect to the increasing peaks, the largest increase was observed for peak 6, followed by peaks 14, 5, 7 respectively. Bars 9 and 18 of 21 days storage are very high because the corresponding CA bars 9 and 18 were very low.

3.4 NIR measurement of Stored Apples

Classification of apples in relation to storage conditions
Apples (Cox) from two harvest times and stored under different conditions were analysed by collecting their NIR spectra in the range 1100-2500 nm. Figure 14 shows an example of the NIR spectra of different samples of apples. The most important differences are in the spectral region characterised by the absorption of water and carbohydrates. PCA was applied for data analysis. Two principal components (PC) explained 92 % of the variation in the data set, with the first factor accounting for 86% of the variation (figure 15). The scores of the samples on the first and second principal components are shown in figure 16. The distribution of the samples along the first axis reflects mainly the difference in water and carbohydrate composition. This distribution shows that there is no significant difference in the samples with respect to the harvest time. However, change in the storage conditions induces changes in the sample composition. The bigger variation is recorded for the samples stored at 0°C. The samples stored under controlled atmosphere conditions are close to each other.

NIR and mealiness of apples
Samples of apples stored at 20°C for different periods of time, varying from zero to 21 days, (corresponding to different degrees of mealiness) were analysed by NIR spectroscopy. The spectral data were analysed with PCA technique. The first two principal components explained 97 % of the variation in the data set (Figure 17). However, as shown in the scores plot (Figure 18), the apples are not well clustered. This is due to the very big variation of the properties of each apple from the batches.

4. Conclusions and future work

4.1 Raw Material

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 turned mealy in an different order than was expected. Because of this it was suspected that these batches may also have a different origin.
The applied protocol for development of mealiness resulted in a broad mealiness range. It is expected that with a more homogenous batch it is possible to improve the results.

4.2 Sensory Investigation

- Panellists were trained on the basic mouth feel descriptors for one apple variety.
- On this storage range mealiness/graininess were negatively correlated with firmness/crispness and dryness was negatively correlated with moistness.
- The differences between mid-range samples (5, 10 and 15 days storage) were not always clear on mouth feel descriptors.
- This range was not suitable to investigate flavour differences in one variety.
- Training with other varieties (Jonagold and Goudreinet) for mouth feel descriptors.
- Training on flavour descriptors (more descriptors). Design will include all the varieties.
- Development of final descriptor list for mouth feel and flavour of three apple varieties.

4. 3 Gas chromatography of aroma compounds

The experiment with Cox apples will be repeated with a more homogeneous batch of apples. Fig. 6 showed that the differences between CA storage and 10 days 20 °C were larger than between 10 and 21 days °C. For this reason storage times shorter than 10 days will be added to the present storage series. A start will be made with the identification of important peaks, with GCMS. Literature research of aroma components of apples with respect the major volatile components found in different varieties of apple will be continued.

4.4 NIRS

There is a large variations in the spectra of individual apples from the same batch. This masks the chances probably introduced by storage under the investigated conditions.
We will conduct a study in which NIRS and sensory evaluation are performed on the same apples.

5. Literature references

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