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Role of yeast proliferation in the quality degradation of strawberries during refrigerated storage

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Abstract

Quality changes of strawberries during storage can be caused both by microbiological and physiological processes. There is little known about the possible contribution of microbiological processes to the quality degradation of strawberries. In this study, quality of strawberries during storage was evaluated by analytical and sensorial analyses. It was the aim to investigate the influence of microbiological activity on the changes of different quality factors of strawberries during storage.

During storage at 7 °C, quality was mainly determined by the odor and by visual defects. Regarding the odor, highly microbiologically contaminated late-season strawberries packaged in air at 7 °C became sensorially unacceptable due to the presence of high amounts of ethyl acetate. This could be attributed to the yeast proliferation: at yeast concentrations above 5.0 log cfu/g, an increase in ethanol was detected in the headspace of the strawberries. It was shown that ethanol was converted to ethyl acetate by strawberries resulting in an unacceptable odor. In an experiment with low microbiologically contaminated early-season strawberries, not reaching the above mentioned yeast counts, less ethyl acetate was detected which resulted in strawberries that were sensorially acceptable during the whole storage period (12 days).

Strawberries packaged in modified atmosphere conditions showed a different quality pattern due to the effect of decreased O_2-concentrations on both microbiological and physiological processes. This paper demonstrates that also microbiological processes on strawberries should be considered as they could play an important role in the sensorial quality when interacting with physiological processes.

Keywords: Quality degradation; Strawberries; Microbiology; Physiology; Storage

1. Introduction

The unique and desirable aroma of strawberries has attracted much research (Zabetakis and Holden, 1997; Boschetti et al., 1999; Fernández-Trujillo et al., 1999; Forney et al., 2000; Bood and Zabetakis, 2002; Olías et al., 2002; Sanz et al., 2003). Ke et al. (1994) proposed a mode of O_2 and CO_2 action on biosynthesis of some aroma compounds in stored strawberries. Other quality attributes like texture and sugar composition have been discussed or investigated (Ke et al., 1991; Shamaila et al., 1992; Kallio et al., 2000). Storage changes of quality attributes have been primarily considered from a physiological point of view. In the case of visual quality, research has also been performed on mould growth (Wszelaki and Mitcham, 2000; Marquenie et al., 2002). New literature on the effect of microbiological processes due to yeast or mould growth on quality attributes, other than visual quality, is very scarce. Van der Steen et al. (2002) described sensorial

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(evaluated by sensory panel) and microbiological quality of strawberries during storage. Ragaert et al. (2006) studied the microbiological activity of yeasts in terms of metabolite production on a simulation medium of strawberries. It was found that yeasts could produce acids, alcohols and esters on the simulation medium. If these compounds are also produced on strawberries, they could affect odor and taste depending on the produced concentrations and threshold values. Moreover, microbiologically produced metabolites could be converted into other compounds by the strawberries. Hamilton-Kemp et al. (1996) showed that exogenous added alcohols were converted to the corresponding acetates by the strawberries. With regard to the effect of this conversion on the odor profile, Larsen and Poll (1992) already determined odor thresholds of some important volatile compounds in strawberries.

In this paper, the effect of microbiological processes on quality of strawberries during storage was investigated by using both analytical and sensorial measurements. Strawberries were stored in air or modified atmosphere conditions at 7 °C. The latter was used to prolong shelf-life by maintaining a constant low O_2-concentration and a constant higher CO_2-concentration in the headspace of the package. This reduces the respiration rate of the produce and the microbiological growth (Phillips, 1996). It was the aim to relate changes of quality factors, measured both by analytical and sensorial measurements, to microbiological and physiological processes.

2. Materials and methods

2.1. Storage experiment

2.1.1. Packaging

A first storage experiment was performed in the month April with a batch strawberries (*Fragaria x ananassa* 'Elsanta') obtained from the auction Hoogstraten (Veiling Hoogstraten, Hoogstraten, Belgium) (early-season). A second storage experiment was performed in the month September with a second batch strawberries (*Fragaria x ananassa* 'Elsanta') obtained from a local wholesaler of fresh produce (Van Landschoot, Ghent, Belgium) (late-season). These batches will be noted as early-season and late-season strawberries. Air packaging at 7 °C was achieved by packaging 500 g of strawberries in perforated high-barrier bags (16 holes of 5 mm diameter per package, 110 μm film thickness, 300 × 400 mm, Euralpack, Wommelgem, Belgium). For storage under modified atmosphere conditions at 7 °C, 500 g of strawberries were packaged in a packaging film (30 μm film thickness, 240 × 250 mm, O_2-permeability: 3529 ml $O_2/m^2 \cdot 24$ h·atm, CO_2-permeability: >20,000 ml $CO_2/m^2 \cdot 24$ h·atm, Hyplast N.V., Klerck's Group, Hoogstraten, Belgium). The dimensions of the film were based on the respiration rate of the '*Elsanta*' strawberries namely 6.36 mlO_2/kg·h at 3% O_2 and 7 °C (Jacxsens et al., 1999). The desired gas atmosphere (3–5% O_2; 5–10% CO_2; rest N_2) was introduced in the packaging by means of a gas-packaging unit (Ragaert et al., 2006).

2.1.2. Measurements

2.1.2.1. O_2 and CO_2 analysis and preparation of samples. During the two storage experiments, two packages per atmosphere were analyzed on Day 0, Day 1 (only late-season strawberries), Day 2, Day 5, Day 7, Day 9 and Day 12. In the case of modified atmosphere conditions, concentrations of O_2 and CO_2 were measured in the two packages as described in Ragaert et al. (2006). Subsequently, each of the four packages was opened aseptically and 150 g strawberries per package were used for volatile organic compound analysis (2.1.2.2), analysis on juice extract (2.1.2.3) and microbiological analysis (2.1.2.4). The rest of the strawberries (350 g per package) were used for sensorial analysis (2.1.2.5).

2.1.2.2. Volatile organic compound analysis

2.1.2.2.1. Detection of volatile organic compounds. The volatile organic compound analysis aimed to detect changes during storage in the composition of the headspace by headspace analysis. These detected changes were then related to other analyses, especially to microbiological analyses and to sensorial analyses. Because of this aim, a semi-quantitative analysis could be applied.

150 g of strawberries were put aseptically in a 500 ml glass recipient, connected with a sorbent sampling tube (Tenax GR, 70 mg, 3 mm ID tube, Alltech, Lokeren, Belgium). This recipient was previously cleaned and disinfected with boiling water and subsequently cooled to room temperature. An internal standard (1 ml of 5 mg/l 4-methyl-2-pentanon in deionized water) (Acros, Geel, Belgium) was included in the recipient in a separate small flask hanging in the headspace. After an initial equilibration time of 10 min at 22 °C, the recipient was flushed at 22 °C with 500 ml helium (Air Liquide, Aalter, Belgium) at a flow rate of 100 ml/min to transfer the volatile organic compounds onto the sorbent sampling tube. Subsequently, a second equilibration time similar to the previous one was applied. Then, the recipient was flushed again but with 15 ml helium and volatile organic compounds were captured onto a second sorbent sampling tube. A flush volume of 15 ml helium did not result in breakthrough losses of ethanol, acetone, methyl acetate and ethyl acetate in contradiction to a flush volume of 500 ml.

Analysis of the sorbent sampling tube was performed with a gas chromatograph (GC) as described in Ragaert et al. (2006). The amount of trapped volatile organic compounds was expressed as the peak area of the compound divided by the peak area of the internal standard.

Fig. 1 gives a chromatogram originating from the headspace of strawberries flushed with 500 ml after 7 days of storage under air conditions at 7 °C. This chromatogram illustrates the presence of some important volatile organic compounds as discussed further in this chapter.

2.1.2.2.2. Identification of volatile organic compounds. Strawberry volatiles were identified by GC coupled with a mass spectrometer (MS) at the moment that off-odors were observed by sensorial analysis. 50 ml of headspace volume was transferred to a sorbent sampling tube (Tenax TA, 200 mg,

4 mm ID tube, Sigma, Bornem, Belgium). All other adsorption conditions (equilibration time, temperature, recipient volume) were the same as described above. The analysis of the trapped volatile organic compounds for identification has been described in Ragaert et al. (2006).

2.1.2.3. Analysis on juice extract. 150 g of strawberries, previously used for the headspace analysis (Section 2.1.2.2), were put in a sterile stomacher bag (180 × 300 mm, Novolab, Geraardsbergen, Belgium). After homogenization in a stomacher (Stomacher Lab-Blender 400, Led Techno, Eksel, Belgium), 30 g of the homogenate was put in a new sterile stomacher bag to be used for microbiological analysis (see Section 2.1.2.4). Another 40 g of homogenate was used to determine pH, total acidity (TA), suspended solids (SS) and the concentration of sucrose, glucose, fructose, citric acid and some metabolites (lactic acid, acetic acid and ethanol) in strawberries. These sugars and citric acid are respectively the main sugars and the main acid in strawberries (Cordenunsi et al., 2002; Pelayo et al., 2003).

To extract the above-mentioned compounds, the homogenate (40 g) of the strawberries was diluted with water to a volume of 100 ml. After filtration (∅ 125 mm, Schleicher and Schwell, Dassel, Germany), the filtrate was heated at 80 °C during 15 min in a warm water bath (type GD100, Grant Instruments, Cambridge, England) to denaturate proteins including enzymes. Subsequently, the filtrate was stored at −18 °C till analysis day where 1 ml of the filtrate (1 ml) was prepared for High Performance Liquid Chromatography (HPLC). This has already been described in Ragaert et al. (2006). The rest of the filtrate, not used for HPLC, was subjected to pH- (Knick pH meter, Berlin, Germany), SS- (Carl Zeiss refractometer, Hamburg, Germany) and TA-measurements. The latter was performed by titrating 10 ml of filtrate with 0.1 N NaOH (Sigma) till pH 8.1.

Calibration curves for sucrose (UCB, Leuven, Belgium), glucose (Sigma), fructose (Sigma), citric acid (Merck), lactic acid (Sigma), acetic acid (VWR) and ethanol (VWR) were made by adding each of these compounds in different known concentrations to strawberry homogenate.

2.1.2.4. Microbiological analysis. 30 g homogenate of strawberries (see Section 2.1.2.3) was diluted (1:10) with sterile peptone saline solution (PPS) in a sterile stomacher bag. PPS consisted of 1 g pepton/l (L34, Oxoid, Hampshire, England) and of 8.5 g NaCl/l (1723, Vel, Leuven, Belgium). After blending the bag with a stomacher, consecutive dilutions (1:10) were made in PPS and plated onto appropriate media. The aerobic psychrotrophic count was determined on PCA (Plate Count Agar, Oxoid), the lactic acid bacteria count was determined on MRS-agar (Man–Rogosa–Sharp, Oxoid) and the yeast and mould count was determined on YGC-agar (Yeast Glucose Chloramphenicol, Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France). All plates were incubated for 5–10 days at 22 °C. Sorbic acid (Sigma) (1.4 g/l) was added to MRS-agar to prevent growth of yeasts and moulds.

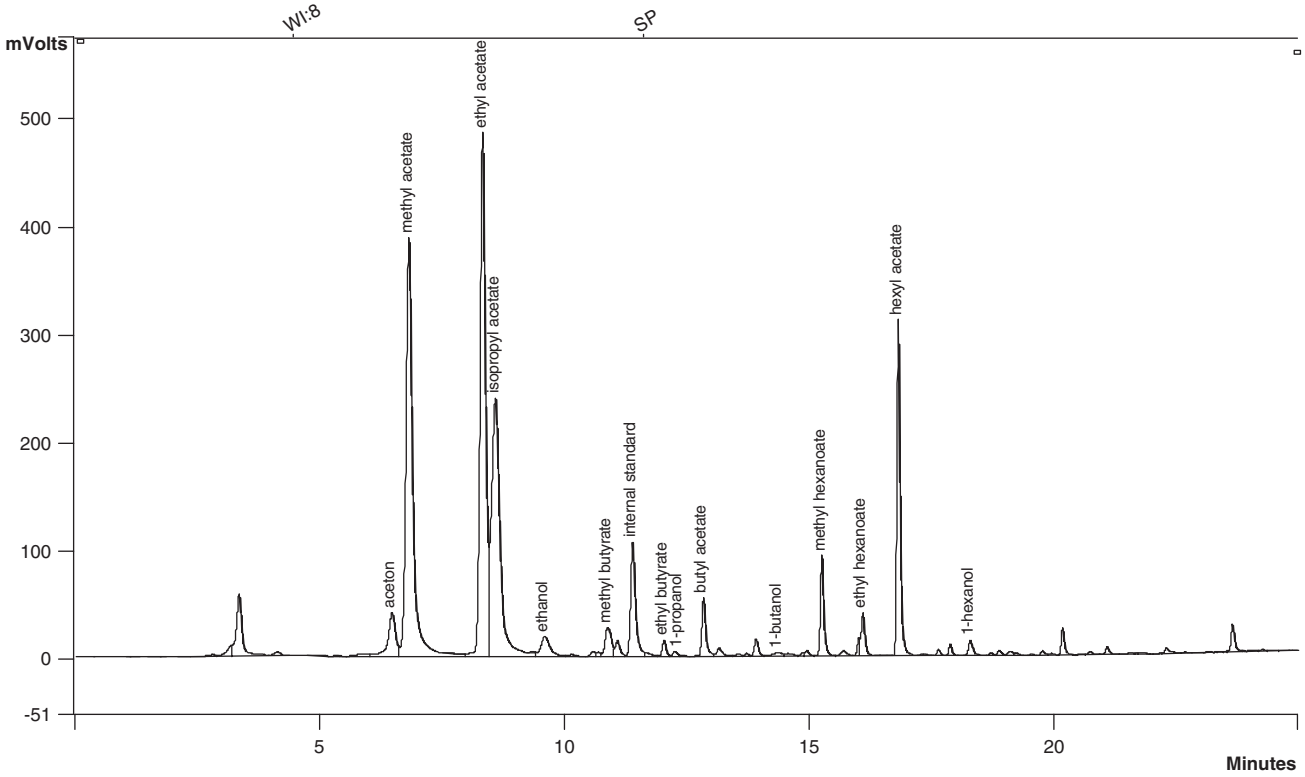

Fig. 1. Chromatogram of the headspace-analysis of strawberries after 7 days of storage under air at 7 °C indicating peak names and peak retention times (flush volume: 500 ml).

Table 1
Shelf-life (days) determined by the sensory panel, together with quality attributes that were scored negatively (score >5 on a scale of 1–9) at the moment the end of the sensorial shelf-life was reached, both for early-season and late-season strawberries, stored in different atmosphere conditions at 7 °C

Conditions	Early-season		Late-season	
	Shelf-life[a]	Negative scores	Shelf-life	Negative scores
Air	>12 days	–[b]	5–7 days	Odor characteristics Color Visual defects
Modified atmosphere	5–7 days	Odor characteristics Deviant taste Color Visual defects	5–7 days	Odor characteristics

[a] The shelf-life of the two packages for each atmosphere condition was reached when 2/3 of the total amount of samples (=8 samples out of 12 samples) of those packages were judged unacceptable for consumption by the sensory panel (sample size: 3 strawberries/sample).
[b] All evaluated quality attributes remained acceptable during the storage period of 12 days (score ≤5).

2.1.2.5. Sensorial analysis. The sensorial analysis was used to determine the sensorial shelf-life of the early- and late-season strawberries, stored in different atmosphere conditions. The end of the sensorial shelf-life was reached when 2/3 of the samples of a particular atmosphere condition (air or modified atmosphere) were judged unacceptable for consumption by the sensory panel. Attributes, negatively scored at that moment, are then determinative for the sensorial quality of the strawberries.

The sensorial analysis was performed on analysis dates by evaluating strawberries that were not used for laboratory measurements. These strawberries were divided among six members of the sensory panel. Each member was asked to evaluate four samples (3 strawberries/sample) corresponding with the four packages of strawberries that were investigated on a particular analysis date (two packages under air conditions and two packages under modified atmosphere conditions). The panel members were previously trained on the sensorial characteristics of fresh strawberries and spoiled strawberries. Scores to be given for the different sensorial characteristics were situated on a scale from 1 to 9.

For sensorial evaluation, samples of strawberries were put in a 250 ml glass recipient that was closed for 10 min (22 °C). Panel members were asked to open the recipient under IR-light and evaluate the characteristics of the odor (1–4: good odor; 5: still acceptable; 6–9: bad, unacceptable odor). Thereafter, strawberries were taken out of the recipient and were evaluated for hardness (1–4: hard, unripe; 5: optimal; 6–9: soft, overripe), juiciness (1–4: juicy; 5: still acceptable; 6–9: dry) and taste. In the case of taste, scores were given for sourness, sweetness and deviant taste. For sweetness and sourness, the scale ranged from 1: intense to 9: poor, while for deviant taste scores 1–5 were associated with no or acceptable degree of deviant taste and scores higher than 5 were associated with an unacceptable degree of deviant taste. Subsequently, color (1–4: light red; 5: optimal red; 6–9: dark red) and possible visual defects (1–5: acceptable degree of visual defects; 6–9: unacceptable degree of visual defects) were evaluated under natural light. Visual defects concern damaged or rotten spots and mould growth. Finally, panel members were asked if the sample was acceptable for consumption. The tasting part of the sensory analysis for evaluating hardness, juiciness, sweetness,

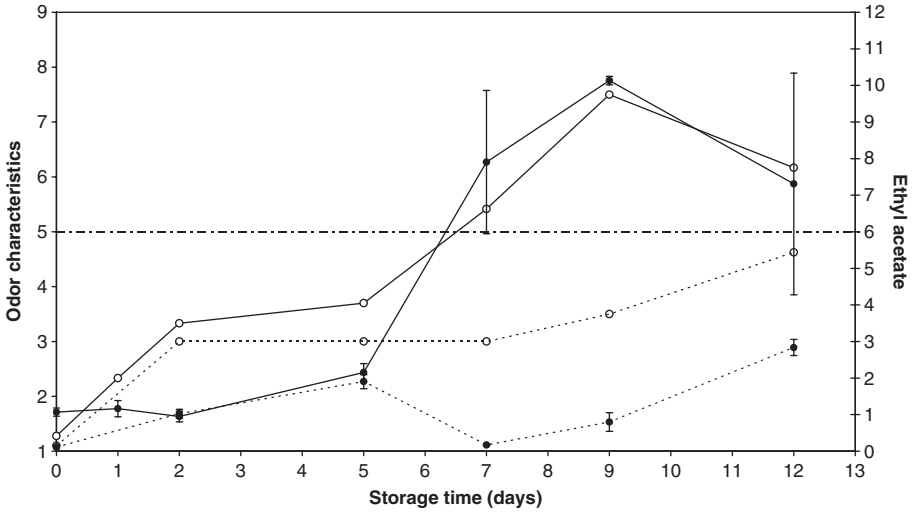

Fig. 2. Evolution of the amount of ethyl acetate (relative to the internal standard), measured in the headspace (●), and the odor characteristics, measured by sensorial analysis (○), for early-season (dashed line) and late-season (full line) strawberries, stored under air at 7 °C (- - -: sensorial limit of acceptability) (error bars represent the standard deviation ($n=2$); points of sensory analysis represent the mean scores, given by 6 panelists on two samples each: $n=12$).

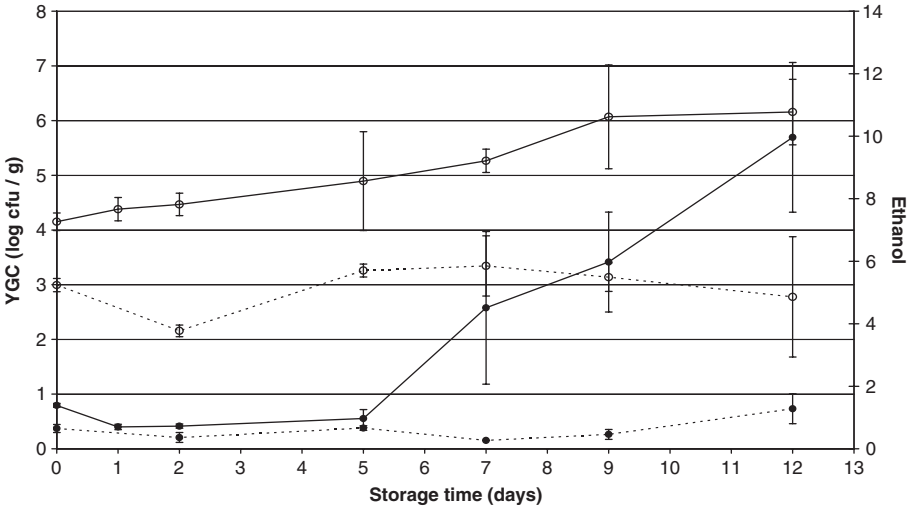

Fig. 3. Evolution of the amount of ethanol (relative to the internal standard), measured in the headspace (●), and the yeast count (YGC) (○) of early-season (dashed line) and late-season (full line) strawberries, stored under air at 7 °C (error bars represent the standard deviation: $n=2$).

sourness and deviant taste was not performed if mould growth or obvious spoilage were observed on the strawberries.

2.2. Dipping experiment

From the results of the storage experiment, it could be hypothesized that increased amounts of ethanol had an effect on the odor quality of the strawberries. To check this hypothesis, dipping experiments were performed. Therefore, a batch of fresh strawberries (*Fragaria x ananassa* 'Elsanta,' early-season) obtained from a local wholesaler of fresh produce (Van Landschoot, Ghent, Belgium) was divided into three subbatches. The first group was dipped into water (reference) and the second and third were dipped for 30 s into an ethanol (VWR) solution of 0.2 g ethanol/l water and 2 g ethanol/l water, respectively. This concentration range was chosen based on the detected ethanol concentrations, found previously on strawberry-agar, inoculated with yeasts (Ragaert et al., 2006). After 4 h, headspace analysis of the three subbatches was performed. Each subbatch consisted of three samples (150 g/sample).

3. Results and discussion

3.1. Odor of strawberries

In all investigated conditions, odor characteristics were a factor that determined sensorial shelf-life of stored strawberries as can be seen in Table 1. There was a difference in shelf-life between early-season and late-season strawberries, both stored at 7 °C in air (Table 1). This difference was not present for modified atmosphere conditions (Table 1).

From Table 1, it can be seen that early-season strawberries stored at 7 °C in air were acceptable for consumption during the whole storage period (12 days). This is also reflected in Fig. 2, from which can be seen that the odor characteristics never reached or exceeded the limit of acceptability (5). This

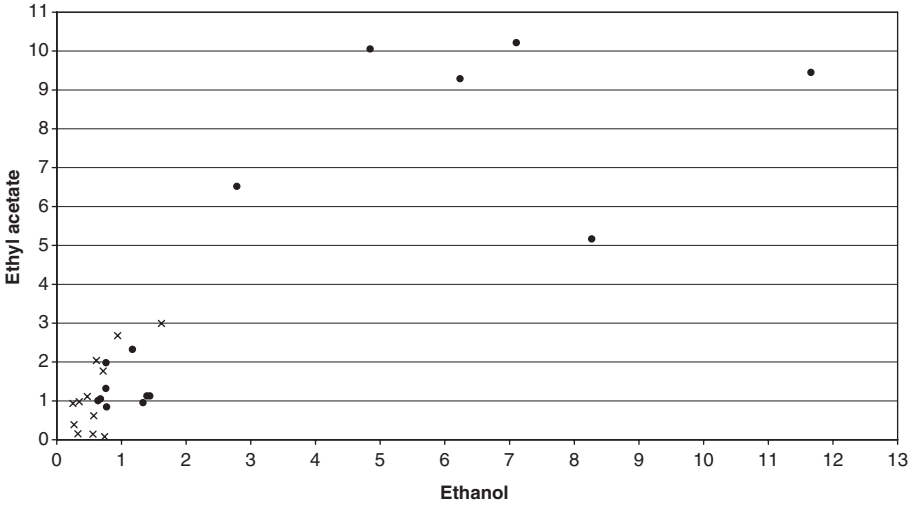

Fig. 4. Relation between ethanol and ethyl acetate (all relative to the internal standard), measured in the headspace of early-season (×) and late-season (●) strawberries during storage under air at 7 °C.

was in contradiction with late-season strawberries, for which these characteristics were found unacceptable from Day 7 on. From the analytical measurements, it could be observed that ethyl acetate was an important odor compound influencing the acceptability of the odor characteristics (Fig. 2). Ethyl acetate has been reported as an off-odor compound in strawberries (Larsen and Poll, 1992; Ke et al., 1994; Larsen and Watkins, 1995). Similar to ethyl acetate, amounts of ethanol also increased fast on Day 7 in the case of late-season strawberries stored at 7 °C in air (Fig. 3). However, ethanol is directly less important in the odor profile of strawberries, due to its very high threshold value (100–800 mg/kg compared to 0.1–1.0 mg/kg for ethyl acetate) (Larsen and Poll, 1992; Larsen, 1994). Both the increase in ethyl acetate as well as in ethanol could not be detected in early-season strawberries.

The difference in ethanol changes between early-season and late-season strawberries were related to a difference in microbiological activity on the strawberries. Yeasts were the dominant flora on the strawberries and were found to have higher levels on the late-season strawberries (Fig. 3). Moreover, an increase in yeast counts was observed on the late-season strawberries (Fig. 3). Ragaert et al. (2006) found that Crabtree-positive yeasts can produce ethanol in air conditions on strawberry-agar from the moment the yeast count reached 5.4–7.6 log cfu/cm^2 (=5.4–7.6 log cfu/g in the case of strawberries). From Fig. 3, it is observed that this range of counts was reached by yeasts on late-season strawberries from Day 5–7. Related to these counts, it should be considered that damaged spots on the strawberries will reach higher microbiological numbers than indicated by the yeast counts in Fig. 3, as high-contaminated spots are diluted by the less-contaminated spots in microbiological analyses.

The above mentioned yeast count could have been responsible for the increase in detected amounts of ethanol on late-season strawberries. Besides ethanol production by yeasts, the increase in amounts of ethanol could also be caused by the fruit itself as a response on the yeast infection. Purvis (1997) stated that fermentative metabolism can be enhanced in fruits by several stress factors including microbial infections. The yeast infection was more intense on late-season strawberries because of the visually perceived higher degree of damage in contradiction to early-season strawberries. In both cases (microbiological or physiological production), ethanol could serve as a precursor for other fermentative metabolites. In Fig. 4, the observed amount of ethanol in the headspace is plotted against the observed amount of ethyl acetate for both early-season and late-season strawberries during the storage period of 12 days. It was apparent that when amounts of ethanol exceeded a certain limit, amounts of ethyl acetate increased. To check this relation between ethanol and ethyl acetate, a dipping experiment was performed. Results of the dipping experiment are presented in Fig. 5 showing that physiological processes of strawberries are affected by externally added ethanol. This is similar to Hamilton-Kemp et al. (1996) who showed that six-carbon alcohols were converted to the corresponding acetates. Next to ethyl acetate, production of ethyl butyrate and ethyl hexanoate is stimulated by ethanol (Fig. 5). These two latter compounds exhibit a positive influence on the odor profile of strawberries (Forney et al., 2000) and could hide the detrimental effect of ethyl acetate on the odor characteristics, due to their low threshold values, compared to ethyl acetate (Larsen and Poll, 1992). However, amounts of ethyl butyrate and ethyl hexanoate did not increase during the storage experiment with late-season strawberries at 7 °C in air (data not shown), resulting in an overall bad odor of the strawberries from Day 7 on due to the increase in ethyl acetate. It should be mentioned that ethyl acetate production during storage of the late-season strawberries in air is not likely to be caused by yeasts because Ragaert et al. (2006) did not find ethyl acetate production on strawberry-agar by yeasts at 7 °C in air.

Besides ethanol, an increase in 1-butanol and 1-hexanol was also found on Day 7 in the case of air-stored late-season strawberries at 7 °C. This increase could also have been caused

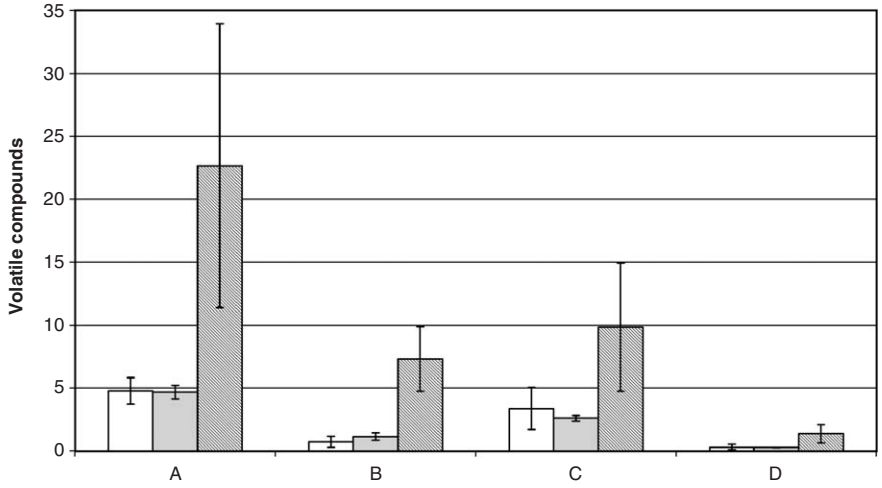

Fig. 5. Effect of dipping fresh strawberries for 30 s in water (white), and ethanol solutions (0.2 g/l (gray) and 2.0 g/l (shaded)) on amounts of ethanol (A), ethyl acetate (B), ethyl butyrate (C) and ethyl hexanoate (D) (all relative to the internal standard), measured in the headspace after 4 h storage under air at 7 °C (error bars represent the 95% confidence interval: $n=3$).

by microbiological or physiological processes. On Day 7, butyl acetate and hexyl acetate increased, probably due to the same enzymatic reaction mechanism as in the case of ethyl acetate, namely the reaction of acetyl-CoA with the corresponding alcohol (Ke et al., 1994; Hamilton-Kemp et al., 1996). Nevertheless, due to the higher amount of ethyl acetate present in the headspace in combination with its high degree of off-odor characteristic compared to butyl and hexyl acetate (Larsen and Poll, 1992), ethyl acetate had the highest influence on the off-odor of the strawberries. Additionally, Ingham et al. (1995) reported that smaller esters such as ethyl acetate persist longer in the human nose space than longer less-polar esters.

The storage under modified atmosphere conditions could not prolong sensory shelf-life of the packaged strawberries compared to storage under air conditions (Table 1). In both storage experiments, the desired equilibrium O_2-concentrations (3–5% O_2) were not reached and the too low O_2-concentrations affected the odor profile of the strawberries. This is illustrated for the late-season strawberries in Table 2. Similar to air conditions, yeasts were the dominant microflora on the strawberries, packaged under modified atmosphere conditions. Thereby, the number of yeasts was not influenced by lower O_2-concentrations, compared with air conditions (*t*-test; $p > 0.05$). This was also found on '*Elvira*' strawberries from Israel (Van der Steen et al., 2002). Contrary to O_2-concentrations, CO_2-concentrations did not change during storage of both early-season and late-season strawberries and ranged between 3% and 6% CO_2.

The too low O_2-concentration could be attributed to an underestimation of the respiration rate due to the occurrence of some damaged spots on the strawberries (certainly on the late-season batch), resulting in higher respiration rates. As specified in Table 1, sensorial shelf-life for both early- and late season strawberries under modified atmosphere conditions at 7 °C was between 5 and 7 days. In this case, shelf-life was not limited by microbiological contamination but was due to too low O_2-concentrations in the package. This caused an increase in physiological production of ethanol and subsequently an increase in physiological production of ethyl acetate by the early-season and late-season strawberries after Day 2. Kader et al. (1989) mention that O_2-concentration <2% result in off-odors by strawberries. In both early- and late-season strawberries an increase in production of the off-odor ethyl acetate was already detected from Day 2 (O_2 <5%), but the resulting off-odor was initially compensated by the production of ethyl butyrate and ethyl hexanoate in the case of the storage under modified atmosphere conditions (data not shown).

Similar to air conditions, also microbiological production of ethanol can be detected under modified atmosphere conditions from the moment microbiological counts are high enough (>5 log cfu/g). Ragaert et al. (2006) found on strawberry-agar that low O_2-concentrations induce a significant increase of ethanol production by yeasts in such a level that detoxification of ethanol by conversion into ethyl acetate and to a lesser extent ethyl butyrate was performed by the yeasts. Stimulation of ethyl acetate and ethyl butyrate in strawberries by low O_2-concentration and/or high CO_2-concentration was described by Ke et al. (1994) from a physiological point of view. Finally, also increases in methyl acetate, butyl acetate, hexyl acetate and 1-propanol were observed during storage of strawberries under modified atmosphere conditions at 7 °C, already from Day 2. So, this was also a physiological reaction on the low O_2-concentration. Further in storage, 1-propanol, 1-butanol, 1-hexanol and hexyl acetate could also be produced by yeasts (Ragaert et al., 2006). It should however be mentioned that ethyl acetate had the highest influence on the off-odor.

3.2. Color and visual defects

As can be seen from Table 1, besides odor characteristics, color and visual defects determined the sensorial shelf-life. No significant differences could be observed between modified atmosphere conditions and air conditions at 7 °C (*t*-test; $p > 0.05$), both for early-season and late-season strawberries. When scores for color and visual defects were compared between early-season and late-season strawberries stored at 7 °C, not taking into account the atmosphere conditions, a significant difference was found from Day 9 on (*t*-test; $p < 0.05$). Thereby, both color and visual defects received a higher score in the case of late-season strawberries. In the case of color, this can be explained by a faster rate of ripening processes (e.g. color change) due to a higher respiration rate caused by the occurrence of more damaged spots on late-season strawberries. These damaged spots also provide more optimal conditions for mould growth compared to early-season strawberries, resulting in a higher level of visual defects from Day 9. No significant differences could be observed on initial contamination with mould spores between early- and late-season strawberries ($p > 0.05$). This is in contradiction with the initial count of yeasts, as mentioned above.

Table 2
Changes of O_2-concentration (%) and amount of ethanol and ethyl acetate (relative to the internal standard) in the headspace of late-season strawberries, stored under modified atmosphere (MA) conditions and air conditions at 7 °C

Day	MA-conditions			Air-conditions	
	O_2	Ethanol	Ethyl acetate	Ethanol	Ethyl acetate
0	6.4 (6.3–6.5)[a]	1.39 (1.32–1.45)	1.07 (0.96–1.18)	1.39 (1.33–1.45)	1.07 (0.97–1.18)
2	3.3 (1.9–4.7)	3.95 (2.40–5.50)	10.00 (9.31–10.70)	0.72 (0.66–0.79)	0.95 (0.80–1.09)
5	1.2 (0.6–1.8)	7.45 (1.81–13.10)	84.14 (15.31–153.0)	0.97 (0.68–1.25)	2.15 (1.91–2.40)
7	0.1 (0.0–0.2)	14.66 (12.70–16.62)	171.0 (122.3–219.6)	4.51 (2.07–6.95)	7.90 (5.95–9.86)
9	0.1 (0.1–0.1)	14.70 (13.52–15.88)	214.2 (208.6–219.8)	5.97 (4.38–7.57)	10.13 (10.02–10.25)
12	0.1 (0.1–0.1)	13.04 (5.35–20.74)	130.2 (101.0–159.3)	9.97 (7.57–12.36)	7.31 (4.28–10.34)

[a] (mean minus standard deviation (SD) – mean plus SD) (n=2).

Related to mould growth, mycelia became visible as a few very small spots on Day 7 on late-season strawberries stored at 7 °C in air. This however did not result in a significant difference in visual defects, compared with modified atmosphere conditions or compared with the early-season strawberries at Day 7. It should also be mentioned that in both storage conditions, color never exceeded a score of 7, which means that strawberries became never really dark-red. Pelayo et al. (2003) found also on some varieties of strawberries limited color changes.

3.3. Other sensorial factors

Related to taste, it can be seen from Table 1 that taste was only deviant in early-season strawberries stored under modified atmosphere conditions, probably related with bad odor characteristics. Only in late-season strawberries stored under air, production of lactic acid and acetic acid was observed. This occurred from Day 9 and concentrations were 8.7±1.3 mg/100g and 0.5±0.1 mg/100 g, respectively at Day 12. Ragaert et al. (2006) show that these acids could be produced by yeasts both under air and modified atmosphere conditions. However, no detection of lactic acid and organic acid occurred in strawberries during storage under modified atmosphere conditions.

In all storage conditions, texture remained acceptable both for early-season and late-season strawberries. This was also mentioned for some other varieties of strawberries by Pelayo et al. (2003).

The sensory panel could not find changes in sweetness or sourness during storage in both storage conditions both for early-season and late-season strawberries. Measurement of SS and TA revealed that the ratio (SS / TA) remained constant during storage in both storage conditions. Contradictory to the decrease in TA, pH remained constant. A difference in evolution of some sugars between early-season and late-season strawberries was observed during storage at 7 °C (data not shown). In early-season strawberries, only a decrease in sucrose concentrations was observed, while in late-season strawberries, also a decrease in glucose concentration occurred. This can be explained by the fact that in late-season strawberries, also microbiological activity was present, resulting in glucose consumption which was also found on simulation medium of strawberries (Ragaert et al., 2006). Moreover, due to stress response of the strawberries to yeast infection, the late-season strawberries could also be responsible for glucose changes.

4. Conclusions and future perspectives

This paper shows that next to physiological processes, also microbiological processes could play an important role in quality degradation of strawberries by producing ethanol as a precursor for the physiological production of the off-odor ethyl acetate. In the future, experiments will be performed to investigate whether ethanol is produced by the yeasts itself or by a physiological reaction of the strawberries, encountering the infection by the yeasts.

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