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Impact of modified atmosphere and humidity packaging on the quality, off-odour development and volatiles of 'Elsanta' strawberries

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Abstract

Development of off-odours, as well as visual quality of packaged fresh produce plays a crucial role in consumer's choice. In this context, this work investigated the odour profile, condensation, gas composition, and postharvest quality attributes of strawberries stored under modified atmosphere and humidity packaging at 5 °C for 14 days. The packages were fitted with fixed area (69, 126.5, and 195.5 cm^2) of different permeable membranes (NatureFlex, Xtend, and Propafilm). No significant changes were detected on the measured physicochemical quality attributes of strawberries and mass loss was below 1.5% across the different packaging systems. Package modification/design had an influence on in-package water vapour condensation, gas composition, and accumulation of secondary volatile organic compounds (acetaldehyde, acetone, ethanol and ethyl acetate).

Keywords: packaging, strawberry, off-odour, quality

1. Introduction

Fresh produce remains metabolically active even after harvest and continues to respire and lose water (Bovi, Caleb, Linke, Rauh, & Mahajan, 2016). This represents a challenge for the development of controlled atmosphere (CA) and modified atmosphere packaging (MAP) systems, since it leads to changes in the package atmosphere over time. Jo, Kim, An, Lee, and Lee (2013) developed a fresh produce container that combines the principles of MAP (atmosphere modification based on produce respiration) and CA (periodic adjustment of atmosphere composition). Their approach consists of a controlled container system fitted with a gas diffusion tube responding to real-time measured O_2 and CO_2 concentration. However, this approach addresses only to optimum gaseous composition and does not take into account the accumulation of water vapour. Water vapour evolution inside fresh produce packages often limits product´s shelf life due to the formation of condensation (Bovi & Mahajan, 2017). Condensation represents a risk to the product quality as water may accumulate on packaging system and/or product surface leading to defects in external appearance and promoting growth of spoilage microorganisms (Bovi et al., 2018; Linke & Geyer, 2013). Thus, the concept of a modified atmosphere and humidity packaging (MAHP) equipped with a humidity control window might represent an innovative approach to avoid or lessen the risk of condensation.

Besides condensation, visual quality, freshness aroma, and development of characteristic off-odour volatiles play a crucial role in consumer's choice, and this influences future decisions to purchase the product. Thus, the identification of characteristic off-odour volatiles during storage life of packaged fresh produce can serve as an indicator of product quality. Around 360 volatile compounds have been identified in the aroma of strawberry (*Fragaria x ananassa* Duch.), however, only a small portion (15-25) of these volatiles are important contributors to the aroma (Jouquand, Chandler, Plotto, & Goodner, 2008; Nielsen & Leufvén, 2008; Zabetakis & Holden, 1997). Some of these compounds include methyl and ethyl esters, furanones, C_6 aldehydes and other C_6 derivative compounds. In addition, strawberries may produce secondary volatile organic compound (VOCs), such as acetaldehyde, ethanol and ethyl acetate during storage. When these secondary volatiles are present in concentrations above their threshold limit they can have a negative effect on the flavour (Pelayo, Ebeler, & Kader, 2003).

Postharvest life of strawberry is short due to physical damage during handling, water loss, physiological disorders, high susceptibility to spoilage microorganisms (Caleb, Wegner, et al., 2016; Chandra, Choi, Lee, Lee, & Kim, 2015; Lara, García, & Vendrell, 2006), and high respiration rate (RR) of 50 to 100 mL CO_2 $kg^{-1}h^{-1}$ at 20 °C (Ozkaya, Dündar, Scovazzo, & Volpe, 2009). Nevertheless, refrigeration in combination with MA systems has been extensively used to extend shelf-life of strawberry. Results have shown that MAP can slow strawberry respiration rate by keeping CO_2 concentration between 10 and 30% (Lara et al., 2006; Nielsen & Leufvén, 2008). In this context, the aim of the study was to design, develop and investigate the effects of modified atmosphere and humidity packaging on: a) its performance in terms of headspace gas composition and moisture condensation; b) the physicochemical quality attributes of strawberries; and c) the shift in VOCs profiles of packaged strawberries during storage.

2. Materials and methods

2.1. Plant materials

Fresh strawberry (cv. Elsanta) was obtained from the commercial grower (Fruchthof Hensen Erdbeerkulturen GmbH & Co. KG, Swisttal-Mömerzheim, Germany), and transported in cooled conditions to the Freshness Laboratory, Department of Horticultural Engineering, Leibniz Institute for Agricultural Engineering and Bioeconomy, Potsdam, Germany. The strawberries were carefully sorted and the damaged, overripe, and poor quality fruit were discarded in order to obtain uniform samples. The strawberries were precooled to the study temperature of 5 °C for 3 hours.

2.2. Design of modified atmosphere and humidity packaging

Polypropylene packages (total 10) of size 13 x 20 x 9 cm (total volume 2.3 L) were used as the base storage container. The lid of each package was modified by cutting windows of different sizes of 33, 66, and 100% of total lid area which is equivalent to absolute area of 69, 126.5, and 195.5 cm^2, respectively. These open windows were hermetically sealed (using double sided hermetic tapes) with different packaging films: i) Xtend (XT) film (StePac, Tefen, Israel), ii) Polypropylene based Propafilm (PP) (Innovia Films, Cumbria, UK), and iii) cellulose-based NatureFlex (NF) polymeric film (Innovia Films, Cumbria, UK). Each packaging film covering the window was perforated with 2 holes of 0.7 mm diameter in order to achieve equilibrium modified atmosphere. Table 1 shows the description of the different packaging window design used in this study. Different window sizes and packaging films were used in order to create different modified humidity conditions. The water vapour transmission rate (WVTR) is 42.79, 19.34, and 0.8 g m^{-2} d^{-1} for NatureFlex, Xtend, and Propafilm, respectively and at 5 °C.

2.3. Package design performance

The packages were filled with strawberries (700 ± 5 g), closed tightly with the designed lids and stored at 5 °C for 14 days. Headspace gas composition (O_2 and CO_2 concentrations) inside each package was monitored daily by using a CheckMate 3 gas analyser (PBI Dansensor, Ringsted, Denmark). A visual documentation of moisture condensation on the lid and window film was recorded after 14 days of storage. In addition, condensation (free/condensed water) and total mass loss (mass loss of strawberry), was quantified at the end of storage on day 14. The amount of water vapour condensed inside the package (g) was quantified by weighing the empty packages before and after the removal of condensed water on the package walls, windows and the lids. The water loss through the film, due to permeability, was also calculated from the difference in the amount of water lost by the strawberry and the amount of water condensed inside the package. One replicate was carried out totalizing 10 packages.

2.4. Physico-chemical quality changes

Fresh strawberry juice was used to measure total soluble solids (TSS), pH and titratable acidity (TA). A digital refractometer (DR301-95, Krüss Optronic, Hamburg, Germany) was used to measure TSS and expressed as %. The TA concentration of the juice sample was measured potentiometrically by titration with 0.1 mol L^{-1} NaOH, to an endpoint of pH 7.0 using an automated T50 M Titra-tor with Rondo 20 sample changer (Mettler Toledo, Switzerland). The TA concentration was expressed as g L^{-1} of citric acid based on fresh mass. The pH was measured with a pH meter (inoLab pH720, WTW Series, Weilheim, Germany) after calibrating with pH buffers 4 and 7. The measurements were done in triplicate on day 0 and on day 14.

2.5. Visual and ortho-nasal quality evaluation

Twelve untrained panelists who are regular consumers and familiar with the quality attributes of strawberry carried out visual and ortho-nasal quality evaluation. Strawberry quality attributes such as texture, odour, and decay were evaluated on a scale of 1 to 5 (Table 2). In addition, visual observation of water vapour condensed on the lid window was also scored on a scale of 1 to 5.

2.6. Evolution of volatile organic compounds

Volatile compounds were extracted by static headspace sampling (SHS). Strawberries from each package were crushed into puree and 5 g of aliquot was placed in 20 mL glass vial with

100 μL of 3-octanol (diluted in absolute methanol to a concentration of 0.1 g L^{-1}) as internal standard. The vials were tightly capped and equilibrated at 80 °C for 20 min in the headspace auto-sampler incubator. Gas sample (1 mL) was automatically withdrawn from the headspace of each vial (HS-20 automated-sampler, Shimadzu Europa GmbH, Duisburg, Germany). Sampling condition for HS-20 auto-sampler was maintained as follows: the oven, sampling line and transfer line temperature was 80 °C, 150 °C and 150 °C, respectively; pressurizing pressure and time was 76 kPa and 2 min, respectively. To increase the sensitivity of the SHS sampling method on the GC–MS, vial shaking level of 3, load time of 0.5 min and injection time of 1 min with single injection parameters were used.

Gas samples were transferred from HS-20 sampler into the GCMS-QP2010 (Shimadzu Europa GmbH, Duis-burg, Germany) for separation of volatile compounds. Due to the volatility, nonpolar character and reactivity of volatile sulphur compounds a mid-polar 1.4 μm film thickness ZebronTM capillary column, with 30 m length and 0.25 mm inner diameter was used (ZB-624, Phenomenex, Aschaffenburg, Germany). Analyses were carried out using helium as carrier gas with a total flow of 16.4 mL min^{-1} and a column flow of 1.22 mL min^{-1}. The GC temperature was held at 50 °C for 1 min, then ramped to 110 °C at 5 °C min^{-1}, then to 180 °C at 20 °C min^{-1}, held for 3 min and finally to 200 °C at 5 °C min^{-1}, and held at this temperature for 1.5 min in total run time of 25 min and split ratio (1:10). The mass selective detector (MSD) was operated in full scan mode and mass spectra in the 35– 350 m/z range were recorded. The ion source and interface temperature were maintained at 200 °C and 230 °C, respectively. Individual volatile compound were identified by their retention time (RT) and calculated Kovats retention index (RI) using *n*-alkane group. The compounds were compared to those registered on the National Institute for Standards and Technology (NIST) mass spectral libraries (NIST v. 08 and 08s, Gaithersbug, MD, USA) and other literature. Only compounds with the square of the correlation coefficient (R^2) above 90% between experimental spectra and NIST MS library were considered. Semi-quantification of the identified compounds was estimated according to Bugaud and Alter (2016) using Eq. (6):

$$\mathrm{RA} = \frac{Ai_c}{A_{its}} C_{its}$$

where RA is the relative abundances of the identified compound (g L^{-1}), Ai_c is the peak area of the identified compound, A_{its} is the peak area of the internal standard, and C_{its} is the final concentration of internal standard in the sample (0.1 mg mL^{-1}).

2.7. Statistical analysis

The data obtained were submitted to analysis of variance (ANOVA) and Tukey's test with significance set at $p < 0.05$ using the Statistica software (version 10.0, StatSoft Inc., Tulsa, USA). In addition, Duncan multiple range test was used to analyse the volatile organic compounds of strawberries in order to determine the difference between mean values at $p < 0.05$. Results were presented as mean ± standard deviation.

3. Results and discussions

3.1. Modified atmosphere and moisture condensation

Gas composition inside the packages varied between 5-14% for O_2 and 8-19% for CO_2 (Figure 1), with exception of the control package. The gas composition of the control package was not shown is Figure 1, nevertheless, it was measured. It reached 1.29% of O_2 already on day 3 of storage and 0% on the remaining days. For CO_2 the concentration reached 23.27% on day 5 and 45% by the end of storage. The PP33, PP66, and NF66 packages had the lowest O_2 steady state conditions (around 6%). However, it was still within the recommended MA conditions for strawberries of 5–10% O_2 and 15–20% CO_2 (Brecht et al., 2003). A decline of O_2 below critical limits (5%) should be avoided as this might lead to in-package anoxia; which in turn results in fermentation and off-odour development (Luca, Mahajan, & Edelenbos, 2016). Overall, the values obtained show similar trends with experimental micro-perforated wild strawberries packed in containers (8-14% O_2) covered with polyethylene terephthalate/polypropylene (PET/PP) multilayer films with three micro-perforations stored for 4 days at 10 °C (Almenar, Catala, Hernandez-Muñoz, & Gavara, 2009). Furthermore, this study showed that the use of fixed window with 2 micro-perforations has the capability of preventing anoxic conditions on packaged strawberries.

Packages fitted with NatureFlex and Xtend windows, independent of the their sizes, effectively prevented water vapour condensation (free/condensed water) in comparison to those fitted with Propafilm and the control package (Figure 2 and 3). This is directly related to the WVTR of the films. Natureflex and Xtend films have very high WVTR, 42.79 and 19.34 g m^{-2} d^{-1} measured at 5 °C, respectively when compared to Propafilm, 0.8 g m^{-2} d^{-1} (Sousa-Gallagher, Mahajan, & Mezdad, 2013). However, the prevention of water vapour accumulation on the package film led to higher mass loss of strawberries. Results show that the type of film and its size had an influence on the rate of mass loss strawberries (Figure 3). The highest product mass loss was observed in the packages covered with NatureFlex (0.57-1.46%), while samples in Propafilm (0.20-0.27%) had the lowest mass loss. The bigger the window size the higher was the mass loss recorded, with the exception of samples PP33 and

P66 that presented similar mass loss independent of the window size, probably, due to the very low permeability of PP film to water vapour. Nevertheless, the overall mass lost by strawberries in this study did not exceed 1.5%, and therefore was significantly below the recommended maximum acceptable loss of 6% (C. N. Nunes & Emond, 2007). Similarly, Caleb, Ilte, Fröhling, Geyer, and Mahajan (2016) investigated the effects of appropriate design of modified atmosphere and humidity packaging (MAHP) systems, with NatureFlex film window on polypropylene film on the postharvest quality of minimally processed broccoli branchlets. Results also showed that the use of the window effectively prevented water vapour condensation on the film surface when compared to bi-axially oriented polypropylene and cling-wrapped commercial control, however, at the expense of a higher product mass loss compared to the control package. Nevertheless, the use of lid window covered with high WVTR films has the capacity of reducing water vapour from the package headspace and therefore, might retard microbial spoilage and increase shelf life. Furthermore, the use of such films as humidity windows is innovative and efficient as these containers are re-usable and there is only the need to change the window film.

3.2. Physico-chemical quality changes

The traditional physical and chemical quality attributes detected no significant ($p \leq 0.05$) changes by the Tukey test in packaged strawberries after 14 days of storage at 5 °C. The range of total soluble solids (TSS), total acidity (TA), and pH obtained in this study was 4.0 - 5.2%, 0.9 - 1.2 g L^{-1}, and 3.9 - 4.1, respectively. TSS and TA are important parameters to determine the fruit quality as they have a direct effect on the flavour. They vary significantly among different strawberry varieties (Kallio, Hakala, Pelkkikangas, & Lapveteläinen, 2000). The authors investigated the sugar and acid composition of six strawberry varieties. They reported that the major acids in strawberries are citric (7.3 -15.8 g L^{-1}) and malic (2.2 - 6.9 g L^{-1}) and total sugar content varied from 5.35 to 10.96%. Nevertheless, both the TSS and the TA obtained in this study were lower than that reported by Kallio et al. (2000), which indicated that the strawberries contained less sugar and were very acid. On the other hand, the pH obtained in this study for cv. Elsanta strawberries was within the range reported for `Sonata` strawberry by Caleb, Wegner, et al. (2016), which was in the range of 3.9 to 4.7. Furthermore, the size and type of film did not affect the physico-chemical quality attributes as there were no significant changes from the initial to the end of storage.

3.3. Visual and ortho-nasal quality evaluation

All sensory attributes received scores below 3 which indicated that all the packages presented compromised quality, especially the control package as it had the lowest score for most of the evaluated attributes (Figure 4). Low scores for the strawberry texture can be associated with mass loss as this leads to shriveling and wilting of the product. Furthermore, our sensorial analysis scores were in accordance with Figure 2 and 3, containers fitted with NatureFlex and Xtend films as lid windows reduced condensation when compared to other packages. This reduction was very important as in-package condensation led to poor quality. Moreover, condensation was quantified as zero, however in the sensory evaluation it was visible. Possibly the films NatureFlex and Xtend absorbed water and formed droplets; therefore, it was visible but could not be quantified. This was due to the fact that the films were not coated with anti-mist and therefore showed droplets adhered to the film as condensed water. On the other hand, Propafilm is a standard material coated with anti-mist; nevertheless, due to the low WVTR the moisture condensation was still visible. It is worth mentioning that anti-mist are chemicals that absorb water and spread it throughout the coated surface. This keeps water droplets from becoming big enough to be visible as condensation.

3.4. Evolution of volatile organic compounds

A total of 8 secondary VOCs were detected at the end of storage day 14 in the different packaging conditions (Table 4). The development of acetaldehyde, acetone, and ethyl acetate are well known to be a result of fermentative metabolism (Nielsen and Leufvén, 2008). Ethanol was below detection limit on day 0. The other fermentative volatiles were detected at low concentrations already on day 0, but further accumulated during the storage of the strawberries. Strawberries kept on the control package had the highest tissue accumulation of ethanol, which indicated that anaerobic respiration was triggered. The increase in ethanol concentration can be associated with the critical gas composition of 45% CO_2 measured on day 14 of storage. High CO_2 concentration could result in the disruption of enzyme activities such as the lipoxygenase pathway (Giuggioli, Briano, Baudino, & Peano, 2015). The production of ethanol and esters varied according to the different modified atmosphere conditions. The influence of headspace gas composition on the accumulation of alcohols and further synthesis of esters was reported by Giuggioli et al. (2015) and Belay, Caleb, and Opara (2017).

Moreover, the strawberries reacted in a different manner to the packaging system conditions. Similar results were found by Nielsen and Leufvén, (2008). Authors pointed out that there can be large differences between strawberry cultivars, especially with regard to the aroma development. Their study indicated that storage in a modified atmosphere affected negatively the aroma development in Korona strawberries; however, the aroma production in Honeoye was not affected in a similar manner. Furthermore, what can be observed from these results is that the traditional physico and chemical properties from strawberries had very little changes within the 14 days of storage compared to the emission of VOCs and the development of off-odour. Thus, this study indicates that the investigation of off-odour during storage can serve as a better indicator of product quality.

4. Conclusion

The overall results indicated that the modified atmosphere and humidity conditions were capable of preventing condensation when the window was covered with films with high permeability to water vapour. Therefore, the concept of such packaging system equipped with a control window represents an innovative approach to minimize the risk of moisture condensation. Moreover, this study showed that the fixed window with micro-perforations was capable of preventing anoxic conditions. Furthermore, the traditional quality parameter detected no significant changes in packaged strawberries, however, the evolution of volatile organic compounds in the package headspace showed significant changes during storage. Therefore, the investigation of off-odour by GC-MS served as a better early indicator of the product quality during storage when compared to the traditional quality parameters (pH, TA, and TSS). Further studies are needed to elucidate the performance of such packaging system under fluctuating temperature conditions, which normally occurs during the long distance supply chain of fresh produce. Measuring actual relative humidity inside the package headspace will also be helpful to understand the dynamics of moisture evolution and condensation on different parts of the package.

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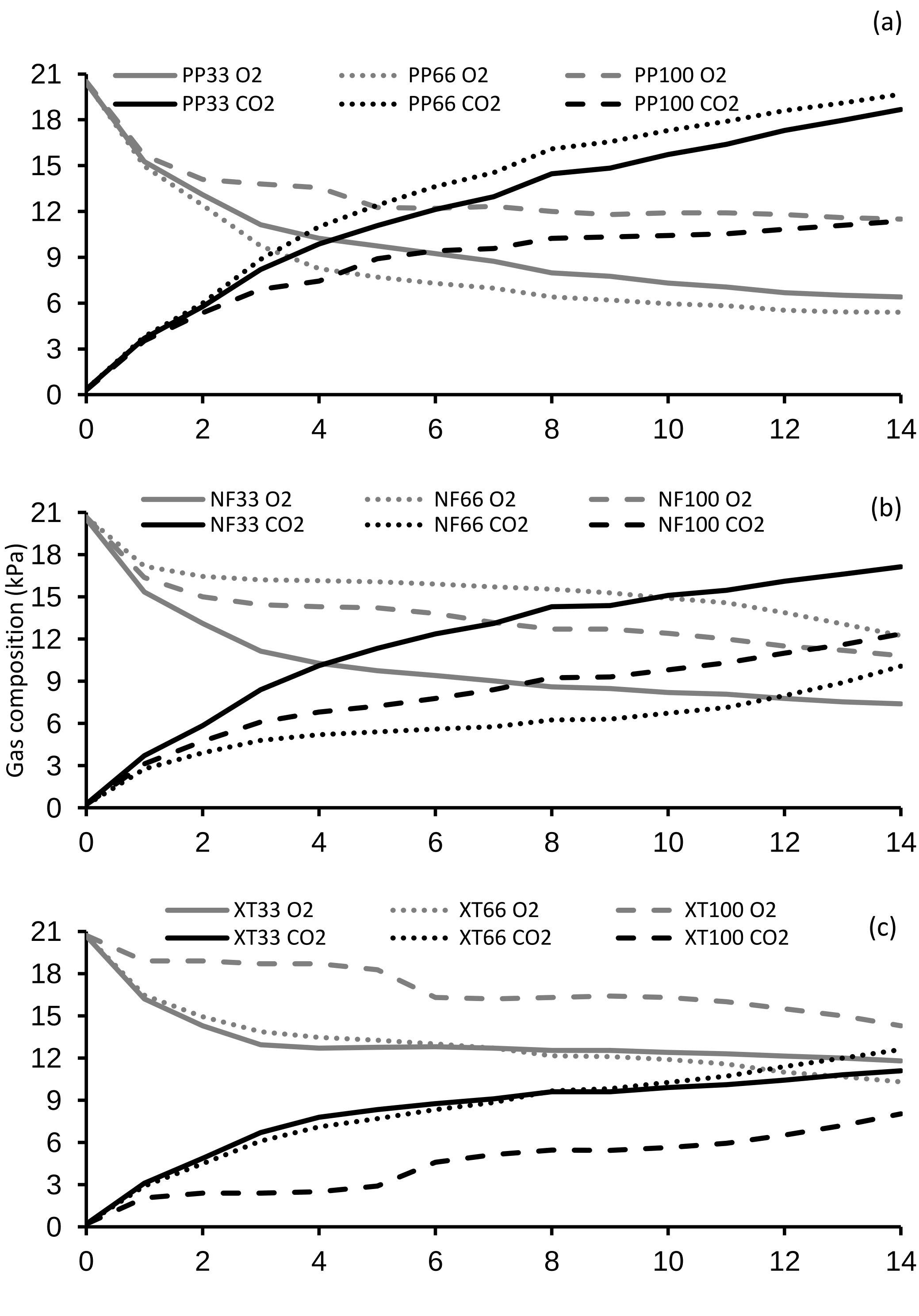

Figure 1. Changes in headspace gas composition for packaged strawberries sealed with fixed window of (a) Polypropylene based Propafilm (PP), (b) NatureFlex (NF), and (c) Xtend films (XT).

Figure 2. Visual documentation of lid and film condensation after 14 days of storage.

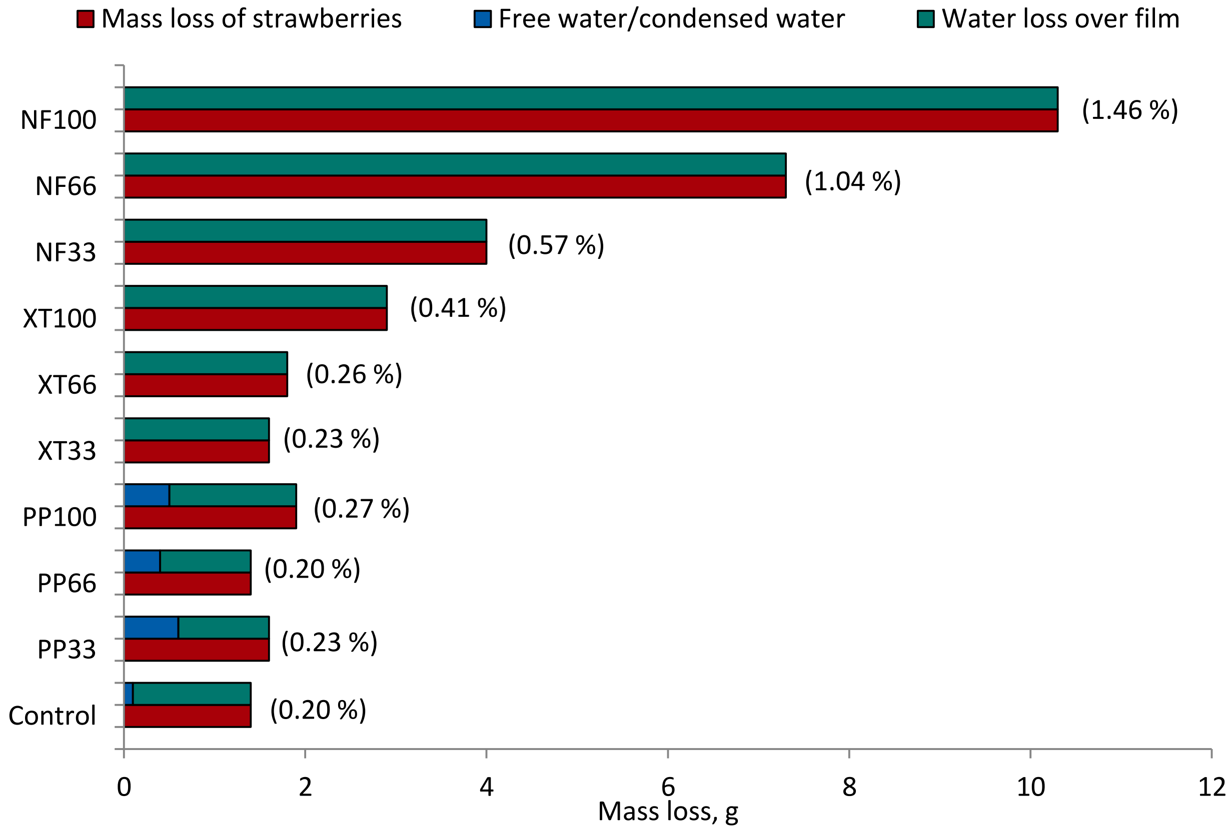

Figure 3. Mass loss of strawberry and in-package condensation during the storage period of 14 days at 5 °C. *The values in bracket represent the percentage strawberry mass loss.

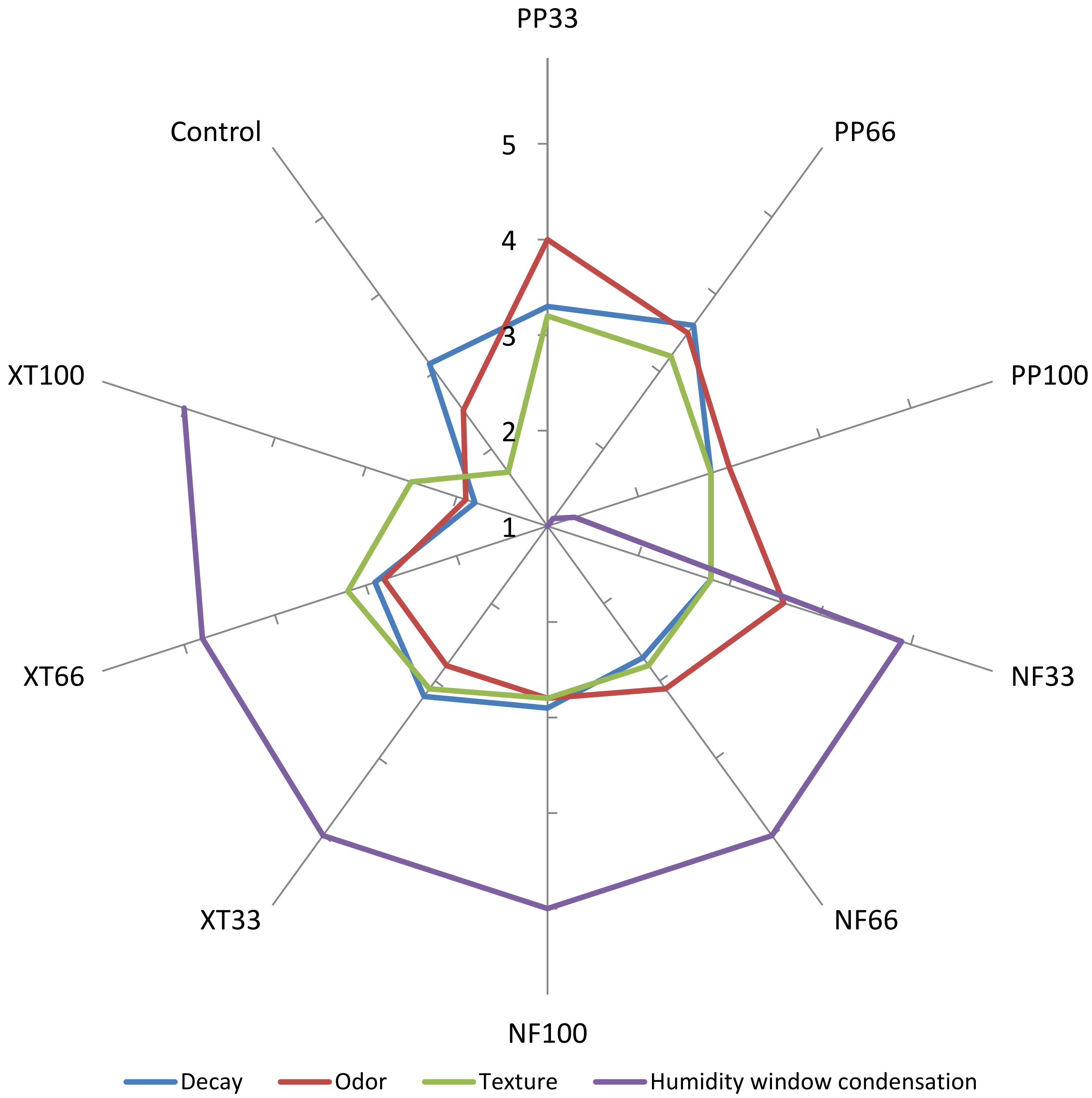

Figure 4. Changes in visual quality attributes of packaged strawberries and observed water vapour condensation on the humidity window after 14 days of storage at 5 °C.

Table 1. Packaging films used and window sizes designed for the storage containers.

Sample	Packaging film	Window size	
		% of lid area	Area of window (cm^2)
Control	Polypropylene lid without perforation	-	-
PP33	Propafilm*	33	69
PP66	Propafilm*	66	126.5
PP100	Propafilm*	100	195.5
XT33	Xtend*	33	69
XT66	Xtend*	66	126.5
XT100	Xtend*	100	195.5
NF33	NatureFlex*	33	69
NF66	NatureFlex*	66	126.5
NF100	NatureFlex*	100	195.5

* With 2 micro-perforations of 0.7 mm

Table 2. Quality scores and descriptors for strawberry

Descriptors	**Scores and description**					**Reference**
	1	**2**	**3**	**4**	**5**	
***Humidity window condensation**	Humidity window is extensively covered with water vapour	Humidity window is partially covered with water vapour $\geq 50\%$	Humidity window is partially covered with water vapour $\leq 50\%$	Humidity window is partially covered with water vapour $\leq 25\%$	Humidity window is completely free of water vapour condensation	Rux, Caleb, Geyer, and Mahajan (2017)
Texture	Very poor (fruit are extremely soft)	Poor (fruit are very soft)	Fair (fruit exhibit minor signs of softness)	Good (fruit are firm)	Very good (fruit are firm and turgid)	(M. C. N. Nunes, Emond, & Brecht, 2003)
Odour	Dislike very much	Dislike moderately	Neither like nor dislike	Like moderately	Like very much	-
Decay	76-100 % decay(extreme decay/completely rotten)	51-75 % decay (moderate to severe decay)	26-50% decay (spots with decay)	1-25 % decay (probable decay)	0 % decay (no decay)	(Rux et al., 2017)

***** Adapted from other studies

Table 3. Volatile organic compounds of strawberries stored for 14 days at 5 °C (mean values (n = 3) ± standard deviation).

Volatiles (mg/mL)	RT	K-RI Est.	K-RI Lit.	Day 0	Day 14									
					Control	PP33	PP66	PP100	XT33	XT66	XT100	NF33	NF66	NF100
Acetaldehyde	4.28	540	518	0.03^{d}	0.25 ± 0.01^{a}	0.1 ± 0.01^{b}	0.09 ± 0.06^{b}	0.03 ± 0.01^{d}	0.06 ± 0.001^{c}	0.12 ± 0.03^{b}	0.04 ± 0.00^{d}	0.04 ± 0.02^{cd}	0.07 ± 0.00^{c}	0.10 ± 0.01^{b}
Ethanol	5.41	602	668	nd	1.98 ± 0.08^{a}	0.29 ± 0.03^{b}	0.06 ± 0.004^{c}	0.05 ± 0.00^{c}	0.02 ± 0.00^{d}	0.02 ± 0.00^{d}	0.03 ± 0.00^{d}	0.18 ± 0.06^{b}	0.07 ± 0.01^{c}	0.02 ± 0.00^{d}
Acetone	6.03	660	455	0.66^{a}	0.02 ± 0.01^{c}	0.09 ± 0.002^{b}	0.10 ± 0.03^{b}	0.08 ± 0.003^{b}	0.10 ± 0.02^{b}	0.09 ± 0.02^{b}	0.04 ± 0.00^{c}	0.08 ± 0.01^{b}	0.09 ± 0.01^{b}	0.09 ± 0.00^{b}
Acetic acid, methyl ester	6.48	554	487	0.001^{e}	0.06 ± 0.002^{c}	0.10 ± 0.04^{a}	0.02 ± 0.00^{d}	0.17 ± 0.06^{a}	0.10 ± 0.04^{a}	0.16 ± 0.08^{a}	0.18 ± 0.04^{a}	0.09 ± 0.05^{abc}	0.12 ± 0.004^{b}	0.18 ± 0.01^{a}
Ethyl Acetate	8.76	632	628	0.002^{f}	1.60 ± 0.07^{a}	1.52 ± 0.50^{a}	0.33 ± 0.03^{b}	0.97 ± 0.06^{a}	0.13 ± 0.02^{d}	0.17 ± 0.00^{c}	0.06 ± 0.00^{e}	1.12 ± 0.54^{a}	0.04 ± 0.01^{e}	0.33 ± 0.05^{b}
Butanoic acid, ethyl ester	15.15	836	785	nd	0.06 ± 0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd
Butanoic acid, 2-methyl-, ethyl ester	16.35	919	820	nd	0.03 ± 0.002	nd	nd	nd	nd	nd	nd	nd	nd	nd
Hexanoic acid, ethyl ester	19.47	1157	984	nd	0.03 ± 0.02	nd	nd	nd	nd	nd	nd	nd	nd	nd

Mean value ± standard deviation in the same row with different lower case superscripts are significantly different based on Duncan (Post-hoc test) at $p \leq 0.05$.
nd: implies that volatile was below detection limit/ not detected; K-RI: kovats retention index Est. (estimated) and Lit. (based on literature)