



Effect of active and modified atmosphere packaging on quality retention of dark chocolate with hazelnuts

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ABSTRACT

The present study investigated the effect of active and modified packaging as well as packaging material oxygen permeability on quality retention of dark chocolate with hazelnuts. Dark chocolate was packaged in: a) polyethylene terephthalate/low density polyethylene (PET/LDPE), and b) polyethylene terephthalate coated with SiOx/low density polyethylene (PET-SiOx/LDPE). Samples were packaged either under, vacuum or N₂ or with an oxygen absorber and stored in the dark at 20 °C for a period of 12 months. “Commercial” control samples for comparison purposes consisted of chocolate packaged in aluminum foil in air while “model” control samples used for sensory evaluation consisted of chocolate packaged in glass jars and stored at –18 °C. Quality parameters monitored were: peroxide value, hexanal content, color, fatty acid composition and volatile compounds. Of the sensory attributes color, texture, odor and taste were evaluated. PV ranged between 0.80 for fresh dark chocolate with hazelnuts and 6.51 meq O₂/kg chocolate fat for commercially packaged samples after 12 months of storage. Respective values for hexanal were 0.53 and 7.56 mg/kg. % Saturated fatty acids (SFA) increased with a parallel decrease in monounsaturated fatty acids (MFA) and polyunsaturated fatty acids (PUFA) after 12 months of storage mainly in least protected samples (commercial package). Likewise, after 12 months of storage an increase in concentration of aldehydes, ketones, alcohols and alkanes ($p < 0.05$) with a parallel decrease in pyrazines were observed especially in case of least protected products after 6 and 12 months of storage. In general after 12 months of storage chocolate showed whitening of the surface resulting to an increase in L^* and a^* values ($p < 0.05$) and a decrease in b^* value. Dark chocolate with hazelnuts retained acceptable quality for ca. 8 months in commercial packages. For samples packaged in PET/LDPE irrespective of storage atmosphere the shelf life was 8 to 9 months and for samples packaged in PET-SiOx/LDPE irrespective of storage atmosphere the shelf life was 11 months. Finally for samples packaged with an oxygen absorber irrespective of packaging material the shelf life was at least 12 months.

Industrial relevance: Chocolate packaged with an oxygen absorber in a barrier packaging material will maintain its aroma, taste and nutritional quality substantially longer than other packaging methods.

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1. Introduction

Chocolate is a complex emulsion based on cocoa, the consumption of which activates pleasure centers of the human brain through its flavor. Chocolate, besides cocoa, usually contains milk, milk powder (Afoakwa, Paterson, & Fowler, 2007); it may also contain sugar and nuts depending on product category. The shelf life of chocolate depends on several parameters including: storage temperature and humidity, availability of oxygen in the immediate environment, directly related to packaging material used, as well as the addition of other ingredients such as fats, nuts etc (Nattress, Ziegler, Hollender, & Peterson, 2004).

Traditionally, almonds and peanuts have been used in confectionery products, such as chocolate in the USA while hazelnuts have been used more in Europe (Nattress et al., 2004). Dark chocolate contains fat in the form of cocoa butter, composed of high levels of saturated fatty acids (SFA), mainly a mixture of stearic (21.21%), palmitic (20.00%) and an amount of unsaturated fatty acids with oleic acid being the predominant unsaturated fatty acid (23.03%) (Mursu et al., 2004). On the other hand nuts, and especially hazelnuts, contain high levels of unsaturated fatty acids such as oleic acid (82.72%), linoleic acid (8.89%) and small amounts of saturated fatty acids such as palmitic acid (4.85%) (Alasalvar et al., 2003). Consumption of SFA has been correlated to an increased risk of coronary heart disease because of their propensity to elevate plasma lipids and lipoproteins and to enhance thrombosis (Lairon, 1997; Grundy, 1994). Conversely, unsaturated fatty acids have been reported to decrease atherogenic factors (Keys et al., 1986; Ascherio et al., 1996). At the same time, high

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levels of unsaturated fatty acids render the product susceptible to oxidation which is the main cause of off-flavor development in nuts (Reed, Gorbet, & O'Keefe, 2000). Furthermore, according to Reed et al. (2000) oxidation of nuts with chocolate coatings show higher oxidation than those stored uncoated under the same storage conditions. According to these authors the chocolate coating probably blocks the passage of air and moisture to the nuts' tissue, resulting in a decrease in relative humidity and therefore increased oxidation rates.

Active packaging, allows packages to interact with food and the environment and play a dynamic role in food preservation (Lopez-Rubio et al., 2004; Brody, Bugusu, Han, Sand, & McHugh, 2008). Ageless® is the registered trade name of the most common active packaging, an oxygen scavenging system based on iron oxidation (Nakamura & Hoshino, 1983) which is an alternative to nitrogen and vacuum packaging techniques for (Grattan & Gilberg, 1993) delayed oxidation and control of microbial growth (Brody et al., 2008).

To the best of our knowledge there is no information in the literature on the use of active and modified atmosphere packaging for the preservation of chocolate. Thus the objective of the present study was to investigate the effect of 1) an oxygen absorber as well as MAP and VP 2) packaging material barrier to oxygen on shelf life extension of dark chocolate with whole hazelnuts. A second objective was to test the experimental PET-SiOx//LDPE laminate as an effective barrier for the protection of dark chocolate.

2. Experimental

Commercial dark chocolate bars with whole hazelnuts were supplied from a local supplier (Athens, Greece) in February 2008 one day after production. Chocolate was packaged in aluminum foil packaged in air. According to the supplier the shelf life of the product is 8 months. Chocolate bars were unpackaged and re-packaged in two different packaging materials: a) PET//PE pouches, 75 µm in thickness and 103 mL/(m² day atm) in oxygen permeability and b) PET-SiOx//LDPE, 62 µm in thickness and 1.4 mL/(m² day atm), measured using the Oxtran 2–20 oxygen permeability tester at 75% RH and 25 °C (MOCON Minneapolis, MN). From these, 3 lots of samples were prepared: the first lot of pouches (PET//LDPE and PET-SiOx//LDPE) containing dark chocolate with hazelnuts (100 g) were evacuated. Lot 2 samples were first evacuated and then immediately injected with N₂ gas produced by a PBI Dansensor MAP Mix 9000 gas mixer (Dansensor, Ringsted, Denmark). Pouches were heat-sealed using a BOSS model NE 48 vacuum sealer (BOSS, Bad Homburg, Germany). In the third lot a ZPT type O₂ absorber (Mitsubishi Gas Chemical Company, Japan) was introduced into each pouch and then pouches were heat-sealed. Samples were stored in the dark at 20 °C. Control samples consisted of chocolate bars with hazelnuts in glass jars flushed with N₂ and stored at –18 °C for up to 12 months. After 0, 2, 4, 6, 8, 10 and 12 months of storage, three separate identical samples were withdrawn from each treatment for chemical and sensory analysis. Duplicate measurements were carried out on each of three replicate samples ($n = 3 \times 2 = 6$).

2.1. Fat extraction—Determination of peroxide value

The fat from chocolate bars was extracted using the Welmann method (G.S.C.L., 1976): Crushed dark chocolate with hazelnuts (5 g) was transferred into a separatory funnel with 100 mL of diethyl ether and 10 mL of distilled water. The separatory funnel was agitated for 2 minutes and subsequently left to equilibrate for 24 h. Fifty milliliter of the sample was transferred to a crystallizing dish and diethyl ether was evaporated in a water bath at 40 °C. The extracted fat was dried in an oven at 105 °C for 3 min, and the residue was used to determine the peroxide value. The peroxide value was determined according to the official Commission Regulation (EC) (1991) method.

2.2. Hexanal determination

2.2.1. SPME procedure

Crushed dark chocolate with hazelnuts samples (0.1 g), along with 1 mL of distilled water and a micro-stirring bar were placed in a 10 mL glass serum vial sealed with an aluminum crimp cap provided with a needle-pierceable polytetrafluoroethylene/silicone septum. Solid-phase microextraction (SPME) was performed with a 75-mm Carboxen/Polydimethylsiloxane (PDMS) fibre mounted to a SPME manual holder assembly (Supelco, Bellefonte, USA). The sample vial was placed in a 60 °C water bath and stirred at high speed. After allowing 10 min for the sample to equilibrate at 60 °C, the needle of the SPME device was inserted into the vial through the septum, and the plunger of the SPME apparatus was pushed down to expose the Carboxen/PDMS fibre to the vial head space. After 10 min of exposure time with constant stirring, the fibre was retracted into the needle assembly, removed from the vial, and transferred to the injection port of the GC unit.

2.2.2. GC-FID analysis conditions

GC analysis of hexanal adsorbed onto the SPME fibre was carried out on a Hewlett-Packard HP 5890 series II GC unit (Wilmington, DE, USA) equipped with a FID detector. A non-polar capillary column (HP-5, J. & W. Scientific, Folsom, USA) 30 m long, 0.32 mm in internal diameter and 0.25 mm in thickness was used. The GC oven was programmed as follows: the temperature was initially set at 40 °C for 5 min, and then raised at the rate of 15 °C per min to 230 °C. The injector temperature was set to 270 °C and detector temperature was kept at 330 °C. Flow rate of the helium carrier gas was 0.8 mL/min. The injector was operated in the split mode (1:2 split ratio) at a temperature of 330 °C. For thermal desorption, the SPME fibre was kept in the injector for 10 min. Data was performed using HP GC Chemstation software for Windows (Hewlett-Packard).

2.3. Semi quantitative determination of volatile compounds

2.3.1. SPME sampling

Crushed dark chocolate with hazelnuts samples (0.1 g), along with 0.1 mL of 0.1 M NaCl of distilled water, 10 µL of 4-methyl-2-pentanone (internal standard) and a micro-stirring bar were placed in a 10 mL glass serum vial sealed with an aluminum crimp cap provided with a needle-pierceable polytetrafluoroethylene/silicone septum. Solid-phase microextraction (SPME) was performed with a 75-mm Carboxen/Polydimethylsiloxane (PDMS) fibre mounted to a SPME manual holder assembly (Supelco, Bellefonte, USA). The sample vial was placed in a 65 °C water bath and stirred at high speed. After allowing 15 min for the sample to equilibrate at 65 °C, the needle of the SPME device was inserted into the vial through the septum, and the plunger of the SPME apparatus was pushed down to expose the Carboxen/PDMS fibre to the vial head space. After 15 min of exposure time with constant stirring, the fibre was retracted into the needle assembly, removed from the vial, and transferred to the injection port of the GC/MS unit.

2.3.2. Gas chromatography–mass spectrometry (GC–MS) identification of volatiles compounds

GC–MS analysis was carried out using an Agilent GC 7890A series combined with an Agilent 5975C Mass Spectrometer in EI (electron Impact) mode. The temperature of ion source and injector was 230 and 250 °C, respectively. The capillary column used was a DB-5 ms (60 m × 0.320 mm i.d and 1 µm, film thickness, J&W Scientific, Agilent Technologies, USA). The oven temperature was programmed at 40 °C for 5 min, then to 115 °C at 10 °C/min for 0 min continuing with increase to 200 °C at 5 °C/min and finally to 280 °C at 10 °C/min at which it remained for 3 min. Helium was used as the carrier gas at a flow rate of 0.8 mL/min. The injector was operated in the split mode

(1:3 split ratio) at a temperature of 330 °C. Identification of volatile compounds was performed using MSD ChemStation E.01.00.237 software for Windows. Peak identification was performed by comparison of retention times and mass spectra of eluting compounds to those of the Wiley library (Wiley7, Nist 05, J. Wiley & Sons Ltd., West Sussex, England).

2.4. Determination of fatty acid methyl esters

The fatty acid composition was determined according to the official method EEC (2568/91) Annex Xa Annex Xb, modified by Rec EEC n. 796/2002 method for the measurement of the characteristics of olive oil and olive-residue oil as described below: approximately 0.1 g of the chocolate fat was weighed in a 5 mL screw-top test tube. To this, 2 mL of heptane was added, and the contents were shaken. Then 0.2 mL of 2 N methanolic potassium hydroxide solution was added, the cap fitted with a PTFE joint was tightened and contents were shaken vigorously for 30 s. Then the screw-top test tube was left to separate into phases until the supernatant phase became clear. The supernatant phase containing the methyl esters was then decanted and gas chromatographic analysis was initiated.

2.4.1. GC-MS fatty acid methyl ester analysis

Chromatographic analysis was carried out using the GC/MS unit mentioned in Section 2.3.2. The temperature of ion source and injector was 230 and 250 °C, respectively. A polar fused silica capillary column was used (30 m × 0.32 mm i.d × 0.5 µm thickness Supelcowax™-10). The oven temperature was programmed at 180 °C for 5 min and then to 240 °C at 3 °C/min. Helium was used as the carrier gas at a flow rate of 1.2 mL/min, with an injector volume of 1 µL using a split ratio of 1:40. Analysis of fatty acid methyl esters was performed using MSD ChemStation E.01.00.237 software for Windows. Identification of fatty acid methyl esters was carried out using the mass spectrum library (Wiley7, Nist 05, J. Wiley & Sons Ltd., West Sussex, England).

2.5. Color measurement

The color of dark chocolate with whole hazelnuts was measured using a Hunter Lab model DP-9000 optical sensor colorimeter (Hunter Associates Laboratory, Reston, VA, USA) and expressed as color L^* (lightness), a^* (redness) and b^* (yellowness) values. Thirty five grams per sample were compressed into a cylindrical (base diameter 11.3 cm, height 2 cm) optical cell. Reflectance values were obtained using a 45 mm viewing aperture. The results reported (L^* , a^* , b^*) are the mean of ten determinations.

2.6. Sensory evaluation

Sensory evaluation (consumer analysis) was carried out by a 51 member untrained panel (20 males and 31 females) consisting of faculty and graduate students of the Laboratory of Food Chemistry and Technology of the University of Ioannina Department of Chemistry. Panelists were chosen using the following criteria: ages between 22 and 60, non-smokers, without reported cases of food allergies who consume chocolate products regularly. Approximately 20 g of dark chocolate with hazelnuts was placed in small plastic containers coded with 3-digit random numbers and tightly capped. The samples were allowed to stand for 0.5 h prior to the evaluation to allow equilibration of volatiles in the headspace. Panelists were served a set of 5 treated samples along with a control sample (stored in glass jars at -18 °C); they were instructed to consume the whole sample and rinse their mouth with sparkling water (room temperature), in between sample evaluation. Sensory attributes evaluated included color, texture, odor and taste. Scoring was carried out on paper ballots using a 9 point hedonic scale where: 9 = extremely like and 1 = extremely dislike for the evaluation of odor, and taste as well as 9 = very crispy and

1 = very soft for evaluation of texture and 9 = no difference compared to control and 1 = product has turned completely white for evaluation of color. A score of 5 was taken as the lower limit of acceptability for color, texture odor and taste.

2.7. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the software SPSS 16 for windows. Means and standard error were calculated, and, when F -values were significant at the $p < 0.05$ level, mean differences were separated by the least significant difference procedure.

3. Results and discussion

3.1. Headspace gas composition

Results showed that for chocolate packaged in PET//LDPE the N_2 concentration fell below 98.1% after 2 months exposure of the product to O_2 while in the presence of the oxygen absorber the N_2 concentration reached 99.1% after 12 months of storage (data not shown). On the other hand the high barrier material PET-SiOx//LDPE even after 12 months of storage retained a N_2 concentration above or equal to 99.8% while in the presence of the oxygen absorber the N_2 concentration remained equal to 100% throughout storage, effectively protecting the product from oxidation.

3.2. Peroxide value (PV)

Formation of primary oxidation products was monitored by determining PV. Changes in PV of dark chocolate with hazelnuts as a function of active or modified atmosphere, packaging material oxygen permeability and time of storage in the dark at 20 °C are shown in Fig. 1. After 12 months of storage, chocolate packaged with the oxygen absorber, irrespective of packaging material permeability, had a very low PV ca. 1.31–1.47 meq O_2 /kg chocolate fat, ($p < 0.05$). Samples packaged in PET-SiOx//LDPE under N_2 or vacuum showed a small increase in PV value (from 0.80 to 2.19 and 2.37 meq O_2 /kg chocolate fat, respectively) while for samples packaged in PET//LDPE under N_2 or vacuum packaged a fivefold increase (from 0.8 to 4.38 and 4.22 meq O_2 /kg chocolate fat respectively) was recorded ($p < 0.05$). Lastly for commercially packaged chocolate higher values of PV were recorded (6.51 meq O_2 /kg chocolate fat). An observation to be made is that for a given packaging material (PET//LDPE or PET-SiOx//LDPE) both atmospheres (vacuum or N_2 atmosphere) showed similar rates of hydroperoxide formation during storage. Reed et al. (2000) studied the effect of chocolate coating (milk chocolate, white chocolate and reduced fat chocolate vs. uncoated) on oxidative stability of normal (NOP) and high oleic peanuts (HOP) stored for 29 weeks at intermediate (0.6) and low (0.19) water activity (a_w) values at 25 °C. They observed an increase in peroxide value of chocolate-coated peanuts during storage especially in chocolate bars coated with NOP compared to HOP. Also, Vercet (2003) studied fat oxidation of almonds coated with white chocolate during storage at 20 °C, at a relative humidity of 65%. They reported a maximum peroxide value of 9.5 meq O_2 /kg fat obtained from almonds coated with white chocolate, after 15 months of storage.

3.3. Hexanal content

Formation of secondary oxidation products was monitored by determining hexanal content. Changes in hexanal content of dark chocolate with hazelnuts as a function of active or modified atmosphere packaging, material oxygen permeability and time of storage in the dark at 20 °C are shown in Fig. 2. Hexanal is directly related to the development of oxidative off-flavors; it has a low odor threshold

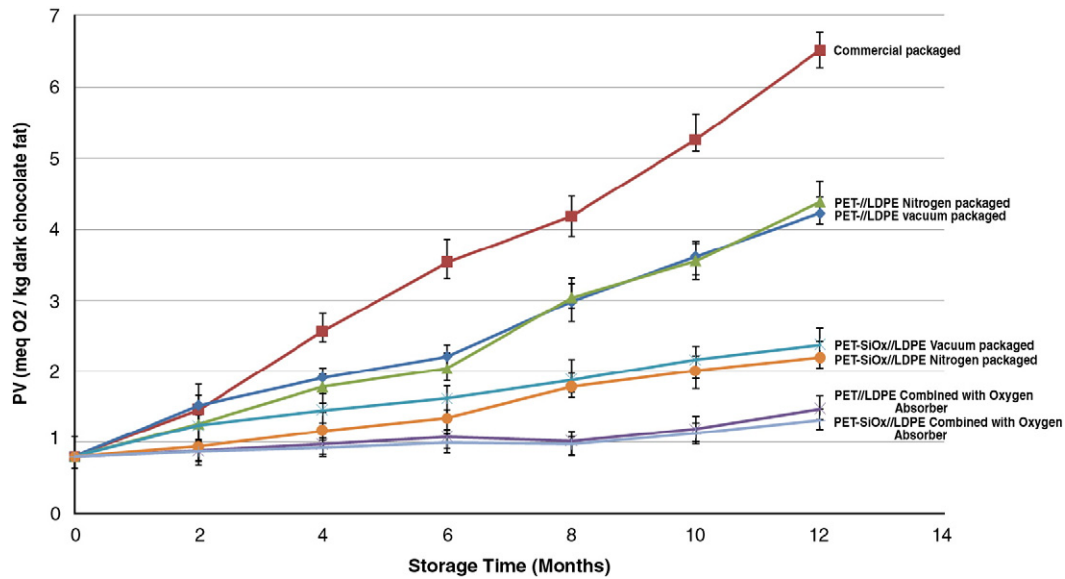


Fig. 1. Peroxide value of dark chocolate with hazelnuts as a function of active and modified atmosphere packaging, packaging material oxygen permeability and storage time in the dark at 20 °C.

(5 ng/g) (Buttery, Turnbaugh, & Ling, 1988) and is thus considered as an indicator of fat quality. Similar to hydroperoxide formation, after 12 months of storage, chocolate packaged with the oxygen absorber, irrespective of packaging material permeability, had a very low hexanal content ca. 1.15–1.62 mg hexanal/kg chocolate, ($p < 0.05$). Samples packaged in PET-SiO_x//LDPE under N₂ or vacuum showed a fourfold increase (from 0.53 to 2.27 and 2.35 mg hexanal/kg chocolate, respectively) while samples packaged in PET//LDPE under N₂ or vacuum showed an eight and nine fold increase (from 0.53 to 4.43 and 4.88 mg hexanal/kg chocolate), respectively ($p < 0.05$). Commercially packaged samples showed the highest hexanal content (7.56 mg hexanal/kg chocolate). For a given packaging material (PET//LDPE or PET-SiO_x//LDPE) both atmospheres (N₂ and vacuum) showed similar rates of hexanal formation during storage. Hashim, Hudiyono,

and Chaveron (1997) studied the oxidation behavior of cocoa butter during storage under day light at room temperature or under accelerated conditions (oxidized in oven at 90 °C) for a period of 12 weeks. Similar to us they reported an increase in hexanal concentration during storage from an initial value of 4 to 580 mg/kg chocolate. Also Kinderlerer and Johnson (1992) reported a large increase in hexanal concentration of hazelnut kernels during storage at ambient temperature in the dark after 3 years. Fadel, Maged, Samad, and Lofty (2006) studied the sensory quality and flavor stability of a cocoa substitute (mixture of chicory roots and carob bean) after storage for 6 months and reported a tenfold increase in hexanal concentration. In contrast, Nattress et al. (2004) studied the effect of different hazelnut pastes (dark chocolate containing either 0, 5 or 10% of roasted or unroasted hazelnuts) on sensory properties and

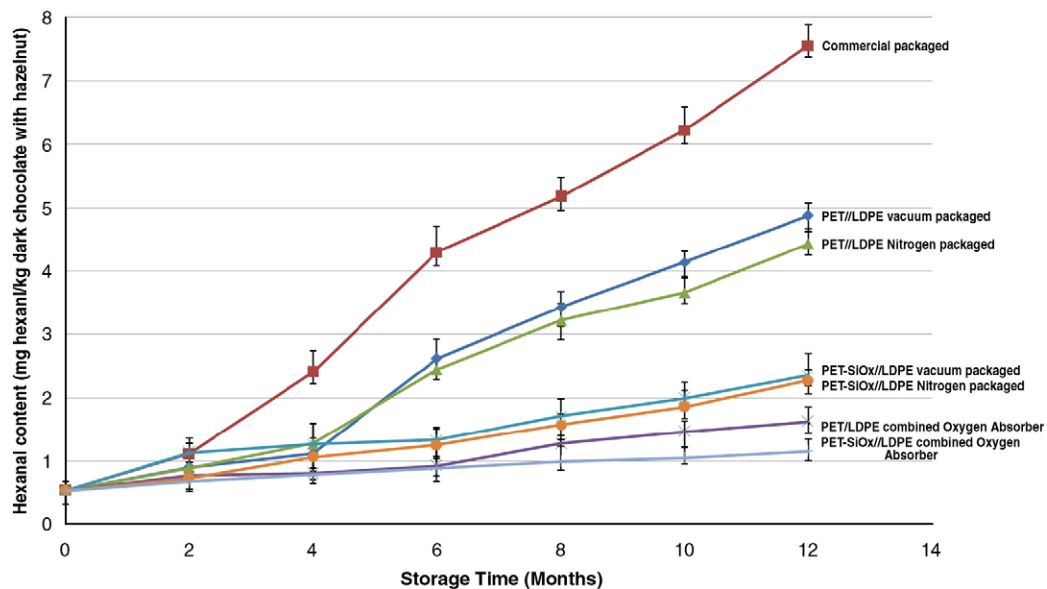


Fig. 2. Hexanal content of dark chocolate with hazelnuts as a function of active and modified atmosphere packaging, packaging material oxygen permeability and storage time in the dark at 20 °C.

shelf life of dark chocolate for a period of 10 months and reported no signs of oxidation in any of the samples as hexanal concentration remained below 0.78 mg/kg in all test samples over storage time.

3.4. Fatty acid composition

Fatty acid composition of dark chocolate with hazelnuts as a function of active or modified atmosphere packaging, packaging material oxygen permeability and time of storage in the dark at 20 °C is shown in Table 1. The initial content (day 0) of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of dark chocolate with hazelnuts was 40.42, 53.11 and 6.47% respectively. According to Karabulut (2007) dark chocolate contains 62.87% SFA of which the predominant fatty acids are palmitic (C_{16:0}) 26% and stearic (C_{18:0}) 35%. Also dark chocolate contains 37.13% unsaturated fatty acids (UFA) with oleic acid (C_{18:1}) being by far the major fatty acid 32.75% and linoleic acid (C_{18:2}) following in quantity (3.44%). In contrast, according to Alasalvar et al. (2003) hazelnut oil contains 7.85% SFA with palmitic acid (C_{16:0}) being predominant (4.85%) and 92.15% UFA with oleic acid (C_{18:1}) being predominant (82.72%) followed by linoleic acid (C_{18:2}) (8.89%).

After 12 months in case of the least protected samples (commercially packaged in air) the SFA, MUFA and PUFA were 53.68, 42.44 and 3.88% while in case of the most protected samples (PET-SiO_x/LDPE with the oxygen absorber) the SFA, MUFA and PUFA were 43.49, 50.68 and 5.83%, respectively. The substantial increase in concentration of saturated fatty acids, myristic, palmitic and stearic is very important as myristic acid is the most atherogenic of all saturated fatty acids and has four times the cholesterol rising effect as palmitic acid (Yilmaz & Gecgel, 2007). Similarly the increase in stearic acid concentration is of significance as it has been shown that its presence increases the risk of coronary heart disease more than palmitic and myristic acids (Hu et al., 1999). Likewise, the increase in palmitic acid content is also important, as experiments have shown that hamsters fed with diets high in palmitic acid raised plasma cholesterol concentrations (Spady & Dietscy, 1988). Jinap, Ali, Man, and Suria (2000) studied the effect of dark chocolate filled with desiccated coconut during storage for 8 weeks at 18, 30 and 35 °C. Similar to us they reported significant changes in fatty acid composition of cocoa butter stored at 18 °C after 8 weeks of storage. In particular palmitic acid increased from 24.56 to

27.17%. As expected, additional changes were observed at 30 and 35 °C as compared to those at 18 °C. In contrast to our results for the least protected samples, Vercet (2003) reported that there were no substantial changes in the fatty acid composition of almonds coated with chocolate during 15 months of storage at 20 °C and at a relatively humidity of 65%. Such differences may be attributed to the lower concentration of unsaturated fatty acids in almonds coated with white chocolate (32.4% UFA) used as compared to hazelnuts coated with dark chocolate (59.6% UFA).

3.5. Production of volatile compounds

In order to determine the optimum conditions for SPME-GC/MS analysis the following parameters were studied: fibre type, absorption and desorption time, sample weight in the vial, extraction time, and temperature. Six fibres with different coating materials were examined: polyacrylate 85 mm in thickness, PDMS 100 mm in thickness; CAR/PDMS 75 mm in thickness, PDMS/DVB 65 mm in thickness, CAR/DVB 65 mm in thickness and DVB/PDMS/CAR 50–30 mm in thickness. The results showed that the 75-mm CAR/PDMS fibre had the highest sorptive capacity. Extraction time was carried from 5 to 30 min. The optimum signal areas were obtained with an extraction time of CAR/PDMS for 15 min into the headspace of the vial after allowing the sample to equilibrate for 15 min at 60 °C. Extraction temperature was varied between 30 and 70 °C. The optimum signal areas were obtained at 60 °C. In order to determine the optimum sample weight samples (0.1 to 0.5 g) were placed in the vial and conditioned as described above. The sample weight was chosen to be 0.1 g chocolate, along with 1 mL of distilled water and 1 mL of 0.1 M NaCl. The addition of water was necessary to generate a homogenous slurry of crushed chocolate and to disperse the internal standard homogeneously throughout the sample. The addition of NaCl to the sample showed a positive effect on the reproducibility and the extraction yield of the headspace compounds. 4-methyl-2-pentanone was chosen as the internal standard as it does not occur naturally in dark chocolate or hazelnut and it does not interfere with flavor compounds of dark chocolate with hazelnuts in the chromatographs. Our experimental conditions with regard to SPME fibre selection contradict those of Ducki, Miralles-Garcia, Zumbé, Tornero, and Storey (2008) who reported that DVB/PDMS/CAR 50–30 mm showed the

Table 1

% Fatty acid composition of dark chocolate with hazelnuts as a function of active and modified atmosphere packaging, packaging material oxygen permeability and storage time in the dark at 20 °C.

Packaging material		Commercially packaged		PET/LDPE packaged under N ₂		PET-SiO _x /LDPE with oxygen absorber	
% area		% area		% area		% area	
Fatty acid	Day 0	6 Months	12 Months	6 Months	12 Months	6 Months	12 Months
C _{14:0}	0.16 ^b ± 0.02	0.47 ^c ± 0.08	1.60 ^c ± 0.02	0.58 ^c ± 0.06	1.10 ^d ± 0.03	0.11 ^a ± 0.02	0.11 ^a ± 0.01
C _{15:0}	0.05 ^a ± 0.01	0.05 ^a ± 0.01	0.03 ^a ± 0.01	0.05 ^a ± 0.02	0.34 ^b ± 0.01	0.06 ^a ± 0.01	0.03 ^a ± 0.02
C _{16:0}	18.82 ^a ± 0.86	21.95 ^b ± 0.74	26.61 ^d ± 0.48	21.39 ^b ± 0.66	22.84 ^{bc} ± 0.51	18.80 ^a ± 1.11	19.95 ^a ± 0.47
C _{17:0}	0.30 ^a ± 0.08	0.50 ^a ± 0.14	1.22 ^c ± 0.07	0.55 ^{ab} ± 0.04	1.21 ^c ± 0.06	0.20 ^a ± 0.07	0.25 ^a ± 0.05
C _{18:0}	20.00 ^a ± 0.46	21.65 ^b ± 0.41	21.98 ^b ± 0.18	20.43 ^a ± 0.32	19.86 ^a ± 0.78	20.30 ^a ± 0.58	21.70 ^b ± 0.75
C _{20:0}	0.85 ^a ± 0.11	1.12 ^b ± 0.16	2.02 ^c ± 0.36	1.10 ^b ± 0.10	1.89 ^c ± 0.14	0.84 ^a ± 0.17	1.23 ^b ± 0.28
C _{22:0}	0.24 ^b ± 0.08	0.29 ^{bc} ± 0.02	0.22 ^b ± 0.05	0.33 ^d ± 0.01	0.14 ^a ± 0.01	0.22 ^b ± 0.04	0.22 ^b ± 0.03
Total SFA	40.42^a ± 1.08	46.03^b ± 0.88	53.68^d ± 1.01	44.43^b ± 0.99	47.38^{bc} ± 1.23	40.53^a ± 1.51	43.49^b ± 1.06
C _{16:1 n-9}	0.41 ^{bc} ± 0.11	0.32 ^{ab} ± 0.09	0.12 ^a ± 0.02	0.44 ^{bc} ± 0.09	0.21 ^{ab} ± 0.06	0.20 ^a ± 0.06	0.29 ^{ab} ± 0.04
C _{18:1 n-9}	52.56 ^d ± 0.74	47.74 ^b ± 1.14	42.21 ^a ± 0.79	49.94 ^b ± 1.14	47.55 ^b ± 1.28	52.65 ^d ± 0.96	50.29 ^{bc} ± 1.25
C _{20:1 n-11}	0.14 ^c ± 0.01	0.15 ^c ± 0.02	0.11 ^{ab} ± 0.01	0.07 ^a ± 0.00	0.13 ^c ± 0.00	0.18 ^{cd} ± 0.04	0.10 ^a ± 0.03
Total MUFA	53.11^d ± 0.89	48.21^b ± 1.28	42.44^a ± 1.25	50.45^{bc} ± 1.51	47.89^b ± 1.02	53.03^d ± 0.97	50.68^{bc} ± 1.28
C _{18:2 n-9,12}	6.18 ^c ± 0.47	5.44 ^b ± 0.25	3.79 ^a ± 0.61	4.92 ^{ab} ± 0.38	4.50 ^a ± 0.61	6.16 ^c ± 0.16	5.63 ^b ± 0.10
C _{18:2 n-9,12,15}	0.29 ^{bc} ± 0.04	0.32 ^c ± 0.02	0.09 ^a ± 0.02	0.20 ^b ± 0.02	0.23 ^b ± 0.04	0.28 ^{bc} ± 0.05	0.20 ^b ± 0.03
Total PUFA	6.47^d ± 0.56	5.76^c ± 0.261	3.88^a ± 0.80	5.12^{ab} ± 0.41	4.73^a ± 0.52	6.44^d ± 0.36	5.83^c ± 0.12

U.D.L. = under detection limit, *t_r* = Retention time, Values are the mean of six determinations (n = 3 × 2 = 6), ± S.D. ^{a-e} Means with different superscripts in the same row are statistically different (p < 0.05).

highest extraction efficiency for chocolate volatiles. As for extraction time and temperature the same conditions were found to give optimum results according to Ducki et al. (2008).

A total number of 33 volatile compounds belonging to the chemical classes of alcohols, ketones, aldehydes, esters, acids, alkanes, sulfur compounds, aromatic hydrocarbons, pyrazines, terpenes and furans were indentified in dark chocolate with hazelnuts on day 0 (Table 2). Present results for flavor profile of dark chocolate with hazelnuts, are in good agreement with those of the literature data (Afoakwa, Paterson, Fowler, & Ryan, 2008). Most of the compounds belong to the basic flavor

profile of cocoa beans, mainly formed during fermentation or during roasting of cocoa (Hoskin & Dimick, 1984; Jinap, Wan, Russly, & Norsin, 1998). For example 3-methylbutanal derives during fermentation of cocoa beans (Frauendorfer & Scieberle, 2008) while 2-methyl-pyrazine, trimethylpyrazine and tetramethylpyrazine increase significantly during roasting (Carnil et al., 1986). A part of the flavor profile determined could be also attributed to hazelnuts. For example compounds such as: 2-heptanone, 2-methyl-propanal, 2-methyl-butanal, 3-methyl butanal, hexanal, 2-heptanone, heptanal, nonanal, toluene (Alasalvar et al., 2003) and hexanoic acid (Matsui, Guth, & Grosch, 1998) have been

Table 2
Volatile compounds (mg/kg) of dark chocolate with hazelnuts as a function of active and modified atmosphere packaging, packaging material oxygen permeability and storage time in the dark at 20 °C.

Packaging material				Commercially packaged		PET//LDPE packaged under N ₂		PET-SiO _x //LDPE with oxygen absorber		
Qf	KlEx	KlLi	Volatile compounds	mg/kg		mg/kg		mg/kg		
				Day 0	6 Months	12 Months	6 Months	12 Months	6 Months	12 Months
			<i>Alcohols</i>							
90	>500	463	Ethanol	10.29 ^a ± 0.82	13.49 ^a ± 1.17	25.54 ^b ± 2.75	10.11 ^a ± 1.91	23.42 ^b ± 1.21	11.26 ^a ± 1.36	13.44 ^a ± 1.50
80	879.4	879	2-Heptanol	0.27 ^a ± 0.03	0.26 ^a ± 0.06	0.74 ^c ± 0.04	0.29 ^a ± 0.08	0.41 ^b ± 0.05	0.27 ^a ± 0.03	0.28 ^a ± 0.04
86	1058.2	1059	1-Octanol	0.39 ^a ± 0.08	0.56 ^{ab} ± 0.06	2.13 ^d ± 0.38	0.42 ^a ± 0.14	1.53 ^c ± 0.09	0.44 ^a ± 0.11	0.51 ^{ab} ± 0.07
			<i>Ketones</i>							
80	>500	500	2-Propanone	1.62 ^a ± 0.11	1.80 ^{ab} ± 0.19	9.26 ^c ± 1.02	5.53 ^c ± 0.77	7.84 ^d ± 0.38	1.53 ^a ± 0.19	1.96 ^b ± 0.09
83	590	592	2-Butanone	0.12 ^a ± 0.02	0.83 ^d ± 0.11	3.78 ^f ± 0.86	0.65 ^c ± 0.06	3.87 ^f ± 0.33	0.38 ^b ± 0.08	1.78 ^e ± 0.16
74	889.2	889	2-Heptanone	0.34 ^a ± 0.04	0.44 ^b ± 0.03	1.46 ^c ± 0.08	0.43 ^a ± 0.07	0.94 ^b ± 0.06	0.27 ^a ± 0.04	0.35 ^a ± 0.04
99	691.8	690	4-methyl-2-Pentanone. ^{1,5}	32.00	32.00	32.00	32.00	32.00	32.00	32.00
72	582	584	2,3-Butanedione	0.35 ^a ± 0.06	0.74 ^b ± 0.12	1.08 ^c ± 0.07	0.61 ^{ab} ± 0.09	0.98 ^c ± 0.10	0.45 ^a ± 0.07	0.54 ^a ± 0.13
			<i>Aldehydes</i>							
86	616.3	615	2-Butenal	0.11 ± 0.02	U.D.L.	U.D.L.	U.D.L.	U.D.L.	U.D.L.	U.D.L.
94	649.5	650	3-methyl-Butanal.	8.18 ^a ± 0.82	10.39 ^b ± 0.56	13.79 ^d ± 0.72	11.62 ^c ± 0.84	11.63 ^c ± 0.34	9.58 ^b ± 0.28	10.14 ^b ± 0.34
86	654.2	660	2-methyl-Butanal.	6.92 ^b ± 0.47	6.35 ^a ± 0.28	6.41 ^a ± 0.32	6.14 ^a ± 0.19	6.25 ^a ± 0.21	6.50 ^d ± 0.14	6.55 ^d ± 0.08
91	540	537	2-methyl-Propanal.	U.D.L.	0.66 ^b ± 0.11	2.04 ^d ± 0.08	0.27 ^a ± 0.12	1.83 ^d ± 0.14	0.98 ^c ± 0.08	1.13 ^c ± 0.19
89	668.9	697	Pentanal	U.D.L.	U.D.L.	3.30 ^c ± 0.16	1.07 ^a ± 0.14	2.40 ^d ± 0.16	U.D.L.	U.D.L.
95	801.4	801	Hexanal	1.15 ^a ± 0.34	4.29 ^c ± 0.81	10.04 ^e ± 0.51	3.20 ^c ± 0.70	7.18 ^d ± 0.80	1.34 ^a ± 0.29	1.91 ^b ± 0.27
97	963	962	Benzaldehyde	3.10 ^a ± 0.53	4.99 ^c ± 0.21	8.51 ^d ± 0.60	3.63 ^b ± 0.48	7.86 ^d ± 0.81	2.35 ^a ± 0.66	3.17 ^a ± 0.53
70	903.3	901	Heptanal	0.25 ^a ± 0.07	0.74 ^c ± 0.13	2.88 ^e ± 0.24	0.96 ^d ± 0.08	1.06 ^d ± 0.15	0.46 ^b ± 0.12	0.50 ^b ± 0.08
72	1004	1004	Octanal	0.69 ^a ± 0.12	1.65 ^c ± 0.07	3.89 ^e ± 0.12	0.98 ^b ± 0.07	2.35 ^d ± 0.11	0.62 ^a ± 0.08	0.71 ^a ± 0.05
91	1107.1	1104	Nonanal	0.72 ^a ± 0.09	1.06 ^b ± 0.11	3.18 ^d ± 0.36	1.48 ^c ± 0.17	3.08 ^d ± 0.22	1.09 ^b ± 0.14	0.93 ^a ± 0.18
95	1056.8	1055	Benzeneacetaldehyde	1.60 ^a ± 0.39	2.13 ^a ± 0.42	3.16 ^d ± 0.11	2.85 ^c ± 0.13	3.48 ^d ± 0.36	1.86 ^a ± 0.28	2.36 ^b ± 0.22
94	835.2	831	2-Furancarboxaldehyde	U.D.L.	0.49 ^{ab} ± 0.07	0.76 ^c ± 0.05	0.49 ^a ± 0.11	0.91 ^d ± 0.04	0.32 ^a ± 0.07	0.44 ^a ± 0.07
			<i>Esters</i>							
86	594	584	Formic acid, ethyl ester	0.26 ^a ± 0.04	0.31 ^a ± 0.12	1.36 ^c ± 0.12	0.31 ^a ± 0.07	0.91 ^b ± 0.11	0.24 ^a ± 0.07	0.31 ^a ± 0.00
90	>500	487	Acetic acid, methyl ester	0.54 ^a ± 0.08	0.93 ^b ± 0.09	0.92 ^b ± 0.11	1.02 ^b ± 0.09	0.96 ^b ± 0.10	0.53 ^a ± 0.07	0.55 ^a ± 0.09
91	588.9	586	Acetic acid, ethyl ester	2.94 ^a ± 0.47	4.50 ^b ± 0.62	9.23 ^d ± 0.36	2.87 ^a ± 0.19	6.60 ^c ± 0.59	2.89 ^a ± 0.61	4.60 ^b ± 0.38
76	756.3	785	Butanoic acid, ethyl ester	0.28 ^a ± 0.06	U.D.L.	U.D.L.	0.31 ^a ± 0.05	0.72 ^b ± 0.07	0.26 ^a ± 0.04	0.34 ^a ± 0.05
			<i>Acids</i>							
70	974	977	Hexanoic acid	U.D.L.	U.D.L.	1.11 ^b ± 0.18	U.D.L.	0.46 ^a ± 0.05	U.D.L.	U.D.L.
			<i>Alkanes</i>							
90	613	618	Hexane	U.D.L.	U.D.L.	2.53 ^d ± 0.38	0.51 ^a ± 0.07	0.99 ^c ± 0.14	0.55 ^b ± 0.05	0.39 ^a ± 0.08
			<i>Sulfur compounds</i>							
93	721.9	722	Dimethyl-disulfide	0.52 ^c ± 0.06	0.31 ^b ± 0.03	0.12 ^a ± 0.04	0.26 ^b ± 0.07	0.13 ^a ± 0.09	0.32 ^b ± 0.06	0.46 ^c ± 0.04
			<i>Aromatic hydrocarbons</i>							
96	619.2	621	Chloroform	0.56 ^c ± 0.04	1.07 ^d ± 0.12	3.36 ^f ± 0.14	0.24 ^b ± 0.02	1.92 ^e ± 0.15	0.11 ^a ± 0.03	0.90 ^d ± 0.08
91	771.8	771	Toluene	1.11 ^b ± 0.09	1.30 ^c ± 0.09	4.97 ^g ± 0.18	1.76 ^d ± 0.14	3.99 ^f ± 0.13	0.92 ^a ± 0.09	2.35 ^e ± 0.07
			<i>Pyrazines</i>							
93	782.9	781	2-methyl-pyrazine	1.29 ^c ± 0.16	0.47 ^a ± 0.10	0.74 ^b ± 0.13	0.61 ^a ± 0.14	0.93 ^c ± 0.03	1.11 ^c ± 0.08	0.97 ^c ± 0.16
90	897.8	894	2,5-dimethyl-pyrazine	2.19 ^c ± 0.07	1.14 ^b ± 0.05	0.58 ^a ± 0.08	1.33 ^c ± 0.09	1.33 ^c ± 0.07	1.94 ^d ± 0.13	1.78 ^d ± 0.17
90	880.3	881	Ethyl-pyrazine	0.54 ^b ± 0.08	0.21 ^a ± 0.06	0.33 ^a ± 0.12	0.33 ^a ± 0.08	0.48 ^{ab} ± 0.09	0.22 ^a ± 0.07	0.30 ^a ± 0.08
86	893.7	894	2,3-dimethyl-pyrazine	0.69 ^c ± 0.05	0.38 ^a ± 0.05	U.D.L.	0.40 ^a ± 0.06	0.37 ^a ± 0.06	0.52 ^b ± 0.04	0.49 ^b ± 0.01
91	1007.8	1004	Trimethyl-pyrazine.	2.27 ^f ± 0.11	0.70 ^b ± 0.06	0.51 ^a ± 0.06	0.96 ^c ± 0.09	0.67 ^a ± 0.10	1.85 ^e ± 0.05	1.38 ^d ± 0.07
90	991	994	2-ethyl-5-mehtyl-pyrazine	1.31 ^c ± 0.15	0.64 ^b ± 0.08	0.55 ^a ± 0.05	0.49 ^a ± 0.02	0.51 ^a ± 0.07	1.40 ^c ± 0.12	1.32 ^c ± 0.09
93	1099.8	1107	3-ethyl-2,5-dimethyl-pyrazine	1.61 ^d ± 0.06	1.25 ^b ± 0.08	0.77 ^a ± 0.11	1.17 ^b ± 0.07	0.84 ^a ± 0.18	1.51 ^c ± 0.04	1.39 ^c ± 0.12
90	1120.7	1121	Tetramethyl-pyrazine	3.31 ^f ± 0.11	1.76 ^c ± 0.20	0.80 ^a ± 0.15	2.25 ^d ± 0.16	1.39 ^b ± 0.14	3.21 ^c ± 0.06	2.62 ^e ± 0.17
			<i>Terpenes</i>							
93	1332.6	1333	Alpha-terpinolene	0.50 ^c ± 0.04	0.26 ^a ± 0.07	U.D.L.	0.40 ^b ± 0.03	U.D.L.	0.31 ^a ± 0.04	0.30 ^a ± 0.07
			<i>Furans</i>							
91	588.1	589	Tetrahydrofuran	U.D.L.	U.D.L.	0.81 ^a ± 0.03	U.D.L.	0.84 ^a ± 0.07	U.D.L.	0.88 ^a ± 0.06

t_r = Retention time. U.D.L. = under detection limit. Values are the mean of six determinations (n = 6) ± S.D. i.s. = internal standard ^{a,b,c,...} Means with different superscripts in the same row are statistically different (p < 0.05), Kl_{Ex} = Kovac Index experimentally determined data, Kl_{Li} = Kovac Index literature data NIST 05, Q_r = Quality factor, Quality factor = % matching of the experimental mass spectra against those found in the Wiley database tentatively identified on the basis of the Wiley7, NIST 05 (J. Wiley & Sons Ltd., West Sussex, England).

identified in raw hazelnuts while compounds such as 2,5-dimethylpyrazine, ethylpyrazine, 2-ethyl-5-methylpyrazine, trimethylpyrazine, 1-heptanol, benzaldehyde and 1-octanol have been identified in roasted hazelnuts (Alasalvar et al., 2003). 3-methyl-butanol, 2-methyl-butanol and trimethylpyrazine were the predominant compounds in concentration and have been characterized as the flavor active compounds with a strong chocolate character (Afoakwa et al., 2008). It must be noted that among aldehydes, 2-methyl-butanol, 3-methyl-butanol and 2-methyl-propranal also have an intense chocolate flavor (Bovheni & Coll, 2002). Similarly, according to Lopez and Quesnel (1974) 3-methylbutanol and dimethyl disulfide together may contribute a cocoa-like odor. In agreement with Bovheni and Coll (2002) we identified tetramethylpyrazine as the most abundant pyrazine in chocolate aroma. Storage time had a significant ($p < 0.05$) effect on volatiles' concentration. Such changes are very important as the development of objectionable odor and taste through oxidation has obvious detrimental effects on food quality and product acceptability by consumers (Frankel, 1982). To the best of our knowledge this is the first time that changes in volatile compounds of dark chocolate with hazelnuts during long term storage are reported.

After 12 months of storage an increase in concentration of aldehydes, ketones, alcohols and alkanes ($p < 0.05$) with a parallel decrease in pyrazines were observed especially in case of least protected products after 6 and 12 months of storage. Compounds such as ethanol, 1-octanol, hexanal, heptanal, octanal, nonanal, which increase in concentration, and pentanal and hexane which formed during storage are secondary oxidation products of lipids (Frankel, 1982). To be more specific 1-octanol, pentanal, heptanal and octanal and nonanal comprise secondary lipid oxidation products and derive from the oxidation of oleic acid. Comparison of data for volatiles' composition with amount of headspace oxygen in pouches, it is clear that in the case of the least protected samples a large increase in concentration of these compounds was recorded, parallel to a large decrease in concentration of oleic and linoleic acids while in the case of the most protected samples minimum changes were recorded in unsaturated fatty acids concentration. Similarly hexanal and hexane derive from the oxidation of linoleic acid which as shown in Fig. 2 increased in concentration with storage time at a rate proportional to the amount of oxygen present in the headspace of pouches. Hexanoic acid formation after 12 months of storage, in least and moderately protected samples, may be attributed to the breakdown of a small amount of hexanal (Lee, Mitchell, & Shibamoto, 2000). It must be noted that compounds such as ethanol and esters such as: formic acid ethyl ester, acetic acid methyl ester and butanoic acid ethyl ester which, to best to our knowledge, are reported for first time in the flavor profile of dark chocolate with hazelnuts. These compounds may be attributed to reactions of secondary oxidation products (Frankel, 1982). Lastly a decrease in concentration of pyrazines during storage, especially in least protected samples, is very important as pyrazines are significant contributors to the flavor of heat-treated foods, especially when processing involves roasting such as in the case of chocolate (Fadel et al., 2006). Similar to us, Hashim et al. (1997) reported a significant increase in concentration of hexanal, heptanal, octanal, nonanal and decanal in cocoa butter during storage up to 12 weeks, under light at room temperature. Also Fadel et al. (2006) reported an increase in aldehydes' and ketones' concentration with a parallel decrease in pyrazines' concentration during storage for 6 months. In contrast to our findings, Nattress et al. (2004) reported no signs of oxidation in dark chocolate with hazelnut paste as octanal concentration remained below 1.95 mg/kg in all tested samples after 10 months of storage.

3.6. Color

Changes in color parameter L^* of dark chocolate with hazelnuts as a function of active and modified atmosphere packaging, packaging

material oxygen permeability and time of storage in the dark at 20 °C are shown in Fig. 3. After 12 months of storage, chocolate packaged under vacuum, irrespective of packaging material permeability, showed the lowest increase in L^* parameter (from 32.89 to 49.00) ($p < 0.05$) while in case of commercially packaged product the highest increase in L^* values was determined (from 32.89 to 56.12). Samples packaged under N_2 or with the oxygen absorber irrespective of packaging material permeability showed an intermediate increase in L^* values (from 32.89 to 53.45) ($p < 0.05$). Color parameters a^* (red to green) and b^* (yellow to blue) showed a small but statistically insignificant ($p < 0.05$) change during storage (data not shown). The observed increase in color parameter L^* can be directly related to the formation of large white spots on the surface of chocolate. This is known in the literature as "fat bloom". It is interesting to note that chocolate samples packaged under vacuum retained their initial dark brown color better than all the rest. As the phenomenon of fat bloom is still not clearly understood (Briones & Aguilera, 2005) it is difficult to provide a satisfactory explanation as to why vacuum packaging retarded this phenomenon. Pressure being applied to the surface of chocolate by the packaging film may be related to "fat bloom" retardation. In any case this postulation requires further study.

Khan and Rousseau (2006) studied the effect of three storage temperatures (11, 20 and 26 °C) on the migration kinetics and equilibrium of a model confectionery product consisting of dark chocolate and a hazelnut based filling during storage for 8 weeks. Similar to findings in this study they reported whitening of surface of chocolate during storage. The rate of migration and the diffusion coefficient increased by 20 and 400 times respectively, when the storage temperature was raised from 11 to 26 °C, respectively. Also, Briones and Aguilera (2005) studied changes in surface color of milk chocolate stored at 28 °C for 52 days. Similar to findings in this study they reported an increase in L^* value after 52 days of storage resulting in surface whitening of the product. It must be noted that these authors reported a lag period before the onset of bloom was observed (36 days of storage) which was not observed in the present study probably due to longer sampling periods as compared to those applied by the above workers (sampling every 2 months vs. sampling every 3 days). Also, Ali, Selamat, Man, and Suria (2001) studied the effect of storage temperature on bloom formation of dark chocolate filled with desiccated coconut during storage at 18 and 30 °C for 2 months. They reported no bloom for samples stored at 18 °C while for samples stored at 30 °C, the onset of bloom was observed after 1 week of storage. Similarly, Jinap et al. (2000) reported no bloom during storage at 18 °C for 8 weeks, while in case of storage at 30 and 35 °C the onset of bloom was observed after 4 and 1 week, respectively, resulting to whitening of the surface of product.

3.7. Sensory evaluation

Significant changes ($p < 0.05$) were observed in color (whitening of dark chocolate with hazelnuts) as a result of fat bloom (data not shown). This finding is in good agreement with the objective measurement of color. Color changes were the least in samples packaged under vacuum. Texture changes ($p < 0.05$) accompanied changes of color as whitening of surface (fat bloom) resulted to softening of chocolate body with most predominant changes observed in commercially packaged samples (data not shown).

Odor and taste changes in dark chocolate with hazelnuts are shown in Figs. 4 and 5, respectively. After 12 months of storage, odor changes (Fig. 4) were the least in products packaged with the oxygen absorber irrespective of packaging material permeability (score 7.6) while the lowest scores were recorded for dark chocolate commercially packaged at 20 °C (score 4.1). In samples with the oxygen absorber significant changes ($p < 0.05$) in odor were observed after 8 months of storage at 20 °C. In contrast, in all other samples significant changes ($p < 0.05$) in odor were observed starting with

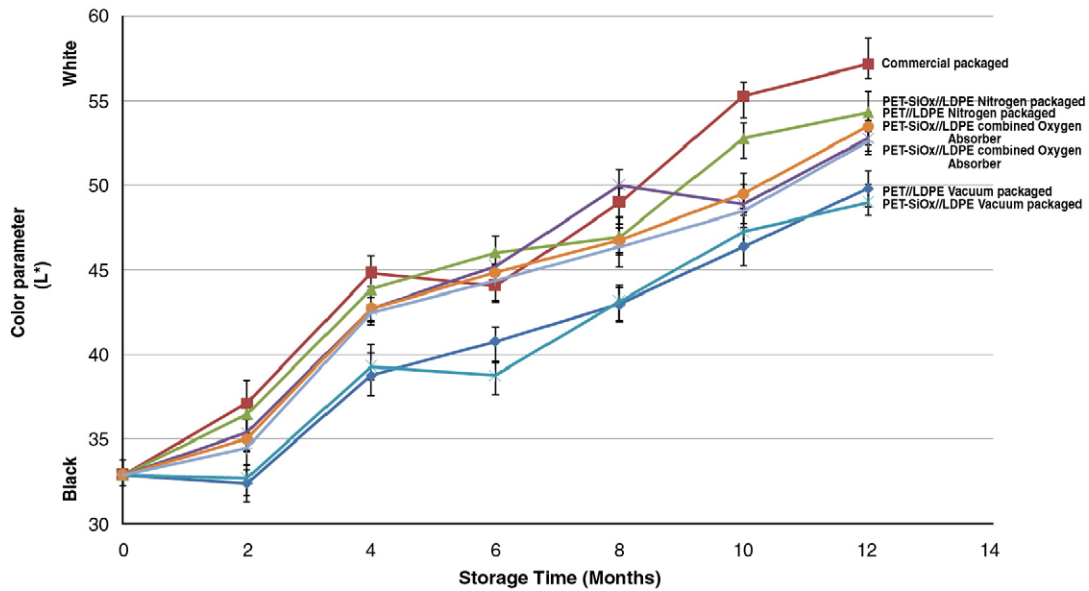


Fig. 3. Changes in L^* color parameter of dark chocolate with hazelnuts as a function of active and modified atmosphere packaging, packaging material oxygen permeability and storage time in the dark at 20 °C.

the second month of storage. This is in general agreement with instrumental determination of volatiles according to which the chocolate volatile compounds decreased with storage time while secondary oxidation compounds increased significantly ($p < 0.05$) for the commercially packaged product, resulting to loss of natural aroma of dark chocolate as recorded by the sensory panel.

As shown in Fig. 4, after 12 months of storage, the smaller taste changes (Fig. 5) were recorded in products packaged with the oxygen absorber stored at 4 °C (score 5.9) while the lowest scores were recorded for the commercially packaged product stored at 20 °C (scores 3.8). In samples with the oxygen absorber significant changes ($p < 0.05$) in taste were observed after 4 months of storage at 20 °C while in all other samples significant changes ($p < 0.05$) were observed starting from the second month of storage. It must be noted that the

sensory panel reported a decrease of bitter taste and an increase of sweet taste during storage, while for samples packaged in PET//LDPE or aluminum foil a strong rancid taste, was recorded. Taste proved to be a more sensitive sensory attribute than odor.

Based on taste, appearance and an acceptability limit of 5 the shelf life of dark chocolate with hazelnuts in commercial packages was equal to 8 months, for samples packaged in PET//LDPE irrespective of storage atmosphere, equal to 8 to 9 months and for samples packaged in PET-SiOx//LDPE irrespective of storage atmosphere equal to 11 months. Finally for samples packaged with an oxygen absorber irrespective of packaging material the shelf life was at least 12 months.

Timms (1984) and Ziegler (1997) reported that typical chocolate deterioration effects, frequently encountered in fat migration are softening and blooming of the surface layer and unacceptable textural

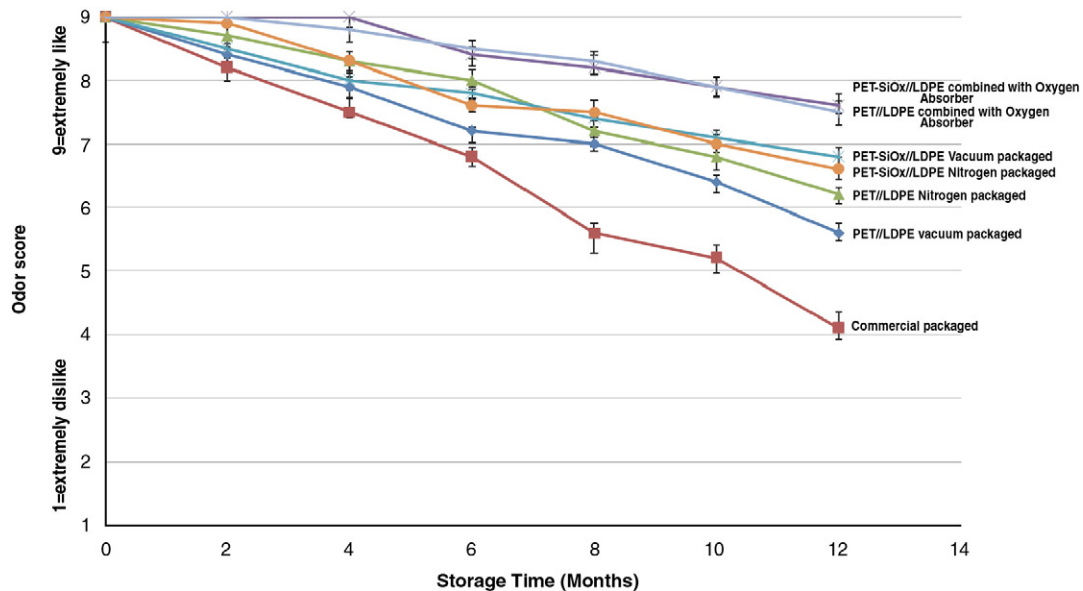


Fig. 4. Changes in odor of dark chocolate with hazelnuts as a function of active and modified atmosphere packaging, packaging material oxygen permeability and storage time in the dark at 20 °C.

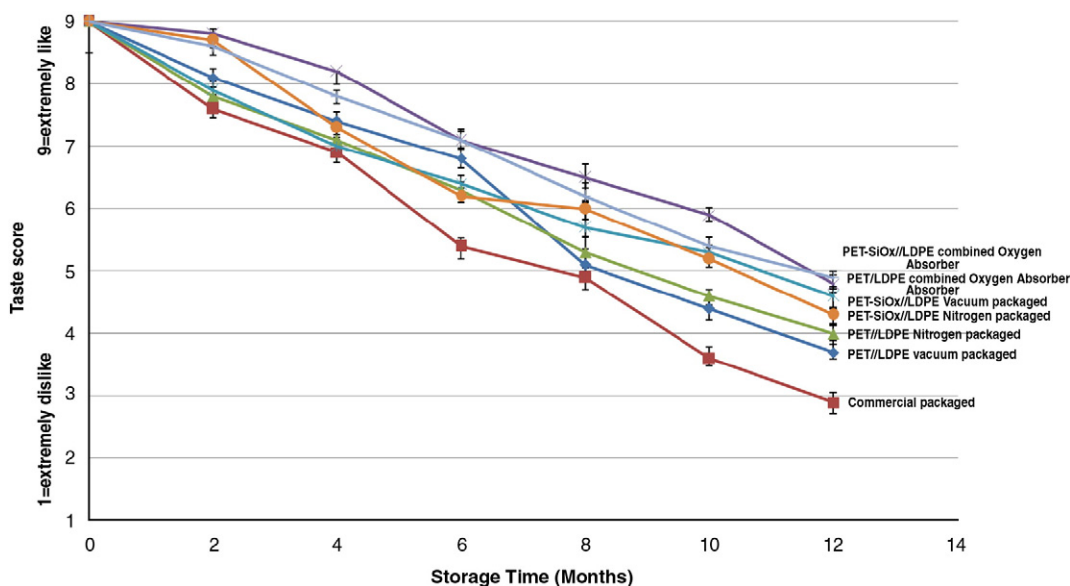


Fig. 5. Changes in taste of dark chocolate with hazelnuts as a function of active and modified atmosphere packaging, packaging material oxygen permeability and storage time in the dark at 20 °C.

changes in the product body, due to the loss of liquid glycerides from the filling centre. Similar to us, Nattress et al. (2004) found that bitterness decreased during storage which is in agreement with the postulation that dark chocolate tends to “mellow” over time. Furthermore these authors reported that storage time significantly influenced the perception of sweet, sour, burnt, rancid, hazelnut, green, metallic, hardness, viscosity, and onset of melt ($p < 0.05$).

Ali et al. (2001) reported that color and texture of filled dark chocolate stored at 30 °C were significantly ($p < 0.05$) less preferred than the control and chocolate stored at 18 °C as a result of fat migration which adversely affected product texture appearance. The flavor of the control samples was significantly ($p < 0.05$) less preferred than that of chocolate stored at 18 and 30 °C. However, chocolate stored at 30 °C scored higher in flavor and lower in overall acceptability compared to the control and that stored at 18 °C. The study indicates that storing chocolate filled with desiccated coconut at 18 °C is the best way to preserve its flavor.

Lastly, Fadel et al. (2006) carried out sensory evaluation of a cocoa substitute sample (mixture of chicory roots and carob bean) in comparison to that of a cocoa sample. Similar to us (for least protected samples), these authors showed a substantial effect of storage time on odor profile of cocoa substitute. The cocoa substitute showed a sharp decrease in earthy/roasty and gradual decrease in sweetish/caramel and cocoa-like notes after 6 months of storage while for chocolate-like attribute reported an opposite trend.

3.8. Conclusion

Dark chocolate with whole hazelnuts retained acceptable quality for ca. 8 months in commercial packages. In samples packaged in PET/LDPE irrespective of storage atmosphere the shelf life was 8 to 9 months and in samples packaged in PET-SiOx/LDPE irrespective of storage atmosphere the shelf life was 11 months. Finally for samples packaged with an oxygen absorber irrespective of packaging material the shelf life was at least 12 months.

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