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Viewpoint

Correlation between sensory and instrumental properties of *Canestrato Pugliese* slices packed in biodegradable films

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This paper compares the performance of three novel biodegradable films having different gas and water vapour permeabilities to the performance of a multilayer film made of polyethylene and EVOH and having high barrier properties. As model food *C. Pugliese* cheese was chosen. The samples were stored for 28 days and, once a week, they were analysed using sensory and instrumental tests.

Sensory data showed samples packed in high permeable biodegradable film were different from fresh cut samples after only 7 days of storage, whereas the other biodegradable films having intermediate water vapour permeability allowed the cheese to keep its sensory properties unchanged for 21 days. The only film which maintained the sensory properties of cheese, with except for texture, during all the investigated time, was the high barrier film. The sensory data are confirmed by acceptability scores.

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Introduction

In the last decades, the massive use of synthetic packaging films has led to serious pollution environmental problems. Synthetic polymer films are largely used as packaging materials, because of their low cost and good barrier and mechanical properties. However, they are totally non-biodegradable (Tharanathan, 2003) and therefore contribute to long-term environmental pollution (Arvanitoyannis & Gorris, 1999). A potential alternative is represented by biodegradable materials, i.e. those materials that undergo physical, chemical, thermal and biological degradation, decomposing ultimately in carbon dioxide and water, and can be used as fertilizers and soil conditioners in agriculture (European, Parliament and Council Directive 94/62/EC, 1994). Unfortunately, the properties of these films are often not compatible with food packaging requirements. Biodegradable films, in fact, are generally made from biological materials such as polysaccharides, polyester, proteins, lipids and their derivates. Films made of polysaccharide and proteins have low barrier properties against water and oxygen and, in addition, have poor moisture stability. On the other hand, films made of lipids or polyesters have good water vapour barrier properties, but they are, usually, opaque and not flexible (Guilbert, Gontard, & Gorris, 1996). However, in spite of these negative performances, biodegradable films may still represent a suitable solution for food packaging. In fact, the package design often overestimates the material performances, thus if design is accurately developed, it could be possible to match more accurately food stability requirements with film performances and use materials which at moment are considered not appropriate.

For example, in the case of cheese, the most important factors that affect its stability are a_w and pH. Water activity depends mainly on moisture and salt contents. During ripening, a_w is not constant but decreases until the cheese surface is in equilibrium with the surrounding atmosphere. During cheese manufacture, the pH decreases for effect of the fermentation of lactose to lactic acid until a level that inhibits the growth of many pathogenic bacteria. Next the pH rises as a consequence of the formation of alkaline N-containing compounds and/or the catabolism of lactic acid (Robertson, 1993). While the packaging does not have influence on the pH of the cheese, the water vapour transmission rate through the packaging material is crucial for controlling the a_w. Additional environmental factors which must be considered in selecting a material for cheese are light and oxygen. Light promotes fat oxidation, which in turn is responsible of off-flavour. The oxygen in contact with the cheese contributes to the oxidation of fats and to the growth of undesirable microorganisms (Robertson, 1993). All these factors affect not only cheese's physical characteristics but also its flavour during storage. In fact many different compounds contributes to cheese flavour and most of them form during cheese ripening. The breakdown of milk proteins, fats, lactose and citrate during ripening gives rise to a series of volatile and non volatile compounds which may be related to total flavour. Sensory experiments confirm the contribution given by fat-derived compounds to cheese flavour (Arora, Cormier, & Lee, 1995; Buchin *et al.*, 1998; Kubickova & Grosch, 1998;).

Known flavour compounds, presumably derived from fat, include fatty acids, methyl ketones, esters, alcohols, lactones and aldehydes (McSweeney & Sousa, 2000; Molimard & Spinnler, 1996). The catabolism of free fatty acids (FFA) leads to the production of flavour and aroma compounds, such as heptan-2-one and nonan-2-one, even though the rate of production of methyl ketones in cheese is affected by temperature, pH, presence of moulds and FFA concentration. Esters and thioesters are other products of fatty acid catabolism, and are common components of cheese volatiles (Urbach, 1993). A great diversity of esters is present in cheese (Molimard & Spinnler, 1996), the most important are ethyl-and-methyl-esters. They are highly fruity flavoured and are formed when FFA react with alcohols. Secondary alcohols can be formed by enzymatic reduction of methyl ketones (Engels, Dekker, De Jong, Neeter, & Visser, 1997), 2-heptanol and 2-nonanol are the main alcohols found in artisanal cheeses.

Some straight-chain aldehydes, e.g. butanal, heptanal and nonanal, may be formed as a result of the bioxidation of unsaturated fatty acids. Straight-chain aldehydes are characterized by 'green grass-like' aromas (Moio, Dekimpe, Etievant, & Addeo, 1993). In this work, the possibility to use biodegradable films (Novamont S.p.A.) for the cheese packaging was explored. As model a typical cheese of Puglia region in Italy, recognized PDO with DPR 10/9/1985, named *C. Pugliese*, was chosen.

For this purpose, sensory and physico-chemical changes of the '*C. Pugliese*' cheese packed by using four different films, were monitored during the storage time. One conventional and three biodegradable films were used as packaging of the samples. Sensory and physico-chemical data were used to predict pleasantness scores given by the consumers to the cheese samples. The objective of the present work was, also, to compare the volatile composition and sensory properties of '*C. Pugliese*' cheese sliced in biodegradable film during storage.

Materials and methods

Materials

The *C. Pugliese* cheese, ripened three months, was provided by Antonio Cordisco S.r.l. It was sliced and packed using four different films. One of them was a multilayer film of polyethylene and EVOH (film A), purchased from Di Mauro Officine Grafiche, Cava dei Tirreni (Italy) and having high oxygen and water barrier properties ($PO_2=6.08\times10^{-16}$ m^3/mPas; WVTR$=6.20\times10^{-12}$ kg/ms), the other three films were biodegradable films, supplied by Novamont S.p.A., Novara (Italy). The three biodegradable films, indicated as B, C and D, had different gas and water permeabilities. Film B was an homopolymer of polyester with oxygen permeability, $PO_2=1.98\times10^{-14}$ m^3/mPas and WVTR$=3.47\times10^{-9}$ kg/ms; film C and D were polymeric blends obtained by mixing two different homopolymers of polyester and a biodegradable aliphatic polymer (1:1 ratio), with $PO_2=2.74\times10^{-15}$ m^3/mPas and WVTR$=9.95\times10^{-10}$ kg/ms and with $PO_2=3.17\times10^{-15}$ m^3/mPas and WVTR$=4.98\times10^{-10}$ kg/ms, respectively.

Methods

The samples were packed and stored, in a confined room, at 8 °C for 28 days and, once a week, instrumental and sensory analyses were performed, as well as acceptability tests. At each storage time the presence of moulds was checked, by means of visual inspection. Cheese just sliced was used as control sample.

Instrumental methods

(1) Colour measurements were performed on the cheese slices by means of tri-stimuli colorimeter (Minolta Chroma Meter, mod CR-300, Long Branch, NJ) and expressed in brightness (L) and blueness/yellowness (b) chromatic coordinates. Colour difference between each packed sample and the control sample (ΔE) [1] as well as whiteness index (WI) [2] were estimated as:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \quad (1)$$

$$\mathrm{WI} = 100-[(100-L)^2 + a^2 + b^2]^{1/2} \quad (2)$$

Where a is the greenness/redness chromatic coordinate.

(2) Relative moisture content was measured by gravimetric analysis. 8–10 g of cheese were spread on the surface of a glass Petri disk and then inserted in an oven (Continental Equipment, PTE LTD, Singapore, China) at 102 °C, for 24 h, next the samples were cooled off in a desiccator and weighed. Relative moisture content was expressed as water content percentage over total weight.

(3) Compression tests were performed by means of an Instron Universal Testing Machine (Instron Ld., mod. 4467 High Wycombe, England), using a crosshead speed of 50 mm/min, equipped with a 60 mm diameter piston and with load bearing cell of 1 kN. From stress–strain curve, Young's modulus was calculated (Masi, 1987).

(4) Lipidic fraction was extracted by means of AOAC procedure (AOAC (1990) method # 920.125) and acidity determination of lipidic fraction was evaluated by means of titration using NAOH 0.1 N and phenolphthalein as indicator. Three grams of fat were dissolved in ethylic alcohol–ether 1:2 v/v solution and, then, analysed. Acidity of lipids was expressed as milligrams of sodium hydroxide required to neutralise 1 g of oleic acid.
(5) Volatile organic compounds (VOCs) evolution during the storage time was assessed by means of static headspace analysis (SHA). VOCs were separated and identified by means of GC–MS. Volatile analysis was performed using an Agilent 7694 headspace auto sampler tied to 6890 Hewlett–Packard gas chromatograph (Agilent Technologies, USA) equipped with a 30 m×0.25 mm ID, film thickness 0.25 μm capillary column (HP-5MS, Agilent Technologies, USA) and a 5973 HP mass spectrometer (Agilent Technologies, USA). Carrier gas was Helium (flow 1.2 ml/min) and split injector ratio was 20/1. Oven temperature was kept at 30 °C for 2 min, risen to 50 °C at 7 °C/min and held for 5 min, risen from 50 to 90 °C at 10 °C/min and held for 5 min, risen from 90 to 120 °C at 10 °C/min and held for 5 min, and risen to the final temperature of 250 °C at 15 °C/min and held for 5 min. Injector temperature was 270 °C. The mass spectrometer operated in scan mode over a mass range from 30 to 400 amu (2 s/scan) at an ionization potential of 70 eV. The peak area of each compound, based on the total ion count, was quantified as percentage of total area and the compounds were identified by comparing their retention times to that of the standards and their mass spectra to the mass spectra library (NIST 98 and WILEY 275).

Three grams of cheese were placed in a sealed vial and were equilibrated in headspace autosampler at 80 °C. After equilibration, carrier gas (helium) flushed and carried the volatiles compounds through the heated transfer line (100 °C) into the GC. Blank vials were run between each sample to clean the column and inhibit carry-over between samples.

Quantitative descriptive analysis

Seven trained judges, selected for their sensory ability and previous experience in performing sensory profiling of other cheeses, evaluated the samples by means of quantitative descriptive analysis. They took part in five training sessions organised in order to let them familiarise with the *C. Pugliese* cheese.

Four samples were evaluated during each session and each sample was tasted in a randomised design with three replications. Samples were served, in monadic way, on white plastic plates identified by three random digit codes chosen by the software FIZZ acquisition (Biosystèmes, Couternon, France). The judges evaluated three visual (colour, greasiness and visual humidity), three texture (deformability, cohesiveness and hardness) and five taste and flavour (pungent odour, rind odour, butter flavour, saltiness and spiciness) attributes, by using continuous unstructured scales (10 cm), in isolated sensory booths under white light.

Acceptability test

Fifty cheese consumers, ranging between 20 and 40 years of age participated in the consumer tests. The participants were randomly selected from a database of consumers living in Campania and recruited by phone on the basis of their interest and availability. All of them were naïve in relation to sensory analysis. The subjects were asked to taste five samples during each session and to indicate the pleasantness of each sample on a nine-point hedonic scale (1, not pleasant at all; 9, extremely pleasant).

Data analyses

The sensory data were collected by means of specific software "FIZZ Acquisition" (Biosystèmes, Couternon, France).

Analysis of variance was performed in order to evaluate the effects of film type and storage time on sensory and physico-chemical properties of the cheese samples. Ratings of pleasantness were transformed to differences (score given to each packed samples at each time minus score given to the fresh sample) and paired *t*-tests were performed in order to evaluate if those differences were significantly different from zero or not (SPSS v10.1).

The relationships between acceptability, sensory properties and instrumental data were studied by partial least squares regression analysis. Partial least squares regression (PLS) is a useful statistical modelling technique used to compare two sets of data by seeking out latent variables common to both data sets (Martens & Martens, 1986). Both sensory and physico-chemical data (the average of three replications) were used as independent variables (X), i.e. predictors of average pleasantness score (Y), (SIMCA-P v.10.0). The variables which did not change during the storage time and for the film effect, were excluded from the PLS analysis.

Results and discussion

Visual inspection proved that moulds were absent on the cheese surface for the entire observation period.

Instrumental analysis

The effects of film and storage time on physico-chemical properties of the cheese are examined by applying two-ways analysis of variance. The storage period has a significant effect on b, ΔE and WI parameters, while the film type does not (Table 1). In particular, the b chromatic coordinate increases during the first 2 weeks and then it remains constant (Fig. 1(a)). As one would expect, the film nature and the time affect strongly the cheese moisture content (Fig. 1(b)). In fact, the cheese moisture variation on time depends on the kinetic with which water molecules

Table 1. Two-ways anova: effect of film and storage time on colorimetric data

Source of variance	Chromatic coordinates	*F* of Fisher
Time	*L* (brightness)	0.53
	b (blueness/yellowness)	8.56****
	WI (index of whiteness)	3.90**
	Δ*E* (colour difference)	19.56****
Film	*l*	1.81
	b	1.48
	WI	2.37
	Δ*E*	0.60

**$p\leq0.01$, ***$p\leq0.001$, **** $p\leq0.0001$.

permeate through the package wall which, in turn, depends on time and barrier properties of the packaging materials (Robertson, 1993). Therefore, the highest is the water vapour transmission rate of the film, the faster is the moisture decrement of the cheese. Consequently, the largest variation overtime of the cheese moisture content is observed for cheese slices packed by using the film B ($F_{3.35}=3.9$; $p<0.05$) which has the highest WVTR. In this packaging condition, the sample moisture content in 28 days passes from 35% to about 31%.

The storage time and the film types does not affect both the Young's modulus of the cheese and the acidity of the lipidic fraction.

Sixty-one volatile compounds are found, they include esters, aldehydes, ketones, alcohols, organic acids, hydrocarbons and lactones. The average percentage areas of the compounds classes as well as the Duncan's test results are summarized in Table 2.

Esters derives from esterification reactions between short- to medium- chain fatty acids and primary and secondary alcohols and have been reported to have 'fruity' odour notes (Molimard & Spinnler, 1996).

Among these, ethyl-acetate is the most important one and it may be responsible of the pungent odour of the cheese.

Aldehydes, such as hexanal and 2-methyl-butanal, are detected in the headspace of all the samples. Those aldehydes derive from the β-oxidation of unsaturated fatty acids (Engels *et al*., 1997). One can observe that, for all the samples, acetaldehyde in the head space of the package increases until 21st day of storage, with except. The concentration of this compound increases after 21 days only in the headspace of the sample packed in film B. Aliphatic aldehydes, characterized by green grass-like and

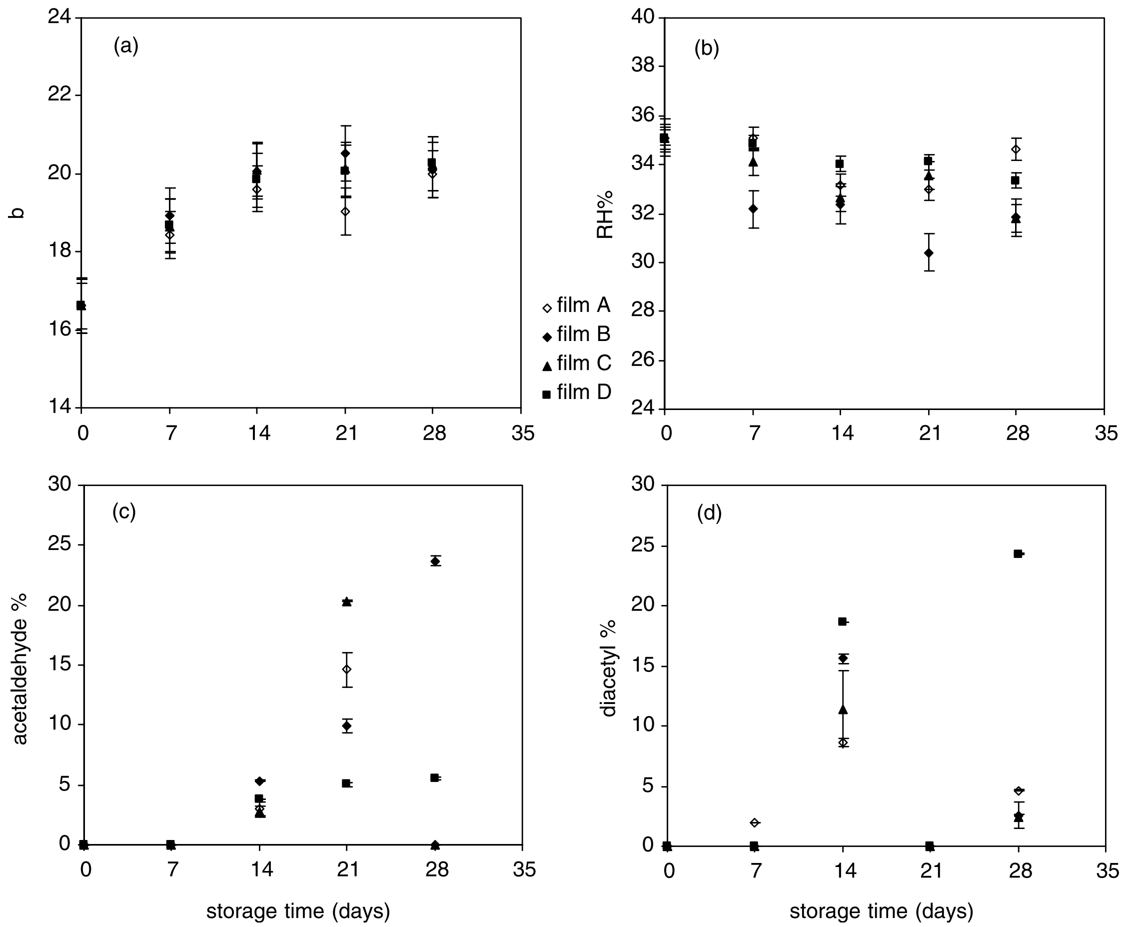

Fig. 1. (a) *b* chromatic coordinate; (b) moisture content; (c) acetaldehyde (%); (d) diacetyl variation (%) variations during the storage time (average values + SEM).

herbaceous aromas (Moio *et al.*, 1993), are the main products of autoxidation of unsaturated fatty acids: autoxidation proceeds via hydroperoxides, further degradation to hydrocarbons, alcohols and carbonyl compounds (Barbieri *et al.*, 1994; Collin, Osman, Delcambre, El-Zayat, & Dufour, 1993; Moio *et al.*, 1993), although lipidoxidation in cheese is limited due to its low redox potential and low levels of unsaturated fatty acids. Engels *et al.* (1997) analysing different types of ripened cheese found that cheeses with high lipolysis, such as Gruviere and Parmigiano, contained a relatively high concentration of linear aldehydes. However, aldehydes do not accumulate in cheese because they are rapidly converted to alcohols or to the corresponding acids (Dunn & Lindsay, 1985; Lemieux & Simard, 1992).

Among the ketones, the presence of diacetyl in the headspace is detected. Diacetyl is produced by the α-ethyl lactate during the metabolism of the citric acid and it confers the characteristic taste of butter to the cheese. The diacetyl evolution in the headspace is not function of the time (Fig. 1(d)).

The alcohols found, (ethanol and 2-methyl ethanol), derive from lactose fermentation or from amino-acid catabolism (Curioni & Bosset, 2002). Secondary alcohol, instead, are due to the enzymatic reduction of methyl ketones, which in turn derive from fatty acids. The most abundant compound of this class, is 3-buten-2-ol, probably originated from butanone which, in turn, was originated from diacetyl. Different alcohols (primary and secondary) have been reported in various cheeses, e.g. Parmigiano, Roquefort, Cheddar (Engels *et al.*, 1997); secondary alcohols, in particular, are important flavour components of blue cheese where they are produced by reduction of methyl ketones. Aliphatic primary alcohols such as 1-butanol, 1-pentanol and1-hexanol contribute with fruity and nutty notes to the flavour of cheese (Gallois & Langlois, 1990).

Other volatile compounds are detected. In particular hydrocarbons as hexane, heptane, ciclo-hexane, which probably originated from lipolysis of fatty acids. Also organic acids, as for example pyruvic acid from lactic fermentation, and δ- and γ-lactones are found. Lactones contribute to cheese flavour giving fruity and sweet creamy fermented notes (Dufossè, Latrasse, & Spinnler, 1994).

Quantitative descriptive analysis

Fig. 2 shows the spider plots representative of cheese slices packed by using the four films. With respect to the control (0 days), the cheese slices, packed in the film A, keep their sensory attributes almost constant for the entire storage period (28 days) (no significant changes are observed) with the only exception of the texture attributes (deformability, $F_{4.97}=5.32$, $p=0.001$; cohesiveness, $F_{4.97}=4.4$, $p=0.003$; and hardness, $F_{4.98}=3.13$, $p=0.018$), which start varying from the 21st days of storage (Fig. 2(a)).

By contrast, the sensory profile of the samples packed in film B differs noticeably from that of the fresh sample just

Table 2. Average percentage areas of volatile compounds classes, extracted by means of SHS

Compounds classes	Fresh sample	Film A 7	Film A 14	Film A 21	Film A 28	Film B 7	Film B 14	Film B 21	Film B 28	Film C 7	Film C 14	Film C 21	Film C 28	Film D 7	Film D 14	Film D 21	Film D 28
Esters	2.99_{b}	nd	nd	nd	4.30_{d}	3.52_{c}	nd	nd	5.07_{e}	nd	nd	nd	4.52_{d}	nd	nd	nd	0.58_{a}
Aldehydes	10.19_{d}	5.76_{b}	3.01_{a}	14.60_{f}	nd	14.12_{f}	9.75_{d}	9.90_{d}	23.99_{h}	10.44_{d}	2.77_{a}	20.35_{g}	7.47_{c}	11.69_{e}	3.83_{ab}	5.03_{b}	5.54_{b}
Ketones	8.52_{cd}	8.98_{d}	18.43_{g}	6.30_{ab}	10.93_{e}	nd	15.59_{f}	8.50_{cd}	5.01_{a}	21.89_{h}	47.09_{m}	12.75_{f}	7.28_{bc}	6.73_{b}	37.97_{l}	6.50_{b}	29.23_{i}
Alcohols	74.82_{i}	15.21_{bc}	16.40_{c}	8.58_{a}	33.65_{e}	87.54_{m}	10.77_{ab}	62.91_{h}	25.05_{d}	79.90_{l}	9.95_{a}	50.09_{g}	42.85_{f}	8.47_{a}	27.34_{d}	51.49_{g}	23.91_{d}
Organic acids	nd	52.57_{de}	51.11_{d}	35.28_{c}	nd	nd	62.21_{f}	nd	2.35_{a}	nd	nd	nd	nd	79.13_{g}	57.60_{ef}	nd	28.65_{b}
Hydrocarbons	3.27_{b}	nd	10.31_{f}	17.90_{g}	52.51_{i}	nd	nd	5.53_{d}	56.04_{l}	nd	2.05_{a}	7.86_{e}	37.91_{h}	nd	4.32_{c}	3.36_{b}	12.10_{f}
Lactones	nd	8.35a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	10.13_{b}	nd	nd	nd

nd, not detected. Means within a row with different letters are significantly different ($p\leq 0.05$).

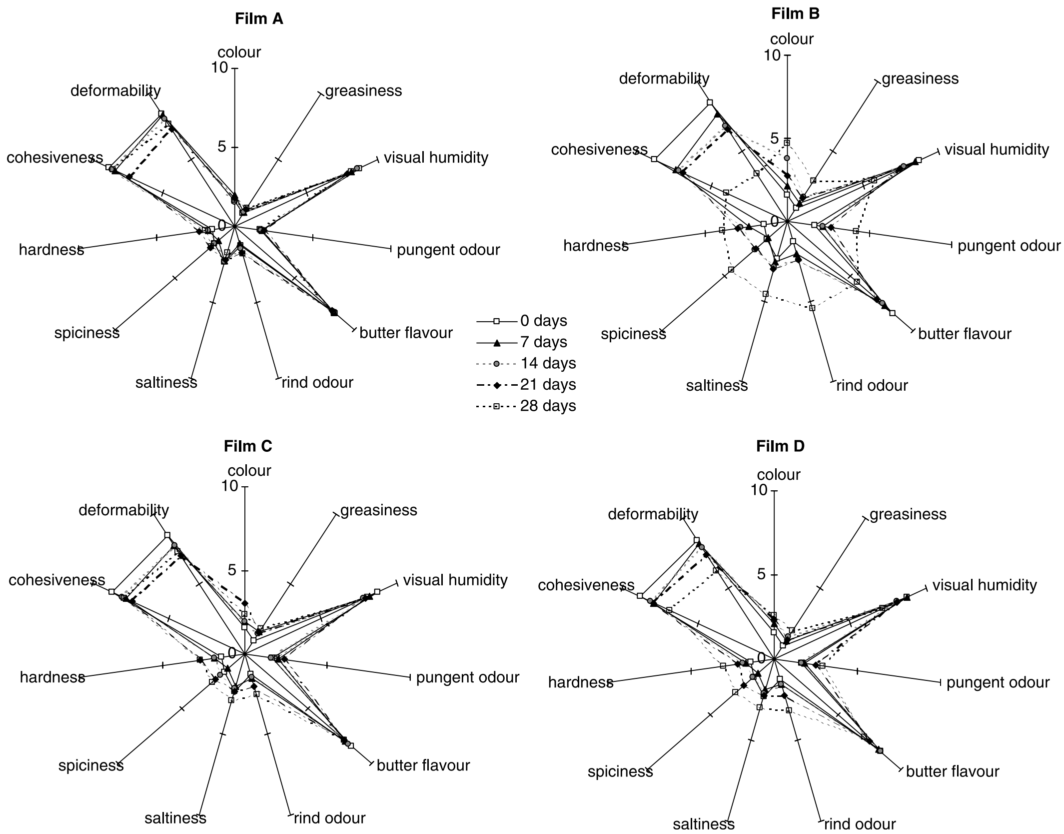

Fig. 2. Spider plot of sensory attributes for film A, film B film C and film D at each storage time ((a)–(d)).

after the first week of storage, and after 28 days, it exhibits the sensory profile that more deviates from the control (Fig. 2(b)), (for sake of brevity *F* and *p* values are not shown).

The sensory profiles of the samples packed in the film C and D remain very similar to that of the control during the first 21 days (no significant difference). About sample C, pungent odour, butter flavour and saltiness do not vary significantly during all the storage time (Fig. 2(c)), while for the sample D only the colour changes are not significant (Fig. 2(d)).

Acceptability data

Only the sample packed in film B exhibits a different behaviour with respect to all the other samples. In particular, consumers evaluated the sample B less pleasant than the fresh sample just after 7 days of storage (7 days sample: $t_{44} = -4.1$, $p < 0.001$; 14 days sample: $t_{49} = -2.4$, $p < 0.02$; 21 days sample: $t_{49} = -3.0$, $p < 0.004$), whereas during the first 3 weeks, the pleasantness scores for all the other samples prepared by using the other packaging solutions are equal to the score attributed to reference sample. After 28 days, all the samples are significantly less pleasant than the fresh sample (sample A: $t_{49} = -3.5$, $p = 0.001$; sample B: $t_{49} = -9.7$, $p \ll 0.0001$; sample C: $t_{49} = -3.9$, $p < 0.001$; sample D: $t_{49} = -3.5$, $p = 0.001$).

PLS analysis

PLS analysis was performed in two steps. In the first step, 11 sensory properties, 61 volatile compounds and nine other instrumental parameters were used as independent variables (*X*) in order to predict pleasantness of the cheese slices (*Y*).

X variables used in the first step, were selected according to their importance in the projection with respect to *Y* (VIP). Thus, 29 variables, having a VIP$\geq$0.8, were used as *X* variables in the second PLS step. Among these all the sensory attributes were selected as well as all the colorimetric parameters. In addition, 12 volatile compounds and moisture content were also, selected.

Table 3 lists the parameters of the two PLS models. In particular, the table reports the number of *X* variables used to predict the pleasantness, the number of latent variables extracted, the explained variance, the predicted variances and the RMSEP (root mean square error of

Table 3. Parameters of two PLS models

	Model 1	Model 2
Variables number	81	29
Extracted components	3	2
Explained variance	95%	92%
Predicted variance	76%	77%
RMSEP	0.14	0.17

prediction). The last figure is the standard deviation of the predicted residuals, therefore it represents an index used to evaluate the model fitting capability. By using the second PLS model rather than the first one, one observes that the number of extracted latent variables decreases and the predicted variance increases to the detriment of RMSEP.

Fig. 3 shows the PLS loadings (a) and scores (b) plots.

By looking at the PLS loadings plot, one can notice

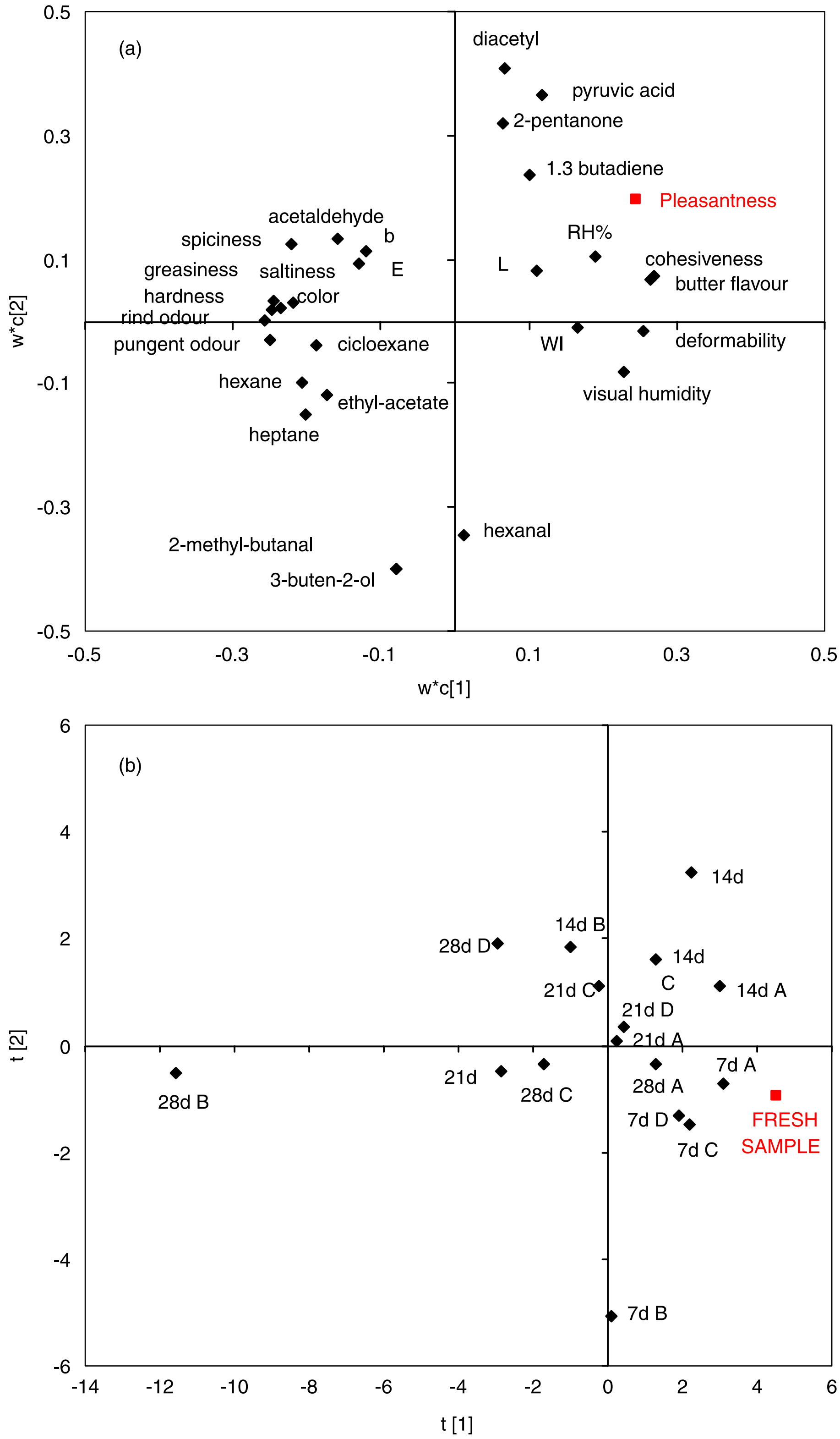

Fig. 3. (a) PLS loadings plot and (b) PLS scores plot.

that consumer judgment depends on some of the evaluated sensory properties of the cheese, only. In particular, the average pleasantness score is related positively to butter flavour and cohesiveness and negatively to pungent and rind odours. In addition, the hedonic judgement is related positively to moisture content, diacetyl, pyruvic acid, 2-pentanone and 1,3 butadiene, whereas the opposite is observed for ethyl-acetate, hexane, heptane and cycloexane.

PLS loadings plot allows one to find, also, correlations between X variables. In particular, several volatile organic compounds are related to sensory characteristics. Ethyl-acetate content is related positively to the pungent and rind odours and acetaldehyde content in the headspace correlates well with the spiciness attribute. These results are in agreement with data reported in the literature (Engels *et al*., 1997).

In addition, PLS suggests that cheese moisture content is positively correlated with cohesiveness and butter flavour and that chromatic parameters ΔE and b are positively related to the colour, as evaluated by the sensory panel, whereas the WI is negatively related to that.

A close examination of Fig. 3(a) and (b) allows one to derive additional information on the film performances. After the first week of storage all the samples are located quite close to the fresh cut cheese, only the sample B is located far from it, although it is still located in the same region. After 2 weeks of storage the sample position moves away from the control sample, however their acceptability improves. With increasing the time, the position of all the sample changes and their distance from the fresh sample increases. Sample B is the only one which moves further from the fresh cut sample while sample A is the one which moves less.

Conclusions

Among biodegradable films, those having intermediate barrier properties may be suitable for cheese packaging; even though their performances are lower than high barrier synthetic films.

PLS suggests that cheese pleasantness can be predicted by sensory properties and also by physico-chemical parameters.

Finally, in order to improve the performance of biodegradable films for packaging application some effort must be done to increase their barrier properties.

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