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Monitoring Changes in Sponge Cakes during Aging by Front Face Fluorescence Spectroscopy and Instrumental Techniques

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ABSTRACT: In the present study, sponge cakes, produced at the pilot scale, were monitored during aging (i.e., 1, 3, 6, 9, 16, and 20 days) by three different analytical techniques. For the texture analyzer, the hardness and elasticity of crumb cakes were found to significantly increase and decrease, respectively, throughout aging. Color parameters (L^*, a^*, and b^*) showed only slight change throughout aging, and a high correlation (R^2 = 0.88) was observed between the whiteness and the yellowness. Tryptophan fluorescence spectra (excitation, 290 nm; emission, 305−490 nm) recorded on cakes exhibited three maxima located at 382, 435, and 467 nm that were attributed to maximum emission of tryptophan (382 nm) and fluorescent Maillard reaction products (435 and 467 nm). The principal component analysis (PCA) applied to the tryptophan spectra allowed a clear discrimination of cakes aged for 1, 3, and 6 days from those aged for 9, 16, and 20 days. Finally, canonical correlation analysis (CCA) performed on the textural and tryptophan fluorescence spectral data sets showed that the two groups of variables were highly correlated because the squared canonical coefficients for canonical variates were 0.99, indicating that cake texture determined at the macroscopic level by texture analyzer is a reflection of its structure at the molecular level determined by fluorescence spectroscopy.

KEYWORDS: *sponge cakes, freshness, texture, fluorescence, chemometric*

INTRODUCTION

The bakery industry is one of the largest organized food industries worldwide and in particular cookies, crackers, and cakes are of the most popular products due to their convenience and ready to eat foam. Thus, particular requirements for determining their quality characteristic during storage have been established.[1] Cakes are generally expected to have shelf life in the range of 1−4 weeks[2] depending on several parameters such as formulation, water activity, packaging, and storage conditions (temperature, relative humidity).

Several factors affect the quality of cakes during storage; among them there are (i) starch retrogradation phenomenon inducing an increase in the hardness; (ii) water migration from the internal to the external zones; and (iii) development of micro-organisms, particularly molds.[3] Staling includes loss of flavor and tenderness, changes in mouth texture, humidity redistribution, and partial dryness of cake. This phenomenon is much slower in cakes than in breads, partly because the latter contain more flour and less fat. From a molecular point of view, the mechanism of cake staling is not clear yet, even though the staling of bread has been studied for more than a century.[4] This could be due to the difficulty in assessing the contribution of each component in cakes because different ingredients interact and affect the cake quality.

The monitoring of cake texture could be determined by different analytical techniques such as universal testing machine, baker compressimeter, texturometer, friabilimiter, penetratometer, and rheometer. Most of these methods have pointed out a strong relationship between the sensory and texture properties.[5−7] Although these techniques are important, they are destructive and time-consuming and need skilled operators.[8,9] To comply with this request, a great number of noninvasive and nondestructive instrumental techniques have been developed for the determination of the quality of food products; among them are front face fluorescence and infrared spectroscopies.[9−11] These techniques are relatively low-cost and pollution-free, given the signals are directly acquired on the samples nondestructively and without any extraction step. The potential of the use of these techniques for the evaluation of the quality of several food products has been increased with the propagated application of chemometric tools. Indeed, it has been pointed out by Karoui and co-workers that fluorescence and infrared spectroscopic techniques could be utilized successfully to (i) authenticate the botanical origin of honey;[12,13] (ii) characterize different varieties of soft cheeses;[14] and (iii) predict some physicochemical and sensory parameters[10,15,16] of European Emmental cheeses.

A wide range of ingredients could be used for producing cakes. Among them, wheat flour, whole egg liquid, and milk powder together comprise a source of proteins. These macromolecules play a huge role in determining both the structure and texture of a matrix such as sponge cake via the protein−protein, water−protein, and/or lipid−protein interactions. Aromatic amino acids and nucleic acids, tryptophan residues of proteins, and neoformed products present in cakes are the best-known fluorescent molecules in dairy and egg products[16] and processed cookies.[17]

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To the best of our knowledge, to date, no study has monitored cake aging by colorimeter, texture, and fluorescence techniques. Thus, the present work aims for the first time to delineate texture, moisture, and color characteristics of sponge cakes packaged in plastic containers and stored at 20 °C with 65% relative humidity for up to 20 days, by instrumental methods and fluorescence spectroscopy. Multidimensional statistical methods such as principal component analysis (PCA) and canonical correlation analysis (CCA) were used to (i) extract information from each data set; and (ii) investigate the relationship between fluorescence spectra and textural parameters.

MATERIALS AND METHODS

Materials. Wheat flour (11−12% protein, 0.50−0.57% ash, and 14−15% humidity) was supplied by NV Dossche Mills (Deinze, Belgium). Crystal sugar (granulated no. 1, 600), and glucose syrup were purchased from Béghin-Say/Tereos (Lille, France) and Cargill (Belgium), respectively. Liquid whole egg was purchased from Fournil artesian (St Laurent de Blangy, France). A mixture of palm and colza fat (shortening DP03) and nonfat powder milk were supplied by ADM-SIO (St Laurent Blangy, France) and ISI (Isigny Ste-Mère, France), respectively. Baking powder was ordered from Panemex S.A.S (Caden, France). Salt was obtained from the local market. Normal tap water was used for all processes.

Production of Cakes. The cake samples were prepared at a pilot scale with wheat flour (23.3%), crystal sugar (20%), glucose syrup (7%), liquid whole egg (18.5%), a mixture of palm and colza fat (14%), water (14%), nonfat powder milk (1.6%), baking powder (1%), and salt (0.6%). The baking process was performed according to internal protocol consisting of, on the one hand, premixing separately sugar and salt and, on the other hand, powder ingredients, specifically wheat flour, glucose syrup, milk, and baking powder. The fat was melted at a temperature of 40 °C for 30 min. Then, using a pilot bakery machine (VMI-Rayner, France) to roll and form the dough at ambient temperature (∼20 °C), melted fat was mixed with the premixed sugar and salt with the speed of the mixer (VMI-Rayner adapted mixer) set at 60 rpm for 2 min. To this mixture were added and mixed successively liquid whole egg with a speed of 60 rpm for 1 min, followed by the premixed powder ingredients with the speed of the mixer set at 20 rpm for 1 min, and water with the speed at 60 rpm for 2.5 min. The batter was molded into aluminum containers of 18 cm long, 8 cm width, and 5 cm deep (approximately 300 g). The pastry was put in the pan and baked in a preheated oven (Four FRIMA, Combi ClimaPlus, FCP 6) at 160 °C for 37 min.

The oven was ventilated during the cooking (speed of ventilation = 1450 rpm, following a cycle of 118 s rotation and 2 s pause). The temperature was monitored during baking with sensors placed horizontally in the core of the sponge cakes and connected to a data logger (CTF 9004, Sonde Ellab SARL, Compiègne, France). Only the four lower levels of grills were used to avoid the interaction with the oven heated superior wall and to ensure the homogeneity and reproducibility of cooking. This procedure allows us to produce 28 sponge cakes, simultaneously. Once baked, the cakes were allowed to cool for 40 min at ambient temperature (∼20 °C) and then packed into plastic bags 180 mm × 280 mm polyamide/polyethylene (PA/PE = 20/70) and presenting thickness of 90 μm and permeability to water vapor (2.6 g m^{-2} d^{-1}), oxygen (40−50 cm^3 m^{-2}), CO_2 (150 cm^3 m^{-2}), and nitrogen (10 cm^3 m^{-2}) (Nagot Busignies, France) using a vacuum sealer (MULTIVAC furnished by Guy Deregnaucourt, Orchies, France) with a level of pressure of ∼738 mbar leaving a space of 1−3 mm between the sponge cakes and the plastic bag. Some cakes were packaged with an oxygen absorber during 20 days to allow the impact of oxygen absorber on quality to be determined.

Cake samples were stored at 20 °C and a relative humidity of 65% for up to 20 days and were analyzed after 1, 3, 6, 9, 16, and 20 days of storage. All of the instrumental measurements were made in triplicate.

Physical Characterization of Sponge Cakes. *Color, Moisture, and Texture Measurements.* Cakes were sliced (1.5 cm thick) with an electrical slicer (Kompernass GMBH, Bochum, Germany). Three slices were taken on both sides of the median part after removing two extremities of 2 cm thick on either side. One disc (⌀ = 3.5 cm) was cut from the center of each slice for color and textural measurements.

The color of cakes was measured with the Minolta Chroma Meter version CR-300 (Konica Minolta Sensing Europe, Roissy Charles De Gaulle, France). Measurements were performed directly on cake crumbs, and the L^*, a^*, and b^* values were determined. A glass plate was placed over the light port of the apparatus and was standardized using black and white reference. The L^*, a^*, and b^* values indicate lightness (0 = dark, 100 = white), redness (+60 = red, −60 = green), and yellowness (+60 = yellow, −60 = blue), respectively. Color measurements were determined at room temperature (∼20 °C) on the surface of each crumb at three randomly selected locations by using diffuse illuminant and 0° angle observer. The total color difference (ΔE^*) between the 1-day-old sponge cake (considered as the reference) and the other sponge cakes was calculated as follows:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

The following criteria were applied to appreciate the evidence of the total color differences: $\Delta E^* < 1$ color differences are not evident to the naked human eye; $1 < \Delta E^* < 3$ color differences are not noticeable to the naked human eye; and $\Delta E^* > 3$ color differences are noticeable to the naked human eye.

The moisture content of the sponge cake crumbs was determined by weighing before and after complete desiccation during 2 h in an oven at 130 °C (Air Concept, FIRLABO, Emerainville, France).

Texture measurements were determined using the TA.XTPlus texture analyzer (Micro Stable System, Goldamin, UK) equipped with a cylindrical probe (⌀ = 3.5 cm) (speed = 0.01−40 mm s^{-1}). Hardness and elasticity were determined on cake crumbs using the "hold until time" technique by fixing the following parameters: test mode, compression; pretest speed, 2.0 mm s^{-1}; test speed, 5 mm s^{-1}; post-test speed, 10 mm s^{-1}; target mode, distance, 5 mm; trigger type, auto (force) for the hardness with a trigger force of 5.0 g; and hold time, 20.0 s.

In terms of physical meanings, the hardness is defined as the force necessary to attain a given deformation and the elasticity as the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed.[1] The hardness corresponds to the maximum force (F_{max}) measured at the beginning of the "hold until time" test, whereas the elasticity is the ratio between the force measured at the end of the hold time (F_{20}) and the force measured at the beginning of the hold time (F_{max}): F_{20}/F_{max}.[1]

Fluorescence Measurements. Fluorescence spectra were recorded using a Fluoromax-4 spectrofluorometer (Jobin Yvon, Horiba, NJ, USA). The incidence angle of the excitation radiation was set at 60° to ensure that reflected light, scattered radiation, and depolarization phenomena were minimized. The spectrofluorometer was equipped with a thermostated cell, and the temperature was controlled by a Haake A25, AC 200 temperature controller (Thermo-Scientific, France). Slices of 3 cm length, 1 cm width, and 0.7 cm thickness were cut off in the middle of the crumb cake. Spectra of cakes mounted between two quartz slides were recorded at 20 °C. For each cake, three spectra were performed using different samples. The sample is illuminated by the photons of excitation (light beam, ∼3 mm in height and ∼0.3 mm width) in its center, limiting the dehydration of the sample. The 305−490 nm tryptophan emission spectra were recorded with the excitation wavelength set at 290 nm. All spectra were corrected for instrumental distortions in excitation using a rhodamine cell in the reference channel.

To reduce scattering effects and to compare the samples, spectra were normalized by reducing the area under each spectrum to a value of 1 according to the method of Karoui et al.[10] Mainly the shift of the peak maximum and the peak width changes in the spectra were considered following this normalization.

Table 1. Evolution of Color and Texture Parameters during Aging of Sponge Cakes Kept at 20 °C and 65% Relative Humidity[a]

	exptl parameters[b]			calcd parameter
aging time (days)	L^*	a^*	b^*	ΔE^c
1	65.89 ± 0.35 a	−1.13 ± 0.17 cd	27.49 ± 0.42 ab	0
3	65.33 ± 0.77 a	−0.92 ± 0.25 bcd	26.81 ± 0.64 b	0.91
6	64.37 ± 1.10 a	−0.62 ± 0.08 abc	27.63 ± 0.17 ab	1.61
9	62.04 ± 0.31 b	−1.53 ± 0.09 d	27.47 ± 0.38 ab	3.87
16	64.23 ± 0.13 a	−0.62 ± 0.12 abc	27.57 ± 0.25 ab	1.74
20	65.48 ± 1.52 a	−0.36 ± 0.07 ab	28.33 ± 0.34 a	1.21
20O[d]	64.66 ± 1.08 a	−0.01 ± 0.05 a	28.37 ± 0.21 a	1.89

[a]The values are mean of three repetitions ± standard deviation. Values in the same column followed by different letters are significantly different ($p < 0.05$). [b]Hunter L^*, a^*, and b^* values for light to dark, red to green, and yellow to blue, respectively. [c]ΔE^*, total color differences. [d]20O, cakes aged for 20 days stored with an oxygen absorber.

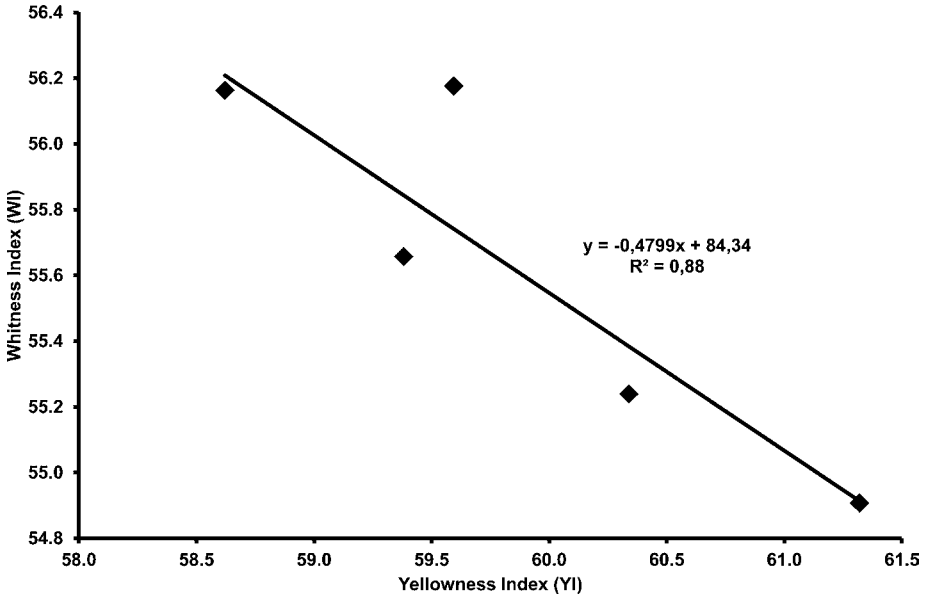

Figure 1. Correlation between whiteness and yellowness of sponge cakes kept at 20 °C and 65% relative humidity during storage.

Mathematical Analyses of Data. PCA was applied separately to tryptophan emission spectra to monitor changes occurring in the cake network during aging. The PCA transforms the original variables into new axes called principal components (PCs), which are orthogonal, so that the data sets presented on these axes are uncorrelated with each other.[18] Therefore, PCA expresses as much as possible the total variation in the data set in only a few PCs, and each successively derived PC expresses decreasing amounts of the variance. The multivariate statistical method was previously used to detect changes occurring during the aging of egg samples,[19] reducing the dimension to two or three PCs while keeping most of the original information found in the data.

In a second step, CCA describing the correlation between two sets of variables recorded on the same samples was applied. The CCA was used with the same samples that have been characterized by two different techniques; it provides a global measure of the connections between the groups of variables and a graphical representation of the existing correlation. The procedure assesses linear combinations of the two groups of variables in such a way that the correlations between these combinations are maxima. CCA was applied to the tryptophan fluorescence spectra and textural data.

The PCA and CCA were performed using XLSTAT 2012 (Addinsoft SARL USA, New York, NY, USA) software.

RESULTS AND DISCUSSION

Evolution of Physical Properties during Cake Aging. *Evolution of Color during Cake Aging.* Except for the sponge cakes aged for 9 days, ANOVA did not show significant difference between the investigated samples regarding the level of lightness (L^*) (Table 1), indicating that this parameter was not affected by the storage time. The same tendency was obtained for a^* values, but a slight evolution could be observed for b^* values. We have no explanation for the high L^* value corresponding to the sample aged for 9 days at the moment. The presence of an oxygen absorber seems not to affect the color parameters because no significant difference was observed between cakes aged for 20 days with or without an oxygen absorber.

The data observed in Table 1 showed also that cakes are located in sector yellow ($+b^*$) with slight tones of green ($-a^*$) and high tones of light ($+L^*$). This observation is in agreement with the findings of Shyu et al.,[20] who found similar values for cakes produced with or without γ-polyglutamic acid.

The only slight evolution of color parameters observed during the storage time could be ascribed to the fact that the formation of color in bakery products known as browning is the result of nonenzymatic chemical reactions that produce colored compounds during the baking process; such reactions are the Maillard reaction and caramelization.[21] Although lipid oxidation occurring during storage would induce changes in the color of the crumb cake surface,[22] no significant difference was observed for the investigated cakes. This observation allows us to

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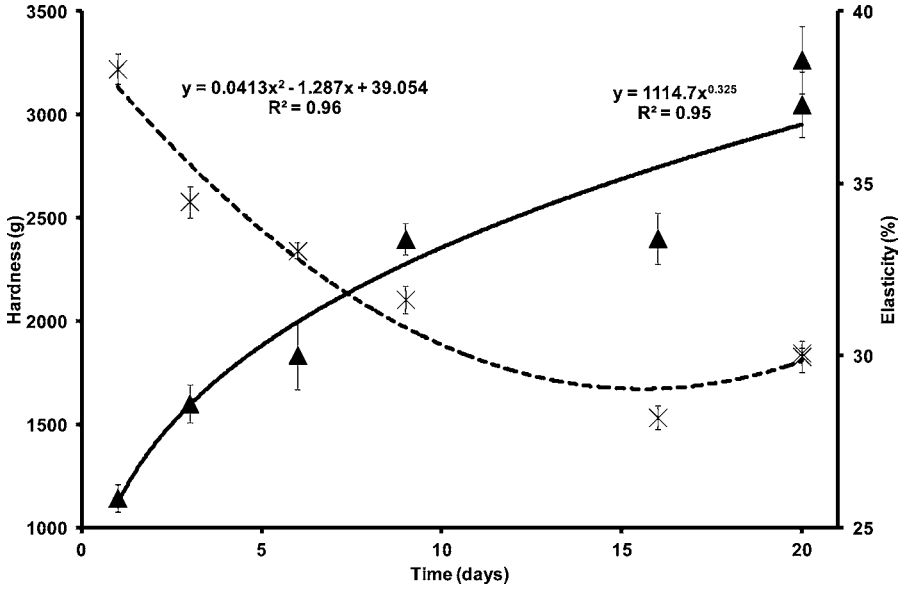

Figure 2. Evolution of hardness (▲) and elasticity (∗) of sponge cakes kept at 20 °C and 65% relative humidity during storage.

propose as one of hypotheses that the level of oxidation is probably low in the present study. Thus, it would be better to monitor cakes for longer periods of time (e.g., 1 month) and/or under accelerated aging process to assess the real impact of oxidation during aging on the evolution of sponge cake color.

To get more information regarding the evolution of color parameters during aging, the relationship between whiteness index (WI)[23] and yellowness index (YI)[23] has been determined, where

$$\mathrm{WI} = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

$$\mathrm{YI} = 142.86 \times \left(\frac{b^*}{L^*}\right)$$

WI decreased throughout aging and passed from the highest value (56.18) corresponding to day 1 to the lowest ones (55.24 and 54.91) corresponding to day 20 with and without an oxygen absorber, respectively. Figure 1 shows that as WI decreases, YI increases. From the same figure, a high correlation was observed between the two parameters because an R^2 of 0.88 was observed, similar to the findings of Ramirez-Navas et al.,[23] who found R^2 of 0.80 between WI and YI in Colombian quesillo cheeses. The observed increase of yellow color could be attributed to the fat globule from vegetable fat and/or egg used in the formulation of the cakes; these components subsequently became more concentrated (more yellowish) upon dehydration occurring during aging. It was reported that the yellow fat globules are responsible primarily for the yellowish tints, where the size of these fat globules from milk influences the color of the globules themselves.[23]

Only a slight color difference between the cakes was observed throughout the storage time, confirming the impossibility of the visual perception of the human eye to detect any color difference. This can be explained by the values obtained for the total color difference (ΔE^*), which were found to be <1.89, not noticeable to the human eye, except for cakes aged for 9 days exhibiting values of 3.8 (Table 1). The low ΔE^* values corresponding to the cakes aged for 20 days without an oxygen absorber could be explained by the mold apparition that might impact the color parameters.

Evolution of Texture during Cake Aging. Results of hardness and elasticity are shown in Figure 2. As expected, the crumb hardness increased, whereas the crumb elasticity decreased throughout the considered storage time. The freshest sponge cakes (aged for 1 day) had the lowest hardness values (i.e., 1144.39 G), whereas the aged ones (20 days) exhibited the highest values (i.e., 3265.44 G), quite similar to cakes kept during 20 days with an oxygen absorber (i.e., 3048.68 G). With regard to elasticity, the freshest sponge cake had the highest elasticity values (38.33%). Most of the texture change (i.e., elasticity decrease and hardness increase) occurred between days 1 and 9. After this storage time, only a slight increase or decrease was observed. The increase in hardness during storage could be attributed to the retrogradation of amylopectin during storage in agreement with Champenois et al.,[24] who pointed out that hardness of white pan bread increased continually with amylopectin retrogradation during storage time, and with Keetels et al.,[25] who reported that the amylopectin recrystallization resulted in an increase of starch gel hardness. Another explanation could arise from the reaction between amylose and amylopectin that induces an increase in the hardness of cake samples throughout storage. Champenois et al.[24] dismissed a total contribution of amylose retrogradation in the hardening process of white pan bread because the linear structure of amylose was supposed to form easily a complex with the lipids during the baking process. The hardness of cake samples aged for 1 day may be mainly attributed to the retrogradation of the noncomplexed amylose, which probably cocrystallized with the amylopectin.[24]

Other components (e.g., protein, lipid, moisture content) could influence the hardness and elasticity properties of cakes during storage. With regard to the moisture content, a significant decrease has been observed during the whole storage time because this parameter passes from 32.2% wet basis at day 1 to 26.7% wet basis after 20 days of storage. This decrease in the moisture content induced an increase in the hardness and a decrease in the elasticity of the investigated cakes. Indeed, the moisture content has been shown to be inversely proportional to the rate of firming.[26] In addition, the decrease of moisture content in cakes accelerates the formation

of cross-links between starch and protein, which could induce an increase in the cake hardness, in agreement with the findings of Gupta et al.,[27] who observed an increase of the cake hardness of the sponge cakes during storage (i.e., it passes from 5.94 at 0 h to 7.57 N after 120 h of storage), and those of Sung and Shyu,[20] who pointed out an increase in the hardness between 0 and 4 days. Water is a plasticizer, making the cake components (e.g., the gluten network) aged for 1, 3, 6, and 9 days more flexible than those aged for 16 and 20 days. Thus, as water is removed (from either gluten or starch) during storage, an increase in the hardness of cakes was observed. Moreover, a gluten and starch complex could induce changes in the texture of the investigated cakes throughout storage because it has been reported that a stable complex with chelation-type hydrogen bonds was observed between gluten and starch.[28]

The use of an oxygen absorber seemed to not allow the initial level of either hardness or elasticity during aging to be maintained. This was expected because the role of oxygen absorber was solely to remove during storage as much as possible the available oxygen surrounding the cake samples. Although it was outside the scope of the present work to reveal the impact of the presence of oxygen absorber on the quality of cake, it would be interesting to more deeply investigate the role of this parameter on the microbiological level because no effect was observed on either the color or texture measurements during 20 days of storage.

To determine the texture evolution of cakes during storage, different models have been developed. The models that fitted the best evolution of hardness and elasticity parameters were respectively polynomial (R^2 = 0.96) and power (R^2 = 0.95) ones as reported in Table 2. These models were in

Table 2. Correlation Coefficients (R^2) of Different Models Used To Explain the Relationship between the Hardness or Elasticity and the Aging Time of Sponge Cakes Kept at 20 °C and 65% Relative Humidity

		R^{2a}	
model	equation[b]	hardness	elasticity
exponential	$y = \exp(a + bx)$	0.77	0.88
linear	$y = a + bx$	0.76	0.92
logarithmic	$y = a + b \ln x$	0.94	0.89
polynomial	$y = a + bx + cx^2$	**0.96**	0.93
power	$y = ax^b$	0.92	**0.95**

[a]In bold are the models with the highest R^2 that could be considered as the best ones explaining the relationship between the considered parameter and storage time. [b]y, textural parameter analyzed, hardness or elasticity; x, time (in days); a, parameter related to the initial value of hardness or elasticity; b and c, parameters related to the variation rate of hardness or elasticity.

disagreement with those of Gómez et al.,[29] who suggested linear models for the evolution of both hardness and elasticity parameters of sponge cakes throughout storage (i.e., 28 days of storage at 20 °C) enabling "a" and "b". Indeed, the "a" parameter was related to the initial value of the hardness or elasticity, whereas the "b" one was related to its evolution. Values of "b" > 0 indicated an increase in the parameter over the storage time and vice versa. The difference between our models and those obtained by Gómez et al.[29] might be due to the difference in the formulation, process baking, and/or storage conditions of sponge cakes. Indeed, Gómez et al.[29] have incorporated an emulsifier (i.e., mixture of lactic acid and acetic esters of mono- and diglycerides of fatty acids) into the formulation of cakes that might induce a delay in the staling phenomenon allowing the initial texture of cakes to be maintained for as long as possible.

Evolution of Tryptophan Fluorescence Spectra during Cake Aging. The normalized tryptophan fluorescence spectra of sponge cakes exhibited three maxima located at 382, 435, and 467 nm (Figure 3) that could be attributed to the

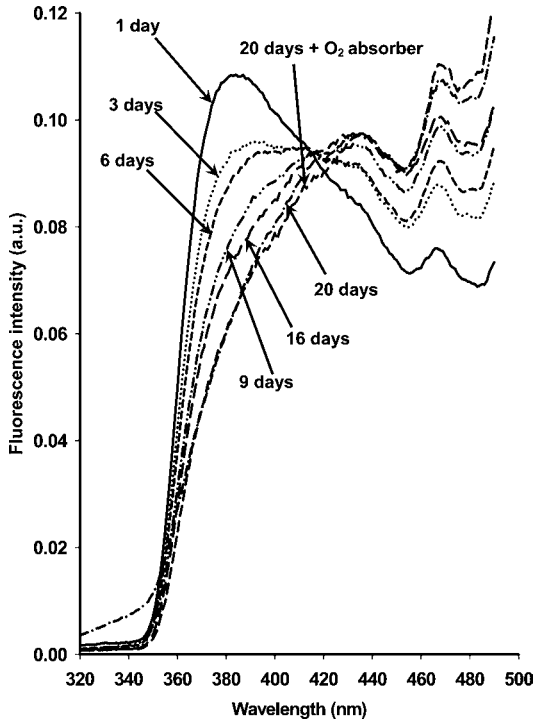

Figure 3. Normalized emission fluorescence spectra acquired after excitation at 290 nm on sponge cakes aged for 1 (—), 3 (···), 6 (- - -), 9 (—··—), 16 (— — —), and 20 days (—·—) and on sponge cakes aged for 20 days with an oxygen absorber (– – –).

maximum emission of tryptophan (382 nm) and fluorescent Maillard reaction products (435 and 467 nm). Around 382 nm, fresh cakes had the highest fluorescence intensity (i.e., 0.11), whereas aged cakes exhibited the lowest one (i.e., 0.06). An inverse trend was observed around 435 and 467 nm because the lowest fluorescence intensity was observed for fresh samples (e.g., 0.8 at 467 nm), whereas aged cakes presented the highest ones (e.g., 0.11 at 467 nm), demonstrating the accumulation of Maillard reaction products such as Schiff bases during the storage of cakes. This observation was in agreement with the findings of Ait Ameur,[30] who pointed out that fluorescent Schiff bases produced by the reaction of lipid-derived aldehydes and the amino groups of the lysine residues of proteins fluoresce at ∼470 nm as well as the oxidized phospholipids (in the 400–435 nm range).

Maximum tryptophan emission wavelength shifted from 382 nm for fresh cakes to 437 nm for aged cakes. The red shift observed in the present study could be explained by the change of (i) tryptophan residues exposure to the aqueous phase in sponge cakes during aging, in accordance with the most plausible hypothesis, namely, retrogradation of amylopectin, explaining the staling phenomenon of cereal products that consists of a release of water distribution from gluten to starch/amylopectin;[4] and/or (ii) starch–protein interactions due to

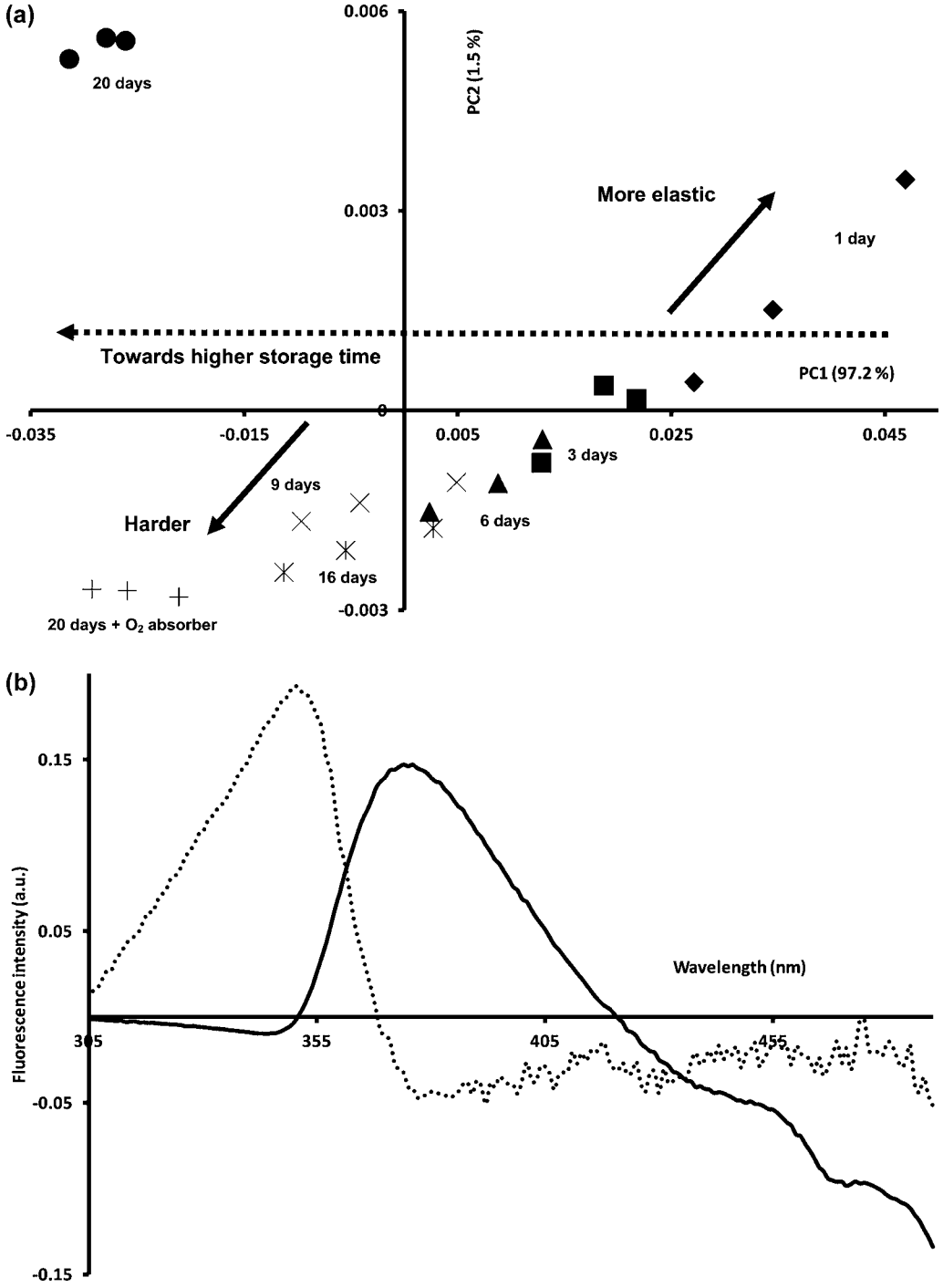

Figure 4. (a) Principal component analysis similarity map determined by principal components 1 (PC1) and 2 (PC2) for the fluorescence spectra recorded after excitation at 290 nm for sponge cakes aged for 1 (◆), 3 (■), 6 (▲), 9 (×), 16 (∗), and 20 (●) days and sponge cakes aged for 20 days with an oxygen absorber (+). (b) Spectral pattern corresponding to PC1 (—) and PC2 (···).

staling phenomenon as pointed out by Every and co-workers.[31] From these observations, it can be concluded that the shape of tryptophan fluorescence spectra could be considered as fingerprints allowing the identification of cake freshness.

Although the tryptophan spectra acquired on the investigated sponge cakes exhibited differences between fresh and aged cakes, univariate analysis is not really appropriate to statistically analyze the data sets. Multivariate statistical techniques such as PCA are more suitable to extract information related to the environment of the intrinsic probes.

Evaluation of the Discriminant Ability of Tryptophan Fluorescence Spectra Recorded on Sponge Cakes throughout Aging. PCA was applied to 21 spectra collected on the 7 sponge cakes at different aging times. The map defined by PCs 1 and 2 (97.2 and 1.5% of the total variance, respectively) showed a clear discrimination of cakes according to their aging time (Figure 4a). Considering PC1, cakes aged for 1, 3, and 6 days had positive score values, whereas the others exhibited mostly negative score values. The observed difference in the tryptophan fluorescence spectra could be related to the changes

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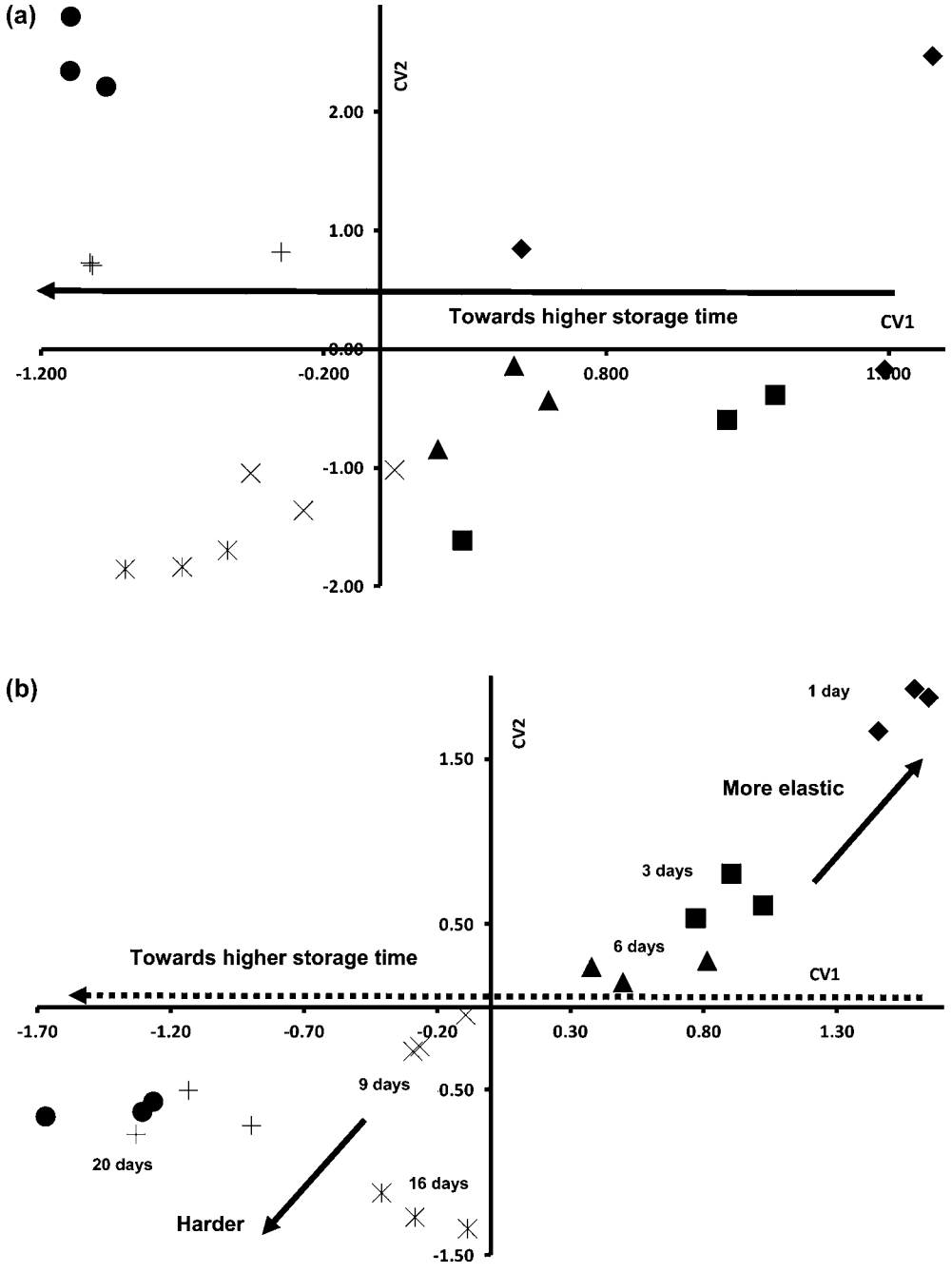

Figure 5. continued

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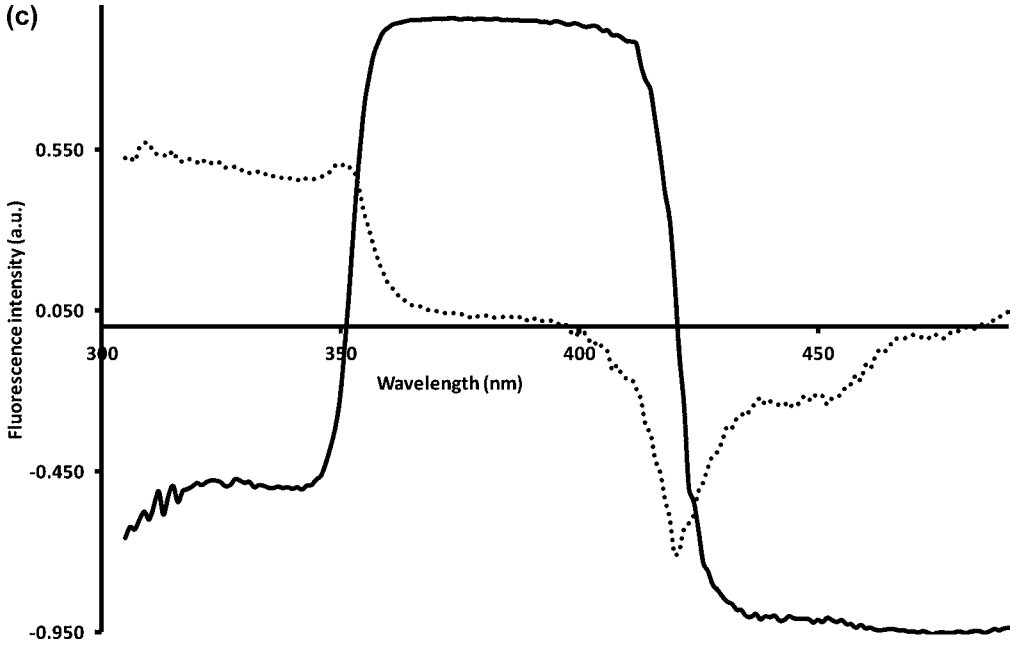

Figure 5. Canonical correlation analysis similarity map determined by the canonical variables 1 (CV1) and 2 (CV2) (a) for the fluorescence spectra recorded after excitation at 290 nm and (b) for the textural data for sponge cakes aged for 1 (◆), 3 (■), 6 (▲), 9 (×), 16 (∗), and 20 (●) days and sponge cakes aged for 20 days with an oxygen absorber (+). (c) Spectral pattern corresponding to CV1 (—) and CV2 (···).

in the protein−protein, protein−water, protein−starch, and/or protein−lipid interactions.

To investigate the basis of the observed spectral discrimination between the sponge cakes according to their aging time, the spectral patterns were analyzed (Figure 4b). From spectral pattern 1, it was concluded that the width of fluorescence spectra was larger for cakes aged for 9 days or more. These spectroscopic differences could be due to different protein−protein interactions and different protein network structures. Spectral pattern 2 indicated a positive peak at 344 nm. Similar results have been previously obtained during the monitoring of egg freshness[32] as well as for different soft cheese varieties.[33]

Correlation between Textural and Fluorescence Data. The correlations between the textural and tryptophan fluorescence spectra have been taken into account to get a better insight into the relationships between the molecular and macroscopic levels of the investigated cakes. CCA can be applied when the same samples have been characterized by two different techniques. The method provides a measure of the link between the groups of variables, and graphical representations of the correlation are revealed.

A high correlation was obtained between the tryptophan and textural data sets (R^2 = 0.99) indicating that the canonical variates provided a common description of the cakes both from the tryptophan fluorescence spectra and from the texture analyzer. The similarity maps of fluorescence and texture analyzer are shown in Figure 5, panels a and b. These maps are similar, which strongly suggested that the texture parameters (hardness and elasticity) and the tryptophan fluorescence spectra make it possible to observe the same phenomenon during the aging of cakes.

As the tryptophan fluorescence spectra were normalized, the spectral information involved in canonical variates 1 and 2 did not address directly the variations of the texture parameters of the investigated cakes, but qualitative differences related to these variations such as protein structures and interactions in the cake matrix. Considering Figure 5b, it appeared that the textural parameters (hardness and elasticity) allowed the separation of the cakes according to the first canonical variate: cake samples aged for 1, 3, and 6 days presenting the highest elasticity and the lowest hardness exhibited positive scores, whereas the other samples (presenting the lowest elasticity and the highest hardness) exhibited negative score values. The nature of the textural parameters linked to the two first canonical variates resulted from different structures that cakes could have during storage.

The high correlation observed between the tryptophan and textural parameters indicated that the former is correlated to one or more continuous phenomenon taking place during the storage and allowing the differentiation between fresh and aged cakes. Spectral pattern 1 (Figure 5c) indicated that the shape of tryptophan fluorescence spectra was broader for aged cakes than for the freshest ones. In fact, spectral pattern 1 from the PCA performed on tryptophan spectra and that of the CCA were similar, suggesting that the changes in the shape of fluorescence spectra were related to that of the protein network structure, resulting from the changes of the chemical and textural characteristics of cakes during aging.

The cakes of the present study were characterized by three analytical techniques (i.e., texture analyzer, colorimeter, and fluorescence spectroscopy) throughout aging. The cakes presenting different texture properties (hardness and elasticity) were found to exhibit different structures at the molecular level determined by fluorescence spectroscopy.

The present study shows that fluorescence spectroscopy and texture analyzer are useful for characterizing changes occurring in cake matrix during aging. As demonstrated by using PCA and CCA, the shapes of the tryptophan spectra are correlated to several characteristics of the protein network, as well as to the physicochemical (moisture content) and texture (hardness and elasticity) parameters. The information derived from the tryptophan spectra makes it possible to characterize the aging stage of cake. The advantage of combining fluorescence spectroscopy and texture analyzer has been demonstrated.

Indeed, applying CCA to the two data sets allowed (i) a clear discrimination of cakes according to their aging time and (ii) an interpretation at the molecular level. Fluorescence spectra and texture analyzer provided different information related to the molecular and macroscopic levels, making possible the characterization of cake in a global way, throughout aging.

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Notes

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