Monitoring quality of UF-soft-white cheeses containing added matured cheeses by biochemical, physicochemical, sensorial, and fluorescence spectroscopy techniques coupled with chemometric tools

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Abstract

The objective of the present work was to investigate by different analytical techniques, the impact of incorporating matured Domiati, Ras, and Cheddar cheeses on UF-soft white cheese's sensorial, physicochemical, biochemical, and molecular structure (fluorescence spectroscopy) features. Results showed that adding matured cheeses to UF-soft white cheese increased soluble nitrogen, free amino, and total volatile fatty acids, thus resulting in modifications of the cheeses' sensorial characteristics. It was demonstrated that cheese flavour was differently enhanced depending on the added matured cheese. The best sensorial properties were obtained up to 20 d of storage with added Cheddar, then Ras, and finally Domiati cheeses. Moreover, fluorescence spectroscopy provided relevant information related to the modification of protein and fat structures, depending on the cheese incorporated. Joint analysis of the experimental data by PLSDA and ComDim demonstrated the interest of those chemometrics tools for providing a global overview of the impact of adding matured cheeses on UF-soft white cheese quality features.

Introduction

UF-soft white cheese (known as Tallaga cheese) is the most widely consumed soft unpickled cheese variety in Egypt due to its cheap price and smooth creamy texture, as well as its less salty flavour (El-Kholy *et al.,* 2016). It is made from ultrafiltered and pasteurised buffalo's milk, cow's milk, or a mixture of both containing 3 - 6% fat without starter culture addition, with different percentages of salt (2 - 3%), and commercial microbial rennet. It is a product ready for consumption within 30 d of storage at refrigeration temperature. However, it often exhibits less pronounced cheese flavour as compared to the most popular soft cheese in Egypt (*i.e.* pickled Domiati cheese), which undergoes metabolic changes during the ripening period.

Several strategies have been studied with the objective to improve UF-soft white cheese quality, like the addition into milk or curd of matured cheese or slurry, cell-free extracts of microorganisms, and commercially available adjunct cultures (Katsiari *et al.,* 2002; Abd-El-Khalek *et al.,* 2008; Calasso *et al.,* 2015; Nezhad Razmjoui Akhgar *et al.,* 2016; Hamdy *et al.,* 2017). The addition of matured cheese is an effective way to enhance flavour intensity, improve body characteristics, and accelerate the formation of soluble nitrogenous compounds, free amino acids (FAAs), and free volatile fatty acids (FVAs) in UF-soft white cheese. This strategy represents the most economical way to improve sensorial characteristics of cheese because it contains microorganisms and bioactive enzymes that will induce chemical and biochemical changes during the cheese process (Nezhad Razmjoui Akhgar *et al.,* 2016; Hamdy *et al.,* 2017). This strategy was also used to accelerate cheese ripening that is a slow and expensive process for the dairy industry (Calasso *et al.,* 2015). For example, addition of ripened cheese slurry to cheese milk has been shown to reduce the ripening time of the resultant cheese by 30% for Ras cheese (Hamdy *et al.,* 2017).

Cheese sensorial characteristics are considered a very complex phenomenon arising from ripening, where biochemical reactions such as proteolysis, glycolysis, and lipolysis occur. Initial milk quality, composition, and cheese making processes results in a defined chemical composition in fresh cheese that also affects the final cheese quality. Therefore, the evaluation of the effect of the addition of slurry or matured cheeses on final cheese attributes and properties cannot be characterised in a simple way. Thus, multiple analytical tools like sensorial,

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physicochemical, biochemical, and rheological techniques were investigated to quantify and evaluate the effects of this procedure on cheese attributes and properties (Nezhad Razmjoui Akhgar *et al.,* 2016).

During the last 15 years, several novel techniques, *e.g.* various spectroscopic technics like NIR (*i.e.* Near Infrared), MIR (*i.e.* Mid Infrared), and fluorescence spectroscopies have been in the spotlight for the evaluation of cheese quality and attributes. Besides their usefulness for automated and online process monitoring, they allow for screening in a non-destructive way, a high number of samples, and obtain relevant information on, for example, molecular structure, texture, and colour attributes. Their application on milk and dairy products has been fully described in different scientific papers, reviews, and books. Among the above mentioned techniques, fluorescence spectroscopy offers several advantages for the characterisation of molecular interactions and reactions, molecule environments, and for the characterisation of molecular and functional properties of cheeses (Loudiyi *et al.,* 2018). This was possible due to its sensitivity to fluorophores (*e.g.* tryptophan and vitamin A) that are indicators of conformational changes of component structures and environments (Dufour *et al.,* 2001).

The evaluation and monitoring of cheese quality during storage are challenging tasks because cheese is a multifactorial biological system containing heterogeneous classes of compounds (*e.g.* fats, proteins, and carbohydrates), interacting to form a complex physical matrix. The complexity of cheeses requires implementation of sensorial and analytical to effectively study the multiple biochemical changes occurring during this process. As far as we know, scientific information about incorporating different matured cheeses (*i.e.* Domiati, Ras, and Cheddar) on combined UF-soft white cheeses quality features (biochemical and sensorial) and molecular structure has been lacking. In this context, the present work proposed to use fluorescence spectroscopy, biochemical, and sensorial analyses, and their combination for the evaluation of the impact of incorporating different matured cheeses on UF-soft white cheese quality features during storage. The combination of the results was performed by Partial Least Square Discriminant analysis (PLSDA) and Common Dimension (ComDim) with the objective to deeply and simultaneously characterise modification of cheese quality attributes (*i.e.* biochemical, structural, and sensorial).

Materials and methods

Matured cheeses

Domiati (4.9 ± 0.01 pH, 55.90 ± 0.13% moisture, 20.75 ± 0.12% fat, 15.82 ± 0.26% protein), Ras (5.40 ± 0.01 pH, 33.24 ± 0.11% moisture, 30 ± 0.21% fat, 25.17 ± 0.31% protein) and Cheddar (5.18 ± 0.03 pH, 36.12 ± 0.44% moisture, 29.83± 0.12% fat, 24.92 ± 0.29% protein) ripened for 90 d were purchased from an Egyptian supermarket. The cheese samples were cut into small pieces, and grated for 2 min in an electric grinder (Polytron® PT 10/35 GT Laboratory Homogenizer, Kinematica, Luzern, Switzerland). The grated cheese samples were stored at 5 ± 2°C until their incorporation in UF-soft-white cheese.

Processing of UF-soft-white cheeses

The experiments were carried out in a pilot dairy plant (Faculty of Agriculture, Fayoum University, Egypt). Fresh whole buffalo's milk obtained from the Faculty of Agriculture Farm was pasteurised (72°C/15 s) and ultrafiltered (concentration factor ~ 4) at 50°C using an ultrafiltration unit (Pasilac, Silkburg, Denmark). The obtained UF milk retentate was cooled to 40°C with a double-jacketed vat containing circulating cold water at 20°C. Calcium chloride (0.02%, 95% purity, Weufang Hongyuam Chemical Co., China), potassium sorbate (0.015%, 99% purity, SUNTRAN Co., China), and NaCl (2.0%, 99% purity, Emisal Company, Fayoum, Egypt) were added to UF milk retentate and mixed (*i.e.* pre-cheese milk). The obtained pre-cheese milk was divided into four equal parts (4 × 1 L) to produce UF-soft cheese. One litre was used to make the control cheese (without the addition of mature cheese) and was named UF-soft white cheese. For the other 3 L, each one was added with 2.5% (w/w) of grated matured Domiati, Ras, and Cheddar cheeses, respectively, based on previous studies (Nezhad Razmjoui Akhgar *et al.,* 2016).

After the incorporation of the matured cheese, the samples were mixed in an electric blender (Polytron® PT 10/35 GT Laboratory Homogenizer, Kinematica, Luzern, Switzerland) at 40°C for 5 min, and filtered through a clean muslin cloth to ensure the complete dispersion in the pre-cheese milk. The pre-cheese milk (1 L) was immediately poured into suitable plastic cups (250 mL volume) containing rennet powder (CHY-MAX, 2280 IMCU/mL, Ch. Hansen, Denmark) dissolved in distilled water (3 g rennet powder/100 kg milk retentate). The cups of cheese were incubated at 40°C (Memmert Co., Schwabach, Germany) until complete coagulation. Then, the cups of cheese were ripened for 10, 20, and 30 d at 5 ± 2°C (relative humidity: 96%). Three cups of cheese from each cheese trial were used for sampling and were analysed during the three periods of storage (*i.e.* 10, 20 and 30 d) for their physicochemical,

biochemical, sensorial, and fluorescence properties (*i.e.* molecular structure).

Physicochemical and biochemical analyses

The cheese samples were analysed in triplicate for acidity, total solids, fat, protein, water soluble nitrogen (WSN), total nitrogen (TN) contents, and pH values according to AOAC (2010). Total volatile fatty acids (TVFA) (0.1 N NaOH/100 g cheese) were determined as previously reported (Kosikowski, 1978). Total free amino acids (FAA) (g leucine/kg) were measured by Cd-ninhydrin analysis as described by Folkertsma and Fox (1992).

Synchronous fluorescence spectra analysis

Emission spectra of tryptophan residues (λ em. 305 - 400 nm with λ ex. 290 nm) and excitation spectra of vitamin A (λ ex. 250 - 350 nm with λ em. 410 nm) were directly recorded on the cheese samples. Measurements were performed at 20°C by using a spectrophotometer (Model FP-6500, JASCO Technologies, MD, USA) equipped with a xenon lamp as source of excitation and presenting a 200:1 signal to noise ratio and a resolution of 1 nm. The spectrometer was equipped with a solid sample holder presenting a 60° incidence angle for excitation radiation. Slices extracted from the centre of cheese samples (2.5 cm length × 0.9 cm width × 0.4 cm thickness) were placed in a sample holder by using a quartz cell (3 × 1 × 1 cm). Three replicates were performed per cheese. Spectral data collection and manipulation were performed using JascoSpetraManager™ software.

Sensorial evaluation

The cheeses' sensory were evaluated by eight expert panellists familiar with UF-soft cheese attributes at the 10th, 20th, and 30th day of cold storage according to IDF (2009). Panellists were selected from the Department's staff based on interest and experience in sensory evaluation of white cheeses. Cheese samples were placed on white plates coded with 3-digit random numbers and then presented to the panellists at 20 ± 2°C in a random order to determine the quality of cheese samples using a 10-point scale, ranging from 0 (poor) to 10 (best) for quality assessment of the attributes described. The panellists evaluated the cheese for texture (the force required to cut through 1 cm thick slice of cheese with a knife), flavour quality (in the mouth), and overall quality. Water was served for mouth rinsing between each sample. The samples were served to the panellists in the same form that they would be served to the consumer.

Statistical analysis

The significance of the differences between biochemical, physicochemical, and sensorial data of the cheese samples was determined by a one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test. Differences were considered statistically significant when $p < 0.05$. All statistical analyses were carried out using the XLSTAT software (Addinsoft, Paris, France).

Fluorescence spectra were normalised by Standard Normal Variate (SNV), smoothed using the savgol algorithm (2nd order polynomial applied with a sliding of 11-point spectral window) and mean centred.

PLSDA was performed after concatenation of the physicochemical, biochemical, and sensorial data in one matrix. PLSDA was performed on MATLAB R2013b (The MathWork, Natick, Massachusetts, USA) using the PLS toolbox 7.0.3. The best number of factors was selected using a leave-one-out cross-validation, by analysing the percentage of cross-validation error and the variance captured error (Wang *et al.,* 2016). Before performing PLSDA, physicochemical, biochemical, and sensorial parameters were mean centred.

To describe in one shot the global characteristics related to cheese quality, ComDim was performed on a 3D data table containing biochemical and physicochemical characteristics (block 1), sensorial features (block 2), tryptophan fluorescence spectra (block 3), and vitamin A fluorescence spectra (block 4), measured on the cheese samples during ripening.

ComDim calculates a weighted sum of the sample 'variance-covariance matrix' and then extracts its first normed principal component as the first "Common Dimension" or "Common Component" (CC). The algorithm uses an initial weighting (or "salience") for each table equal to 1, but then iteratively recalculates the salience of each block for the calculated CC. Thus, the percentage of variability extracted by the CC can be calculated. After computation of the first CC, each original data block matrix is deflated, and the procedure repeated for the calculation of the second CC, and so forth. Therefore, each CC is the first principal component of a weighted sum of the samples ''variance-covariance'' of deflated matrices (Jouan-Rimbaud Bouveresse *et al.,* 2011). Before performing ComDim analysis, each data block (*i.e.* 1 to 4) was pre-processed as indicated for PLSDA. ComDim analysis was performed in MATLAB R2013b (The MathWork, Natick, Massachusetts, USA) (Qannari *et al.,* 2001).

Results

Biochemical, physicochemical, and sensory

characteristics of experimental cheeses

Table 1 presents the biochemical and physico-chemical characteristics obtained for the control (*i.e.* UF-soft white cheese) and cheeses containing matured Domiati, Ras, and Cheddar cheeses during refrigerated storage (*i.e.* 10, 20, and 30 d). As compared to the control, the results showed that adding matured chees-es had no significant effect ($p > 0.05$) on moisture, fat, and protein contents whatever the ripening stage. How-ever, significant differences were observed ($p < 0.05$) for the acidity and pH values between cheese samples during storage. After 10 d of storage, it is noticeable that the UF-soft white cheeses containing matured cheese had higher acidity and lower pH as compared to the control. The highest acidity values were recorded with cheeses enriched by 2.5% of matured Cheddar cheese (*i.e.* 5.90 to 18.30 g.kg^{-1} for 10 and 30 d, respec-tively). On the other hand, the addition of matured cheese affected the ripening indices (*i.e.* WSN/TN, FAA, and TVFA) of the cheeses, and presented signifi-cant differences ($p < 0.05$) for those parameters during storage. As compared to the control, all samples containing matured cheeses presented higher levels of WSN/TN, FAA, and TVFA.

Results concerning sensorial analysis (*i.e.* texture, flavour, and overall quality parameters) of the different cheeses during cold storage are presented in Table 2. Texture, flavour, and overall quality scores gradually decreased during storage for all samples, except for the flavour attribute in samples containing

Table 1. Biochemical and physicochemical characteristics of the UF-soft white cheeses incorporated with different mature cheeses (*i.e.* Domiati, Ras, and Cheddar) after 10, 20, and 30 d of storage.

Property	Storage period (day)	Control	UF- soft cheese made with 2.5% of mature cheese		
			Domiati	Ras	Cheddar
pH	10	6.33 ± 0.60^{aA}	5.43 ± 0.60^{cA}	5.37 ± 0.60^{cA}	5.87 ± 0.60^{bA}
	20	5.73 ± 0.10^{aB}	4.90 ± 0.60^{bB}	4.90 ± 0.60^{bB}	4.67 ± 0.60^{cB}
	30	5.47 ± 0.60^{aC}	4.20 ± 0.10^{dC}	4.57 ± 0.60^{bC}	4.30 ± 0.10^{cC}
Acidity (g.kg^{-1})	10	3.00 ± 0.10^{cC}	5.30 ± 0.20^{bC}	6.30 ± 0.10^{aB}	5.90 ± 0.40^{aC}
	20	5.40 ± 0.20^{cB}	13.00 ± 0.10^{bB}	13.30 ± 0.40^{bA}	14.70 ± 0.20^{aB}
	30	7.40 ± 0.10^{cA}	17.60 ± 0.20^{aA}	13.70 ± 0.20^{bA}	18.30 ± 0.90^{aA}
Moisture (g.kg^{-1})	10	678.10 ± 1.50^{aA}	674.70 ± 3.90^{aA}	682.90 ± 0.75^{aA}	681.20 ± 0.50^{aA}
	20	669.00 ± 1.70^{aA}	668.80 ± 1.30^{aB}	666.70 ± 0.57^{aB}	667.40 ± 0.71^{aB}
	30	666.90 ± 3.40^{aA}	656.50 ± 2.90^{bB}	657.60 ± 0.53^{bB}	662.90 ± 0.50^{abB}
Fat (g.kg^{-1})	10	158.30 ± 0.29^{abA}	158.30 ± 0.31^{bB}	162.00 ± 0.29^{abA}	161.70 ± 0.58^{aB}
	20	161.70 ± 0.29^{bA}	162.00 ± 0.45^{bC}	163.30 ± 0.50^{aA}	163.30 ± 0.29^{aA}
	30	163.30 ± 0.29^{aA}	165.00 ± 0.56^{aA}	166.70 ± 0.28^{aA}	168.30 ± 0.29^{aAB}
Protein (g.kg^{-1})	10	104.10 ± 0.95^{bB}	118.20 ± 0.41^{aA}	116.50 ± 0.49^{aA}	117.10 ± 0.14^{aB}
	20	118.70 ± 0.08^{aA}	120.70 ± 0.45^{aA}	121.90 ± 0.39^{aA}	121.80 ± 0.33^{aAB}
	30	119.50 ± 0.34^{aA}	123.80 ± 0.81^{aA}	124.70 ± 0.29^{aA}	126.10 ± 0.29^{aA}
Water soluble nitrogen / total nitrogen WSN/TN (%)	10	16.74 ± 0.15^{bB}	20.23 ± 0.87^{aA}	20.77 ± 0.70^{aB}	21.51 ± 0.90^{aB}
	20	18.04 ± 0.15^{bB}	22.14 ± 0.02^{aA}	22.06 ± 0.57^{aB}	24.01 ± 0.96^{aB}
	30	20.48 ± 0.17^{bA}	23.05 ± 0.48^{bA}	27.86 ± 0.49^{aA}	28.68 ± 0.76^{aA}
Free amino acids % (g leucine.kg^{-1})	10	0.41 ± 0.01^{bC}	0.48 ± 0.06^{bB}	0.62 ± 0.02^{aB}	0.65 ± 0.05^{aC}
	20	0.49 ± 0.02^{cB}	0.65 ± 0.02^{bA}	0.66 ± 0.03^{bAB}	0.93 ± 0.07^{aB}
	30	0.54 ± 0.03^{dA}	0.68 ± 0.02^{cA}	0.76 ± 0.07^{bA}	1.21 ± 0.01^{aA}
Total volatile fatty acids % (0.1 N NaOH.100 g^{-1})	10	16.00 ± 1.00^{cB}	18.33 ± 1.53^{bcB}	20.00 ± 1.00^{bC}	27.00 ± 2.00^{aC}
	20	23.67 ± 1.53^{cA}	30.67 ± 1.15^{bA}	31.33 ± 3.21^{bB}	39.67 ± 0.58^{aB}
	30	26.67 ± 2.08^{cA}	33.00 ± 2.65^{bA}	35.33 ± 1.15^{bA}	44.67 ± 3.06^{cA}

Values are means ± standard deviations. Means with lowercase superscript in a row indicate significant difference in LSD test ($p < 0.05$). Means with uppercase superscript in a column indicate significant difference in LSD test ($p < 0.05$).

Table 2. Sensory attributes (*i.e.* texture, flavour, and overall quality) of the UF-soft white cheeses incorporated with different mature cheeses (*i.e.* Domiati, Ras, and Cheddar) after 10, 20 and 30 d of storage.

Sensory attribute	Storage period (day)	Treatment			
		Control	UF-soft cheese made with 2.5% of mature cheese		
			Domiati	Ras	Cheddar
Texture (1 - 10 points)	10	9.0 ± 2.5[aA]	9.0 ± 1.5[aA]	9.0 ± 1.2[aA]	9.0 ± 1.6[aA]
	20	8.0 ± 0.5[aAB]	7.0 ± 0.5[bB]	7.0 ± 0.2[bB]	7.0 ± 0.6[bB]
	30	7.0 ± 0.5[aB]	5.0 ± 0.5[bC]	5.0 ± 0.2[bC]	5.0 ± 0.6[bC]
Flavour (1 - 10 points)	10	7.0 ± 2.1[cA]	6.0 ± 0.6[dA]	9.0 ± 0.68[bA]	10.0 ± 0.55[aA]
	20	6.0 ± 1.6[cA]	5.0 ± 0.55[dA]	9.0 ± 0.66[bA]	10.0 ± 0.45[aA]
	30	6.0 ± 1.5[dA]	5.0 ± 0.26[cA]	9.0 ± 0.67[bA]	10.0 ± 0.28[aA]
Overall quality (1 - 10 points)	10	8.0 ± 1.6[cA]	7.0 ± 2.6[bA]	9.0 ± 2.6[aA]	10.0 ± 1.5[aA]
	20	7.0 ± 2.3[bA]	5.0 ± 3.5[cB]	8.0 ± 3.8[aA]	8.0 ± 1.8[aB]
	30	7.0 ± 2.5[aA]	4.0 ± 3.6[cB]	6.0 ± 2.7[bB]	6.0 ± 2.5[bC]

Values are means ± standard deviations. Means with lowercase superscript in a row indicate significant difference in LSD test ($p < 0.05$). Means with uppercase superscript in a column indicate significant difference in LSD test ($p < 0.05$).

Ras (*i.e.* 9 points) and Cheddar (*i.e.* 10 points) cheeses. No significant differences ($p > 0.05$) among cheese samples were observed regarding texture after 10 d of storage. Nonetheless, this parameter changed significantly ($p < 0.05$) after 20 and 30 d. Maximum flavour and overall quality scores were obtained in cheeses containing Cheddar, followed by Ras, and finally Domiati cheeses.

Evaluation of the discrimination ability of sensorial, biochemical, and physicochemical data

In order to extract relevant information concerning similarity and dissimilarity among sensorial, biochemical, and physicochemical characteristics of cheeses during storage, PLSDA was jointly applied to the different data sets. This combined analysis was performed after matrix concatenation consisting in juxtaposing the different data matrix in order to obtain one matrix gathering all the data sets.

Before performing PLSDA, the cheese samples were divided into four groups corresponding to each cheese's category (*i.e.* control, cheeses with added Cheddar, Ras, and Domiati, respectively). The results obtained after performing PLS-DA are shown in Table 3, Figure 1a (*i.e.* loadings plot), and Figure 1b (*i.e.* scores plot). The analysis of scores plot highlighted that a good discrimination between the four cheese categories could be assessed by using only two loading vectors (LV1 and LV2) (Table 3 and Figure 1b). Accuracy values for classification of the four groups were 96.30, 100, 92.5, and 88.89%, respectively (Table 3). Concerning storage time, similarity and loading maps (Figure 1) demonstrated that increasing

Table 3. Confusion matrix and percentage of accuracy obtained after PLSDA applied jointly on sensory and physicochemical data sets recorded during storage (*i.e.* 10, 20 and 30 d) for discrimination of the UF-soft white cheeses control, and UF-soft white cheeses containing mature cheeses (*i.e.* Domiati, Ras, and Cheddar).

Predicted as	Actual class				Total	Accuracy (%)
	Control	Cheddar	Domiati	Ras		
Control	9	0	1	1	11	96.30
Cheddar	0	9	0	0	9	100
Domiati	0	0	8	1	9	92.59
Ras	0	0	0	7	7	88.89
Total	9	9	9	9	36	94.44

Control: UF soft-white cheese; Cheddar: UF soft-white cheese containing added Cheddar; Domiati: UF soft-white cheese with containing Domiati; Ras: UF soft-white cheese containing added Ras.

storage time decreased the overall quality and texture of the cheeses, pH, and moisture; while an inverse trend was noted for fat, protein, acidity, FAA, TVFA, and WSN/TN contents. Loading plot (Figure 1a) demonstrated that samples containing added Cheddar clearly presented higher scores for flavour, followed by the cheeses containing added Ras, then the control cheeses and finally the cheeses containing added Domiati.

When analysing LV1 axis, it can be noted that the cheeses stored for 30 d presented a high flavour, fat content, FAA, WSN/TN, TVFA, protein, and acidity. Nonetheless, the opposite was noted for the other biochemical (moisture and pH) and sensorial (texture)

parameters. When considering the LV2 axis of the loading plot (Figure 1a), it can be highlighted that the cheeses presenting the highest flavour and overall sensory acceptability presented higher moisture, texture, and pH values, while exhibiting lower values for FAAs, WSN/TN, TVFAs, acidity, and proteins.
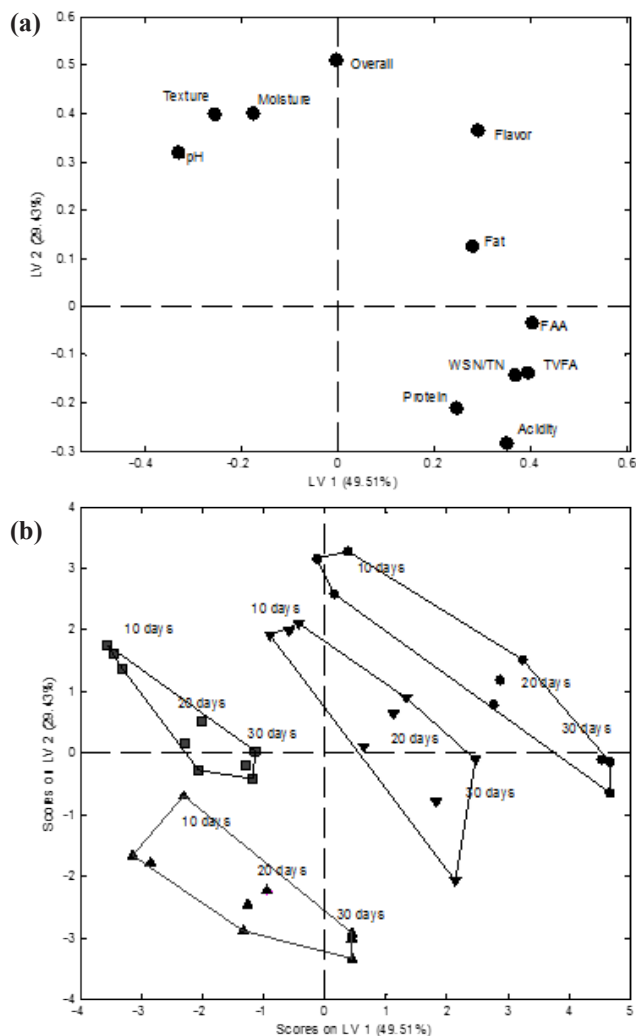
(a)

(b)

Fig. 1. Two first LV (LV1 and LV2) of loading (a) and similarity (b) maps obtained after PLSDA applied to concatenated data matrix (biochemical, physicochemical and sensory) of UF-soft cheeses during storage (10, 20 and 30 days).
Control cheese (■); cheese containing Domiati (▲); cheese containing Cheddar (●); cheese containing Ras (▼).TVFA: Total Volatile Fatty Acids; FAA: Free Amino Acids; WSN/TN: Water Soluble Nitrogen/Total Nitrogen

Fluorescence spectra analysis of cheeses

Figure 2 presents the normalised fluorescence spectra recorded at a fixed excitation (*i.e.* 290 nm) and emission (*i.e.* 410 nm) wavelengths on the cheeses containing added matured cheeses (*i.e.* Domiati, Cheddar, and Ras) at 10 d of storage. The shape and intensities of spectra largely varied from one cheese to another. Equivalent results were noted for the other days of storage.
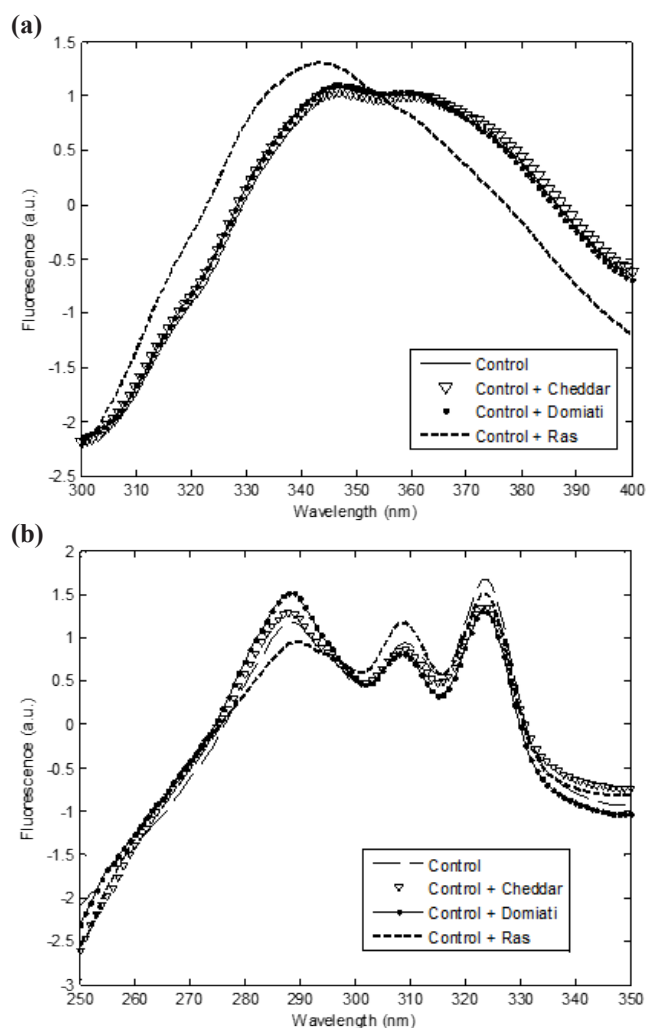
(a)

(b)

Fig. 2. Effect of adding mature cheeses (Cheddar, Domiati and Ras) on normalized fluorescence spectra: (a) tryptophan emission spectra; (b) vitamin A excitation spectra

Joint analysis of cheeses' biochemical, physicochemical, sensorial, and fluorescence data

Before performing ComDim analysis, a 3D combination of the data blocks was performed. The 1st block of the 3D matrix contained physicochemical and biochemical data, the 2nd one is the scores of sensory attributes, the 3rd one is the spectra obtained after excitation at 290 nm, and the last one is the spectra recorded at fixed emission (*i.e.* 410 nm). Four common dimensions (99.01% of variance explained) were identified as optimum for extraction of the overall variation of the data blocks.

Figure 3 (a-i) depicts the ComDim results. More specifically, Figure 3 (a-c) illustrates the scores with information on the similarities and differences among the samples. Figure 3 (d-f) presents the saliences, identifying which block contains the information responsible for the scores pattern in each CC. Figure 3 (g-i) presents the loadings that provided information about which variables in each table are important for the patterns seen in the scores.
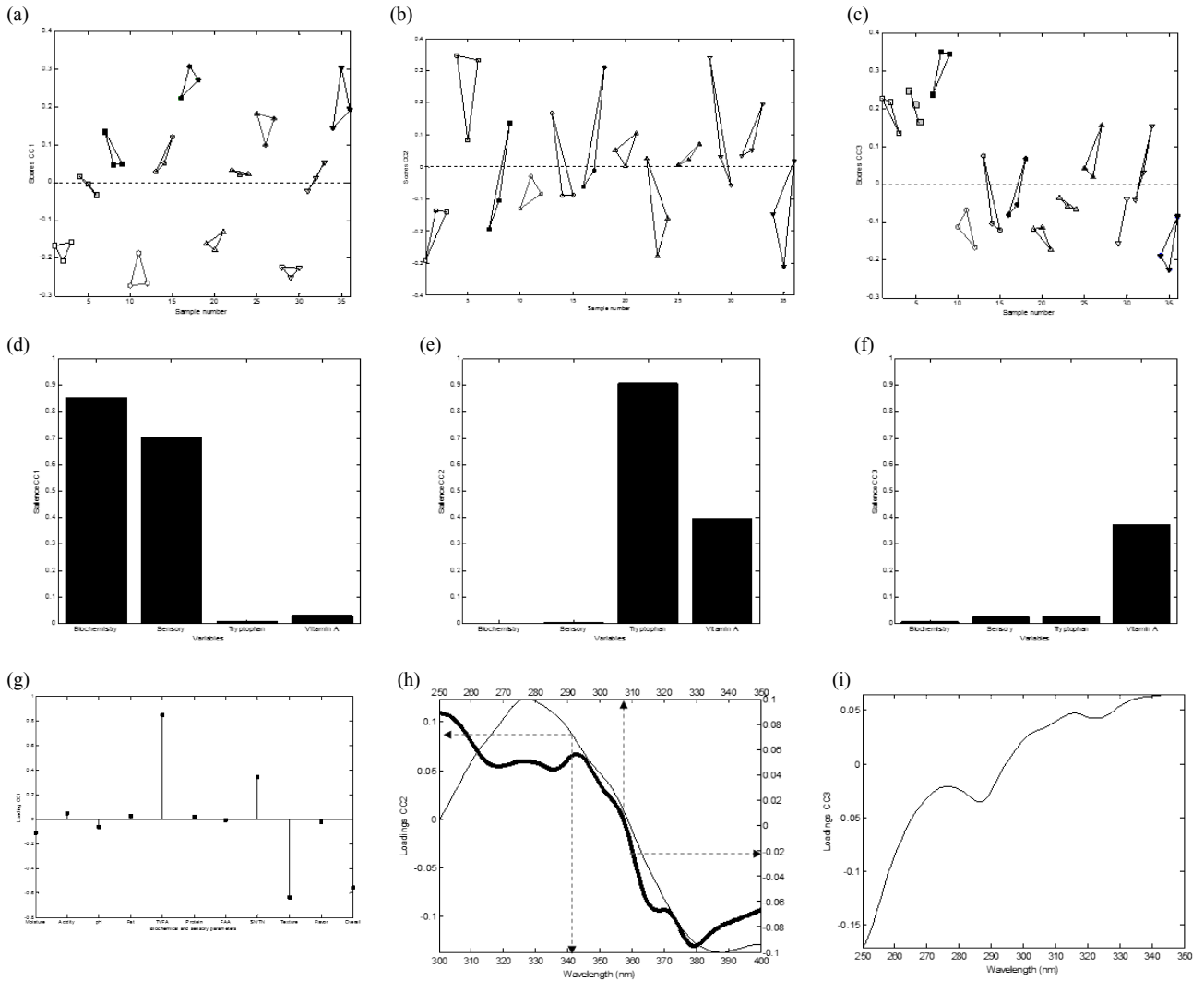
Fig. 3. ComDim results. (a-c) Scores, (d-f) Saliences and (g-i) Loadings Control cheese (□), UF-soft cheese containing Cheddar (O), UF-soft cheese containing Domiati (Δ), UF-soft cheese containing Ras (▽). White symbol: 10 days of storage, gray symbols: 20 days of storage and black symbols: 30 days of storage. TVFA: Total Volatile Fatty Acids; FAA: Free Amino Acids; WSN/TN: Water Soluble Nitrogen/Total Nitrogen; CC: Common Component; the arrows indicated the axes to consider for each CC2 loading pattern.

For common component 1 (CC1), the saliences (Figure 3d) revealed that biochemical and sensorial parameters (*i.e.* Blocks 1 and 2) had the highest influence on the score patterns. Concerning the scores (Figure 3a), the CC1 showed a clear discrimination between cheeses stored at 10 d (negative score values), and cheeses stored for 20 and 30 d (positive score values). Moreover, an increase of the scores is observed for each cheese during the storage period (30 > 20 > 10 d). As compared to the control, the highest contrasts are noted between the cheeses with matured Cheddar and Ras cheeses at 30 d of storage, while the cheeses containing matured Domiati cheese did not exhibit huge differences. The loadings for CC1 (Figure 3g) demonstrated that the samples are essentially differentiated by TVFA, WSN/TN, texture, and the overall quality. On the one

hand, samples on the positive side of the CC1 scores plot are distinguished from the others based on their TVFA and WSN/TN contents. On the other hand, samples on the negative side of the CC1 scores plot presented similarities for texture and overall quality score. The loadings also highlighted that (i) WSN/TN and TVFA of all experimental cheeses gradually increased during storage; (ii) the addition of matured cheeses affected the WSN/TN and TVFA during storage; and (iii) the level of the TVFA and WSN/TN of cheese containing Cheddar was higher as compared to the other cheeses.

Concerning sensory attributes, the loadings for CC1 (Figure 3g) demonstrated that the quality of the control gradually decreased above 10 d of storage. Moreover, adding matured cheeses has different effects depending on both cheese type and

storage period. For cheeses stored for 10 d, adding matured Cheddar and Ras cheeses increased the overall quality and texture scores of the control; while adding matured Domiati cheese decreased those parameters.

Based on the saliences for common component 2 (CC2) (Figure 3e), the predominant contribution to the distribution of samples was due to fluorescence obtained after excitation at 290 nm (*i.e.* Block 3) and to a lesser extent to the fluorescence spectra obtained at 410 nm fixed emission (*i.e.* Block 4). The loading pattern associated with block 3 (Figure 3h) presented a positive landscape between 250 to ~ 305 nm and a negative one between 305 to ~ 350 nm describing a shift to lower wavelengths for spectra obtained after excitation at 290 nm and at a 410 nm fixed emission, respectively. Concerning the score plot, no clear pattern depending on storage time or on the added cheese is noted. Nonetheless, the analysis of CC2 score plot (Figure 3b) demonstrated that as compared to the control (*i.e.* 10 d of storage), an increase of the scores is clearly visible for samples containing Cheddar, Domiati, and Ras cheeses (*i.e.* Ras > Domiati > Cheddar > Control). After 20 d of storage, a decrease of the scores was observed from the control to samples containing Cheddar, Domiati, and Ras cheeses (control > Cheddar = Ras > Domiati).

The saliences for the common component 3 (CC3) (Figure 3f) showed that the predominant contribution to this CC was due to excitation spectra (Blocks 3) recorded at 410 nm fixed emission. At first, we could identify a clear discrimination of the control (positive scores) from the other samples, containing Cheddar, Domiati, and Ras (almost negative scores). Moreover, a slight increase of the scores for each cheese could be observed during the storage period, except for Ras cheese.

The fourth component is not depicted due to the lack of discrimination between samples observed for this CC.

Discussion

Effect of adding matured cheeses on physicochemical, biochemical, and sensorial characteristics of UF-soft white cheese

First of all, physicochemical and biochemical results obtained for the control are in the same range as those previously reported for UF-soft white cheeses (Erdem, 2005; Fathollahi *et al.,* 2010). Furthermore, the ANOVA and PLSDA outcomes highlighted differences in pH, composition, and sensorial features between the control and the cheeses containing added matured cheeses. Those

observations were previously reported by Nezhad Razmjoui Akhgar *et al.* (2016) on Iranian UF white cheese. The difference in pH and acidity values observed between the control and cheeses containing added matured cheeses might be attributed to a difference in composition and the content of glycolytic bacteria provided by Domiati, Ras, and Cheddar cheeses (Nezhad Razmjoui Akhgar et al., 2016).

During storage, an increase of protein and fat contents were noted and principally attributed to a continuous loss of moisture ($p < 0.05$) (Mostafa *et al.,* 2000). Concerning pH, different authors (Sheehan and Guinee, 2004; Nezhad Razmjoui Akhgar *et al.,* 2016) highlighted also an equivalent decrease during storage. Those observations were attributed to the metabolism of lactose to lactate by lactic acid bacteria resulting in the decline of pH and increase in acidity.

The three ripening indices evaluated *i.e.* WSN/TN, FAAs, and TVFAs were affected by the addition of matured cheeses. The differences noted for WSN, TVFAs, and FAAs in the cheeses containing added matured cheeses could be related to differences in the proteolytic and lipolytic activities due to *i.e.* residual coagulant, indigenous milk enzymes, starter or non-starter microflora, and secondary microorganisms provided by the added matured cheeses (Beuvier and Buchin, 2004).

It is accepted that ripened soft cheeses are viscoelastic materials, and their viscoelastic behaviour can be influenced by changes in their formulation (Lobato-Calleros *et al.,* 2000) caused by the incorporation of matured cheeses, whose components can interact with the casein matrix in the curd. Moreover, during ripening, the breakdown of the protein network can be involved in the modification of cheese texture, and the formation of flavour and/or off-flavour compounds with secondary catabolic changes of the proteolysis products (*i.e.* peptides and FAAs) (McSweeney and Sousa, 2000). The lipolysis also results in modifications by releasing FFAs and TVFAs, which directly or indirectly contribute both to modification of cheese flavour and texture. Due to the effect of adding matured cheeses on ripening indices, the study of sensory attributes of cheeses containing added matured cheeses is of utmost importance.

UF-soft white cheeses containing added matured Ras and Cheddar cheeses were generally more appreciated by the panellists for their flavour, texture, and overall quality among the cheese samples up to 20 d of cold storage (*i.e.* 10 and 20 d). This could be attributed to the added Cheddar and Ras cheeses permitting a rapid development of the

best sensorial characteristics of UF-soft white cheeses. This is probably associated with the combination of the higher concentrations of TVFAs and FAAs, and soluble peptides which act as a flavour precursor in UF-soft white cheeses containing added matured cheeses. Therefore, using matured Ras and Cheddar cheeses may induce better sensory, texture, and overall sensorial properties in UF-soft white cheese up to 20 d of storage. The lower level of flavour, texture, and overall quality of the control cheese observed up to 20 d of storage may be correlated with lower levels of nitrogen fractions and FAAs (Abd-El-Khalek *et al.,* 2008; Nezhad Razmjoui Akhgar *et al.,* 2016).

Inversely, the lower score given for sensorial parameters (*i.e.* texture, overall quality, and flavour) for the cheeses at 30 d of storage, particularly for the cheeses containing added matured cheeses, could be explained by an excessive development of peptidase and lipolysis activities, which could lead to a high accumulation of FAAs, TVFAs, acidity development, and lower pH. This can lead to pungent odour, irritant volatile fatty acids, ammonia odours, and a lack of texture (Diezhandino *et al.,* 2016), thus resulting in a soft, weak, and pasty body.

The present results suggested that the addition of matured Cheddar and Ras cheeses might contribute to a faster development of UF soft white cheese quality attributes concerning texture, overall quality, and flavour in UF-soft white cheese only up to 20 d. Moreover, the results obtained confirmed that the concatenation technique coupled with PLSDA enabled the differentiation between the four groups of cheeses depending on storage time and added matured cheeses.

Joint analysis of biochemical, sensorial, and fluorescence spectra data sets of UF-soft white cheeses

An advantage of applying ComDim as compared to low and mid-level data fusion approaches, is that it provides information about the relation between individual data blocks (*i.e.* common variables) and their contribution to each CC. ComDim can be usefully applied in order to study the complementarities and differences between the information gathered by the various techniques used for UF-soft white cheese analysis (*i.e.* biochemistry, fluorescence spectroscopy, and sensorial analysis). Therefore, applying ComDim to all the data sets may lead to a better understanding of the specificities and the links between biochemical, physicochemical, structural, and sensorial features of the different UF-soft white cheese formulations. Moreover, a better insight into the relationships between the data at both molecular (biochemical, fluorescence) and macroscopic

(sensorial features) levels can be assessed.

The salience for CC1 clearly underlined a high contribution of biochemical and sensory attributes during both storage and after adding matured cheeses on the discrimination of cheese samples. This probably highlighted a high correlation of biochemical and sensory attributes of the cheeses in agreement with Kraggerud *et al.* (2014). Contrary to the PLSDA results, the ComDim outcomes demonstrated that the discrimination of samples was essentially assigned to TVFA, WSN/TN (positive loading values), texture, and overall quality (negative loading values), while the other parameters contributed little. This is probably due to the effects of lipolysis and proteolysis on both overall quality and texture of cheeses (McSweeney and Sousa, 2000).

This was confirmed by the observations of both fluorescence spectra modifications and ComDim results noted for CC2 and CC3 that can be related to cheese molecular structure and molecular interactions. It appears that the addition of matured cheese and storage period had an effect on both tryptophan and vitamin A fluorescence spectra. Indeed, fluorescence spectra obtained after excitation at 290 nm presented emission characteristics to tryptophan, while excitation fluorescence spectra recorded at 410 nm emission wavelength can be assigned to vitamin A (Ozbekova and Kulmyrzaev, 2017) localised in the core and membrane of the fat globules. Fluorescence spectroscopy is known to be sensitive to characterising molecular interactions and reactions, alteration of molecular environments of fluorophores, and has been used to characterise molecular structure and texture of cheeses (Karoui and Dufour, 2006). Moreover, it is accepted in the literature that the emission of tryptophan residues in protein and vitamin A can be used as indicators of protein conformational changes, the physical state of triglycerides, and protein lipid interactions in dairy products (Dufour *et al.,* 2001).

Based on the saliences for CC2, the predominant contribution to the distribution of samples is due both to fluorescence of tryptophan residue (*i.e.* Block 3) and to a lesser extent, to vitamin A (*i.e.* Block 4). This suggested that the phenomenon depicted by the two fluorophores reported in CC2 was dependent. It was highlighted by Kulmyrzaev *et al.* (2005a) that the shape of tryptophan fluorescence can be altered by fluorescence spectra of vitamin A due to its sensitivity to the physical state of triglycerides and to casein/fat globule interactions in cheese matrix, protein structure, and protein/protein interactions. Therefore, the sole common phenomena that can be observed by vitamin A fluorescence and tryptophan

fluorescence are the interaction of the fat globule membrane with the protein network. It was previously reported that the variation of the environment polarity, viscosity, and conformational changes of protein can affect the shape, the maximum, and the position of the tryptophan emission band (Ladokhin, 2000). It is accepted that milk proteins contain at least one tryptophan residue that can be used to monitor protein modification during ripening. This can be confirmed by the observation of the CC2 pattern of tryptophan indicating that the associated cheese spectra shifted to lower wavelengths describing a higher hydrophobic environment of tryptophan for samples located in the positive side of the CC2 plot scores compared to samples located in the negative side. Therefore, the observed phenomenon was assigned to proteolysis of milk proteins and pH modification during cold storage altering the structure of milk proteins, and thus the environment of tryptophan residue (Dufour *et al.,* 2001). Our observations are consistent with Christensen *et al.* (2003) and Kulmyrzaev *et al.* (2005b) who noted both a variation in the intensity and width of tryptophan emission spectra during storage of cheese samples. Those observations are in accordance with pH decrease, WSN/TN ratio, and FAAs increase observed for all the cheeses in the present work.

The loading associated with vitamin A underlined modifications of band intensities between cheeses related to conformational changes assigned to proteins and their interactions with fat from one cheese to another. Those observations confirmed previous investigations of Dufour *et al.* (2000) who noted that changes in the shape of vitamin A excitation spectra is highly correlated to changes of fat globule/protein interaction. It may be concluded that both spectral data sets allowed describing changes in fat globule/protein interactions and the microstructure of cheeses. It is acknowledged that interactions between the casein network and fat globules play an important role in textural properties of cheese due to the plasticising effect of fat (Madadlou *et al.,* 2006).

The saliences for the CC3 showed that the predominant contribution to this CC is due exclusively to vitamin A excitation spectra (*i.e.* Blocks 3), and that this phenomenon is strictly independent from the one observed in CC2. This pattern could highlight modification in fat globule characteristics, like changes in lipid structures and their environments at a molecular level. The modification in the shape and intensity of vitamin A excitation spectrum was correlated with the physical state of triglycerides in fat globules, changes in the environment, and solvent viscosity (Dufour and Riaublanc, 1997). This is consistent with modifications in lipid viscosity generally observed during ripening time due to their partial crystallisation (Dufour *et al.,* 2000), and to differences of TVFA and fat contents noted from one cheese to another and during storage period in the present work.

Conclusion

The present work investigated the potential of combining different analytical techniques to assess the impact of adding matured Cheddar, Ras, and Domiati cheese into pre-cheese milk on the sensory, biochemical, and structural properties of UF soft white cheese. It was demonstrated that the addition of matured cheeses enhanced cheese sensory properties by modifying pH, acidity, soluble nitrogen compounds, and total volatile fatty acids from the beginning of storage. As compared to the control, cheese quality was enhanced with matured Cheddar cheese followed by Ras cheese and then Domiati cheese. Moreover, the application of chemometric methods (PLSDA and ComDim) can be considered a powerful strategy to provide a full picture of the global characterisation of UF-soft cheese during storage and to get valuable information about the relative contribution of each data block to the samples' similarities and dissimilarities. This approach could be used to study the overall impact of other strategies (*e.g.* slurry addition) to improve the quality of fresh cheese.

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