Study of NaCl and KCl effects on molecular structure of cheese by Mid-infrared spectroscopy

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Abstract

In this study, five Cantal-type cheeses with different salts (NaCl and KCl) and two ripening times (5 and 15 days) were analyzed for their physicochemical characteristics, their structure at a molecular level and their rheological properties during heating (20 to 60 °C). The analysis of the molecular structure of cheeses was investigated by MIR spectroscopy coupled with ICA (Independent Components Analysis) and rheological properties by small-amplitude oscillatory rheology. ICA on physicochemical characteristics showed a good discrimination of the cheeses as a function of their chemical characteristics and ripening time. ICA applied to MIR spectra gave Independent Components (ICs) that were attributed to the molecular characteristics of protein, water and fat. Signal proportions of each IC depicted information regarding changes in those ICs with salts, heating and ripening. In addition, similar fat melting temperatures were obtained, regardless the technique used (oscillatory rheology and MIR) for all cheeses. This study demonstrated that MIR spectroscopy coupled with ICA is a promising tool to monitor and characterize modification of cheeses at a molecular level depending on temperature, salt content, and ripening time.

Key words. Cheese, salt, ripening, MIR, ICA

1. Introduction

The reduction of the NaCl content of cheese has received considerable attention by research teams during the past decades and numerous investigations have been carried out in order to reduce the Na levels of cheese by e.g. reducing added NaCl, partial or complete substitution of NaCl by other salts like KCl, MgCl2, and CaCl2 (McMahon et al., 2014). Nonetheless, NaCl is a major determinant of water activity, and thereby exerts control over microbial growth, enzyme activity, biochemical changes during cheese ripening, and the simultaneous development of the desired flavor and aroma. In addition, it presents a recognized role in improving the texture, and color of cheese (Guinee & Fox, 2004, chap. 7).

The modern lifestyle of consumers enforces new models of cheese consumption, e.g. in fondue, pizza, French bread pizzas or sauces. Thus, apart from the flavor value, much more attention has been focused recently on the cheese texture and functional attributes, such as meltability (Guinee, Pudja, Miočinović, Wiley, & Mullins, 2015). Meltability is one of the most important physical properties of cheeses used for their heat induced functional properties. Indeed, on cooking, cheese may be required to melt, flow, stretch, and oil-off to varying degrees depending on its application. Effect of salt on cheese melting seems to vary according to cheese type (e.g., Mozzarella and Muenster), age (e.g., few days compared with several weeks old), content of salt (e.g., 0.5% compared with 2.0%) and composition (e.g., calcium 0.4% compared with 0.7% and fat contents) (Pastorino, Hansen, & McMahon, 2003).

Due to the importance of meltability, several methods were used individually or jointly to evaluate the effect of cheese composition and manufacturing on this attribute. Different authors have used empirical methods such as Schreiber and Arnott tests (Mounsey & O'Riordan, 1999). Other authors used dynamic low amplitude oscillatory rheology, for determining rheological properties of cheeses and the effect of formulation (e.g., concentration of NaCl and KCl) and process (e.g. heating) on its meltability (Guinee et al., 2015). In recent decades, spectroscopic techniques like Mid Infrared (MIR) spectroscopy have been used increasingly to characterize raw and food products. The potential of MIR spectroscopy for monitoring and characterizing cheese origins, chemical parameters, texture, physico-chemical modifications during ripening has been the subject of several research papers (Kulmyrzaev et al., 2005). Those studies demonstrated that MIR spectroscopy is a suitable method for analyzing texture and molecular structure of cheeses. Nonetheless, MIR spectroscopy was never used for studying the effect of salts on the molecular structure and meltability of cheese. Therefore, the objectives of the present study are: (i) to investigate the ability of MIR spectroscopy coupled with Independent Components Analysis (ICA) to monitor the molecular structure changes of uncooked pressed model cheese (Cantal-type cheese, a French Cheddar-like cheese) during gentle heating and ripening; (ii) to study the reliability and accuracy of MIR spectroscopy compared to dynamic testing rheology to evaluate the meltability of fat in cheeses and of cheese matrix and (iii) to gain some insight into the relation between the physicochemical, molecular structure, and meltability characteristics of investigated cheeses.

2. Materials and methods

Cantal-type cheese manufacture

This study was performed on five cylindrical model cheeses (size: 12 cm× 5.5 cm and weight: 861 ± 27 g) with different salt contents: 0.5% NaCl (A), 1% (B), 2% NaCl (considered as the control and labelled (C)), 1.5% NaCl/0.5% KCl (D) and 1% NaCl/1% KCl (E). Cheeses were manufactured as described previously (Loudiyi et al., 2017) and analyzed after 5 (noted A, B, C, D and E in the text) and 15 days (noted A′, B′, C′, D′ and E′ in the text) of ripening (relative humidity (RH): 96% and temperature (θ): 9 °C). Two cheeses per formulation were analyzed (i.e. a total of 20 cheeses).

Cheese chemical composition

Cheese chemical composition was measured from the center part of the cheeses. Moisture, protein, fat, water soluble nitrogen (WSN), proteolysis, ash, Ca, P, Mg, K, Na, Cl, P and lactate contents, pH values and Water activity values were measured as described in detail elsewhere (Loudiyi et al., 2017). All the physicochemical measurements were performed in triplicate.

MIR spectroscopy

All MIR spectra were recorded between 3800 and 900 cm−1 at a 4 cm−1 resolution on a Tensor II Series Fourier transform spectrometer (Bruker, Billerica, MA, USA) mounted with a thermostated ATR accessory equipped with a grip. The ATR cell is six reflections and was made of a horizontal ZnSe crystal which presented an incidence angle of 45°. The temperature was controlled by a Specac temperature controller series 4000 (Eurolabo, Paris, France). Slices of cheese samples (6.8 cm length ×0.7 cm width× 0.4 cm thickness) extracted from the center of the cheeses were placed on the crystal after exerting a slight pressure on the grip ensuring a good contact between the two elements. Thirty two scans were recorded per spectrum in order to improve the signal to noise ratio. All spectra were recorded on the same sample during heating from 20 to 60 °C with a temperature step gap of 5 °C. Three replicates were performed per cheese.

Dynamic oscillatory experiment

The dynamic oscillatory parameters were performed under the same condition as those of Loudiyi et al. (2017a,b). A rheometer (Kinexus pro+, rSpace for Kinexus 1.61 Sofware, Malvern Instruments, Malvern, Worcs, UK) equipped with a plate geometry of 20 mm diameter and a serrated probe was used to perform measurements. Before analysis, cheeses were sliced in their central part into thin disks (2 mm ± 0.05 thick and 20 mm diameter) with a cheese slicer equipped with a millimeter guide and a die cutter of 20 mm diameter (TA, Instruments, leatherhead, UK).

Oscillation experiments were performed in the linear viscoelastic region, determined before performing real experiment, by applying a shear strain ranging from 0.001 to 100% at a constant frequency of 1 Hz. All analyses were carried out on the same sample during heating from 20 to 60 °C (rate: 3 °C.min−1) by using a Peltier heating element equipped with an Active Hood Peltier Plate cartridge permitting to prevent thermal loss from the sample environment and hence minimized sample thermal gradient. Six experiments were performed per cheese formulations. Measurement was performed at a shear strain of 0.05% and a constant frequency of 1 Hz. The recorded data included the elastic component G′ (storage modulus), the viscous component G″ (loss modulus), the tangent of the loss angle (tan δ=G″/G′) and the complex viscosity (η*) versus temperature. The log (η*) and the tan (δ) vs temperature (°C) curve were the only parameters considered for further analysis in order to determine respectively (i) the fat melting temperature as previously reported by Boubellouta and Dufour (2012) and (i) the cheese melting points (tan δ= 1).

Independent Components Analysis

Independent components analysis (ICA) is a powerful technique for blind source separation. It aims to extract the pure underlying signals from a set of mixed signals by estimating a linear transformation that maximizes the statistical independence among the extracted source signals (Bouveresse, Moya-González, Ammari, & Rutledge, 2012). In order to perform ICA and to extract the source signal (i.e. ICs) and their proportions, the Joint Approximate Diagonalization of Eigen matrices algorithm was used (Rutledge & Bouveresse, 2013). The ICA-by-blocks procedure was used to identify the number of independent components (Bouveresse et al., 2012). In order to avoid interference from noise and mechanical drifting on ICA results, the MIR spectra were centered and normalized by the probabilistic quotient normalization (PQN). All computations were performed using MATLAB R2013b (The MathWork, Natick, Massachusetts, USA).

Statistical analysis

Two-way analysis of variance (ANOVA) using the general linear model (GLM) procedure was investigated to compare the temperature values of fat melting and cheese melting obtained with the two methods (i.e. MIR and rheology). Multiple comparison of means was performed using Tukey's test. Before analysis, the assumption of normality using Shapiro-Wilk's test and homogeneity of variance using Levene's test were checked. The two tests were performed using univariate and GLM procedures respectively. The data distributions followed a normal distribution and the variances within salt levels were similar. All the functions were carried out using the SAS statistical software package (Version 9.3, SAS institute Inc., Cary, NC). All differences were considered as statistically significant at $p < 0.05$.

3. Results and discussion

Cheese chemical composition

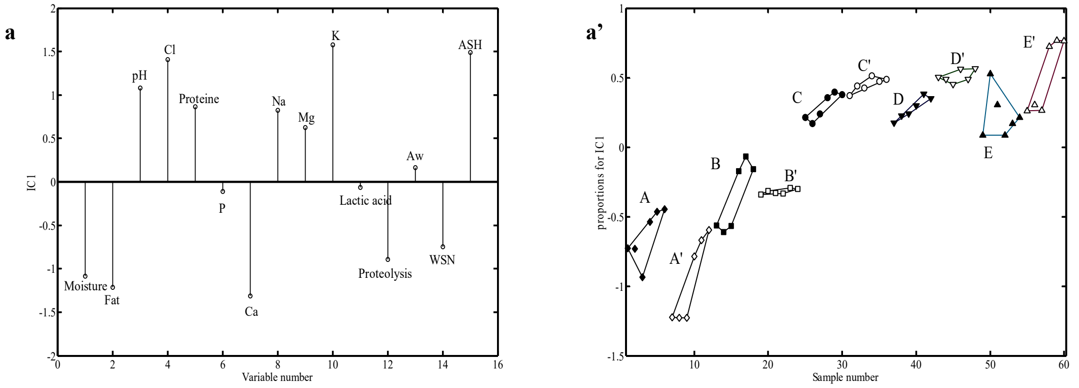
Three ICs, as determined by Random_ICA procedure were selected after using B=2 blocks, F max = 10 and k =30 repetitions. **Figure 1** presents results obtained after ICA applied jointly on chemical parameters, cheese formulations (A, B, C, D and E) and ripening time (i.e. 5 and 15 days). The IC plots were used to interpret the chemical variation contained in the data, while proportions of each IC were used to visualize the relation between cheeses in the corresponding IC. More precisely, these figures depicted the relevant source signals (ICs) (Fig. 1(a–c)) and the proportions of each source signal (Fig. 1(a′–c′)).

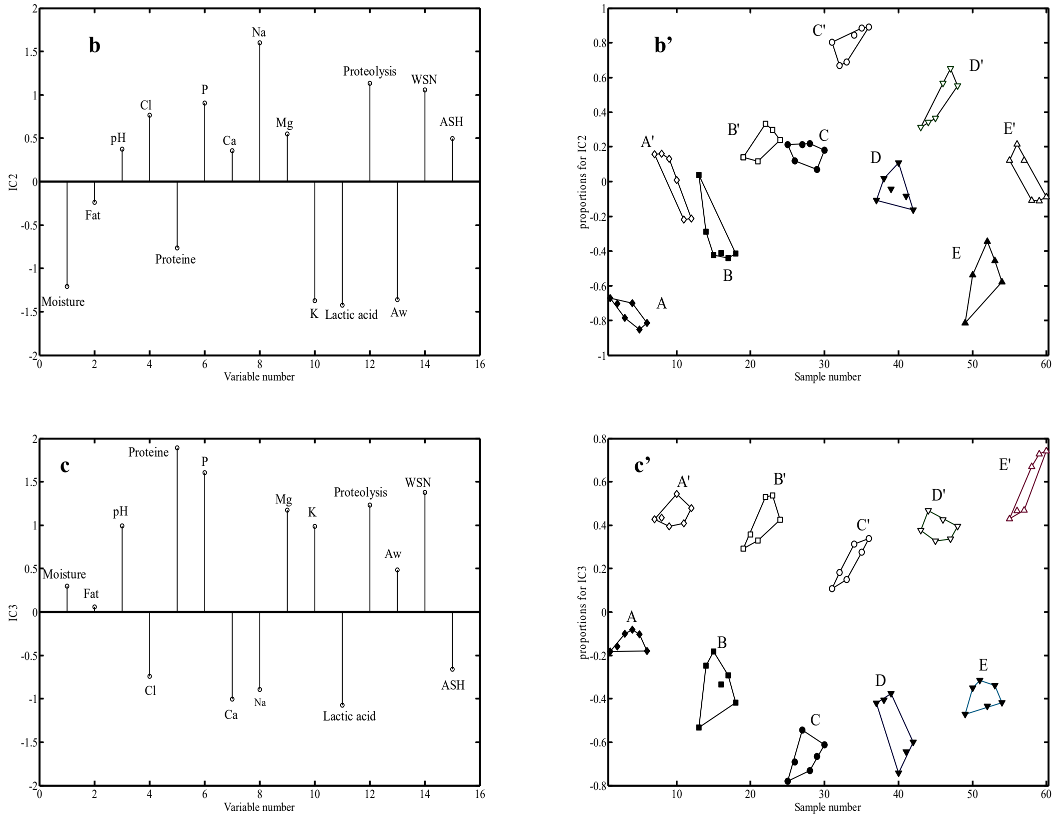
The proportion values on IC1 (Fig. 1a′) showed that samples with high salt content (i.e. C-D-E and C′-D′-E′) were in the positive side, while those with low salt content (A-B and A′-B′) were in the negative side. Moreover, IC1 discriminated slightly cheeses depending on ripening time. Chesses C′, D′ and E′ presented higher values for IC1 compared to C, D and E cheeses; while the opposite was noted for cheeses containing low added NaCl (A-B and A′-B′). Based on IC1 (Fig. 1a), cheeses containing low added NaCl showed a combined lower pH, Cl, protein, K and ash values and higher moisture, fat, Ca, proteolysis and WSN contents compared to cheeses containing high salt content.

The similarity map defined by IC2 (Fig. 1b′) did not show a clear discrimination between the different cheeses. Nonetheless, an increase of the proportion values were observed from the cheeses reduced in NaCl (A-B and A′-B′) to the controls (C and C′) and a subsequent decrease of the proportion values from the controls to the cheeses containing KCl (D-E and D′-E′). The discrimination between samples (Fig. 1b) is principally due to a combination effect of Na, proteolysis and WSN that presented high positive values and moisture, K, lactic acid and Aw that presented negative values on IC2.

The map defined by IC3 (Fig. 1c′) showed a good discrimination of cheeses based on ripening time. After 5 days of ripening cheeses presented negative values, while after 15 days of ripening cheeses presented positive ones. Moreover, the proportion values on IC3 decreased with increasing NaCl content and increased with increasing KCl content, whatever the ripening time. The IC3 source signal (Fig. 1c) showed that cheeses after 15 days of ripening presented a combined higher protein, P, WSN, proteolysis, Mg, K, and pH and lower lactic acid, Ca, Na, ash and Cl contents compared to the cheeses ripened during 5 days.

Fig. 1.ICA source signals (i.e. "loadings"; a, b, c), and proportions of source signal (i.e. "scores": a',b',c') obtained after ICA on chemical parameters of the five cheese formulations ripened for 5 days (A: 0.5% NaCl (♦), B: 1% NaCl (■), C: 2% NaCl (●); D: 1.5/0.5% NaCl/KCl (▼); E: 1/1% NaCl/KCl (▲)) and 15 days (A': 0.5% NaCl (◊), B': 1% NaCl (□), C': 2% NaCl (○), D': 1.5/0.5% NaCl/ KCl (▽) and E': 1/1% NaCl/KCl (△)).





ICA applied to MIR spectra

ICA is an efficient tool to study sets of spectral data for identifying independent chemical phenomenon (i.e. wavelengths) involved in the changes of complex spectral landscape. In the present study the method was applied to the data matrix containing MIR spectra, in order to identify jointly the effect of heating (i.e. 20–60 °C), salts (i.e. NaCl and KCl) and ripening time (i.e. 5 and 15 days) on the molecular structure of cheese.

The number of ICs was defined by the Random_ICA procedure with 2 blocks, 20 factors and 30 repetitions (Bouveresse, Benabid, & Rutledge, 2007). Four ICs were selected based on the correlation boxplot, which depicted that after extracting 4 ICs the correlation values started to decrease. **Figure 2** depicted the results obtained after ICA on MIR spectra of the different cheeses (A-B-C-D-E and A′-B′-C′-D′-E′) during ripening. The relevant source signals are presented in Fig. 2 (a–d) and the proportions of each source signal are reported in Fig. 2 (a′–d′).

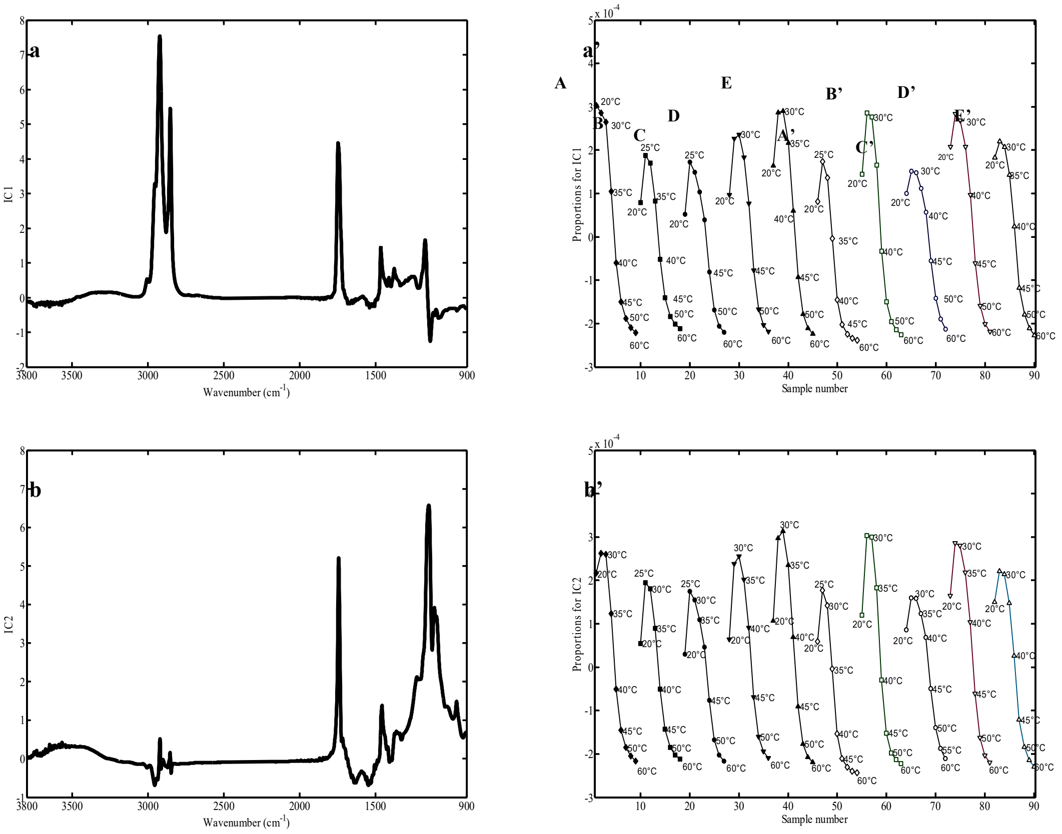
IC1 (Fig. 2a) presented three bands (2923, 2853 and 1744 cm−1) previously related to the modification of physical state of lipids (Bertrand and Dufour, 2006, chap. 6). The variation of the IC1 proportions (Fig. 2a') presented two distinct linear regions whatever the cheese formulation. These findings could indicate a transition zone, for which the cheese softened and started to flow in accordance with previous studies (Boubellouta & Dufour, 2012; Loudiyi et al., 2017a). Therefore, it was reported that the greater change observed between 20 and ~40 °C was the result of fat liquefaction and more precisely of the melting of the triglycerides, which are fully liquid at 35–40 °C (Lefevere, Dewettinck, & Huyghebaert, 2000). While, the limited change shown from ~40 to 60 °C, could be due to a mechanical resistance of the cheese network in relation to the thermal stability of the proteins. The transition zone was noted slightly different between the cheese reduced in NaCl (A-B and A′B′) compared to the cheeses containing high salt content (C-D-E and C′-D′-E′) for the two ripening times. This indicated that reducing NaCl content has probably more impact on the thermal behavior as well as the structural modification at a molecular level of cheeses than the NaCl substitution by KCl. This is probably due to changes in protein-protein and water-protein interactions that modify structural properties of the cheese matrix. These agree with previous investigations on Parmesan, Raclette, Comté and Cheddar cheeses (Reparet & Noël, 2003).

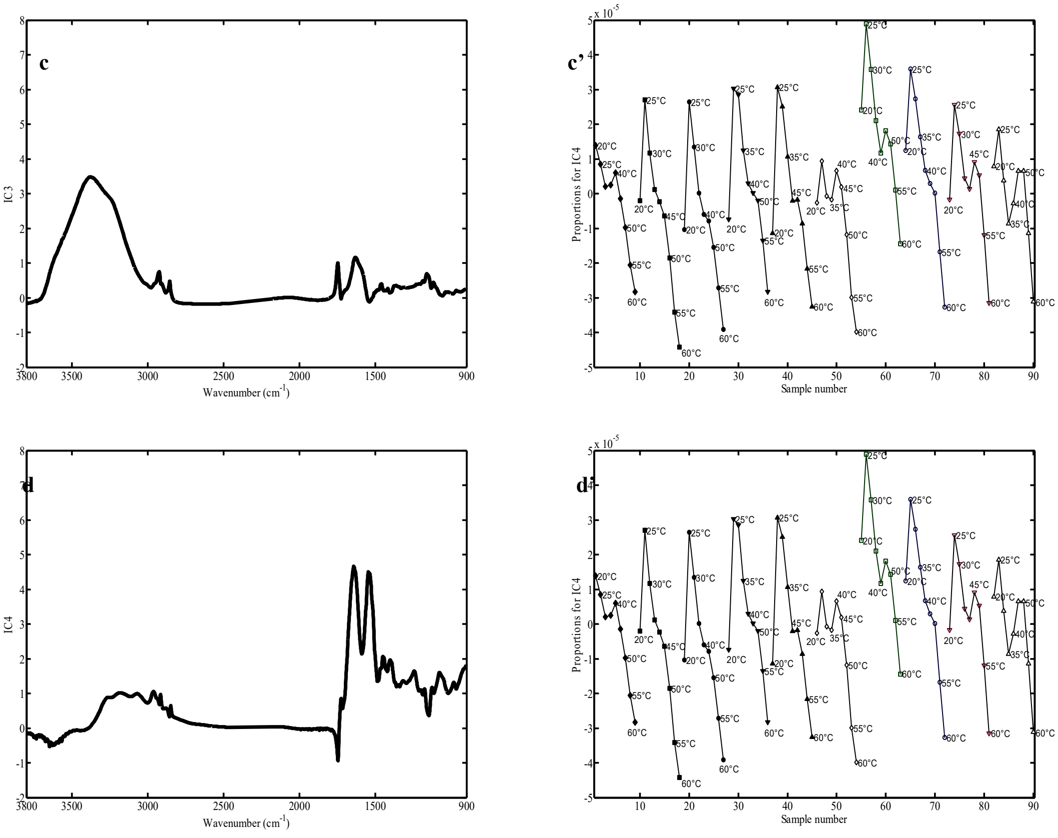
For IC2, two bands at 1744 and 1149 cm−1 were observed (Fig. 2b). However, the assignment of the IC2 profile to a specific chemical modification is quite difficult. Since, this profile was associated to the triacylglycerol esters stretching mode (Bertrand & Dufour, 2006, chap. 6), lactose, monosaccharide (Petibois, Melin, Perromat, Cazorla, & Déléris, 2000) and lipid vibrations (Martín-del-Campo, Picque, Cosío-Ramírez, & Corrieu, 2007).

The third IC (Fig. 2c) exhibited bands previously associated to O-H stretching mode of water molecule in Cheddar cheese (Chen et al., 1998).

The fourth IC (Fig. 2d) showed two well-defined peaks related to the Amide I and Amide II bands (1641 and 1547 cm−1, respectively). These bands give molecular information on the protein and on their interactions with other components such as water, ions, and proteins (Olchowicz, Coles, Kain, & MacDonald, 2002). They were associated with changes in casein secondary structure, protein aggregation and protein-water interaction in semi-hard and experimental soft cheeses (Mazerolles et al., 2001). Almost the same profile for the IC4 proportions (Fig. 2d’) were noted for all cheeses during ripening except for cheeses containing the lowest Na concentration. These results indicated that reducing NaCl above 50% changed drastically the thermal behavior of cheese. This finding is in accordance with McCarthy, Wilkinson, Kelly, and Guinee (2016) on Cheddar cheese, reporting that reducing salt below critical levels (e.g.< 1.2%) impairs cooking properties. Changes observed during ripening, depending on the salt content, could suggest that the effect of salt on cheese is enhanced by ripening time. Indeed, during ripening, a very complex series of reactions occurs (i.e. glycolysis, lipolysis and proteolysis) predetermined by the manufacturing process, especially by the levels of moisture, NaCl and pH, residual coagulant activity, the type of starter and, in many cases, by the secondary microflora (added or adventitious) (Park & Haenlein, 2013, chap. 17).

Fig. 2. ICA source signals (a, b, c, d), and proportions of source signal (a”,b”,c”, d”) obtained after ICA during heating from 20 to 60°C on MIR spectra ofcheeses ripened for 5 (A: 0.5% NaCl (♦), B: 1% NaCl (■), C: 2% NaCl (●); D: 1.5/0.5% NaCl/KCl (▼); E: 1/1% NaCl/KCl (▲)) and 15 days(A’: 0.5% NaCl (◊), B’: 1% NaCl (□), C’: 2% NaCl (○), D’: 1.5/0.5% NaCl/ KCl (▽) and E’: 1/1% NaCl/KCl (△)).





Melting temperatures

In rheology, the melting temperatures of fat in cheese and cheese matrix were calculated respectively from the log η* and tan δ plots versus temperature. For the melting temperature of fat, log η* presented two distinct linear regions with temperature, and the intersection point was identified as the fat melting temperature (Loudiyi et al., 2017a). The temperature at which tan δ= 1 indicates the point where the viscous behavior of cheese begins to prevail (i.e. cheese matrix melting). Regarding MIR, it has been reported that the fat and the cheese melting temperatures can be derived respectively by plotting the intensity at 2924 and 1634 cm−1 of MIR spectra versus temperature (Boubellouta & Dufour, 2012). The plots showed two distinct linear regions and the intersection points were identified as the fat and the cheese melting temperatures respectively.

Table 1 presents fat and cheese melting temperatures calculated from MIR spectra and dynamic oscillatory experiments for the different cheeses. Regarding fat melting, rheological data showed that, compared to the control, decreasing NaCl content decreased fat melting temperature, while increasing its substitution by KCl tended to increase this temperature. These differences could describe the effect of NaCl on lipolysis (Cruz et al., 2011) for the cheeses with low NaCl content and to higher contents of free fatty acids and extensive lipase activity for cheeses containing KCl (Lindsay, Hargett, & Bush, 1982). Moreover, similar fat melting temperatures were generally observed with the two methods (i.e. MIR and rheology). Therefore, MIR spectroscopy permitted to characterize changes in lipid viscosity during heating in accordance with previous studies conducted on Comté and Raclette cheeses (Boubellouta & Dufour, 2012).

Regarding cheese melting, calculated temperatures agree with those reported for Emmental and Comté cheeses (Famelart, Le Graet, Michel, Richoux, & Riaublanc, 2002). However, in contradiction with Boubellouta and Dufour (2012), drastic differences were noted between values obtained with the MIR spectroscopy and rheology. Contrary to fat melting, these results suggested that using MIR spectra for predicting the melting temperature of cheese matrix was probably not suited for the present cheeses. These differences could be assigned to the absorption of other components such as water which could affect the properties of the amide I band (Boubellouta & Dufour, 2012) and to the difference in the processing of cheeses. Those results agree with our previous investigation performed on the same cheeses with fluorescence spectroscopy (Loudiyi et al., 2017b). Moreover, compared to the cheeses reduced in added NaCl (i.e. A-B and A′-B′ cheeses), generally higher values of cheese melting were noted for the cheeses containing KCl. This indicated that changing the cation from Na to K might lead to increase the cheese melting temperature, probably due to the impact of salts on both pH and protein solubilization in accordance with Pastorino et al. (2003) and chemical observations reported in the present study.

During storage, no significant difference was observed in terms of cheese melting temperatures between cheeses with the same salt treatment, regardless of the method used (i.e. rheology and MIR spectroscopy). These results are similar to previous one reported for Cheddar cheese (Guinee, Auty, Mullins, Corcoran, & Mulholland, 2000). However, generally significant difference was noted in terms of fat melting after 15 days of storage, in accordance with Lefevere et al. (2000). This difference could be assigned to changes of lipid viscosity following their partial crystallization (Boubellouta & Dufour, 2012; Dufour et al., 2000) and to the protein breakdown (Lefevere et al., 2000) during ripening, in accordance with our chemical observations.

Table1. Evaluation of the melting temperatures of cheese and fat in cheese by MIR spectra and dynamic low amplitude oscillatory rheology.

Ripening (day)	Salt treatment^{1}	Cheese melting temperature (°C)		Fat melting temperature (°C)	
		Rheology (tan δ)	MIR (1634 cm^{-1})	Rheology (log η*)	MIR (2924 cm^{-1})
5	A	53.00 ± 0.88^{d}	45.75 ± 0.29^{h}	34.20 ± 0.67^{i}	$34.68 \pm 0.39^{g,h,i}$
	B	55.51 ± 0.38^{c}	47.36 ± 0.30^{g}	$35.10 \pm 0.64^{f,g,h,i}$	$34.80 \pm 0.16^{g,h,i}$
	C	59.89 ± 0.05^{a}	$50.98 \pm 0.40^{e,f}$	$35.87 \pm 0.32^{d,e,f}$	$36.13 \pm 0.26^{c,d,e}$
	D	59.32 ± 0.22^{a}	$50.94 \pm 0.44^{e,f}$	$35.49 \pm 0.51^{d,e,f,g}$	$35.15 \pm 0.29^{e,f,g,h}$
	E	$57.17 \pm 0.38^{b,c}$	50.17 ± 0.21^{f}	$34.64 \pm 0.46^{h,i}$	$36.26 \pm 0.43^{b,c,d}$
15	A'	$52.34 \pm 0.25^{d,e}$	45.76 ± 0.11^{h}	34.14 ± 0.11^{i}	33.08 ± 0.37^{j}
	B'	56.41 ± 1.39^{c}	48.70 ± 0.50^{g}	$35.02 \pm 0.33^{f,g,h,i}$	$35.15 \pm 0.44^{e,f,g,h,i}$
	C'	58.91 ± 0.47^{a}	50.58 ± 0.86^{f}	$37.16 \pm 0.17^{a,b,c}$	$37.22 \pm 0.22^{a,b}$
	D'	$58.57 \pm 0.86^{a,b}$	50.52 ± 0.44^{f}	$37.17 \pm 0.29^{a,b,c}$	$37.10 \pm 0.63^{a,b,c}$
	E'	56.46 ± 0.75^{c}	50.70 ± 0.67^{f}	37.26 ± 0.37^{a}	$37.25 \pm 0.31^{a,b}$

^{a,b}Means in each column with the same letter did not differ significantly (P > 0.05).
^{1} Salttreatmentsare: A-A'(0.5% NaCl); B-B'(1% NaCl); C-C'(2% NaCl); D-D'(1.5/0.5% NaCl/KCl); E-E'(1/1% NaCl/KCl)

4. Conclusion

This study permitted to highlight differences in chemical composition, molecular structure and functionalities (fat melting and cheese melting) through ripening and salt content (NaCl and KCl) on experimental Cantal-type cheeses. We also demonstrated that MIR spectroscopy has a huge potential to delineate through ripening and heating the molecular structure changes of cheeses with different salt contents (KCl and NaCl). In addition, generally similar fat melting temperatures for each kind of cheese were obtained regardless the technique used (dynamic rheology and MIR spectroscopy); while MIR spectroscopy shows its limit to predict the melting temperature of cheese matrix.

In spite of this encouraging results, our hypothesis still remain to be validated due to the high variability and complexity of the cheese following the effect of seasonality (lactation) and batch-to-batch variability (cheese making process) on molecular structure. However, this primarily investigation certainly underlines that MIR spectroscopy coupled with ICA seems to be a promising methodology to characterize modification of the molecular structure of cheeses depending of salt content and heating.

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