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Monitoring of lactic acid fermentation process using Fourier transform near infrared spectroscopy

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The aim of this paper was to evaluate the suitability of Fourier transform near infrared (FT-NIR) spectroscopy, combined with multivariate data analysis, to monitor milk lactic acid fermentation as an indication of possible deviations in quality parameters. Fermentation trials performed with different inocula (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* as single or in mixed cultures) at three incubation temperatures (37°C, 41°C and 45°C) were monitored by FT-NIR spectroscopy. Rheological and conventional quality parameters (microbial counts, pH, titratable acidity, lactose, galactose and lactic acid concentrations) were used as reference values to assess the findings with FT-NIR spectroscopy. Principal component analysis was applied to spectra to uncover molecular modifications. PC1 scores, rheological data and conventional quality parameter values were modelled as a function of fermentation time to designate critical points all along the process. Results showed that FT-NIR spectroscopy is a useful tool for real-time assessment of curd development during fermentation, offering crucial information in agreement with rheology and conventional quality parameters.

Keywords: FT-NIR, lactic acid fermentation, modelling, rheology

Introduction

Fermentation is one of the earliest methods adopted to obtain value-added milk products with an extended shelf life. Although about 400 different names have been found all over the world for traditional and industrial fermented milks, these products are quite similar, with only a few variations.[1] Three broad categories of fermented milk products have been identified (i.e. lactic fermented, yeast–lactic fermented and mould–lactic fermented milks) depending on the kind of milk used in the production process, the predominant microbial species in the inoculum and their metabolic products.[2]

Fermentations carried out by lactic acid bacteria are the most widespread in the dairy industry for milk acidification and flavour development,[3] of which yoghourt is the most common. The Codex Alimentarius Commission defines yoghourt as a symbiotic culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* which should be viable, active and abundant in the product to the date of minimum durability.[4] The sour taste characterising lactic acid fermented dairy products originated from the conversion of lactose into lactic acid and the consequent milk pH reduction from around 6.7 to values lower than 4.5.

An at-line control of the fermentation processes usually involves pH measurement and titratable acidity determination. Other key indicators of fermentation progress are lactic acid, carbohydrates (i.e. lactose and galactose) and bacterial concentrations, but they are not routinely measured, being expensive and time-consuming.

The final quality of fermented milk products is strictly related to the exact determination of the optimal incubation

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time. Milk composition variations, an anomalous behaviour of starter microorganisms, an incorrect control of the incubation temperature, as well as a number of other process variables, can yield end products with low overall quality. The risk of product failure can be reduced only by a complete understanding and an accurate monitoring of the process.[5]

An effective control at all stages of the fermentation process requires fast methods, providing real-time information. An easy, fairly innovative, inexpensive and non-destructive technique is near-infrared (NIR) spectroscopy, which has been proposed as an alternative to conventional analyses for in-time monitoring of various products and processes. Absorptions in the spectral range between 14,000 cm^{-1} and 4000 cm^{-1} are associated with the main chemical components of foodstuff, such as water, proteins, carbohydrates and fats. In particular, NIR vibration and combination overtones of the fundamental C–H, N–H, O–H and C=O bonds are the main recordable phenomena.[6]

The use of NIR spectroscopy for dairy product analysis is well documented.[7–10] In particular, during recent years, the interest in dairy fermentation monitoring by means of NIR spectroscopy has increased. Navratil *et al.* described how the fusion of NIR spectroscopy and electronic nose data can be applied to the on-line monitoring of industrial fermented milk production.[11] Moreover, Ntsame Affane *et al.* developed models to predict acidity parameters in Kefir by using NIR reflectance spectroscopy, demonstrating its adequacy for screening purposes.[10]

One of the main features of NIR radiation is that it is composed of broad and mostly overlapped bands, which hinder the direct extraction of information from the raw spectra, making necessary the use of multivariate data analysis.[10,12,13] One of the most widespread chemometric tools for qualitative data analysis is principal component analysis (PCA). It employs an orthogonal transformation to convert the original dataset into a sub-set of linearly uncorrelated highest-variance components (principal components—PCs). The PCs are linear combinations of the original variables and each component explains a part of the total variance of data; in particular, the first significant component accounts for the largest source of total variance, while the further PCs explain the residual variation.[9,14]

The aim of this paper was the evaluation of the suitability of NIR spectroscopy, joined with multivariate data analysis (i.e. PCA), for the monitoring of lactic acid fermentation in milk. In particular, the possibility of developing an easy and fast protocol able to promptly detect possible defects in product quality parameters (for example, acidity and texture) due to deviations from the regular processing trend was investigated. The availability of this protocol could be useful in the assessment of the best strategy for the management of nonconformities. In order to identify the main changes occurring during fermentation, conventional quality parameters and rheological characteristics were analysed and modelled as a function of fermentation time for the identification of the kinetic critical points.

Materials and methods

Materials

Skimmed milk powder (Merck, Darmstadt, Germany) was reconstituted to 10% w/v with distilled water, distributed in 1 L bottles and subjected to heat treatment at 112°C for 15 min and then stored at 4°C until use.

Bacterial strains were isolated from the commercial yoghourt culture YO-MIX 305 (Danisco A/S, Copenhagen, Denmark) and identified, by sequencing the amplified 16S rDNA region, as *Streptococcus salivarius* spp. *thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*. Pure cultures of the two strains were stored at –20°C in appropriate media (see below) with added glycerol (20% v/v).

Fermentation trials

Frozen stocks of *S. thermophilus* and *L. bulgaricus* were activated by incubation at 37°C overnight in M17 broth (Merck, Darmstadt, Germany) and de Man, Rogosa, Sharpe (MRS) broth (Becton Dickinson & Co., Franklin Lakes, NJ, USA), respectively, both modified by adding 10% w/v lactose. After centrifugation for 15 min at 3000 g, cells were harvested and resuspended in sterilised skimmed milk. For each fermentation trial, 800 mL of skimmed milk was inoculated with a single culture or with a mixed culture containing both microorganisms (1 : 1). A calibration curve obtained through the correlation between cell number and optical density measured at 600 nm with a spectrophotometer (Jasco V650, Jasco Europe, Cremella, Italy) was used to set the inoculum supply at approximately 10^6 CFU mL^{-1}. Aliquots of about 60 mL of the inoculated skimmed milk were then aseptically distributed into sterile glass bottles and placed in a circulating water bath at a specific fermentation temperature (37°C, 41°C or 45°C), for 7.5 h. Every 45 min, a bottle was taken to analyse the fermented milk, while spectroscopic and rheological evaluations were carried out continuously.

A total of nine different fermentation trials, twice replicated, were performed (18 experimental trials). Analytical results are reported as the average of the two technological replicates for each fermentation condition.

Microbiological analysis

From the incubated bottles, aliquots of about 10 g of sample were aseptically collected and resuspended in a 2% sodium citrate solution to obtain the first decimal dilution. After homogenisation, appropriated serial dilutions (1 : 10) were made in duplicate, plated on modified (0.5% sucrose, 0.5% lactose) homo- and heterofermentative differentiation (HHD) agar (Biolife, Milano, Italy) and incubated at 37°C for 48 h. The modified medium is able to differentiate between the two strains on the basis of their acidifying abilities. Results are expressed as the number of colony forming unit per gram of fermented milk (CFU g^{-1}).

pH and titratable acidity

pH values were potentiometrically measured using a pH meter 3510 (Jenway, Dunmow, UK) equipped with a glass electrode.

Titratable acidity was determined according to the IDF/ISO Standard n° 150 and expressed as a percentage of lactic acid.[15] Both the analyses were performed in duplicate.

Sugars and organic acids

Lactose, galactose and lactic acid were determined by high-performance liquid chromatography (HPLC), using an instrument (ThermoFinnigan, Milano, Italy) fitted with a Carbo H4 pre-column (3.0 mm ID, Phenomenex, Castel Maggiore, Italy) followed by an Aminex HPX-87H cation exclusion column (300 × 7.8 mm, BioRad Laboratories, Richmond, CA, USA). Elution was performed isocratically at 65°C with 5 mM H_2SO_4 (Merck, Darmstadt, Germany) as mobile phase, at a flow rate of 0.8 mL min^{-1}. Peaks were detected using a refractive index detector RI-71 (Showa Denko, Europe GmbH, Munich, Germany) and registered by the Empower 2 chromatography data software (Waters Corporation, Milford, MA, USA). For peak identification and quantification, calibration curves of each component were calculated by analysing standard solutions in mobile phase as follows: lactose monohydrate (Merck, Darmstadt, Germany), ranging from 0.1 g L^{-1} to 10 g L^{-1}; galactose (Sigma–Aldrich, St Louis, MO, USA), ranging from 0.02 g L^{-1} to 10 g L^{-1}; L(+)-lactic acid (Merck, Darmstadt, Germany), ranging from 0.02 g L^{-1} to 3 g L^{-1}.

Five grams of sample was added to 10 mL of acetonitrile (Merck, Darmstadt, Germany) and centrifuged at 5000 g for 12 min at 4°C (Rotina 380R, Hettich Zentrifugen, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The supernatant was filtered through a 0.45 µm PDVF-filter (Alltech, Milano, Italy) and injected in the 20 µL loop of the HPLC system. Sample preparation and injection were performed in duplicate.

Rheological measurements

Curd development was monitored at regular intervals on the same sample using a Physica MCR 300 rheometer (Anton Paar GmbH, Graz, Austria), supported by the software Rheoplus/32 (v. 3.00, Physica Messtechnik GmbH, Ostfildern, Germany). A dynamic oscillatory test was performed, using concentric cylinders (CC27) and applying a constant 1% strain at a fixed 1 Hz frequency. The inoculated skimmed milk (19 mL) was inserted in the measurement element, pre-heated at the desired fermentation temperature. Sample evaporation during the test was prevented by a solvent trap filled with deionised water. Storage (G′) and loss (G″) moduli were measured at 2 min intervals throughout the whole fermentation process.

FT-NIR spectroscopy

Near infrared spectra of the inoculated skimmed milk during the fermentation process were collected at regular intervals from the same sample in transflectance mode, using a Fourier Transform (FT)-NIR spectrometer equipped with a fibre-optic probe with a 1 mm pathlength (MPA, Bruker Optics, Milano, Italy). The probe was inserted directly into the inoculated skimmed milk contained in a glass bottle placed in a water bath to maintain the defined fermentation temperature. Spectral data were collected every 15 min over the 12,500–4000 cm^{-1} range, with a resolution of 16 cm^{-1}, 64 scans for both background and samples and a scanner velocity of 10 kHz. Instrument control and data acquisition were performed by the OPUS software (v. 6.5, Bruker Optics, Milano, Italy).

Data processing

After smoothing (moving average method, segment size = 11 data points), FT-NIR data were transformed into first derivative (Savitzky–Golay method, polynomial order = 2, gap size = 11 data points) to minimise the effect of baseline shifts and reduced to the range 8900–5555 cm^{-1} in order to eliminate useless or saturated variables from spectra. PCA was applied to the averaged spectral data obtained by the two technological replicates of each fermentation condition, by using the Unscrambler v. 9.8 software (Camo Software AS, Oslo, Norway). All spectral data sets were mean-centred before performing PCA calculations.

The scores of PC1 were modelled as a function of fermentation time, using the sigmoid Equation (1) implemented in Table Curve software (v. 4.0, Jandel Scientific, San Rafael, CA, USA):

$$y = a + b \cdot exp\left\{-exp\left[-\left(\frac{\left\{x - d\,ln\left[ln(2)\right] - c\right\}}{d}\right)\right]\right\} \qquad (1)$$

Also, the average data obtained by conventional analyses and rheological evaluations were modelled as a function of fermentation time, applying the same sigmoid function, in agreement with the microbial nature of the transformation studied.[16,17]

In order to identify kinetic critical points during fermentation—i.e. time related to the maximum rate and acceleration or deceleration of the phenomena—the first and second derivatives of the sigmoid functions were calculated.

Results and discussion

Microbiological analysis

The use of modified HHD, which provides a morphological differentiation of colonies and recovery close to those obtained by separated specific media (MRS pH 5.4 and M17), permitted the enumeration of the single species on a unique plate.[18] The change in viable counts of *S. thermophilus* and *L. bulgaricus* during fermentation trials are presented in Figure 1. The initial mean counts of *L. bulgaricus* for the three different fermentation trials [Figure 1(a)] ranged from 1.33 to 2.97×10^6 CFU g^{-1}, increasing then to 2.00–3.07 × 10^8 CFU g^{-1} after 5 h of fermentation without significant changes till the end of the experiments (2.52–2.98 × 10^8 CFU g^{-1}). As regards *S. thermophilus* [Figure 1(b)], the initial concentrations ranged from 2.67 to 4.23×10^6 CFU g^{-1}, reaching a plateau at about 2.81–3.00 × 10^8 CFU g^{-1} after 5 h. In the trials performed with mixed cultures [Figure 1(c)], *L. bulgaricus* grew faster than *S.*

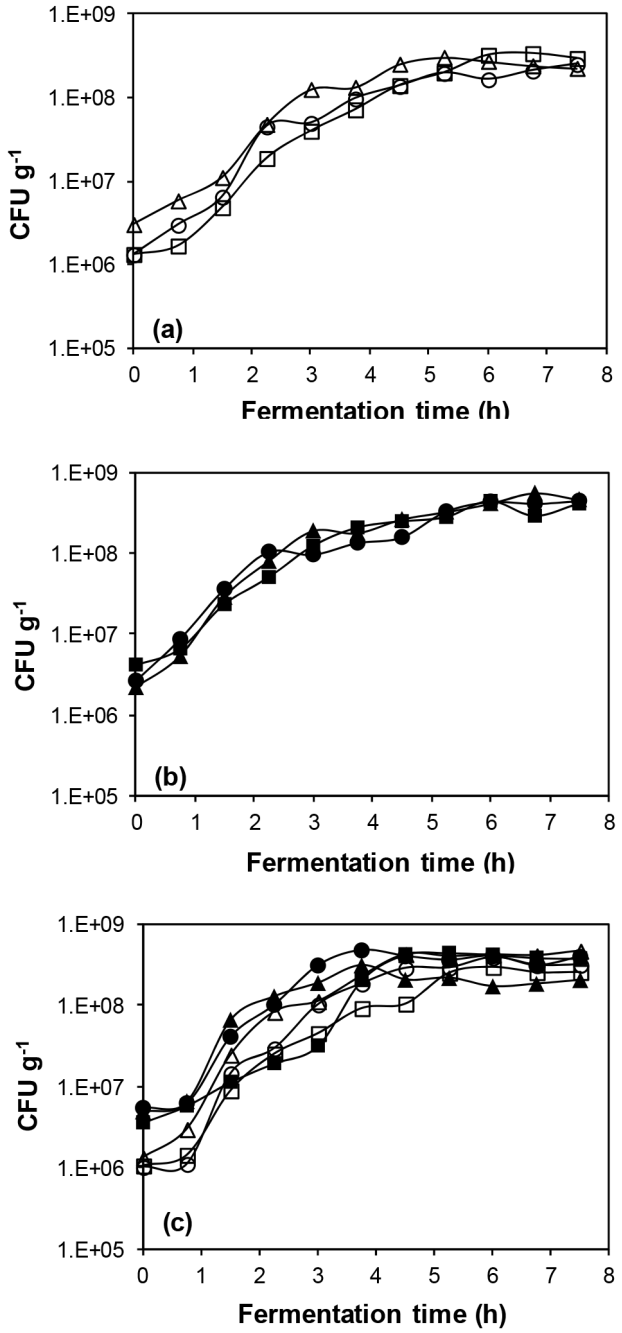

Figure 1. Growth of (a) *L. bulgaricus*, (b) *S. thermophilus* and (c) the mixed culture during lactic acid fermentations carried out at different temperatures. □ *L. bulgaricus*, 37°C; ○ *L. bulgaricus*, 41°C; △ *L. bulgaricus*, 45°C; ■ *S. thermophilus*, 37°C; ● *S. thermophilus*, 41°C; ▲ *S. thermophilus*, 45°C.

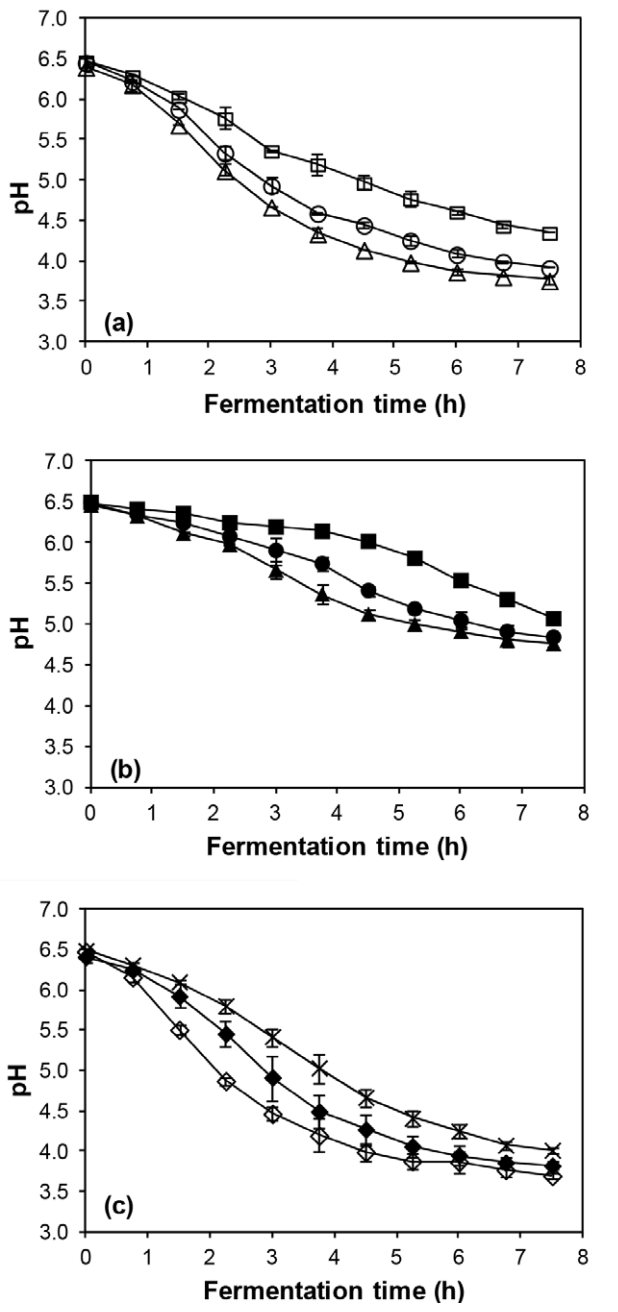

Figure 2. Evolution of pH values during lactic acid fermentations carried out at different temperatures with (a) *L. bulgaricus*, (b) *S. thermophilus* and (c) the mixed culture. □ *L. bulgaricus*, 37°C; ○ *L. bulgaricus*, 41°C; △ *L. bulgaricus*, 45°C; ■ *S. thermophilus*, 37°C; ● *S. thermophilus*, 41°C; ▲ *S. thermophilus*, 45°C; × mixed culture, 37°C; ♦ mixed culture, 41°C; ◇ mixed culture, 45°C.

thermophilus, but after 5 h both microorganisms reached their plateau, at about $2.17–4.43 \times 10^8$ CFU g^{-1}.

pH and titratable acidity measurements

Results concerning pH values of fermented milks produced by single and mixed cultures are shown in Figure 2. The mixed culture was the most efficient in lowering pH values, followed by single cultures of *L. bulgaricus* and then *S. thermophilus*.

The mixed culture [Figure 2(c)] lowered the pH from 6.5 to 3.8, within 7.5 h at 41°C, which is the common temperature used for milk lactic acid fermentation; whereas the single

Table 1. Changes in titratable acidity (TA), lactose, galactose, and lactic acid concentrations during lactic acid fermentations carried out under different processing conditions.

Inoculum[a]	Temperature (°C)	TA increase (% lactic acid)	Lactose consumption (g 100 g^{-1})	Galactose increase (g 100 g^{-1})	Lactic acid production (g 100 g^{-1})
I1	37	0.62	1.22	0.33	0.30
	41	0.81	0.71	0.51	0.50
	45	0.87	1.25	0.50	0.60
I2	37	0.24	0.66	0.15	0.19
	41	0.34	0.60	0.15	0.20
	45	0.40	0.40	0.16	0.28
I3	37	0.81	1.38	0.34	0.48
	41	0.88	1.51	0.40	0.50
	45	1.02	0.90	0.47	0.65

[a]I1, *L. bulgaricus*; I2, *S. thermophilus*; I3, *S. thermophilus* and *L. bulgaricus* (1:1)

cultures of *L. bulgaricus* [Figure 2(a)] and *S. thermophilus* [Figure 2(b)] lowered the pH value to 3.9 and 4.9, respectively, due to the higher acidification activity of lactobacilli.[19]

The change in titratable acidity, expressed as a percentage of lactic acid, is illustrated in Table 1. The titratable acidity of reconstituted skimmed milk (0.13–0.16%) increased till a maximum of 1.02% in milk fermented at 45°C with the mixed culture. The lowest value was reached when inoculating *S. thermophilus* at 37°C (0.24%). Our results are in accordance with those reported by Rasic and Kurmann,[19] showing that *S. thermophilus* produces a maximum lactic acid concentration of 0.7–0.8%, while *L. bulgaricus* is a homo-fermenting lactic bacteria producing up to 1.7% of lactic acid. The titratable acidity developed by *S. thermophilus* and *L. bulgaricus* increased with the rise in incubation temperature, as reported by Tamime and Robinson.[5]

Sugars and organic acids

Table 1 shows, for each fermentation trial, the changes in the main chemical constituents involved in lactic fermentation (lactose, galactose and lactic acid). Both *L. bulgaricus* and *S. thermophilus* ferment lactose: during the homolactic fermentation, the disaccharide is transported into the bacterial cell and transformed by a lactase into glucose and galactose. Glucose is then catabolised to lactic acid, while galactose is exported from the cell. For this reason, a galactose accumulation in the medium is expected, as observed in this work.

Within the 7.5 h of fermentation with *L. bulgaricus*, lactose consumption ranged from 0.71 g 100 g^{-1} to 1.25 g 100 g^{-1}, while with *S. thermophilus*, because of its lower acidification capacity, the decrease was only 0.40–0.66 g 100 g^{-1}. As regards the mixed culture, a maximum lactose concentration reduction of 1.51 g 100 g^{-1} was registered. At the end of the fermentation process, the highest increase in galactose content was observed with the *L. bulgaricus* inoculum, whereas the highest lactic acid production was noticed when the mixed culture was tested. Similar results of lactic acid production were also reported by Dave and Shah for yoghourt made from commercial starter cultures.[20] The lowest values of lactic acid in the final product were obtained after inoculating a single culture of *S. thermophilus*, this species being less acid tolerant than the other one.

Rheological behaviour

Rheological behaviour was studied to monitor the curd development during fermentation as the textural characteristics of fermented milk gel are of paramount importance for the quality of the final product. A small amplitude oscillatory test was used in this research, in order to follow the development of the curd structure without system perturbation. Average storage modulus (G′) and loss modulus (G″) values over the fermentation times in the different trials are illustrated in Figure 3. The G′ value defines the degree of the solid-like character of the gel, whereas the G″ value indicates the degree of the liquid-like behaviour. Thus, at the beginning of fermentation, when milk was still liquid, G″ values were always higher than G′ values. When the gel began to form, G′ and G″ values rapidly increased, with a higher rate for G′. Usually, the time required for G′ to cross-over G″ is considered as the onset of gelation.[21,22] When the mixed culture [Figure 3(c)] or the *L. bulgaricus* [Figure 3(a)] inoculum was used, the time needed for curd development was shorter than in trials carried out with *S. thermophilus* [Figure 3(b)]. At the optimum temperature of 41°C, the onset of gelation occurred after 2 h, 2.5 h and 5.3 h of fermentation for the associative, the *L. bulgaricus* and the *S. thermophilus* inoculum, respectively. Moreover, trials carried out with *S. thermophilus* resulted in a gel weaker than those developed in the other runs, as can be noticed by the lower final values of G′ and G″. These results are in agreement with those reported for pH, titratable acidity and lactic acid development, which showed a better performance of the mixed and the *L. bulgaricus* cultures in comparison with the *S. thermophilus* inoculum.

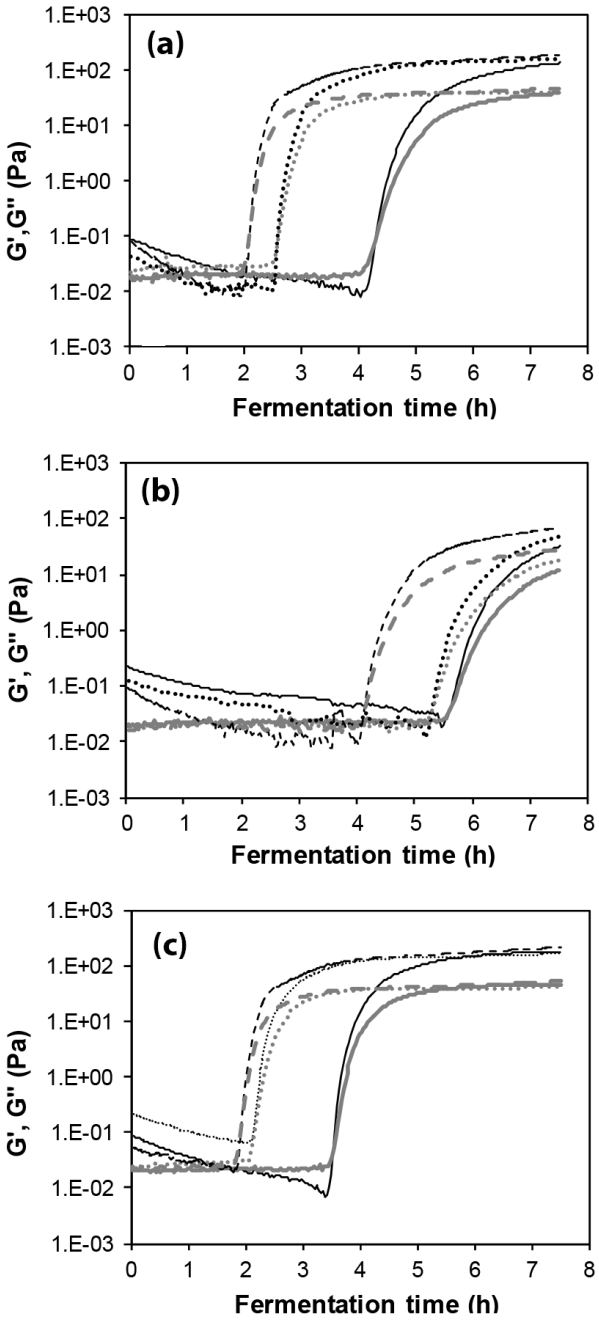

Figure 3. Evolution of storage (G′) and loss (G″) modulus during the lactic acid fermentations carried out at different temperatures with (a) *L. bulgaricus*, (b) *S. thermophilus* and (c) the mixed culture. — (G′) 37°C; — G″, 37°C; ····· (G′) 41°C; ····· G″, 41 °C; - - - (G′) 45°C; - - - G″, 45°C.

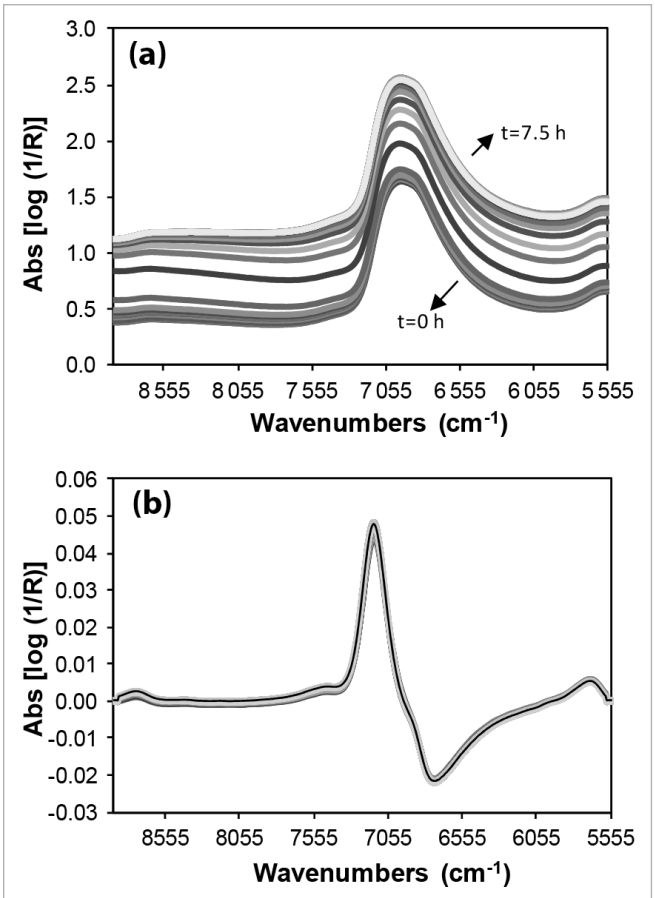

Figure 4. Reduced FT-NIR spectra collected during the lactic acid fermentation carried out at 45°C with the mixed culture of *L. bulgaricus* and *S. thermophilus*: (a) raw spectra (*t* = fermentation time); (b) spectra after transformation with first derivative.

FT-NIR spectroscopy

In this study, spectra were acquired in transflectance mode, that combines transmission and reflectance principles, because during milk fermentation the physical and chemical properties of the matrix evolve. The use of transmitted light is necessary at the beginning of the fermentation when the viscosity of milk is low, whereas a reflectance measurement is required with the progress of the bioprocess leading to a weak gel behaviour of the system.[12]

Figure 4 shows an example of the reduced (8900–5555 cm^{-1}) FT-NIR spectra collected during the fermentation process carried out with the mixed culture at 45°C and the corresponding signals transformed into the first derivative. Similar results were obtained for all the fermentation trials (results not shown). The dominant peak at about 6900 cm^{-1} in the FT-NIR spectra is related to the O–H first overtone. Qualitative evaluation of the spectra showed a remarkable trend in the FT-NIR spectra during the fermentation time: spectra collected from milk at the beginning of the bioprocess were characterised by absorbance values lower than those of the spectra acquired at the end of fermentation from coagulated milk. This change could be due to physical effects, such as casein micelle size, which heavily influences the NIR spectrum.[23] Although the absorption increased with fermentation time, it was difficult to extract information regardless of scattering effects causing the baseline drift. For this reason, a first derivative transformation was applied to the spectral signals.

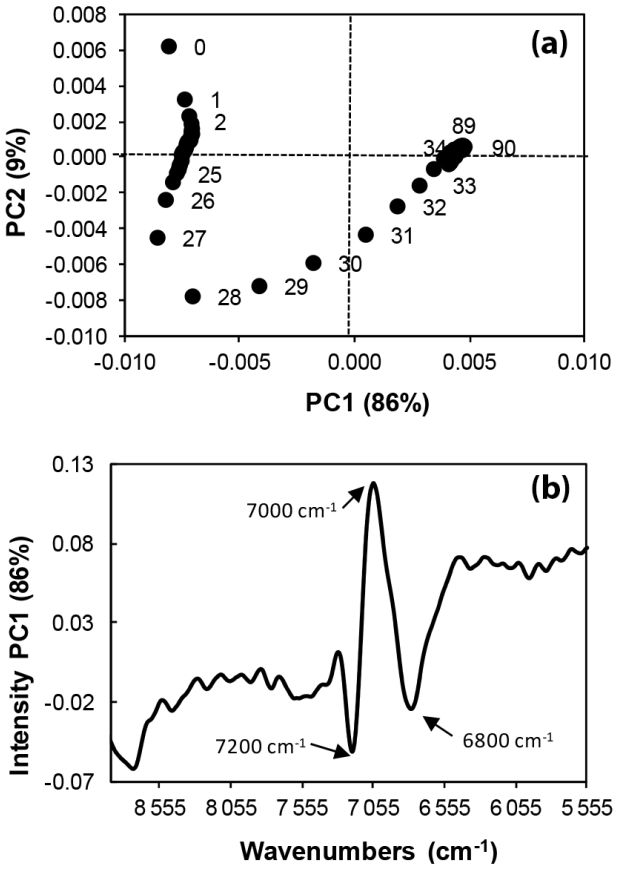

Figure 5. Plots obtained from PCA applied to FT-NIR spectra collected during the lactic acid fermentation carried out at 41°C with the mixed culture of *L. bulgaricus* and *S. thermophilus*: (a) PC1 vs PC2 score plot; (b) loading line plot for PC1. In the score plot, point label numbers increase with fermentation time.

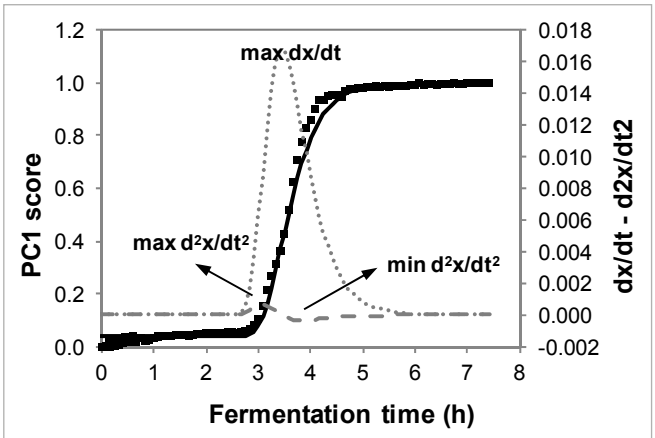

Figure 6. Example of PC1 scores modelling as a function of fermentation time. ... *dx/dt* (first derivative, h^{-1}); --- d^2x/dt^2 (second derivative, h^{-2})].

In order to uncover changes related to the time occurring during milk fermentation, reduced and pre-treated FT-NIR data were processed by PCA. Figure 5 shows the score plot and loading line plot obtained for the fermentation trial performed at 41°C with the mixed culture. Similar results were obtained for the other fermentation trials (results not shown). In the score plot, a suitable distribution of the samples in the area defined by the first two principal components according to the fermentation time was noticed. The intensity of loadings highlighted that the main wavenumbers responsible for the sample separation were mainly associated with the O–H bands of water.[24]

Kinetic models

In order to evaluate the ability of NIR spectroscopy to follow the milk fermentation process, kinetic PC1 scores obtained from spectra elaboration by PCA, as well as chemical and rheological data, were modelled as a function of the fermentation time, fitting the sigmoid function shown in Equation (1). PC1 scores were previously normalised from 0–1, in order to compare the results obtained in different trials. Figure 6 shows an example of the PC1 scores fitted by Equation (1) and the first and second derivatives of the curve. Derivatives were used to calculate the critical points related to the maximum rate (maximum value of first derivative), acceleration (maximum value of second derivative) and deceleration (minimum value of second derivative) of the phenomena. Scores obtained in all the fermentation trials were well fitted by Equation (1), always giving r^2 values higher than 0.99. The sigmoid model was also reliable for G′, pH, titratable acidity and lactic acid data ($r^2 > 0.94$).

The curves obtained by NIR-PCA and rheological data showed a very similar trend, meaning that both techniques detected the evolution of parameters strictly related to the curd development. The described phenomenon could be related to the casein micelles becoming bigger as a consequence of their aggregation, occurring at pH values of 5.5–5.2.[25] In fact, also the times corresponding to the critical points of the fermentation were practically the same for these two analytical techniques (Table 2) and corresponded to the development of pH values ranging from 5.5 to 5.2 (Figure 2). A good agreement was also observed with the critical points calculated considering the other analytical parameters. Only the times related to the maximum acceleration of the process, i.e. to the beginning of the fermentation, were lower if calculated on the basis of pH, titratable acidity (Table 2) and lactic acid values (data not shown). These results could be ascribed to the different phenomena associated with the evaluated parameters: pH, acidity and lactic acid concentration are related to the acidification operated by the microorganisms, which generally precedes the curd development described by rheological and spectral data.

Times related to the critical points confirmed that a lactic fermentation carried out by *L. bulgaricus* alone, or in association with *S. thermophilus*, is more efficient than the process performed only by *S. thermophilus*, showing

Table 2. Times corresponding to maximum acceleration (max d^2x/dt^2 or min. d^2x/dt^2), maximum rate (max dx/dt) and maximum deceleration (min d^2x/dt^2 or max d^2x/dt^2) of the lactic acid fermentation processes.

Inoculum[a]	Temperature (°C)	FT-NIR (PC1)		Rheometer (log G″)		pH		Titratable acidity (% lactic acid)		FT-NIR (ABS at 7100 cm^{-1})		
		Max d^2x/dt^2 (min)	Max dx/dt (min)	Max d^2x/dt^2 (min)	Min d^2x/dt^2 (min)	Min d^2x/dt^2 (min)	Max dx/dt (min)	Max d^2x/dt^2 (min)	Max dx/dt (min)	Min d^2x/dt^2 (min)	Max d^2x/dt^2 (min)	Max dx/dt (min)	Min d^2x/dt^2 (min)
I1	37	184	202	251	287	0	132	277	105	423	450	175	202	225
	41	135	148	147	174	18	114	209	36	186	314	121	130	144
	45	108	108	120	142	23	100	186	82	173	264	94	103	112
I2	37	306	337	341	373	95	263	427	168	314	450	292	328	360
	41	283	306	314	355	64	209	350	150	255	368	265	297	324
	45	233	265	241	278	50	155	255	105	223	345	234	279	324
I3	37	184	198	206	233	59	168	282	118	227	341	180	193	202
	41	130	144	124	151	50	123	241	114	209	309	112	126	144
	45	103	112	111	133	23	100	186	64	155	245	90	99	108

[a]I1, *L. bulgaricus*; I2, *S. thermophilus*; I3, *S. thermofphilus* and *L. bulgaricus* (1 : 1)

earlier starting and maximum rate points. As expected, maximum deceleration times, corresponding to the end of the fermentation, showed a wide range, depending on the inoculum and incubation temperature considered.

In order to verify the possibility of simplifying the NIR instrumentation, the evolution of the processes at a fixed wavenumber was also tested. In particular, absorbance values of the spectra at 7100 cm^{-1}, corresponding to the OH combination band, were plotted against fermentation time and modelled applying Equation (1). Also, in this case, r^2 values higher than 0.99 were obtained for all the fermentation conditions considered and the times corresponding to the critical points were practically the same as those obtained from the PC1 score modelling (Table 2).

Conclusion

The obtained results demonstrated that FT-NIR spectroscopy combined with PCA is a valid, simple, cheap and robust tool for the in-line monitoring of milk lactic acid fermentation. FT-NIR spectroscopy gave crucial real-time information in agreement with rheology and conventional quality parameters. In particular, spectroscopic kinetic models were able to describe the curd development during lactic acid fermentation, thus giving the opportunity to easily follow in-line an important quality parameter of fermented milks such as texture.

These models may be used to effectively monitor and control lactic acid fermentation, in order to detect if the process is moving out of control and to establish the best strategy for its management, before operating costs and quality deterioration of the product make the process unprofitable.

Moreover, the good results obtained using only spectral data at a fixed wavenumber (7100 cm^{-1}) suggest that a simplified and cheaper NIR device could be developed for the industrial monitoring of the lactic acid fermentation process.

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