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Spectroscopic Prediction of Rheological Properties of Stirred Yoghurt

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ABSTRACT

A set of stirred yoghurt samples of identical chemical composition, and only differing in processing conditions, were submitted to an array of spectroscopic methods (NIR, fluorescence and low-field NMR) with the aim of evaluating the ability of these methods to predict rheological parameters.

INTRODUCTION

Spectroscopic methods, in particular near infrared spectroscopy (NIR), have a long history in the dairy field where they have proven useful in quantitative chemical analysis. By contrast, the notion of predicting sensory properties from spectroscopic data is quite recent[1]. Due to their high reproducibility and precision they can be an attractive alternative to more cumbersome rheological and even sensory methods, e.g. descriptive analysis. The application of spectroscopic methods to food texture studies is contingent on the construction of multivariate calibration models using large data sets. Most work has been done on near-infrared spectroscopy (NIR), e.g. on processed cheese[2] and Danbo cheese[3] over the course of ripening. Fluorescence spectroscopy distinguishes itself by its outstanding sensitivity (100-1000 times more sensitive than other spectroscopic methods). In addition, fluorescent molecules are very sensitive to their environment, e.g. to the fat globule-protein interaction in a dairy system[4]. Front-face fluorescence spectroscopy, where the surface of the sample is probed, has been used to discriminate sensory and rheological properties in soft cheese[5].

One particular problem with studies concerned with relating spectroscopy and food texture is the presence of spurious variables. For instance, the viscosity might be predicted well in one particular study, but frequently this can be ascribed to a design of nuisance variable, e.g. protein level, which is well predicted by the spectroscopic method in question. Whether additional information is present is an important issue; for low-field NMR data on raw potatoes this has indeed been found to be the case[6]. NMR was found to be able to predict certain descriptors such as *Springiness* and *Adhesiveness*, ostensibly due to its sensitivity to the state of moisture in the sample. For validation purposes it is thus of interest to evaluate these spectroscopic methods on a set of products (or model systems) with a constant chemical composition, but varying processing conditions (homogenization pressure, heat treatment etc.).

We have evaluated a set of stirred yoghurt samples by both spectroscopic and rheological methods. Stirred yoghurt is attractive because a great deal of variation can be accomplished without varying the chemical composition, and thus eliminating lurking variables. In this way we have produced a set of samples with similar chemical composition, and widely differing rheological properties.

MATERIALS AND METHODS

Methods

Stirred yoghurt samples were manufactured in pilot scale (2 kg) at the facilities of the Department of Food Science, University of Copenhagen. Yoghurt milk base was made by blending (per batch) 1.593 L of tap water, 0.222 kg of low-heat skimmed powder (MILEX 240 LH, Arla Foods Ingredients, Viby, Denmark) and 0.184 kg of full cream (38 g fat/100 g; Borup A/M, Gørløse, Denmark) in a Scanima Type SRB-20 mixer (Scanima A/S, Aalborg, Denmark) and subsequently store the milk base at 5°C overnight to allow for a proper hydration of the milk powder. The following day the milk base was homogenized at 60°C, heat treated aproppriately, and inoculated at 42°C with a frozen, concentrate yoghurt starter culture (CH-1, Chr. Hansen A/S, Hørsholm, Denmark).

Experimental design

Processing variables were: 1) homogenization pressure (50 and 200 bar), 2) heat treatment temperature (72, 82 and 82 °C as well as no heat treatment) and 3) inoculation level (high/low), amounting to a total of 16 samples; these were manufactured in triplicate following a randomized experimental design, for a grand total of 48 samples. Eight samples were produced each day, and tested seven days later.

Instrumental characterization

The yoghurts were evaluated instrumentally by steady shear viscometry, dynamic oscillation, near-infrared (NIR) spectroscopy, fluorescence spectroscopy and low-field NMR spectroscopy. All measurements were done at 22°C.

Rheological characterization (shear viscometry and dynamic oscillation testing) was done with a 25 mm cup-and-bob measurement system using C-VOR controlled stress rheometer (Malvern). Flow curves of shear stress vs. shear rate were recorded at 24 logarithmically spaced shear rates between 0.0001-1000 s^{-1}. Dynamic oscillation testing (frequency sweep) was performed at 20 logarithmically spaced frequencies between 0.01-10 Hz, with a strain of 0.002.

VIS/NIR reflectance spectra (400-2500 nm) were recorded on a NIR-Systems 6500 spectrophotometer (FOSS NIRSystems, Inc., Silver Spring, Maryland, USA). A circular sample holder with a diameter of approximately 4 cm was used. The measurements were obtained as the average of 32 scans using a transport module (NR-6506).

Fluorescence spectra were measured on a Perkin Elmer LS50 B spectrofluorometer equipped with a Front-Face Accessory. The sample holder was the same as the one used for VIS/NIR, see above. For every sample, one excitation-emission matrix (EEM) was measured with excitation at every 10 nm from 260 nm to 360 nm. The emission spectra ranged from 260 nm to 600 nm. Each measurement started with the highest excitation wavelength and ended with the lowest in order to minimize photodecomposition of the sample.

LF-NMR relaxation measurements were performed on a 23.2 MHz Maran bench top pulsed ^1H NMR analyzer (Resonance Instruments Inc., Witney, UK). The transverse relaxation time constants (T_2) of the samples were measured using the Carr-Purcell-Meiboom-Gill (CPMG) pulse experiment. The CPMG experiments were carried out using a relaxation delay of 6 s and 16 consecutive scans for noise reduction along with a filter width of 0.1 MHz.

Spectra were recorded on both milk base and yoghurt; rheological measurements were done only on the yoghurts.

Data analysis

The relationships between spectroscopic and rheological data were explored by PLS regression using Unscrambler Ver. 9.2 (CAMO Software, Oslo, Norway) whereas the effect of design variables was evaluated by 50/50 MANOVA using 50/50 MANOVA for MATLAB Ver. 2.0 with MATLAB Ver. 6.5 (Mathworks, Natick, MA). Fifty-fifty MANOVA[7] was used for finding significant effects in the spectral and rheological data of the design variables. In 50/50 MANOVA the data is reduced by Principal Component Analysis (PCA) and subsequently ordinary Multivariate Analysis of Variance (MANOVA) is applied on the principal components. 50/50 MANOVA is particularly suitable for spectra and rheological data, which tend to be highly collinear. In addition to the test of significance, the method provides the explained variance of each effect, which is the sum of this effect's sum of squares of the response divided by the sum of the total sum of squares of the response variables.

PLS models were cross-validated using randomized segments of four samples. The resultant Root Mean Square Error of Cross-Validation (RMSECV) were benchmarked against values obtained by regressing the design variables on the multivariate data matrices (ANOVA PLSR[8]). Mean-centered NIR spectra were submitted to scatter correction (MSC and EMSC) and regressed on the dependents variables G'[0.01 Hz] and $\sigma[247\ s^{-1}]$ (elastic modulus at 0.01 Hz and shear stress at 247 s^{-1}). Fluorescence data used were emission spectra at 320 nm, while LF-NMR data were expressed by the transversal relaxation time constants, T_2.

RESULTS AND DISCUSSION

NIR data from Day 2 had to be disregarded because of equipment failure.

Results from 50/50 MANOVA on rheological data and spectra are tabulated in Tab. 1-3. Table 1 displays the explained variances and significance levels of each design variable. Elastic moduli are better modelled than shear stresses, and heat treatment temperature has the highest explained variance. No significant interaction between homogenization pressure and heat treatment temperature.

Elastic moduli and shear stressed were found to be correlated; the latter could be predicted from the former (R^2 = 79%) using PLS2 regression (multivariate X = elastic moduli and Y = shear stresses).

Table 1: Effect of design variables on rheological data of yoghurt (elastic moduli and shear stresses): explained variance (%) and significance level from 50/50 MANOVA

YOGHURT	G'	σ
Homogenization pressure (A)	34.5***	38.9***
Heat treatment temperature (B)	56.0***	34.3***
Inoculation level (C)	$0.0^{n.s.}$	$0.7^{n.s.}$
A x B	$0.0^{n.s.}$	$0.5^{n.s}$
A x C	$0.1^{n.s.}$	$0.8^{n.s.}$
B x C	$0.1^{n.s.}$	$0.1^{n.s.}$
A x B x C	$0.0^{n.s.}$	$0.5^{n.s.}$
Total model	90.7	75.8

G': elastic moduli; s: shear stresses. n.s.: not significant

Similar results for the three spectroscopical methods are given in Tab. 2-3. Yoghurt spectra are much better modelled than milk spectra (higher significance levels and explained variances). This implies that the spectra capture information about the physical makeup of the gelled milk structures that is not present in the milk bases. Still, NIR as well as LF-NMR is able to capture some variability caused by the design variables homogenization pressure and heat treatment temperature already in the milk - the total explained variance is nearly the same for both methods. NIR captures the effect of the heat treatment temperature well, which might be ascribed to the progressively higher degree of protein denaturation. LF-NMR, being sensitive to the mobility of water, captures the effect of both design variables equally well.

Table 2: Effect of design variables on spectroscopical data from milk samples: explained variance (%) and significance level from 50/50 MANOVA

MILK	NIR	Fluorescence	LF-NMR
Homogenization pressure (A)	13.7*	$1.9^{n.s.}$	14.3**
Heat treatment temperature (B)	4.3***	$1.3^{n.s.}$	4.3**
A x B	$4.2^{n.s.}$	$13.7^{n.s.}$	$4.0^{n.s.}$
Total model	22.2	16.9	22.6

Homogenization pressure and heat treatment temperature are both remarkably well represented by both NIR and LF-NMR. Both are highly significant, but whereas homogenization pressure is captured better by NIR, LF-NMR does a better job in representing the effect of homogenization pressure and heat treatment temperature. This is possibly because NIR is more sensitive to light scattering effects, which are clearly influenced by the homogenization pressure, while LF-NMR, as mentioned above, better reflects the effect of the heat treatment temperature, due to the increased water holding capacity induced by the heat treatment[9].

The effect of inoculation level was not captured by any method.

Table 3: Effect of design variables on spectroscopical data from yoghurt samples: explained variance (%) and significance level from 50/50 MANOVA

YOGHURT	NIR	Fluorescence	LF-NMR
Homogenization pressure (A)	45.1***	11.4**	18.1***
Heat treatment temperature (B)	9.7***	6.4**	46.8***
Inoculation level (C)	$0.5^{n.s.}$	$0.5^{n.s.}$	$2.0^{n.s.}$
A x B	3.8**	$4.0^{n.s.}$	$1.2^{n.s.}$
A x C	$0.4^{n.s.}$	$3.0^{n.s.}$	$1.8^{n.s.}$
B x C	$0.5^{n.s.}$	$0.0^{n.s.}$	$0.5^{n.s.}$
A x B x C	$1.8^{n.s.}$	$2.3^{n.s.}$	$0.0^{n.s.}$
Total model	61.8	27.6	70.4

The effect of pretreatment of NIR spectra is shown in Tab. 4. The dependent variable is G' measured at 0.01 Hz. Multiplicative Scatter Correction gives the lowest explained variance, but is deemed the more attractive option because fewer PLS components (latent variables) are needed - this is normally seen as an attractive feature of a multivariate model.

Table 4: Predictive ability of NIR spectra regressed on G'[0.01 Hz] as effected by pretreatment.

	UNTREATED	MSC	EMSC
Explained variance R^2	0.94	0.88	0.96
RMSECV	1.57	1.87	2.39
PLS components	8	4	8

RMSECV: Root Mean Square Error of Cross-Validation

Tab. 5-7 show the actual predictive preformances of PLS models of the selected rheological parameters G'[0.01 Hz] and [247 s^{-1}] based on NIR, fluorescence and LF-NMR, respectively. Again, fluorescence is outperformed by NIR and LF-NMR in terms of explained variance. However, the actual predictive ability is better represented by the Root Mean Square Error of Cross-

Validation (RMSECV). The RMSECV is given in the original units (Pa), and should be benchmarked against values obtained by ANOVA PLSR. In our case we find RMSECV = 1.20 Pa for elastic moduli for and RMSECV = 7.83 Pa for shear stresses. These are the maximally attainable RMSECVs, given the quality of the rheological data. The NIR-based models are actually not far off.

In all cases G'[0.01 Hz] is clearly modelled better than $\sigma[247\ s^{-1}]$, which is to be expected, as G' is a direct manifestation of the microstructure of the sample, while σ probes the large-scale deformation properties.

Another way to twist the data is to try and predict G'[0.01 Hz] in yoghurt from the NIR spectra on milk (this is similar to predicting the properties of boiled potatoes by raw potato spectra[6]). The outcome is poorer, with $R^2 = 0.69$ and RMSECV = 4.05. This lends further credence to the notion that spectroscopy data captures unique microstructural information, i.e. not just chemistry.

Table 5: Predictive ability of NIR spectra regressed on G'[0.01 Hz] and $\sigma[247\ s^{-1}]$

NIR	PLS comp.	R^2	RMSECV
G'[0.01 Hz]	4	0.88	1.87
$\sigma[247\ s^{-1}]$	4	0.58	8.33

Models based on fluorescence do not fare that well, but are more parsimonious (require fewer PLS components).

Table 6: Predictive ability of fluorescence data regressed on G'[0.01 Hz] and $\sigma[247\ s^{-1}]$

Fluorescence	PLS comp.	R^2	RMSECV
G'[0.01 Hz]	2	0.81	3.43
$\sigma[247\ s^{-1}]$	2	0.73	10.44

The performance of LF-NMR is in between NIR and fluorescence. Again, fewer PLS components are needed.

Table 7: Predictive ability of LF-NMR data regressed on G'[0.01 Hz] and $\sigma[247\ s^{-1}]$

LF-NMR	PLS comp.	R^2	RMSECV
G'[0.01 Hz]	1	0.83	2.87
$\sigma[247\ s^{-1}]$	1	0.62	10.97

CONCLUSIONS

In terms of predictive ability, NIR came out on top, followed by LF-NMR, and with fluorescence being a distant third. On the other hand NIR-based models required more PLS components.

The purpose of the paper was to elucidate if the studied spectroscopical methods capture anything but chemical information. The results show convincingly that they actually do just that. The design variables were much better reflected in the yoghurt samples compared to the milk samples. Furthermore, the prediction of G' was much poorer when milk spectra were used.

As expected, homogenization pressure and heat treatment temperature turned out to be highly significant - inoculation level was not.

It would be interesting to repeat the work with whey protein gels, whose microstructure can be manipulated more precisely by varying the addition of Na^+ and Ca^{2+} ions[10].

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