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QUANTITATIVE ASSESSMENT OF MAPLE SYRUP PROPERTIES BY MEANS OF FLUORESCENCE SPECTROSCOPY

ALAIN CLÉMENT[1], BERNARD PANNETON[2], LUC LAGACÉ[3]

[1] Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Ouest, St-Hyacinthe, Qc., Canada, alain.clement@agr.gc.ca
[2] Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, 430 boul. Gouin, St-Jean-sur-Richelieu, Qc., Canada, bernard.panneton@agr.gc.ca
[3] Centre de Recherche, de Développement et de Transfert Technologique Acéricole Inc. 142 rang Lainesse, St-Norbert d'Arthabaska, Qc., Canada, luclagace@centreacer.qc.ca

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ABSTRACT Simple and portable tools that can speed up quality assessment and monitoring are of interest in the food industry. The progress made over the last decade in electro-optics makes fluorescence spectroscopy a potential tool as it is fast, requires minimal sample preparation, and it is now relatively low cost. Maple syrup is a high value commodity that is currently classified in terms of its color. It was recently found that maple syrup displays intrinsic fluorescence under excitation wavelength ranging from 250 nm to 500 nm and above. The objective of this study was to determine if the fluorescence signal could be used to assess routine physico-chemical parameters, determine its typicity, detect off-flavors or even distinguish flavor classes that can add further value to the product. It was possible to predict the harvesting period of maple sap within the season from its spectra (R^2 = 0.88 in 2003 and 0.81 in 2004). Color of syrup (R^2 = 0.91 and 0.88) and bacterial counts in sap (R^2 = 0.75 and 0.78) were also predicted from syrup spectra. Discriminant analysis revealed that between 71% and 100% of syrup samples were correctly classified according to the farm of origin in 2003 and 2004. Mixed data factorial analysis was used to explore the link between fluorescence, physico-chemistry parameters and flavor. This analysis found a well structured topology and a partial least square discriminant analysis showed a relationship between this topology and fluorescence. This was a strong indication that fluorescence of maple syrup can be used to assess its flavour.

Keywords: Maple syrup, fluorescence, flavour, prediction, rapid methods, spectroscopy, PLS regression, discriminant analysis, automated quality control.

INTRODUCTION Maple syrup is produced by heat evaporation of maple sap collected from maple sugar trees (*Acer saccharum* Marsh) during the early spring season in North America. The harvesting season generally occurs between early February and the end of April and lasts four to six weeks. Maple sap is mainly composed of sugars (sucrose, glucose, fructose) (van den Berg et al., 2006) but also contains a variety of other nutrients such as organic acids (Stuckel and Low, 1996), amino acids (Morselli and Whalen,

1986), phenolic compounds (Kermasha *et al.*, 1995), and vitamins (Morselli and Whalen, 1975). Aromatic compounds present in maple syrup originate from maple sap; they are mostly lignin derivatives (Filipic *et al.*, 1969). They are also synthesized during the heating process, as amino acids react with reducing sugars to produce such compounds as 5-hydroxymethylfurfural (HMF) and pyrazine through Maillard's reaction (Filipic et al., 1969; Kermasha et al., 1995).

Some data are available on maple syrup, showing that various spectroscopic methods (NIR, Raman and FTIR) have an excellent potential to determine the presence of adulterants (Paradkar *et al.*, 2002) and possibly distinguish them (Paradkar *et al.*, 2003). Routine methods of analysis of maple syrup (soluble solids, transmittance at 560 nm, pH, acidity, viscosity), although needed for quality control, offer limited means to highlight specific quality profiles. Unlike honey, that gets much of its character from the floral species in surrounding fields (Corbella and Cozzolino, 2005), maple syrup quality is influenced by the timing of sap harvest. Early season syrups tend to be light-colored, while late season batches are much darker, with a more robust taste (Morselli and Whalen, 1991). Optical spectroscopy is of interest not only for routine physico-chemical testing, but also to highlight characteristics such as the moment of harvest and typicity, defined here as the specificity of maple syrup physico-chemistry with respect to the location of the production farm, which has been little studied. It could offer a potential for specific quality labelling and refined marketing.

The aim of the present study was to determine the potential of fluorescence spectroscopy to assess the major physico-chemical characteristics of maple syrup in a rapid and non-destructive way, and to explore new possibilities of automated classification, including typicity and the moment of sap harvest.

MATERIALS AND METHODS Samples of maple sap and corresponding syrup were obtained from 5 and 7 maple syrup producers in Quebec, Canada, during the 2003 and 2004 seasons respectively. Farms "JH", "MH", "DL", "AC" and "RB" are located in South Eastern Quebec. Farms "AB", "EX" and "NB" are located in South Western Quebec. A new batch of syrup was made daily with sap freshly collected by a tubing network installed in the maple grove. There were a total of 98 and 125 syrup samples in 2003 and 2004. From this sampling, a variable named «Period» was calculated. This variable corresponds to a fraction of the total number of sap flow days, which was site specific, from the beginning until the end of the harvesting season.

Syrup viscosity was measured with a digital rheometer, and color was determined as transmittance at 560 nm. Microbial counts (bacteria, yeast and moulds) in sap were determined by anaerobic incubation on acidified potato dextrose agar (Difco) and expressed in colony forming units per mL (CFU/mL). Log values were used in calculations. Syrup flavour profiles were determined by a trained sensory panel that determined, by consensus, if samples had one or many of the following characteristics on a presence / absence basis: Woody, Empyreumatic (slightly burned flavour), Confectionery (sugary taste), Maple (well balanced typical taste), or presence of any off-flavour.

Fluorescence profiles of syrups were measured at three excitation wavelengths (230, 275 and 360 nm). Fluorescence emission spectra are designated by their excitation

wavelength. For example, a spectrum obtained under excitation at 275 nm is named E275. Similarly, fluorescence observed at 350 nm is designated as F350. E275-F350 refers to the fluorescence measured at 350 nm under excitation at 275 nm. Fluorescence spectra were measured by a Shimadzu spectrofluorometer (model 5301PC). Emission and excitation slits were set at 5 nm, except for syrup at E275 (10 and 3 nm). The excitation light beam was directed at a 1 cm quartz cuvette containing syrup that was maintained at 25°C by circulating water from a thermostatic bath in the measurement chamber. Syrup samples were measured after dilution with distilled water (1/25 w/v). Fluorescence emission was collected at a 90° angle and measured at 1 nm intervals between Rayleigh and second order scattering.

PLS regressions for prediction of physico-chemical and microbial variables were computed with the Unscrambler, version 9.7 (Camo software AS, Oslo, Norway). Residual predictive deviation (RPD), which is the ratio of variable standard deviation to RMSECV (Arana et al., 2005) was calculated, providing an indication of the potential for practical application of spectroscopy, higher values indicating a better prediction ability. Factorial analysis for mixed data (FAMD) was performed under R (R Development Core Team, 2008) using the SensoMineR package (Husson et al. 2008). The classification algorithm linking flavor to fluorescence spectra was based of the partial least-square discriminant analysis (Barker and Rayens, 2003) as implemented in the PLS_Toolbox 4.0 (Wise et al. 2006). Custom Matlab™ and R scripts were developed for data extraction and plotting. No pre-treatment was applied on the X (fluorescence spectra) or Y matrices. The number of latent variables was chosen using the estimated signal-to-noise ratio (function "estimatefactors" of the PLS_Toolbox).

RESULTS AND DISCUSSION. Raw fluorescence spectra. Intrinsic fluorescence emission profiles of maple syrup were characterized by two major regions of fluorescence. The first was best observed at E275 nm, and produces two peaks, one at F320 and the other at F460 nm. The second major fluorescence emission peak was observed around F460 nm with E360 nm (Fig. 1). Early season samples, which are occasionally Woody flavoured had a higher E275-F320 peak amplitude, while late season samples, mostly Empyreumatic generally displayed relatively high E360 nm fluorescence. In fact, data collected at specific farms all displayed a gradual decrease of E275-F320 with a parallel increase in of E360-F460 throughout the harvesting season.

Attribution of fluorescence spectra to specific aromatic compounds can only be tentative at this stage, considering the complex aromatic make-up of maple syrup and the weak knowledge of individual classes of phenolics as sap harvesting season progresses. However, the E275-F320 emission region could correspond to amino acids, particularly tryptophan, which fluoresces at about 350 nm in pure water, but whose emission is highly dependant on the matrix (Lakowicz, 2007). The E360-F460 peak in syrup could correspond to Maillard reaction complexes. In thermal processing of milk, E360-F440 is considered suitable for such measurements (Schamberger and Labuza, 2006). Other possible sources of the second fluorescence peak are the main phenols identified in sap: vanillin, syringaldehyde, coniferol, and sinapic, ferulic, p-coumaric acids (Kermasha *et al.*, 1995), suggesting that lignin-derived phenolics are major contributors to maple products fluorescence.

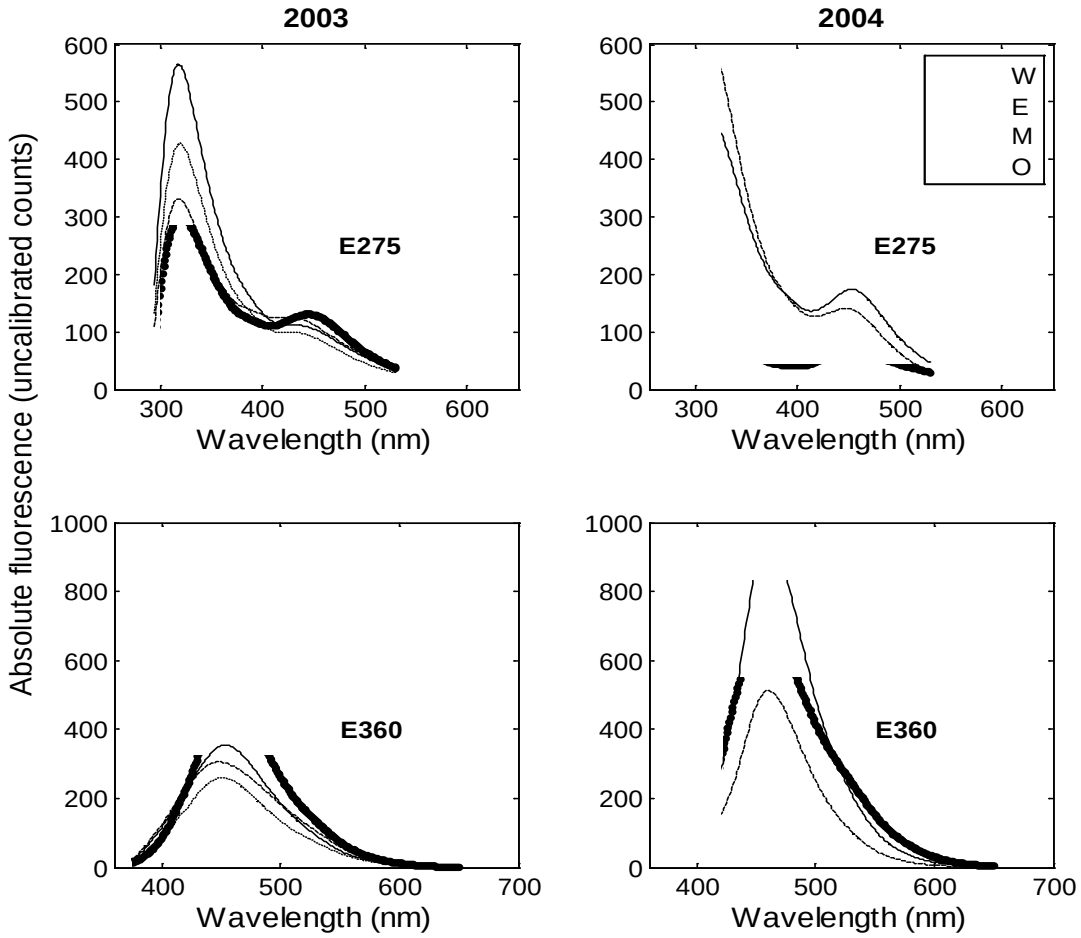

Figure 1. Some examples of fluorescence spectra of representative maple syrup flavours in 2003 and 2004. W: Woody; E: Empyreumatic; M: Maple; O: off-flavour.

Predicting physico-chemical and sap flow period. Maple syrup color (% light transmittance at 560 nm) is a key variable used in current classification systems. Color was accurately predicted applying PLS regression using syrup fluorescence spectra as the X matrix. R^2 values of 0.91 and 0.88 were obtained for 2003 and 2004 respectively. RPD values were close to 3.0, (Table 2) suggesting a good potential for practical application. The best excitation wavelength for prediction was 360 nm (Table 1). A PLS regression of bacterial counts in sap, resulted in a R^2 of 0.75 in 2003, and 0.78 in 2004, under excitation at 230 nm, giving RPD values in the 2.1 – 2.2 range (Table 1), which we suggest is sufficient for semi-quantitative measurements. These results were slightly superior to those obtained for yeasts and moulds. A number of compounds in microbial biomass including proteins and nucleic acids, are known to produce fluorescence, which allows monitoring of bacterial growth (Leblanc and Dufour, 2004).

Prediction of sap harvesting period from fluorescence measurement at E275 were quite precise, with R^2 values reaching 0.88 in 2003 and 0.82 in 2004 (Table 1, Fig. 2), at an excitation wavelength of 275 nm. This resulted in RPD values above 3.0 in 2003 and above 2.2 in 2004. The performance of Period prediction from syrup spectra in 2004 was

only slightly inferior to that of 2003 even though the second season was not typical, as there was only a loose relationship between color and period variables. This suggests that fluorescence spectroscopy has a greater potential than color for syrup characterization. Actually, attempting to predict Period from syrup color measurements with a linear regression gives very poor results (R^2 = 0.28 in 2003 and 0.00 in 2004).

Table 1. Prediction of various physico-chemical quality parameters of maple syrup by PLS regressions.

Parameter	Excitation	Pre-process.[a]	Out.[b]	LV[c]	RMSECV	R^2	RPD[d]
			2003				
Color (syrup % transmit. at 560 nm)	E360	SG 11-2	0	10	4.70	0.91	3.36
Bacterial counts in sap (log)	E230	SG 21-2	2	11	0.68	0.75	2.14
Yeasts & moulds in sap (log)	E275	SG 17-2	1	12	0.63	0.55	1.52
Period (proportion, sap harvest season)	E275	SG 17-2 D1	0	11	0.099	0.88	3.16
			2004				
Color (syrup % transmit. at 560 nm)	E360	SG 11-2	2	10	7.52	0.88	2.87
Bacterial counts in sap (log)	E230	SG 21-2	2	10	0.50	0.78	2.19
Yeasts & moulds in sap (log)	E275	SG 17-2	1	9	0.65	0.62	1.61
Period (proportion, sap harvest season)	E275	SG 17-2 D1	0	7	0.126	0.82	2.28

a: Pre-processing: SG 21-2 D1 = Savitstsky-Golay with 21 points smoothing and polynomial order 2, first derivative; b: Out. = number of outliers removed from analysis; c: LV = number of latent variables used in the model; d: RPD = residual predictive deviation.

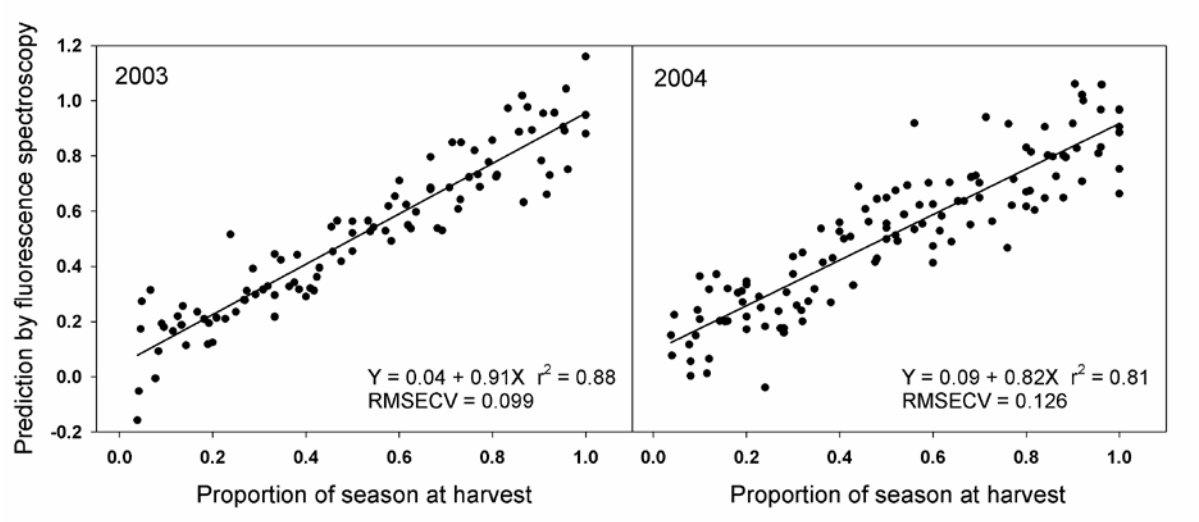

Figure 2. Prediction of the proportion of harvesting season of sap by fluorescence spectroscopy of syrup samples in 2003 and 2004.

Prediction of the moment of harvest opens the possibility for maple syrup classification according to flavour categories. Early season syrups tend to have a mild or Woody flavour, mid season syrups a more sugary/Confectionery or typical Maple flavour, whereas late season syrups have a much stronger Empyreumatic flavour (Lagacé et al., 2002; Morselli and Whalen, 1975). However, a common industrial practice of syrup lot mixture would break the traceability linkage with initial sap timing of harvest.

Prediction of production site (typicity). Besides the effect of the timing of sap harvesting on syrup quality, little is known on the effect of local environmental conditions, including soil and climate, on syrup quality. If overall fluorescence emission profiles were characteristic of specific production sites, this could be a valuable tool to support claims of value-added syrups produced in different areas.

As a first step, separate analyses were performed for 2003 and 2004. In 2003, the PLS-DA model had 9 latent variables. The confusion matrix (Table 2) showed that for 4 out of 5 production sites, 85 % or more of the samples were assigned to the correct production site. Interestingly, DL and MH farms are close to each other, but in a different geographical region as compared to AB. In 2004, performances were generally higher with success rates larger than 85 % for all sites, except DL (78 %). Again, the source of confusion is not related to geographical proximity. In honey, the botanical origin, determined by its aromatic profile, was successfully determined by fluorescence spectroscopy (Ruoff *et al.*, 2006).

Prediction of flavours. A mixed data factorial analysis was performed on both flavour scores (nominal) and physico-chemical data (ordinal values) to outline any underlying structure in the data. The first two axes accounted for 48.14% of the overall variance. In the case of flavours, the group representation isolated two groups. The Woody and Empyreumatic flavours were related mostly to the second axis, while the Maple, Confectionery and off-flavour formed a group linked to the first axis (Fig. 3).

On the correlation circle, the transmittance was opposed to time which was consistent with the well known fact that syrup tend to be darker as the harvesting season progresses. Also, the yeast and mould, and bacterial counts of the sap increased in time, which is consistent with the fact that as the season progresses, the mean ambient temperature increases resulting in conditions more favourable to increased microbial growth (Fig. 3). On one hand, the Woody and Empyreumatic flavor group was roughly on the same axis as the main axis for the physico-chemical variables. On the other hand, the Maple, Confectionery and off-flavours group was in a dimension nearly perpendicular suggesting that the expression of the flavors in this last group was independent of time in the season, syrup color and microbial counts in sap.

The observations of the raw fluorescence spectra (Fig. 1), the fact that syrup physico-chemistry and microbiology can be predicted by fluorescence, and the results from the mixed data factorial analysis suggests that there was a link between the fluorescence spectra and the flavours. These results provided scope for attempting to predict flavors from fluorescence spectra using the partial least-square discriminant analysis for N classes (NPLSDA). Separate analysis for excitation at 275 and 360 nm were performed. Six latent variables were retained corresponding to a signal-to-noise ratio greater than 2.

Table 2. Confusion matrices (years 2003 and 2004) for prediction of production site from maple syrup fluorescence spectra (E275). Column and row headers are production site code. True values in rows and predicted in columns (*e.g.* in 2003, 71 % of the AB samples were predicted as coming from AB and 14 % as coming from MH). White on black indicates percentage of well predicted. Black on grey indicates major sources of confusion (>10 %).

2003	AB	AC	DL	JH	MH
AB	**71%**	0%	**10%**	5%	**14%**
AC	5%	**90%**	5%	0%	0%
DL	**14%**	0%	**86%**	0%	0%
JH	0%	0%	0%	**95%**	5%
MH	**10%**	0%	0%	5%	**85%**

2004	AC	DL	EX	JH	MH	NB	RB
AC	**100%**	0%	0%	0%	0%	0%	0%
DL	0%	**78%**	0%	0%	0%	**11%**	**11%**
EX	0%	0%	**96%**	4%	0%	0%	0%
JH	0%	0%	0%	**100%**	0%	0%	0%
MH	0%	4%	0%	4%	**88%**	4%	0%
NB	0%	0%	0%	**11%**	0%	**89%**	0%
RB	0%	0%	0%	0%	5%	9%	**86%**

The regression coefficient vectors were similar for both years and the data were pooled. For the off-flavours at E275 nm, the profile was a mirror of the ones for the Confectionery (Fig. 4) and the Maple flavors indicating that the off-flavour and the Confectionery or Maple flavors were mutually exclusive. Part of profile of the regression coefficients for the Woody flavor was a mirror of the profile for the Empyreumatic flavor

for (>375 nm). Around F320, the profile was typical of a function for detecting a peak shift for the Empyreumatic flavor with a shift towards the higher wavelength being positively correlated with the Empyreumatic flavor. For the Woody flavor around F320, the profile was close to a curvature function (second derivative), a narrower and higher peak being positively correlated with the Woody flavour. The peaks and valleys in the profiles of regression coefficients were on different location when comparing the profiles for Confectionery, Maple and off-flavour to the profiles for the Woody and Empyreumatic flavors. The results from the NPLSDA were in agreement with the results from the mixed data factorial analysis that indicated that the five basic flavors can be summarized on a two dimensional map.

In all cases, the classification success rates were lower than 80% (Table 3). The success rates tended to be higher for the off-flavour, the Confectionery and the Woody flavors. As the NPLSDA model was calibrated for each individual flavor, a success rate of 50% corresponded to pure chance. Results from the NPLSDA analysis revealed that there was a connection between the flavor and the fluorescence spectra and that this was more pronounced for the off-flavour, Woody and Empyreumatic flavors.

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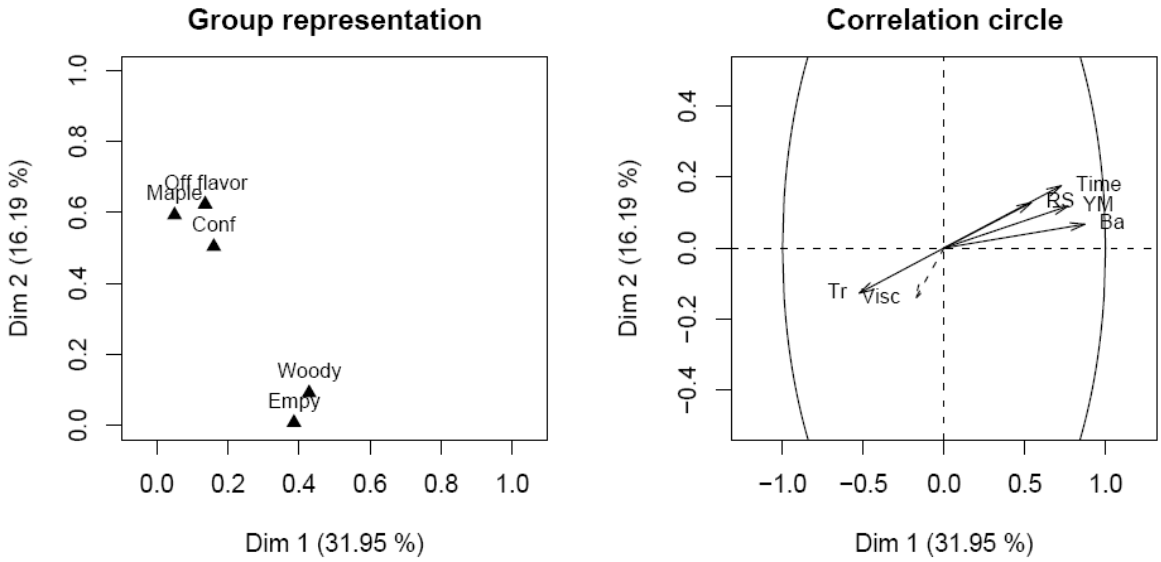

Figure 3. Group representation and correlation circle from Mixed data factorial analysis. The vertical axis of the correlation circle was stretched for readability. Tr: transmisttance; RS: reducing sugars; Ba: bacterial counts; YM: yeast and mold contamination; Visc: viscosity

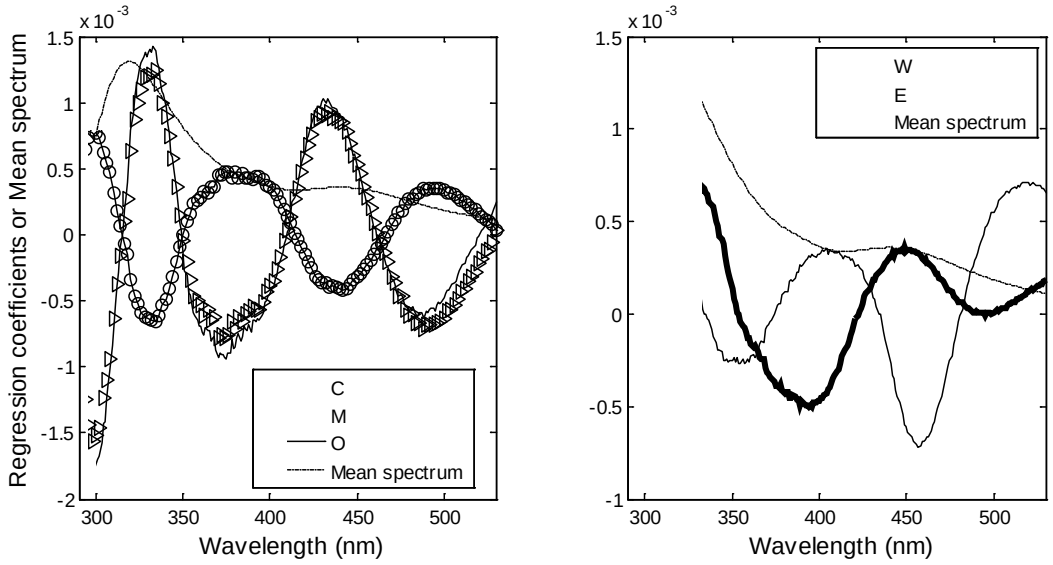

Figure 4. The regression coefficients for each flavor. NPLSDA with 6 latent variables on E275 spectra. Data from 2003 and 2004 pooled. C: Confectionery; M: Maple; O: off-flavour; W: Woody; E: Empyreumatic.

Five latent variables were retained in the NPLSDA model for E360 nm. The profiles of the regression coefficients were similar for 2003 and 2004 and again, the data sets were pooled. Within a scaling factor, the profiles for all flavors were similar and within a sign, corresponded to the second derivative of the spectra. In the F435-F500 range, the model computed the curvature or the spread of the main peak (Fig. 5). For a narrow and high peak, the score of the corresponding sample will be high. A similar parameter was computed by the model for the F400-F435 range showing that in this area, there was some significant differences in curvature at about F415. Therefore, discrimination was based by adding the effect of the curvature at about F415 that changed the apparent spread of the main peak at F460 and could induced a slight peak shift.

Table 3. Performance of the NPLSDA in predicting a single flavor – E275 and E360.

	Confectionery	**Woody**	**Empy**	**Maple**	**Off flavor**
	E275				
2003	64%	71%	77%	70%	75%
2004	70%	77%	69%	67%	72%
Pooled	65%	72%	73%	67%	72%
	E360				
2003	69%	64%	74%	71%	72%
2004	65%	73%	79%	69%	70%
Pooled	67%	73%	78%	61%	69%

The performances of the NPLSDA model for E360 were similar to the ones for the model at E275 (Table 3 and 4). The success rate and the specificity were consistently better for the Empyreumatic flavor indicating that the change in curvature around F415 might be specific to this flavor.

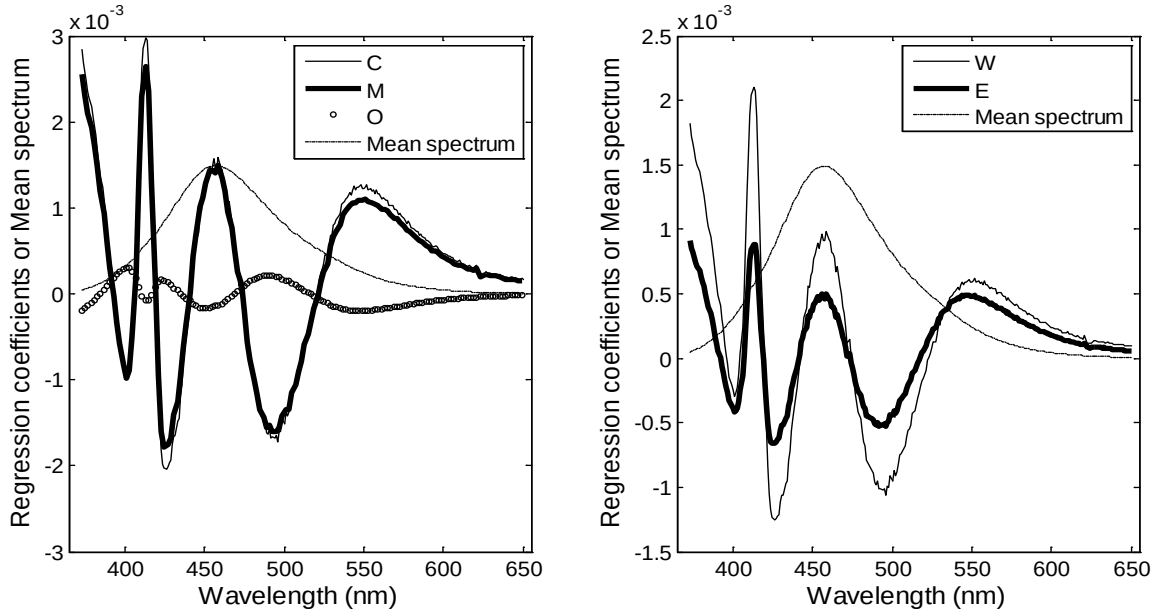

Figure 5. The b-coefficients for each flavor. NPLSDA with 5 latent variables on E360 spectra. Both years pooled.

CONCLUSION

Fluorescence spectroscopy is a valuable tool for measurement of maple syrup properties. The overall interest of the method is the simultaneous assessment of a number of parameters that can increase the market value of the product, including calibration of sensory variables, which are virtually impossible to measure otherwise on a large scale. Future research focuses on simplified measurement geometry, and automatic assessment of product authenticity.

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