

# Near infrared reflectance analysis of molasses and fats used in compound feed

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## Introduction

Molasses and fats are today widely used in feedstuff manufacture, chiefly as energy sources but also because they share certain technological and nutritive advantages as feed components. Since these simple ingredients are derived by agroindustrial processing from a basic commodity, their composition varies depending, *inter alia*, on agroclimatic and technology-related factors. In view of this variability, it is recommended that analysis includes measurement of moisture and sugars in molasses and MIU (moisture + impurities + unsaponifiable), free fatty acids and both saturated and unsaturated fatty acids in fat.<sup>1-3</sup> Liquid samples are generally more awkward to analyse, in terms of both laboratory handling and the need for more expensive, more sophisticated but also slower equipment than that traditionally used for the analysis of solid ingredients. For that reason, quality controls of these key ingredients may often be inadequate and, in some cases, certain parameters of nutritional interest may not be determined (for example fatty acids).

The purpose of the present study was to demonstrate the viability of NIR for quality analysis of fat and molasses used in the manufacture of feedstuffs.

## Material and methods

### Experimental material

A total of 42 samples of molasses (cane and beet) and 61 samples of fats (animal and oleins) were supplied by a Spanish compound feed manufacturer (SAPROGAL S.A.).

### Collection of NIR spectra

Samples of molasses and of melted fat were analysed using a Foss NIRsystems 6500 SY-I scanning monochromator fitted with a spinning cup, working in reflectance mode in the spectral range 400–2500 nm. Measurements were made in folded-transmission gold reflector cam-lock cups, with a pathlength of 0.1 mm. Two spectra were measured per sample, the mean spectrum being used for subsequent analysis.

## Reference analyses

### Molasses

The following parameters were determined: moisture [(1) infrared balance and (2) oven-dried with marine sand), crude protein (Kjeldahl), ash (heating furnace oven at 550°C), sodium chloride (potentiometric method) and total sugars (Luff–Schoorl).

### Fats

The following parameters were determined: moisture (oven drying), acidity (volumetric method expressed as % oleic), insoluble impurities (petroleum ether filtration), unsaponifiable (ethyl ether + KOH method) and fatty acids profile (gas chromatography).

## Chemometric treatment of data

Spectroscopic and chemical data were subjected to chemometric treatment using WinISI ver. 1.04 software.<sup>4</sup> NIR calibration equations were obtained and evaluated following Shenk and Westerhaus<sup>5</sup> and Williams and Sobering.<sup>6</sup> The following statistical parameters were used to select the best calibration equations: standard error for the calibration set (*SEC*), standard error of cross validation (*SECV*), coefficient of determination for the calibration process ( $R^2$ ) or the cross-validation process ( $r^2$ ) and ratios *RPD*<sup>6</sup> (*DT/SECV*) and *RER* (*Range/SECV*).

## Results and discussion

Table 1 shows statistics for the sample population and results for NIR calibration equations for predicting the chemical composition of molasses.

Calibrations obtained for crude protein (*SECV* = 0.14%;  $r^2$  = 0.99) and NaCl (*SECV* = 0.05%;  $r^2$  = 0.99) afforded a high degree of precision and accuracy. The equations obtained for sugars and ash accounted for 86% and 84%, respectively, of variability within the sample set used; and although in both cases the *RPD* ratio fell short of the value of three recommended by Williams and Sobering<sup>6</sup> for routine analyses, it is likely that a larger sample set would yield better statistics. In any case, the equation clearly enables swift and precise analysis of a parameter which is of great value in characterising molasses and which, at present, prompts considerable inter-laboratory error.<sup>7</sup> The NIR equation obtained for measuring moisture content, using the infrared balance as reference method, displayed poor predictive capacity (*SECV* = 1.69%,  $r^2$  = 0.42). This may be due to the use of an indirect reference method, leading to a non-error-free prior calibration. To confirm this hypothesis, a new calibration was

**Table 1. Characterisation of molasses from the calibration set and resulting calibration statistics.**

	Mean	Range	<i>SD</i>	<i>SECV</i>	$r^2$	<i>RPD</i>	<i>RER</i>
Moisture <sup>a</sup>	23.06	19.3–28.6	2.10	1.69	0.42	1.2	5.5
Moisture <sup>b</sup>	22.40	18.5–28.1	1.81	0.34	0.96	5.3	28.2
Protein	8.37	3.9–10.3	2.64	0.14	0.99	19.1	45.7
Ashes	10.61	8.0–13.8	1.47	0.60	0.84	2.5	9.7
NaCl	2.17	1.16–4.03	1.11	0.05	0.99	20.9	57.4
Total sugars	48.48	43.9–53.9	2.83	1.04	0.86	2.7	10.0

<sup>a</sup>Reference method: infrared balance

<sup>b</sup>Reference method: heating oven with marine sand

obtained for moisture content using oven heating with marine sand as the reference method; this afforded an excellent predictive capacity ( $SECV = 0.34\%$ ;  $r^2 = 0.96$ ).

Gillespie<sup>7</sup> obtained  $SECV$  values of 0.42%, 0.63%, 0.78% and 0.79% for crude protein, moisture, total sugars and ash, respectively, using a molasses sample set whose mean values for these parameters were very similar to those recorded here.

Chemical and NIR calibration statistics for the fats studied are shown in Table 2. In overall terms, NIR technology predicts a large number of quality parameters in fats destined for use in animal feeds. Of all the parameters studied here, only insoluble impurities correlated poorly ( $r^2 = 0.31$ ) with spectroscopic data. This is not surprising, given that we are not dealing with a chemically-defined entity<sup>8</sup>. For practical commercial analysis of fat, the parameter generally used is MIU, which jointly covers values for insoluble impurities, moisture and the unsaponifiable fraction. This analytical parameter provides considerable information on the non-nutritional fraction of a fat,<sup>2</sup> and is one of the criteria to be used in energy evaluation.<sup>3</sup> As Table 2 shows, the equation for predicting MIU affords a high degree of precision and accuracy ( $SECV = 0.38\%$ ,  $r^2 = 0.94$ ). The NIR equation obtained for predicting moisture content ensures precise evaluation of this parameter ( $SECV = 0.14\%$ ,  $r^2 = 0.88$ ). Moisture assessment is of major importance, since moisture accelerates oxidation by reacting with the equipment (tanks) and releasing oxidation-catalysing metals.

There are no studies in the NIR literature addressing the use of this technology for the analysis of fats used in the feed industry, so some comment is required on the precision and accuracy of the equations obtained in fats and oils for human consumption, an area where NIR applications are becoming

**Table 2. Characterisation of animal fats from calibration set and resulting calibration statistics.**

	Mean	Range	<i>SD</i>	<i>SECV</i>	$r^2$	<i>RPD</i>	<i>RER</i>
Moisture	0.44	0.07–2.10	0.42	0.14	0.88	3.0	14.0
Free fatty acids	7.22	0.65–11.40	1.90	0.83	0.82	2.3	13.0
Insol. impurities	0.32	0.01–0.69	0.16	0.13	0.31	1.2	5.1
Unsaponifiable	1.45	0.18–5.80	1.23	0.45	0.87	2.7	12.5
MIU <sup>a</sup>	2.26	1.04–7.59	1.60	0.38	0.94	4.2	17.33
Lauric acid	0.11	0.06–0.13	0.02	0.009	0.67	2.2	7.8
Miristic acid	1.95	0.11–3.01	0.75	0.13	0.97	5.8	22.1
Palmitic acid	21.72	7.16–25.89	5.47	0.76	0.98	7.2	24.5
Palmitoleic acid	2.27	0.09–3.34	0.86	0.33	0.86	2.6	10.0
Stearic acid	13.65	4.05–20.38	4.60	0.74	0.98	6.2	22.2
Oleic acid	37.73	24.59–45.55	3.43	0.97	0.92	3.5	21.7
Linoleic acid	13.55	3.82–55.95	14.56	0.65	0.99	22.4	80.4
Margaric acid	0.67	0.05–1.13	0.28	0.06	0.96	4.7	18.6
Margaroleic acid	0.42	0.19–0.58	0.08	0.039	0.77	2.1	10.0
Araquidic acid	0.19	0.14–0.43	0.06	0.024	0.86	2.5	12.1
Behenic acid	0.21	0.01–0.72	0.21	0.035	0.97	6.0	20.3

<sup>a</sup>MIU: moisture + impurities + unsaponifiable

increasingly important. Although the acidity equation displayed satisfactory predictive capacity ( $SECV = 0.83\%$ ,  $r^2 = 0.82$ ), greater precision and accuracy have been obtained in oils for human consumption.<sup>9</sup> Precise knowledge of the proportion and/or the ratio of saturated to unsaturated fatty acids is among the most crucial criteria when assessing the energy and nutritional value of fats.<sup>8</sup> In practice, the high cost of fatty acid analysis has led analysts to prefer the so-called iodine index as a measure of the degree of unsaturation. This index enables assessment of the fatty acid profile and of the saturated/unsaturated ratio. The results obtained here (Table 2) show that NIR technology affords a high degree of precision and accuracy for predicting the major fatty acids, such as palmitic, stearic, oleic and linoleic acids, although even greater precision has been obtained for rendered Iberian pig fat using a similar analytical method.<sup>10,11</sup> Differences in precision and accuracy may be accounted for by the greater presence of impurities in fats used in the feed industry and the small number of samples displaying the calibrations shown in Table 2. NIR technology also enables excellent, or at least acceptable, prediction of other fatty acids present in smaller amounts.

## Conclusions

NIR technology provides a viable method of chemical and, therefore, nutritional characterisation of two major liquid ingredients in animal feed. Given the current crisis of consumer confidence in ingredients of animal origin, characterisation of animal fats could usefully be extended; NIR technology offers a perfectly feasible means of doing this.

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