Determination of the Tanning Degree of Vegetable-Tanned Leather by Infrared Spectroscopy (FTIR)

by
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Abstract

We have developed an analytical method to determine the tanning degree (TD) and the parameters associated with its calculation by means of the instrumental technique of Fourier Transform mid-infrared spectroscopy (FTIR) with Attenuated Total Reflection Mode Module (ATR) and the chemometric treatment of spectral data. Early studies show that it is possible to make the determination of several parameters simultaneously (TD, combined tannins, hide substance and leather substance) using FTIR spectra recorded directly on the leather tanned with a mixture of mimosa and quebracho, thus reducing dramatically the time of analysis and avoiding the use of pollutants.

Resumen

Se ha desarrollado un método analítico para determinar el índice de curtición (IC), así como de los parámetros asociados a su cálculo, mediante la técnica instrumental de la espectroscopía de infrarrojo medio con Transformada de Fourier (FTIR) con un módulo Módulo de Reflexión Total Atenuada (ATR) y el tratamiento quimiométrico de los datos espectrales. Los primeros estudios realizados ponen de manifiesto que es posible realizar la determinación de varios parámetros simultáneamente (IC, taninos combinados, sustancia piel y sustancia cuero) utilizando los espectros FTIR registrados directamente sobre las pieles curtidas con una mezcla de mimosa y quebracho, reduciendo espectacularmente el tiempo de análisis y evitando la utilización de productos contaminantes.

Introduction

Physical tests, chemical analyses, and fastness tests serve the purpose of assessing the quality of leather. The International Union of Leather Technologists and Chemists (IULTCS) recognizes a number of methods of chemical analysis as official for leather assessment. These methods have the abbreviations IUF, IUC and IUP followed by a number and many of them have their equivalent in the ISO and EN Standards. Some of these methods determine the amount of metal (chromium, aluminium, etc.) present in the leather. In a vegetable-tanned leather, the equivalent to the amount of metal in leather is called tanning degree (TD), or also the combined tannin. It is known that the amount of tannin present in the leather affects some of its properties[1], which justifies our interest in such methods of analysis. The traditional analyses to determine the combined tannins and subsequently calculate the tanning degree are very time-consuming and require considerable experience to get replicable[2] results. The combined tannins, expressed on the weight of the dried and degreased leather, are calculated by subtracting from 100 the sum of hide substance, inorganic non-washable ash and total washable materials (organic and inorganic). This is a process that involves several independent experiments. However, it has several drawbacks, as is quite slow and involves the use of pollutants. Also, the reproducibility of the results depends on the experience of the analysts performing the experiments. The results obtained from traditional methods of analysis are rather empirical.

In recent years, analytical techniques have advanced greatly. The synergy of different spectroscopic techniques with the powerful ability to perform mathematical calculations thanks to advances in computer technology have enabled new methods of analysis that are faster, more sensitive and cost-effective. These analyses enable to determine more parameters simultaneously and a great deal of samples[3]. Several research teams have studied the possibilities of these analytical techniques to determine the tannins present in different substances. Donkin[4] determined the raw material used to make vegetable extracts by means of near infrared spectroscopy (NIR). Cuadros et al.[5] began to study the application of NIR in the determination of tannin and non-tannin in vegetable extracts with good results. In this line, Gutterres[6]

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performed a study on the diffusion and degree of fixation of tannins in vegetable tanning by NIR. Nakagawa[7] studied the characterization and the molecular weight of vegetable tannins by using various spectroscopic techniques. Ozgunay et al.[8] investigated the molecular structure of valonea tannin by means of mid-infrared spectroscopy with Fourier Transform (FTIR).

In this current paper we intend to move forward in the research and study the possibility of determining directly the tanning degree of vegetable-tanned leather, without having to manipulate or destroy the sample. To this aim we have used the FTIR technique and a module of Attenuated Total Reflection Mode (ATR) to record the spectra of the vegetable-tanned leather samples. We have calculated the parameters with the help of multivariate calculation methods. This test system is suitable for systematic control of different batches of production due to the fact that the sample is never destroyed. In addition, this system is fast and cost-effective, as it does not require expensive instrumental techniques. This method can also be very useful for monitoring on-site the development of a vegetable-tanning process, especially in the penetration and fixation stages. By cutting a sample of leather, drying it, splitting it and analysing every layer the precise condition of the tanning can be monitored in less than 10 minutes.

THEORETICAL FRAMEWORK

Mid-infrared spectroscopy by Fourier transform (FTIR)

The origin of absorption in the infrared region lays in the vibration movements of the molecule, which can be either of stretching type (when a change in the interatomic distance along the axis of the bond between the two atoms takes place) or of bending type (in which a change in the angle of the bond occurs). The wave number ranges between 650 to 4000 cm^{-1}. Besides the vibrations described above, in polyatomic molecules, vibronic coupling or interaction of vibrations can occur. In this vein, the mid-infrared spectra of a pure substance consists of a set of narrow bands of absorption corresponding to the vibration transitions of the molecules, whose position is influenced by their surroundings. Therefore, each substance will have a very characteristic infrared spectra, which makes this technique very suitable for qualitative analysis. The positions of the bands, in the infrared and in the vibrations originating them, are well documented[9]. Likewise, the information generated by these bands can be used for quantitative analysis through adequate mathematic treatment of spectral data.

Chemometrics

Instrumental techniques, especially spectroscopic techniques, generate large amounts of data from the samples in short intervals of time. However, this does not automatically imply that the more data obtained, the more information is available in the system. These data become valuable for analysts only after having been interpreted correctly, thereby becoming information. Chemometrics is defined as an area of chemistry that designs procedures to optimize the final results of experiments or processes by using mathematical methods, formal logic and statistics.

Chemometrics covers many objectives, such as signal filtering, pattern recognition methods and calibration methods (multivariate techniques through which it seeks a relationship between analytical signal and sample properties). In this study, quantitative analysis is performed in two stages. First, calibration has the purpose of selecting the model that describes the relationship between the reflectance values of the FTIR spectrum and the concentrations of the analytes studied. The second stage is prediction, which uses the calculated model to predict concentrations of an unknown sample, based on its spectrum. The calibration method used in this study was Partial Least Squares Regression (PLSR)[10]. We selected linear combinations of the predictor variables that were highly related to the response variables and were able to explain the variation in the predictors. If the answer was a single variable it was called PLS1, whereas when the response was multivariate it was called PLS2.

EXPERIMENTAL

Samples

The analyses were performed on bovine hides vegetable tanned with the following extracts:

Quebracho extract Ato Unitan. 72% of tannins. pH (6.9 ºBé) = 4.3-4.8

Mimosa extract Clarotan. 68% of tannins. pH (6.9 ºBé) = 4.0-4.5.

The formulation below was used to tan the hides (Table I):

TABLE I
Formulation

(on pre-tanned weight):

Tanning	100% H_2O 20% Mimosa + 20% Quebracho	Mixed at 50% in three doses at 30 min. intervals r – 12 h. Check through tannage Overnight rest
Fatliquoring	100% H_2O 3% Sulphated oil 0.5% Raw oil 1.5% Sulphited oil 0.5% HCOOH (1:10)	 T = 40ºC r – 45 min. r – 30 min. 1 day rest
Toggling		

Instrumentation and Software

The FTIR spectra of the leather samples were recorded with a Nicolet (Impact 4000) spectrophotometer equipped with an ATR cuvette that allowed us to obtain the complete spectra in the wave number interval of 4000 to 650 cm^{-1}, 32 spectra on average. The calculations were conducted using the Unscrambler software, version 7.5, which allows the calculation of a single PLS1 variable and PLS2 multivariate calculation.

Reference Methods

All bovine leather samples were analyzed in triplicate using the official methods recognized by the IULTCS to compare results with those obtained using spectroscopic analysis.

The chemical analyses and physical tests carried out, together with the methods followed[2], are detailed below:

- IUC 4. Determination of matter soluble in dichloromethane and free fatty acid content
- IUC 5. Determination of volatile matter
- IUC 6. Determination of water soluble matter, water soluble inorganic matter and water soluble organic matter
- IUC 7. Determination of sulphated total ash and sulphated water insoluble ash
- IUC 10. Determination of nitrogen and hide substance

From the results of these analyses we calculated the values of the leather substance, combined tannins and tanning degree. These amounts are expressed in relation to dried and degreased leather weight and were calculated according to the following formulas:

Leather substance (%) = 100 – Water soluble matter – Sulphated total ash (1)

Combined tannins (%) = 100 – Water soluble matter – Sulphated total ash – Hide substance (2)

*Tanning degree (%) = (Combined tannins / Hide substance) * 100 (3)*

Obtained results will be called "reference values."

Obtaining spectra

FTIR spectra were recorded with a ATR cuvette that allows the reading directly on the leather samples. This technique enables the analyst to obtain spectra of opaque leather samples due to the reflection of infrared radiation on the surface of the solid. When a leather sample is placed in contact with the reflective surface of a crystal of high refractive index, radiation is not totally reflected but penetrates slightly into the leather surface to a depth of about 1-5 microns, producing an absorption spectrum that provides information on the vegetable extracts used in vegetable tanning and on other parameters such as hide and leather substance. To obtain representative spectra for each sample the possible variability of the distribution of these vegetable extracts has been taken into consideration. Therefore, measurements on each side of all leather samples (grain and flesh) were performed. Figure 1 shows the simplicity of recording of FTIR spectra from leather samples.

Figure 1. Data acquisition

Obtaining the spectrum takes a very short time (seconds), which emphasizes the overall simplicity and the high speed of the analysis. Besides, the concentration of an individual analyte or of several analytes simultaneously can be obtained from two spectra of each of the samples.

Calculation of Results

The two mathematical methods PLS1 and PLS2 enable analysts to determine the following parameters directly from the FTIR spectra: tanning degree, combined tannins, hide substance and leather substance, individually with the former method and simultaneously with the latter method.

We compared the results of the official method and the FTIR methodology through the joint test of slope and intercept at origin. This test is based on calculating, via linear regression, the equation of a straight line whose independent variable is the predicted value and the dependent variable is the reference value. To pass the test, the range of values of the slope, within the accepted margin of error, must include 1, and the cutting of the intercept, must include 0. If the joint test is passed, this means that the proposed methodology yields results comparable to those of the reference methods, with no systematic errors. It also means that the single errors of each sample should not be taken into account, as the whole prediction sample passes the statistical test.

With the official methods, the value of the analysed parameter throughout the whole leather volume is obtained, as the analysis is performed with ground leather. With our method, we propose measurements to be carried out primarily on the two leather surfaces (grain and flesh). We wanted to make sure that an uneven distribution of vegetable extracts would not influence the comparison of results between the two analytical methods under study. To this end, we verified the degree of penetration and distribution of the vegetable extract in the leather by splitting some of the leather samples and recording the FTIR spectra obtained.

RESULTS AND DISCUSSION

To determine the tanning degree and the other parameters involved in the numerical calculation of this degree, the Partial Least Squares Regression (PLSR) has been applied as multivariate calibration method. Calculations have been carried out from FTIR spectra and the reference values obtained for the samples have been described previously. Figure 2 shows an example the FTIR spectra of two of the leather samples. The spectral differences between the leathers can be observed, with a TD calculated value of 43.3 and 63.1 respectively.

Table II shows the average results obtained using the official methods.

The set of available samples is divided into two parts, the calibration set, with 49 samples, and the prediction set, with 18 samples. The calibration set consists of a limited number of samples that represents the whole set. This allows us to establish relationships between the properties to be determined and the analytical measurements. The prediction set consists of samples that have not been used in the construction of the model. It enables us to determine the predicting capacity of the model.

TABLE II
Official methods: Analysis results

Number of samples = 67

Parameter	Average (%)	Range (%)
Hide substance	60.82	56.78-66.23
Leather substance	92.11	88.3-95.80
Combined tannins	31.32	24.37-34.89
Tanning Degree	51.01	43.30-63.10

The different models were calculated in absorbance mode, first and second derivatives in the measurement interval (650 a 4000 cm^{-1}) to determine the best quantification conditions.

Table III shows the values of relative mean square error of calibration (RSEPC) and prediction (RSEPP) obtained in a PLS1 model calculated in absorbance mode with 11 or 12 principal components (PC) for the different analytes, which were suggested by Unscrambler software as optimal, based on the minimization of y-residual variance. Table III also shows the results obtained in a PLS2 model in absorbance mode and with 17 PC. These models allow the calculation of parameters such as TD, hide substance content, leather substance content and tannins absorbed in the leather.

RESPC and RSEPP values are calculated from the values in the official methods and the values provided by the PLS1 and PLS2 programs both for calibration and prediction samples. Tables IV and V show the predicted values for the two models with the optimal number of factors and the reference value for

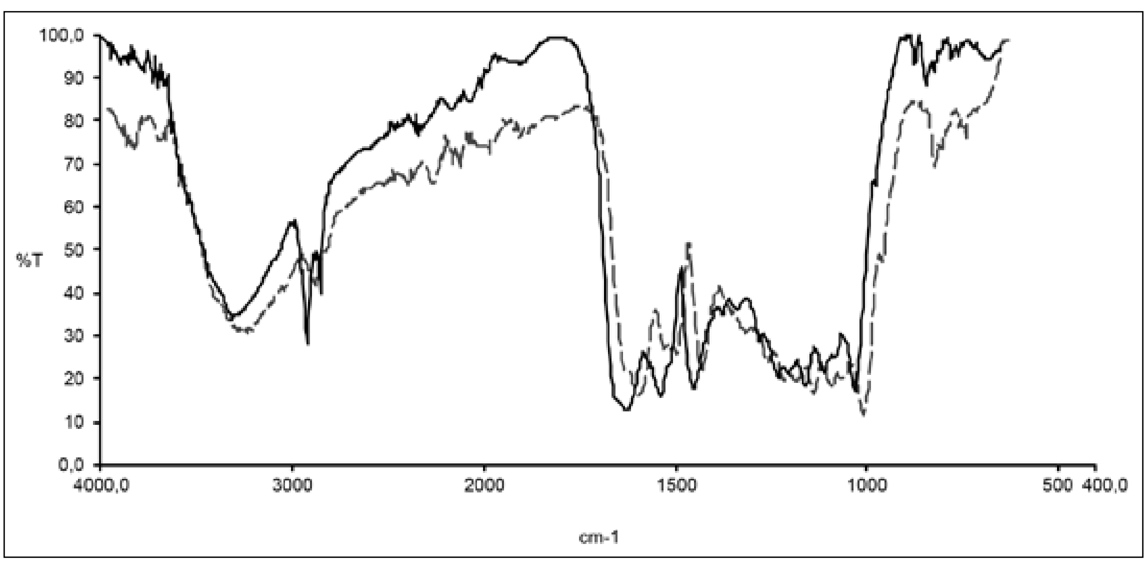

Figure 2. Example of hte FTIR spectra of vegetable -tanned leather samples with a calculated tanning degree of 43.30% and 63.10% respectively.

TABLE III
RSEPC and RSEPP values

Parameter	RSEPC (%)		RSEPP (%)	
	PLS1	PLS2	PLS1	PLS2
Hide Substance	0.75	1.46	3.56	3.27
Leather Substance	0.36	0.90	2.01	2.24
Combined tannins	1.10	1.72	5.27	5.70
Tanning Degree	2.00	2.24	5.72	7.17

each of the 18 prediction samples, in the case of TD, and 15 samples in the case of the tannins, together with the value of relative error (%), which makes it possible to calculate the RSEPP final value. The high number of principal components (17) does not imply any problem given both the strength of the predicted values and the utilization of an only model to calculate the indicated parameters, in the case of PLS2. When the calculations are carried out through PLS1 the calculated models use 11 PC.

Figure 3 shows the graphics with the values predicted with PLS2 versus the reference values in the case of TD. Figure 4 shows this in the case of the combined tannins.

The points adjust fairly well to the regression line of slope one (1.07 ± 0.16 in the predicted values in the calculation of TD and 1.07 ± 0.18 in the predicted values in the calculation of the combined tannins) and to the ordinate at the zero origin (-4.35 ± 8.42 in the TD and -2.73 ± 5.53 in combined tannins).

The application of the joint test of slope and ordinate in the origin of both data sets results in no statistically significant differences between predicted and reference values, with a significance level of 95%. Thus, the proposed methodology gives results comparable to those of official methods, with no systematic errors.

Figure 5 shows the graphics of predicted values versus reference values in the case of TD, whereas figure 6 shows this in relation to combined tannins calculated through PLS1.

Applying this calculation the points adjust fairly well to the regression line of slope one (1.14 ± 0.25 in the predicted values in the calculation of TD and 1.20 ± 0.38 in the predicted values in the calculation of the tannins) and zero intercept (-7.04 ± 12.90 in the TD, and -6.80 ± 11.59 in the tannins). The application of the joint test of slope and intercept sets results in no statistically significant differences between the predicted and the reference values, with a significance level of 95%. Thus, the proposed methodology also yields results comparable to those of official methods, with no systematic errors.

In addition, when assessing the degree of penetration and fixation of vegetable extracts, the results of the analytes for the different horizontal cuts of the same sample have values similar to those obtained in the grain layer and flesh. Therefore, the results from the registration of the spectra are representative of the entire sample.

CONCLUSIONS

This paper presents the results of the first studies on the possibility of simultaneously determining different analytes (TD, combined tannins, hide substance and leather substance) directly on samples of vegetable-tanned leather with mimosa and quebracho extracts. The techniques used are mid-infrared spectroscopy (FTIR) and multivariate calibration.

An appropriate model of PLS2 and the PLS1 models calculated for each analyte yielded good results in the samples studied in

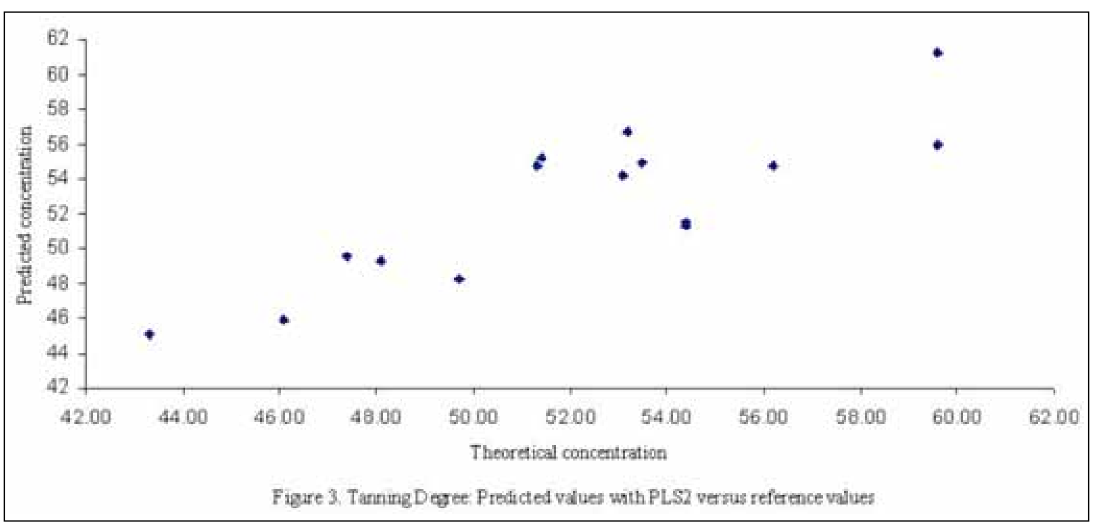

Figure 3. Tanning Degree: Predicted values with PLS2 versus reference values.

TABLE IV
RSEPP values of each sample (PLS2)

Sample	Tanning degree (%)			Combined tannins (%)		
	Reference	Predicted	Error (%)	Reference	Predicted	Error (%)
1	47.40	49.52	4.5	29.7	30.38	2.3
2	51.30	54.77	6.8	-	-	-
3	59.60	55.98	6.1	-	-	-
4	59.60	61.28	2.8	-	-	-
5	45.30	38.43	15.2	28.4	24.37	14.2
6	45.30	40.91	9.7	28.4	26.50	6.7
7	49.70	48.26	2.9	29.7	29.33	1.2
8	48.10	49.24	2.4	29.1	29.40	1.0
9	43.30	45.03	4.0	27.4	29.03	5.9
10	46.10	45.92	0.4	28.6	28.88	1.0
11	53.10	54.18	2.0	31.8	29.83	6.2
12	54.40	51.27	5.8	33.7	32.62	3.2
13	56.20	54.73	2.6	33.5	33.57	0.2
14	53.50	54.89	2.6	33.4	33.56	0.5
15	54.40	51.47	5.4	31.7	30.21	4.7
16	47.60	38.26	19.6	29.3	26.12	10.9
17	53.20	56.74	6.7	32.9	34.21	4.0
18	51.40	55.24	7.5	31.8	33.66	5.9
Average			7.17			5.70

TABLE V
RSEPP values of each sample (PSL1)

Sample	Tanning degree (%)			Combined tannins (%)		
	Reference	Predicted	Error (%)	Reference	Predicted	Error (%)
1	47.40	48.65	2.6	29.7	29.63	0.2
2	51.30	55.41	8.0	-	-	-
3	59.60	56.73	4.8	-	-	-
4	59.60	39.15	5.0	-	-	-
5	45.30	41.20	13.6	28.4	24.81	12.6
6	45.30	41.19	9.1	28.4	26.50	6.7
7	49.70	47.73	4.0	29.7	29.14	1.9
8	48.10	48.54	0.9	29.1	28.18	3.2
9	43.30	47.68	10.1	27.4	29.60	8.0
10	46.10	46.71	1.3	28.6	28.87	0.9
11	53.10	55.05	3.7	31.8	30.26	4.8
12	54.40	51.85	4.7	33.7	32.92	2.3
13	56.20	54.73	1.3	33.5	34.30	2.4
14	53.50	56.94	2.4	33.4	33.29	0.3
15	54.40	55.43	1.9	31.7	30.58	3.5
16	47.60	48.70	2.3	29.3	26.51	9.5
17	53.20	56.55	6.3	32.9	33.99	3.3
18	51.40	55.34	7.6	31.8	33.48	5.3
Average			5.72			5.27

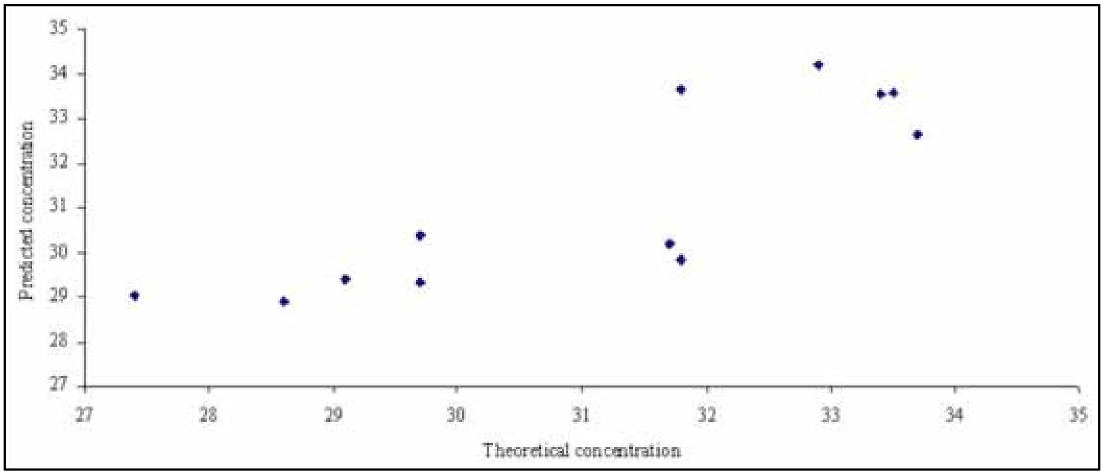

Figure 4. Combined tannins: Predicted values with PLS2 versus reference values.

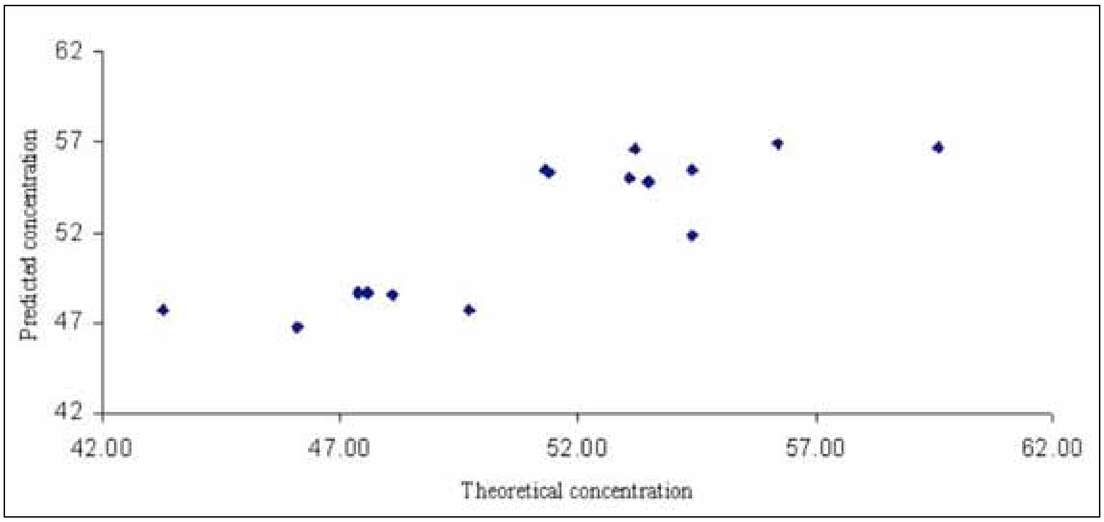

Figure 5. Tanning Degree: Predicted values with PLS1 versus reference values.

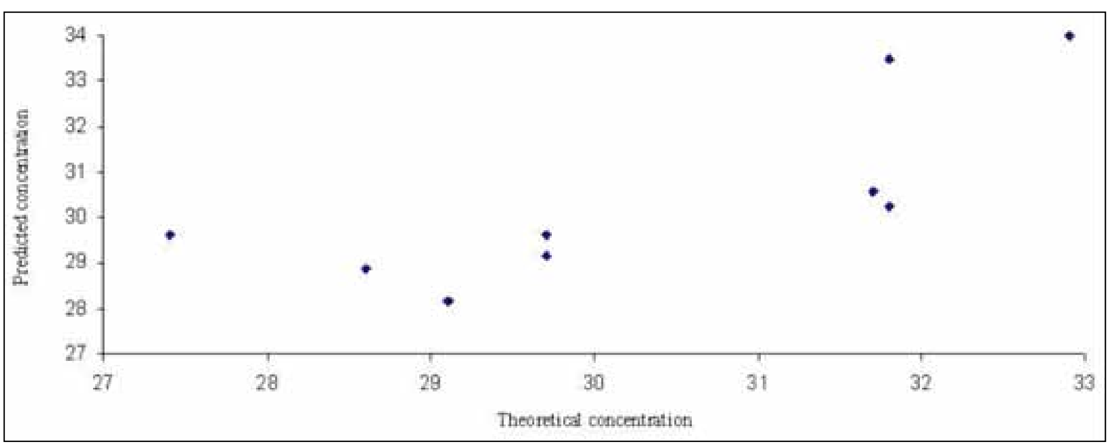

Figure 6. Combined tannins: Predicted values with PLS1 versus reference values.

the prediction. Quantification errors were of an order of magnitude accepted in the tanning industry. It can therefore be stated that there are no significant differences in values obtained through both calculation methods.

The possibility of a fast quantification of analytes from a large number of samples, compared to official methods that require much more time-consuming and very often empirical procedures, leads us to conclude that the proposed method is an advantageous alternative to the official method. This is specially the case regarding the systematic control of the evenness of production batches and the on-site control of the development (penetration and fixation) of vegetable tanning.

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