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Enzymatic-spectrophotometric determination of sucrose in coffee beans

Ángela Alcázar, J. Marcos Jurado, M[a] Jesús Martín, Fernando Pablos*, A. Gustavo González

Department of Analytical Chemistry, Faculty of Chemistry, University of Seville, E-41012 Seville, Spain

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Abstract

A spectrophotometric method for determining sucrose is proposed. Sucrose is hydrolyzed by invertase into glucose and fructose. Then, glucose is oxidized in presence of glucose oxidase and the produced hydrogen peroxide reacts with phenol-4-sulfonic acid sodium salt and 4-aminoantipyrine in presence of peroxidase, yielding a pink dye with an absorption maximum at 505 nm. This method was validated following the EURACHEM and VAM project guidelines for method validation. Trueness, precision, robustness, sensitivity and linearity were considered. The method was applied to the determination of sucrose in green and roasted coffee beans. A comparison with the HPLC method with pulsed amperometric detection was carried out.
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Keywords: Sucrose; Coffee; Enzymatic determination; Spectrophotometry

1. Introduction

Coffee flavour is developed during the roasting process from aroma precursors present in green beans. The aroma formation is very complex and includes Maillard and Strecker reactions and flavour precursors degradations [1]. Among these precursors, sucrose is one of the most important [2]. Sucrose is the major free sugar present in green coffee, in actual amounts dependent upon the species and sources of the coffee [3]. Sucrose has been quantitated in coffee beans by using ion chromatography with pulsed amperometric detection (IC-PAD) [4]. The ion chromatography separation of simple carbohydrates using anion exchange columns and alkaline mobile phases is relatively straightforward. However, the detection presents several handicaps and drawbacks [5]. Only electrochemical detection involving a series of potential pulses, intending to avoid the poisoning effect of the carbohydrates on the working electrode, is suitable. From a validation point of view, this methodology involves a number of experimental factors to be controlled, such as detection, oxidation and reduction potentials, delay, integration, oxidation and reduction times [6–8]. This fact limits dramatically the robustness of the method just because the high number of experimental factors. Moreover, the obtained values of intermediate precision are rather disperse. Thus, due to these handicaps we call on another procedure to determinate sucrose in coffee beans.

The determination of low molecular weight sugars, specially glucose or sucrose, can be easily carried out by using enzymatic procedures, some of them being based on the use of invertase and glucose oxidase [9,10]. In this case, instead of using a chromatographic separation, selectivity is achieved from the features of the enzymatic process, leading to the quantitation of sucrose without interferences from other glucides.

The aim of this paper is to develop an enzymatic spectrophotometric method for determining sucrose in samples of coffee beans. This procedure is easy to handle, speed enough and cheaper than those involving a chromatographic separation with pulsed amperometric detection.

2. Outline of the method

Sucrose is a disaccharide that can be hydrolyzed by invertase into glucose and fructose. β-D-glucose is oxidized in presence of glucose oxidase (GOD) to D-gluconolactone.

* Corresponding author. Tel.: +34 754 557 173; fax: +34 954 557 168.
E-mail address: fpablos@us.es (F. Pablos).

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The hydrogen peroxide produced in this reaction is determined by means of phenol-4-sulfonic acid sodium salt and 4-aminoantipyrine in presence of peroxidase (HRP), yielding a coloured product with an absorption maximum located at 505 nm. The reactions sequence is shown in Fig. 1. The amount of the dye formed is a measure of the sucrose concentration in the sample.

3. Experimental

3.1. Apparatus

An Unicam UV series 500 (Thermo Spectronic, Cambridge, UK) equipped with quartz-cells of 1.0 cm was used for absorbance measurements.

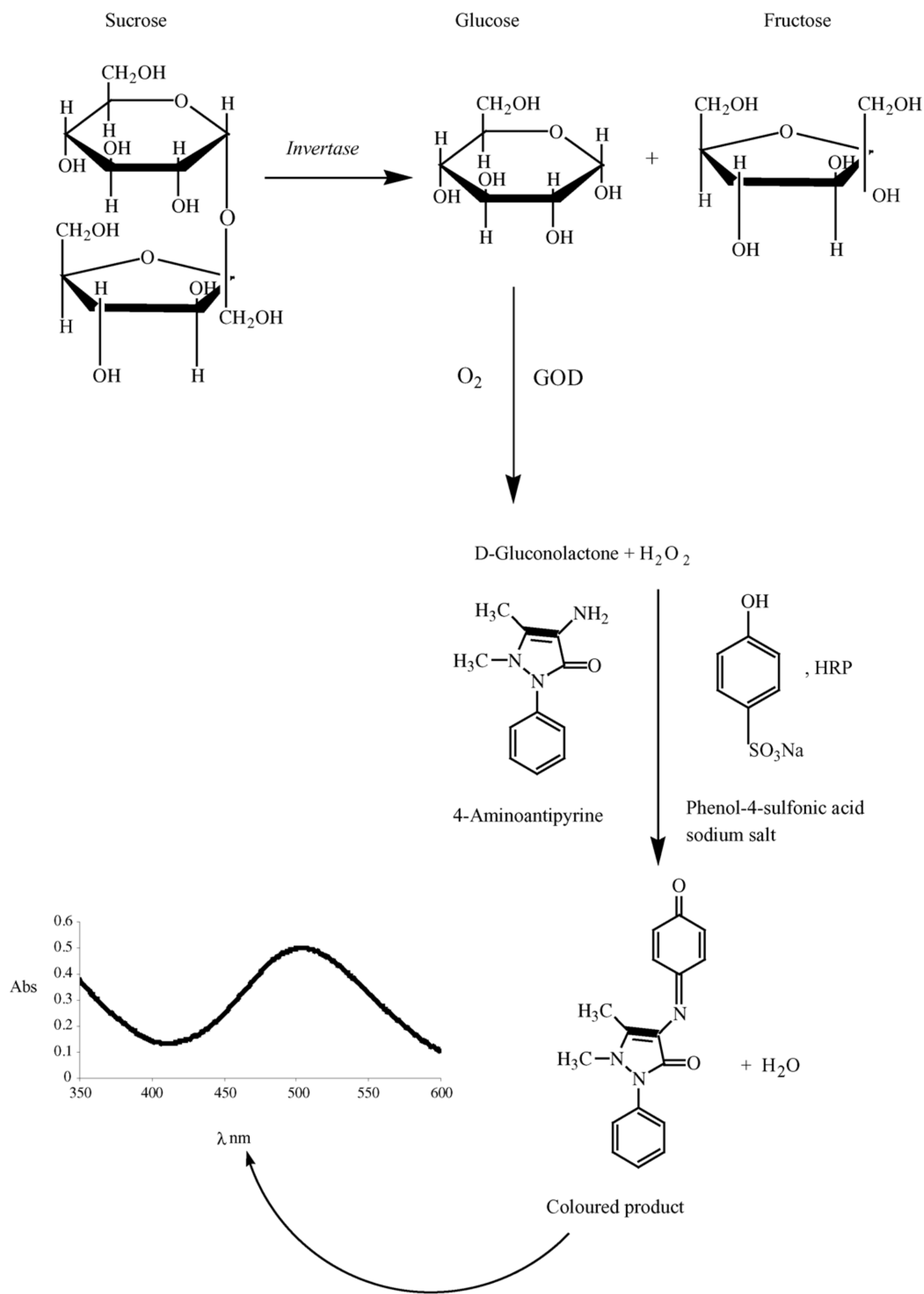

Fig. 1. Sequence of reactions to obtain the coloured product.

An IKA WERKE M20 grinder & crusher apparatus (GmbH & CO. KG Staufen, Germany) fitted with M23 star-shaped cutter, specially designed for vegetal samples, was used for processing coffee beans. The apparatus enables refrigeration of crushing chamber by means a flow of liquid nitrogen.

A Techne Tempette TE-8D thermostat assembled to a Techne Refrigerated bath RB5 (Techne, Duxford, Cambridge, UK) was used for controlling the temperature of solutions with a confidence of about ±0.1 °C.

3.2. Reagents and solutions

Invertase from baker's yeast (*Saccaromyces cerevisiae*), peroxidase from horse radish (HRP), glucose oxidase from *Aspergillus niger* (GOD), 4-aminoantipyrine, D-(+)-sucrose, D-(+)-glucose anhydrous, α,α,α-Tris-(hydroxymethyl)-methylamine, phenol-4-sulfonic acid sodium salt dihydrate, citric acid, sodium citrate, acetic acid, zinc acetate and potassium hexacyanoferrate(II) were of analytical grade and were obtained from Fluka (Buchs, Switzerland).

Trinder solution was prepared dissolving 4-amino-antipyrine (0.5 mM), phenol-4-sulfonic acid sodium salt (20 mM), GOD (15 U/mL) and HRP (10 U/mL) in α,α,α-Tris-(hydroxymethyl)-methylamine buffer (0.02 M, pH 7). Invertase solution (72 U/mL) was prepared dissolving invertase in citrate buffer (0.1 M, pH 4.6). Carrez I solution was prepared dissolving 21.9 g of zinc acetate and 3 mL of acetic acid in 100 mL of water. Carrez II solution consisted of 10.6 g of potassium hexacyanoferrate(II) dissolved in 100 mL of water.

Milli-Q treated water with resistivity higher than 18.2 MΩ cm^{-1} was used throughout.

3.3. Samples

Green coffee samples were supplied from Centre National de Recherche Agronomique (CNRA) (Ivory Coast). Roasted coffee samples were obtained from local stores. Before analysis, laboratory samples were ground and sieved (0.2 mm) to ensure the homogeneity of the assay portions. The samples were dried at 103 °C until attain constant weight for determining their moisture.

3.4. Procedure

Each coffee sample was extracted in a 50-mL capped tube. Each tube contained about 300 mg of coffee powder to which 25 mL of distilled water were added. Extraction of sucrose was achieved by heating the tubes at 60 °C for 15 min, tubes being hand shaken every 5 min. Colloidal material present in the aqueous extract was precipitated by adding 0.5 mL of Carrez I solution plus 0.5 mL of Carrez II solution. The solution was then filtered (0.22 μm) and transferred into a 100-mL volumetric flask, diluting with water till the mark. An aliquot of 4 mL of the obtained solution plus 1 mL of invertase was heated at 55 °C for 15 min to hydrolyze the sucrose. About 2 mL of the hydrolyzed solution were taken to a test tube and then 2 mL of Trinder solution were added. This mixture was allowed to stand for 18 min to let the coloured product be formed. The absorbance was measured at 505 nm.

Table 1
Results of repeatability and reproducibility study

Determination	Repeatability[a]	Reproducibility[a]
1	6.8	6.3
2	6.7	6.4
3	6.8	6.5
4	6.5	6.7
5	6.9	6.4
6	6.7	6.5
7	6.6	6.7
8	6.8	6.8
9	6.8	6.5
Mean	6.7	6.5
R.S.D. (%)	1.8	2.5

[a] Sucrose in % (w/w) dry base.

4. Method validation

In the following the procedure will be validated for the matrices involved in the coffee samples according to the EURACHEM [11] and the VAM project [12].

4.1. Precision

The first parameter to be evaluated is repeatability in order to consider a rather pure random variation. A control sample was selected for evaluating repeatability. The control sample was analysed nine times without changing any operational module in a short period of time. The results are presented in Table 1. The relative standard deviation in repeatability conditions ($R.S.D._{\cdot repeat}$) was 1.8%. Once repeatability was studied, the reproducibility was estimated in terms of intermediate precision conditions. The control sample was split into nine assay portions, which were analysed two times per week during 2 months from randomized selection. The results obtained are gathered in Table 1. Accordingly, the relative standard deviation in intermediate precision conditions ($R.S.D._{\cdot ip}$) was 2.5%. This finding is compatible with the recommendations of AOAC (2.7–2.8%) for the analyte level assayed [13].

Moreover, by applying the *F*-test:

$$F = \frac{(R.S.D._{\cdot ip})^2}{(R.S.D._{\cdot repeat})^2} = 1.93 \leq F_{crit}(8, 8, 0.05) = 4.43$$

Then, it can be concluded that there is no significant difference between the repeatability and the intermediate precision variance.

Table 2
Recoveries and uncertainties

w (mg)	C_{spike} (mg/L)	U^2 (C_{spike})	$\bar{R}_m$ (%)	$u(\bar{R}_m)$ (%)
10	200	16.660	106	2.5
20	400	18.645	105	1.7
30	600	21.952	103	1.5
45	900	29.390	100	1.4

4.2. Trueness

The trueness study was carried out following the Barwick and Ellison recoveries method [12]. The control sample together with the spiked ones were analysed by quadruplicate at four fortification levels as indicated in Table 2.

The recovery ($\bar{R}_m$) and its uncertainty $u(\bar{R}_m)$ at every spike were evaluated as

$$\bar{R}_m = \frac{\bar{C}_{obs} - \bar{C}_{native}}{C_{spike}}$$

$$u(\bar{R}_m) = \bar{R}_m \sqrt{\frac{s^2_{obs} + s^2_{native}}{n(\bar{C}_{obs} - \bar{C}_{native})^2} + \frac{u^2(C_{spike})}{C^2_{spike}}}$$

Being S_{obs} and S_{native} the corresponding standard deviations of the quadruplicate ($n=4$) determination of a given level. The uncertainty $u(C_{spike})$ is the standard uncertainty in the concentration of the spike solution:

$$C_{spike} = \frac{wP}{V}$$

where w is the mass of sucrose standard weighed and P its purity. V is the volume of the volumetric flask used for preparing the spike solution. The corresponding uncertainty will be

$$u(C_{spike}) = C_{spike}\sqrt{\frac{u^2(w)}{w^2} + \frac{u^2(P)}{P^2} + \frac{u^2(V)}{V^2}}$$

$u(w)$, $u(P)$and $u(V)$ being the corresponding uncertainties for w, P and V, respectively. The uncertainty in the mass determination was computed after calibration of the balance. The analytical balance was calibrated by using a 100 g standard weight E2 OIML with a tolerance ±0.15 mg. The standard mass was weighed up to 10 times within repeatability conditions. The repeatability standard deviation (S_{rep}) was 0.000078 g. By following the ASTM requirements [14,15] the relative uncertainty of calibration assuming negligible contributions of environmental factors and for determinations of mass lower than the maximum load of the balance (100 g) is given by

$$u_{rel}(\text{cal}) = \sqrt{\frac{S^2_{rep} + \frac{a^2_{read}}{12} + \frac{a^2_{std}}{6}}{M^2_{std}}}$$

where S^2_{rep} is the repeatability variance, already evaluated, a_{read} the readability specification of the balance (0.1 mg), a_{std} the weight tolerance (±0.15 mg) and M_{std} the nominal value of standard mass (100 g). For these values, $u_{rel}(\text{cal}) \approx 10^{-6}$. This uncertainty is then used to obtain the combined uncertainty for the mass determination, $u(w)$ when the weight is measured by the difference: $w = w_{gross} - w_{tare}$

$$u(w) = \sqrt{u^2_{rel}(\text{cal})w^2 + \frac{2}{3}a^2_{lin} + 2S^2_{rep} + \frac{1}{6}a^2_{read}}$$

Here w is the weighed object mass, a_{lin} the linearity specification of the balance (±0.2 mg) and u_{rel}(cal), a_{read} and S_{rep} were already introduced. By setting w as the maximum weighed mass (45 mg), the combined uncertainty for mass determination ($u(w)$) was found to be 0.0002 g. The purity of the reagent was 99.5% minimum from HPLC assay. This means that purity spans over the interval [0.995, 1] in fractional scale, and can be rewritten in terms of purity tolerance interval: 0.9975 ± 0.0025. Accordingly and assuming that purity follows a rectangular distribution, the corresponding type B uncertainty is given by

$$u(P) = \frac{0.0025}{\sqrt{3}} = 0.00144$$

The uncertainty in the 50-mL volumetric flask can be split into three contributions, namely the calibration tolerance, the mark repeatability and the temperature bias. The calibration tolerance according to the manufacturer's information is ±0.12 mL, which assuming a triangular distribution leads to the uncertainty

$$u_{cal}(V) = \frac{0.12}{\sqrt{6}} = 0.049$$

Volume repeatability was obtained by weighing the volumetric flask filled with water to the mark. Assuming the uncertainty in the water density negligible, the type A uncertainty calculated as a standard deviation was of ±0.18 mL. Thus, $u_{rep}(V)$ was 0.18. The bias temperature effect on volume arises from departures from the calibration temperature (20 °C). The maximum gap (ΔT) observed was about 4 °C. This temperature tolerance is then converted into volumetric one by using the water thermal expansion coefficient ($k = 2.1 \times 10^{-4}\,°\text{C}^{-1}$) as follows, $\Delta V = \Delta TkV$, and our case the value was 0.042. The corresponding uncertainty evaluated from a rectangular distribution produces:

$$u_{temp}(V) = \frac{0.042}{\sqrt{3}} = 0.024$$

The total volumetric uncertainty was $u(V) = \sqrt{u^2_{cal}(V) + u^2_{rep}(V) + u^2_{temp}(V)} = 0.19$.

In Table 2, the results of the recovery study at each fortification level are gathered.

In order to assess the trueness, the Student's t-test was applied to each intermediate recovery:

$$t = \frac{|\bar{R}_m - 100|}{u(\bar{R}_m)}$$

Table 3
Determination of sucrose[a] in coffee beans by IC-PAD and the proposed method

Sample	IC-PAD	Proposed method
1	8.78	8.85
2	7.36	7.41
3	6.91	6.80
4	5.19	5.20
5	4.58	4.61
6	3.43	3.37

IC-PAD: ion chromatography with pulsed amperometric detection.
[a] % (w/w) dry base; average of triplicate measurements.

The obtained values were 2.4, 2.9, 2.0 and 0, respectively, for each fortification level. The critical value t (3, 0.05) is 3.18. Accordingly, all recoveries did not differ from 100% and hence, traceability was assessed.

Aside from this evaluation of the trueness a comparison with a currently practiced method was also performed. Ion chromatography with pulsed amperometric detection (IC-PAD) is used for determining sucrose in coffee beans. In our case we use the chromatographic conditions proposed by Ky et al. [4]. An anion-exchange column with a highly alkaline mobile phase was used, and the amperometric detection was carried out using a gold working electrode operated in a pulsed mode. Coffee beans, after the sample treatment, were filtered (0.2 μm) and injected into the IC-PAD system. For the sake of comparison, six samples, with sucrose content uniformly distributed between the working range extremes, were analysed by both methods. The results obtained are gathered in Table 3. In order to perform the comparison of the two methods two different statistical approaches were applied: the paired t-test for dependent samples [16] and the non-parametric regressive method of Passing and Bablok [17].

The paired t-test, gives a mean difference of 0.0017 with a standard deviation 0.0694, which leads to a t-value of about 0.06. The two-tailed tabulated Student's t statistic for 5 degrees of freedom and a 95% confidence level is 2.57. Accordingly, there is no statistical difference between the two methods.

The method of Passing and Bablok is a non-parametric regression based in ranks but taking into account the errors of both axes [18,19]. By taking X for the IC-PAD method and Y for the proposed in this work, the non-parametric regression line is $Y = -0.0767 + 1.0167X$, as depicted in Fig. 2. The 95% confidence interval obtained for the intercept was (−0.5740, 0.3135) and for the slope (0.9399, 1.0963). These intervals include the ideal values 0 and 1, respectively, and hence it can be concluded that there is no significant difference between the two methods.

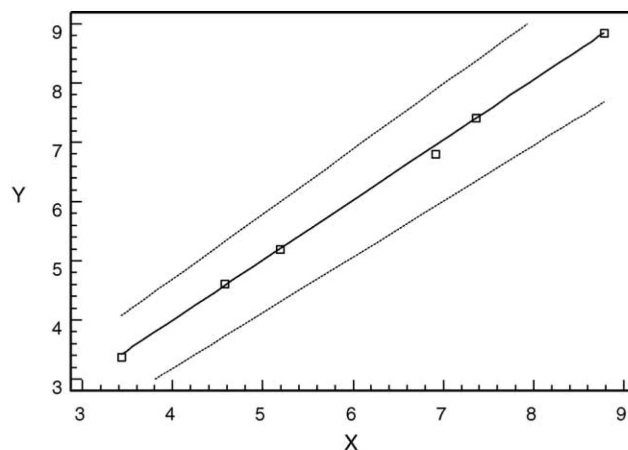

Fig. 2. Passing and Bablock plot for two method comparison. X (IC-PAD), Y (proposed method).

4.3. Linearity and sensitivity

Response linearity was studied from external calibration by using aqueous standards of sucrose. Concentration range covers from 100 to 1200 mg/L sucrose. The standard deviations of the signal at each calibration point did not differ according to the Cochran test and consequently, homocedasticity is achieved and we can apply conventional least squares procedures for calibration. The corresponding fitted straight line was $y = (-0.00416 \pm 0.00072) + (0.0001 \pm 1.05 \times 10^{-6})x$. The correlation coefficient was 0.9994 and the on-line linearity [20]:

$$\%\text{R.S.D.}_b = 100\left(1 - \frac{s_b}{b}\right) = 98.95$$

where b and s_b are the slope and its standard deviation, respectively.

The linear dynamic range [21] was obtained by plotting the response factors $R_i = y_i/x_i$ in the y-axis against the corresponding concentrations x_i on the x-axis. In this case R_i values were obtained as the ratios absorbance/concentrations. The obtained line should be with zero slope through the full linear range. Parallel horizontal action lines are drawn at 95 and 105% of the target line. The method is linear up to the point at which the plotted R_i versus x_i intersects any action line. In our case, the linear working interval is from 200 to 900 mg/L as depicted in Fig. 3.

The limit of detection derived from the calibration line was calculated according to the IUPAC convention [22] and was 20.3 mg/L.

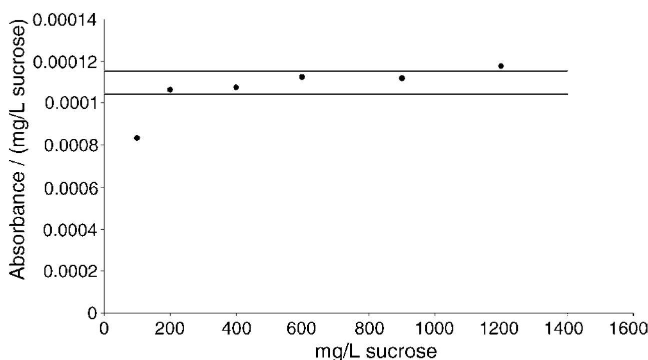

Fig. 3. Linear working interval.

Table 4
Coded and absolute values for the experimental studied variables

Experimental variables	Level "+1"	Level "0"	Level "−1"
T (°C)	65	60	55
t_{ex} (min)	15.5	15	14.5
t_h (min)	15.5	15	14.5
t_r (min)	18.5	18	17.5
pH_1	7.2	7.0	6.8
pH_2	4.8	4.6	4.4

T: hydrolysis temperature; t_{ex}: extraction time; t_h: hydrolysis time; t_r: reaction time; pH_1: pH Tris–buffer; pH_2: pH citrate buffer.

4.4. Robustness

From the perspective of internal validation, robustness deals with the effect of experimental factors of the analytical method on the results [23,24]. Plakett–Burmann designs are useful tools for the study of robustness of analytical procedures, especially when the possible factor interactions can be considered negligible [25].

The procedure to carry out the robustness study was as follows [26]: (a) Select the factors to be studied. (b) For every factor, define the nominal, maximum and minimum values expected in routine work. These values are encoded as 0, +1 and −1, respectively. (c) Build a two level 2^{7-4} fractional Plakett–Burmann matrix. (d) Perform the experiments in random order on a control sample with the analyte concentration halfway the concentration of working range.

In our case, the selected factors were: hydrolysis temperature (T), extraction time (t_{ex}), hydrolysis time (t_h), reaction time (t_r), pH of Tris–buffer (pH_1) and pH of citrate buffer (pH_2). The assays values corresponding to the selected levels are gathered in Table 4. The design matrix is depicted in Table 5. The 8 runs were split into two sets of 4 runs according to the levels +1 or −1, Each factor (x) is evaluated as the difference of the average result obtained at level +1 compared with the obtained at level -1:

$$D(x) = \left(\frac{\sum_i Z_i}{4}\right)_{x=+1} - \left(\frac{\sum_i Z_i}{4}\right)_{x=-1}$$

Here Z_i is the result of the run expressed as % sucrose.

Table 5
Results obtained for the design matrix

Experiment	T	t_{ex}	t_h	t_r	pH_1	pH_2	Mute	% Sucrose $(\%(w/w))_{db}$
1	+1	+1	+1	+1	+1	+1	0	4.04
2	+1	+1	−1	+1	−1	−1	0	4.19
3	+1	−1	+1	−1	+1	−1	0	4.00
4	+1	−1	−1	−1	−1	+1	0	4.15
5	−1	+1	+1	−1	−1	+1	0	5.52
6	−1	+1	−1	−1	+1	−1	0	6.08
7	−1	−1	+1	+1	−1	−1	0	5.20
8	−1	−1	−1	+1	+1	+1	0	5.82

Table 6
Significance of the estimated effects on the analytical response

Variable	D	t	95% significance	99% significance
T	1.56	18.2	Yes	Yes
t_{ex}	0.16	1.90	No	No
t_h	0.37	4.31	Yes	Yes
t_r	0.13	1.49	No	No
pH_1	0.22	2.51	Yes	No
pH_2	0.02	0.14	No	No

The effect of the factor change on the method performance was evaluated by using a student's t-test [27]:

$$t(x) = \frac{|D(x)|\sqrt{n}}{s\sqrt{2}}$$

s is the standard deviation coming from the intermediate precision study (R.S.D. = 2.5%). By considering the average of 8 runs as the expected value, we obtained 4.88% of sucrose and then s equals to 0.12. The different t-values obtained for every factor are presented in Table 6. These t-values were compared with the tabulated ones for 7 degrees of freedom at 95% ($t = 2.36$) and 99% ($t = 3.50$) significance levels. According to these results, there are some factors that must be controlled with special caution: T and t_h at a 99% significance level and T, t_h, and pH_1 at a 95% significance level.

5. Determination of sucrose in coffee beans

In order to evaluate the applicability of the method, samples of green and roasted coffee were analysed. Since the method is based on glucose released by invertase activity we considered possible interferences due to the presence of endogenously reducing sugars. Simple sugars, including reducing ones, are present in green coffee, but the quantities are very small [4]. For monitoring possible interferences coffee samples were analysed following the described procedure but without the invertase hydrolysis step. No signal was observed in any of the analysed samples and hence it can be concluded there is no interference from endogenously reducing sugars. In the case of green coffee samples the obtained results are depicted in Table 7. Values ranging between 7.6 and 4.6% $(w/w)_{db}$ were obtained. These results are in accord with the reported values in Ref. [3]. There is some variability in the obtained data, but the sucrose content in the green coffee samples tend to be variable. Varietal and cultivar differences, state of maturity, processing and storage conditions produce differences of the sucrose content [4]. Roasted coffee samples were also analysed. In this case sucrose was not found in any of the analysed samples. Sucrose is lost rapidly during roasting process, so that at a medium or dark type of roast sucrose is completely lost [4].

Table 7
Sucrose content in green coffee samples

Sample	Sucrose[a]
1	5.9 ± 0.2
2	4.8 ± 0.1
3	5.0 ± 0.1
4	5.2 ± 0.2
5	4.7 ± 0.1
6	4.6 ± 0.2
7	7.6 ± 0.1
8	4.6 ± 0.2
9	4.8 ± 0.1
10	4.6 ± 0.1
11	5.4 ± 0.1
12	5.9 ± 0.1
13	5.1 ± 0.1
14	7.3 ± 0.1
15	6.5 ± 0.1

[a] % (w/w) in dry base; average of triplicate measurements.

6. Conclusions

The proposed method has been shown to be useful for determining sucrose in coffee beans. The proposed procedure is suitable, easy to apply and does not need expensive instrumentation. The most critical variables were the temperature of the extraction step, the time of the hydrolysis reaction and pH of the reaction that yields the coloured product. In any case these variables can be easily controlled in order to obtain reproducible results. No differences have been found between the proposed method and the currently practiced IC-PAD method.

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References

[1] C.A.B. De Maria, L.C. Trugo, R.F.A. Moreira, C.C. Werneck, Food Chem. 50 (1994) 141.
[2] M.N. Clifford, in: M.N. Clifford, K.C. Wilson (Eds.), Coffee, Botany, Biochemistry and Production of Beans and Beverage, Publishing Company Inc, Westport, 1985, pp. 305–374.
[3] L.C. Trugo, in: R.J. Clarke, R. Macrae (Eds.), Coffee, Chemistry, vol. 1, Elsevier, London, 1985, pp. 83–114.
[4] C.L. Ky, S. Doulbeau, B. Guyot, S. Akaffou, A. Charrier, S. Hamon, J. Louarn, M. Noirot, Plant Breed. 119 (2000) 165.
[5] M.B. Jensen, J. Chem. Ed. 79 (2002) 345.
[6] W.R. La Course, Pulsed Electrochemical Detection in High-performance Liquid Chromatography, Techniques in Analytical Chemistry Series, Wiley, New York, 1997.
[7] W.R. La Course, Analysis 21 (1997) 181.
[8] D.C. Johnson, W.R. La Course, Anal. Chem. 62 (1990) 589A.
[9] L. Rotariu, C. Bala, V. Magearu, Anal. Chim. Acta 458 (2002) 215.
[10] P. Trinder, Ann. Clin. Biochem. 6 (1969) 24.
[11] EURACHEM-CITAC Guide, 2000. Quantifying Uncertainty in Analytical Measurements, 2nd ed. On-line document http://www.vtt.fi/ket/eurachem/quam2000-pl.pdf.
[12] V.J. Barwick, S.L.R. Ellison, Protocol for Uncertainty evaluation from validation data, 2000. Report No. LGC/VAM/1998/088.
[13] K. Helrich (Ed.), Peer Verified Methods Program, Manual on Policies and Procedures, Association of Official Analytical Chemists, Arlington, VA, USA, 1993.
[14] American Society for Testing & Materials (ASTM), 1991. Standard specifications for laboratory weights and Precision Mass Standards, E 617.
[15] ASTM. West Conshohocken PA. (1993). Standard Method of Testing Top-Loading, Direct Reading Laboratory Scales and Balances, E 898.
[16] J.N. Miller, J.C. Miller, Statistics and Chemometrics for Analytical Chemistry, 4th ed., Pearson Education Ltd, 2000.
[17] H. Passing, W. Bablok, J. Clin. Chem. Biochem. 21 (1983) 709.
[18] H. Passing, W. Bablok, J. Clin. Chem. Biochem. 22 (1984) 431.
[19] W. Bablok, H. Passing, R. Bender, B. Schneider, J. Clin. Chem. Biochem. 26 (1988) 783.
[20] L. Cuadros, A.M. García, C. Jiménez, M. Román, Anal. Lett. 26 (1993) 1243.
[21] L. Huber, LC-GC Int. 11 (1998) 96.
[22] IUPAC. Compendium of Analytical Nomenclature, Definitive Rules 1987. Blackwell Scientific Publications, Oxford, 1997.
[23] L.C. Rodríguez, R. Blanco, A.M. García, J.M. Bosque, Chemom. Intell. Lab. Syst. 41 (1998) 57.
[24] W.J. Youden. Statistical Techniques for Collaborative Tests, AOAC, Washington, 1967.
[25] A.G. González, Anal. Chim. Acta 360 (1998) 227.
[26] I. García, M.C. Ortíz, L. Sarabia, C. Vilches, E. Gredilla, J. Chromatogr. A 992 (2003) 11.
[27] Y. Vander Heyden, K. Luypaert, C. Hartmann, D.L. Massart, J. Hoogmartens, J. De Beer, Anal. Chim. Acta 312 (1995) 245.