COMPARING TOTAL NITROGEN AND CRUDE PROTEIN CONTENT OF GREEN COFFEE BEANS (*Coffea* spp.) FROM DIFFERENT GEOGRAPHICAL ORIGINS

Carla Isabel Rodrigues[1,a], Rodrigo Maia[1], Cristina Máguas[1]

(Recebido: 11 de junho de 2009; aceito 25 de novembro de 2010)

ABSTRACT: A combustion - TCD (thermal conductivity detection) elemental analysis method has been applied for the first time for total nitrogen and crude protein determination of 72 green coffee bean samples from 20 different geographic origins distributed over North America, South America, Africa, Asia and Oceania. Crude protein content varied among different coffees from distinct geographic origins.
In this study we aimed at applying an elemental analysis method for total nitrogen determination in green coffee and calculation of crude protein content according to AOAC Official Method 992.23. Elemental analysis by combustion - TCD presented itself as a fast, accurate, high precision and reproducible approach (intermediate precision 1.37% RSD; E_n 0.17) for the determination of the total nitrogen in green coffee.

Index terms: Crude protein determination, total nitrogen, green coffee, elemental analysis, thermal conductivity detection.

COMPARANDO PERCENTAGEM TOTAL DE NITROGÉNIO E PROTEÍNA EM GRÃO VERDE DE CAFÉ (*Coffea* spp.) DE DIFERENTES ORIGENS GEOGRÁFICAS

RESUMO: *O método de análise elementar por combustão - DCT (detector de condutividade térmica) foi aplicado pela primeira vez para a determinação do nitrogênio total e proteína bruta de 72 amostras de café verde de 20 origens geográficas diferentes, distribuídas ao longo da América do Norte, América do Sul, África, Ásia e Oceânia. O teor de proteína bruta variou entre cafés de diferentes origens geográficas. Neste estudo, objetivou-se aplicar o método de análise elementar para determinação de nitrogênio total em café verde e o cálculo do teor de proteína bruta, de acordo com o método AOAC 992,23. A análise elementar por combustão - TCD apresentou-se como um processo rápido, preciso e com boa reprodutibilidade (intermediário precisão 1,37% RSD; Pt 0,17), para a determinação do nitrogênio e proteínas totais em café verde.*

Palavras-chave: Café verde, análise elementar, nitrogênio total, determinação de proteína total, detecção por condutividade térmica.

1 INTRODUCTION

Coffee is consumed by a large proportion of the human population (about 70 to 80%) (SCHILLER et al., 2001) and is one of the world's most valuable export commodity grown in many of the most bio diverse regions of globe (MARCONE, 2004; RICKETTS et al., 2004). Commercial available roasted coffees derive from two species, *i.e. Coffea Arabica* L. and *Coffea canephora* Pierre ex Froehner var. *robusta* that are cultivated in all continents except Europe. Usually, consumers buy coffee blends composed by a mixing of Arabica and Robusta. Different compositions originate different blends whose aroma and flavour can be quite distinct and exquisite (MENDES et al., 2001). The amino acids and proteins of the green coffee bean are known as precursors of many important aroma compounds found in roasted coffee (HOMMA, 2001). During the roasting process, pyrolitic reactions take place leading to the formation of particular volatile and semi-volatile aroma compounds responsible for the sensory qualities of roasted coffee (GROSCH, 2001; HERNANDÉZ et al., 2007). In this sense, the crude protein content (CPC) of the green coffee beans may constitute important information when deciding the best roasting conditions and blend composition. Several methods have been applied for routine total nitrogen (TN) and protein determination in food such as the Kjeldahl method and near-infrared spectroscopy (KAMIZAKE et al., 2003; KIM et al., 2007). Kjeldahl method allows the determination of coffee total nitrogen, and if a multiplying factor is applied, it is possible to convert total nitrogen to crude protein content. An alternative analytical

[1]University of Lisbon, Faculty of Sciences, Stable Isotopes and Instrumental Analysis Facility, Center for Environmental Biology, Campo Grande Ed. ICAT, 1749 – 016 Lisboa, Portugal
[a] cirodrigues@fc.ul.pt

method for the TN and CPC determination in food is the Organic Elemental Analysis (OEA). The advantages of the OEA are that this method does not require organic solvents and allows the analysis of up to 60 different samples per day. Different types of elemental analyzers display a fast and reliable way of multi-element analysis from carbon and nitrogen determinations to a wider determination set of elements such as carbon (C), hydrogen (H), nitrogen (N) and sulphur (S), although different combustion-reduction system layouts are involved. A combustion furnace coupled with a thermal conductive detector (TCD) is the primary approach for the determination of C, N and S in a variety of organic substances. The determination of N by combustion-TCD is a fast and highly reproducible method that avoids many sample clean-up procedures and minimizes interferences from some elements (e.g. K, Hg and Cu).

In this work, we aimed at applying OEA for TN determination of 72 green coffee bean samples from 20 different geographic origins. With the value of TN of each green coffee it was possible to calculate CPC according to AOAC Official Method 992.23 (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC, 2000). This study allowed the comparison of TN and CPC of different greens coffees originated from different geographical origins distributed by North and South America, Africa, Asia and Oceania.

2 MATERIALS AND METHODS

2.1 Biological Material

Green coffees from 20 different geographic origins in a total of 72 samples (table 1) were provided from Novadelta, Comércio e Indústria de Cafés, S.A. (Campo Maior, Portugal). Samples were divided in three groups: *gourmet coffee* (high market value), *certified coffee* (with at least one kind of certification) and *other* (origins imported by Novadelta, S. A. in major quantities that don't have any certification). All samples are arabica type of coffee (*Coffea Arabica* L.) except green coffees from Angola that are robusta (*Coffea canephora* Pierre ex Froehner).

2.2 Reagents and Materials

For C and N elemental analysis, grinded coffee was weighted in folded tin capsules 5 x 9 (EuroVector, Milano). The combustion was done in excess oxygen (H_2O < 3 ppm, C_nH_m < 0.5 ppm) (AirLiquide, Portugal) and gas chromatography carrier was helium (He) (H_2O < 3 ppm, O_2 < 2 ppm, CnHm < 0.5 ppm) (AirLiquide, Portugal). Chromium oxide (20 – 50 mesh) (EuroVector, Milano) and silvered cobalt (II, III) oxide (20 – 50 mesh) (EuroVector, Milano) were used as oxidation catalysts. Water was removed with a magnesium perchlorate (6 – 18 mesh) (EuroVector, Milano). Reduction of NO_x and removal of excess O_2 was achieved with copper reduced (fine wire ϕ 0.7 mm) (EuroVector, Milano). The calibration was performed with wheat flour standard OAS (certified value for carbon: 39.53% w/w with uncertainty of +/- 0.26%; certified value for nitrogen: 1.47% w/w with uncertainty of +/- 0.07%) from Elemental Microanalysis Ltd, United Kingdom. The wheat flour standard was chosen for being a plant material with certified value for nitrogen close to the observed values in green coffee beans. The matricidal similarity and approximate elemental composition between the wheat flour standard and the green coffee beans are important to avoid matrix effects during the analysis.

2.3 Apparatus

Elemental analysis was performed in a EuroEA 3000 Elemental Analyser (EuroVector, Milano) with a TDC detector. Separation was done on gas chromatography column EVR 8 x 6 mm, 2.0 m (EuroVector (Milano). Carrier gas (He) pressure was set to 70 KPa. Sample combustion was carried at 1025 ºC with an oxygen pressure of 20 Kpa with a sample delay time of 15.7 sec. NO_x reduction was achieved at 650 ºC. Oven temperature was set to 120 ºC. The detector signal was acquired and processed on Callidus 4.1.21 software (EuroVector, Milano).

2.4 Assay procedure

Green coffee beans were grinded in a mill (Retsch, Germany), 3 times 5 minutes to reach a particle size of less than 1 mm. After grinding, samples were dried overnight at 60 ºC and weighted in tin capsules, folded and weighted again. The folded capsule weight was registered and used for C and N percentage calculation. The elemental analysis was

Table 1 – Origin of the 72 green coffees **(*Coffea* spp.)** included in this work (producer estate indicated whenever known).

Gourmet coffee				Certified coffee				Other			
Continent	Country	Location	Sample	Continent	Country	Location	Sample	Continent	Country	Location	Sample
Oceania	Papua New Guinea	Western Highlands	1	Africa	Malawi	Luwinga	34	South America	Peru	Yanesha	56
			2				35				57
Africa	Ethiopia	Yirgacheffe	3		Angola	Lubango	36		Ecuador	Hel Cafetal Estate	58
			4			Amboim	37				59
			5				38	North America	Mexico	Magarogype	60
			6				39			Chiapas (Tuxtla)	61
	UR Tanzania	Unknown	7	North America	Guatemala	Huehuetenango	40		El Salvador	San Miguel	62
		Lunji	8			Chimaltenango	41			San Antonio	63
		Unknown	9	South America	Brazil	Fazenda Da Terra	42		Nicaragua	Santa Rita	64
		Mount Kilimanjaro	10			Fazenda São Benedito	43	Africa	Zambia	Mubuyu Estate	65
			11			Fazenda Muzambo	44				66
			12			Mogiana	45		Zimbabwe	Peruzu	67
			13			Zona da Mata	46				68
	Kenya	Mount Kenya	14			Mogiana	47		Rwanda	Gatare	69
			15			Zona da Mata	48				70
			16			Fazenda Lagoa	49	Asia	Indonesia	Mandeling	71
			17			Fazenda Nossa	50			Sulawesi	72
			18	Oceania	East Timor	Ermera	51				
			19				52				
			20				53				
			21				54				
			22				55				
			23								
		Kirimiri	24								
			25								
North America	Hawaii (U.S.)	Greenwell Estate	26								
		Kona	27								
		Kona	28								
	Costa Rica	San Marcos de Tarrazu	29								
			30								
			31								
	Jamaica	Sierra del Centro	32								
			33								

performed in triplicates and the average and standard deviation was calculated. Certified Reference Material (CRM) for method validation was Wheat Flour Standard OAS. The certified values for C and N of the CRM were determined with an elemental analyser calibrated to acetanilide 141d from National Institute of Standards and Technology (NIST), Maryland, USA.

2.5 Total nitrogen determination

Chromatographic peak area (μV.sec,) allows N weight (μg) determination from the calibration function (table 2) and TN (%, w/w) is calculated based on the initial weight (mg) of the sample.

2.6 Crude protein determination

CPC (%, w/w) was determined by multiplying TN (%, w/w) by the general factor for cereal protein determination of 6.25 as described in AOAC Official Method 992.23 (AOAC, 2000).

2.7 Statistical analysis

Analysis of variance (ANOVA and Levene's test for homogeneity of variances) was performed with Statistica software, version 9.0 (Statsoft, US).

3 RESULTS AND DISCUSSION

3.1 Organic Elemental Analysis (OEA)

The proposed method allowed green coffee TN and CPC determination. The N limit of detection (LOD) was determined based on the standard error or the standard deviation of blank (S_0) and the calibration slope (S) (Validation of analytical procedures: Methodology Q2B (INTERNATIONAL CONFERENCE OF HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE - ICH, 1996). The linear dynamic range was determined for seven different calibration levels (N amounts) of CRM, Wheat Flour Standard OAS (Elemental Microanalysis, U.K.). Each calibration level was determined six times. The calibration test was performed in triplicate. The calibration plot was performed applying the least-squares method to N peak area (μV.sec) versus N weight (μg). Table 2 lists the parameters and correlation coefficients obtained for the linear dynamic range. Linearity range of work in which the method demonstrates acceptable level of precision, accuracy and linearity was achieved. N weight average values of 14.7 mg

Table 2 – Summary of analytical parameters of elemental analysis.

Analytical Parameters	Value
Linear dynamic range	
Plot Equation	N (μg) = 0.0075 N Peak Area (μV.sec) – 3.7112
R^2 (n = 7)	0.9995
R (n = 7)	0.9997
LOD (μg)	0.18
LOQ (μg)	0.54
CRM measurements	
Precision	
Repeatability (% RSD)	2.18
Intermediate Precision (% RSD)	1.37
Accuracy	
CRM (%)	1.47 ± 0.07
μ_{lab} (%)	1.46
S_{lab} (%)	0.03
RSD_{lab} (%)	1.85
E_n	0.17

LOD – limit of detection; LOQ – limit of quantification; RSD – relative standard deviation.

to 102.9 μg was well adjusted (test value (PG) = 1.163 < F (6, 6; 99%) = 8.466) and could be used as our linear range of work.

Comparison between linear and polynomial calibration curves (Mandel test) was done and differences between the two models were not significant (PG = 1.06 < F (6, 6; 99%) = 8.466). We conclude that the linear model leads to a good adjustment of experimental values for calibration. Calibration linear model parameters are shown in table 2. In order to express the precision under the same operating conditions over a short interval of time (INCLEDON; LAM, 2004), repeatability of N determination in green coffee was determined and was 2.18% RSD (table 2). The results indicate that the present method can be applied to N determination in green coffee (*Coffea* spp.) bean.

Intermediate precision expresses within-laboratory variation and is generally performed on different days using different analysts, equipment and sample preparations (INCLEDON; LAM, 2004). Intermediate precision of N determination in our laboratory was 1.37% RSD. "Trueness" of the method was calculated (CRM, six replicates). Normalised deviation was 0.17 showing an excellent accuracy of our method (E_n = 0.17 < 1). Chromatographic peak areas (µV.sec) for green coffee samples allowed N weight (µg) determination from calibration function (table 2) and TN percentage calculation based on the initial weight (mg) of the sample. Whenever the number of samples per origin was $n \geq 3$, average and standard deviation of total N was calculated.

3.2 TN and CPC of Green Coffees from Different Geographical Origins

Total N and CPC of the 72 green coffee samples are shown in table 3. We obtained for the total 72 samples a TN average value of 2.14% (w/w) with a standard deviation of 0.28% and a CPC average of 13.41% (w/w) with a standard deviation of 1.75%.

The determination of protein content of green coffee bean has been performed on the basis of TN content and this approach is based on two assumptions: that dietary carbohydrates and fats do not contain nitrogen, and that nearly the entire N in the diet is present as amino acids in proteins (KIM et al., 2007). Differences in green coffee CPC were found within the same geographic origin. In some cases, variation of CPC among coffees of the same country (e.g. average CPC for UR Tanzania coffee was 13.1% with standard deviation of 3.6%; table 3) was higher than the overall variation found for the total 72 green coffees analysed (average CPC of the 72 green coffee samples analysed was 13.41% with standard deviation of 1.75%; table 3). Variations in the TN and CPC of the 72 green coffees are shown in figure 1.

Variations in TN and CPC were observed between coffees derived from North and South America (figures 1a and b), Asia/Oceania (figure 1c) and Africa (figure 1d). The lowest and highest values of CPC were observed in the African continent, in UR Tanzania (samples 8 and 13, table 3). In Asia, CPC values were slightly lower, ranging from 10.5 to 14.8% (w/w) in comparison to American coffees. Whether in South America or North America, the lowest CPC value was 11.1% (w/w) (samples 58 and 61, table 3) raising up to the 14% range with two highest values of 15.8% (w/w) (sample 50, table 3) and 18.6% (w/w) (sample 29, table 3). However, in spite of the observed variations, an analysis of variance (one-way ANOVA and Levene's ANOVA test for homogeneity of variances) do not shows significant differences in CPC that would eventually lead to the discrimination of origin of the green coffee bean samples.

TN and CPC were similar between the Robusta and Arabica coffees included in this study. In the case of Arabica coffee, and depending on the country of origin, differences in TN and protein can be very small (i.e. Kenya) or very large (i.e. UR Tanzania). Climate, altitude, soil type, cultivar and breeding techniques (VOSSEN, 2001) are parameters with important impact in coffee quality, taste and aroma. Although in our study we cannot show which factors are influencing the measured differences in TN, since protein content is related to aromatic quality, we estimate that the above mentioned factors can be related with differences in TN of the bean and aroma quality. TN of washed green coffee (i.e. Guatemala and Malawi), harvested through a process that includes a washing step, was not different from the values found for non-washed coffees. Although Guatemala washed coffee originated from very high altitudes, its TN content was not distinct from the values we measured in low altitude coffees (i.e. Papua New Guinea and Ethiopia).

Table 3 – Total nitrogen (TN) (%, w/w) and crude protein content (CPC) (%, w/w) of green coffee **(*Coffea* spp.)** (n = 72) (TN and CPC ANOVA observed means and standard deviation indicated whenever n e ≥3; the average standard error for TN and CPC was 1.1 and 0.2, respectively; TN

Country	Sample	TN	Mean TN (stdv; std err)	CPC	Mean CPC (stdv; std err)	Country	Sample	TN	Mean TN (stdv; std err)	CPC	Mean CPC (stdv; std err)	Country	Sample	TN	Mean TN (stdv; std err)	CPC	Mean CPC (stdv; std err)
P. N. Guinea	1	2.00	2.2	12.5	13.7	Malawi	34	2.15	2.3	13.4	14.2	Peru	56	2.18	2.3	13.6	14.6
	2	2.37		14.8			35	2.38		14.9			57	2.49		15.6	
Ethiopia	3	2.12	2.1 (0.1)	13.3	13.2 (0.3)	Angola	36	2.01	2.4 (0.3)	12.6	14.7 (1.6)	Ecuador	58	1.77	1.8	11.1	11.1
	4	2.10		13.1			37	2.61		16.3			59	1.85		11.6	
	5	2.05		12.8			38	2.29		14.3		Mexico	60	1.79	1.8	11.2	11.2
	6	2.18		13.6			39	2.47		15.4			61	1.77		11.1	
UR Tanzania	7	1.94	2.1 (0.6)	12.1	13.1 (3.6)	Guatemala	40	2.13	2.2	13.3	13.7	El Salvador	62	2.14	2.2	13.4	13.8
	8	1.49		9.3			41	2.24		14.0			63	2.25		14.1	
	9	2.04		12.8		Brazil	42	1.98	2.2 (0.3)	12.4	13.9 (1.4)	Nicaragua	64	2.11	-	13.2	-
	10	1.93		12.1			43	2.49		15.6		Zambia	65	2.23	2.2	13.9	13.6
	11	2.08		13.0			44	2.31		14.4			66	2.11		13.2	
	12	1.89		11.8			45	2.15		13.4		Zimbabwe	67	2.40	2.3	15.0	14.5
	13	3.32		20.8			46	2.19		13.7			68	2.23		14.0	
Kenia	14	1.91	2 (0.1)	11.9	12.6 (0.9)		47	1.80		11.3		Rwanda	69	2.62	2.4	16.4	15.0
	15	2.18		13.6			48	2.23		13.9			70	2.17		13.6	
	16	1.91		11.9			49	2.30		14.4		Indonesia	71	2.30	2.2	14.4	13.5
	17	2.12		13.3			50	2.52		15.8			72	1.99		12.5	
	18	2.04		12.8		East Timor	51	2.16	2 (0.3)	13.5	12.7 (1.5)						
	19	2.04		12.8			52	1.99		12.4							
	20	2.12		13.3			53	2.34		14.6							
	21	1.78		11.1			54	1.68		10.5							
	22	2.07		12.9			55	1.96		12.3							
	23	1.78		11.1													
	24	2.17		13.6													
	25	2.06		12.9													
Hawaii	26	2.00	2 (0.1)	12.5	12.8 (0.6)												
	27	1.97		12.3													
	28	2.16		13.5													
Costa Rica	29	2.97	2.4 (0.5)	18.6	15.1 (3.1)												
	30	2.12		13.3													
	31	2.13		13.3													
Jamaica	32	2.39	2.3	14.9	14.1												
	33	2.12		13.3													

TN average for the 72 green coffee samples was 2.14% with standard deviation of 0.28%. CPC average for the 72 green coffee samples was 13.41% with standard deviation of 1.75%.

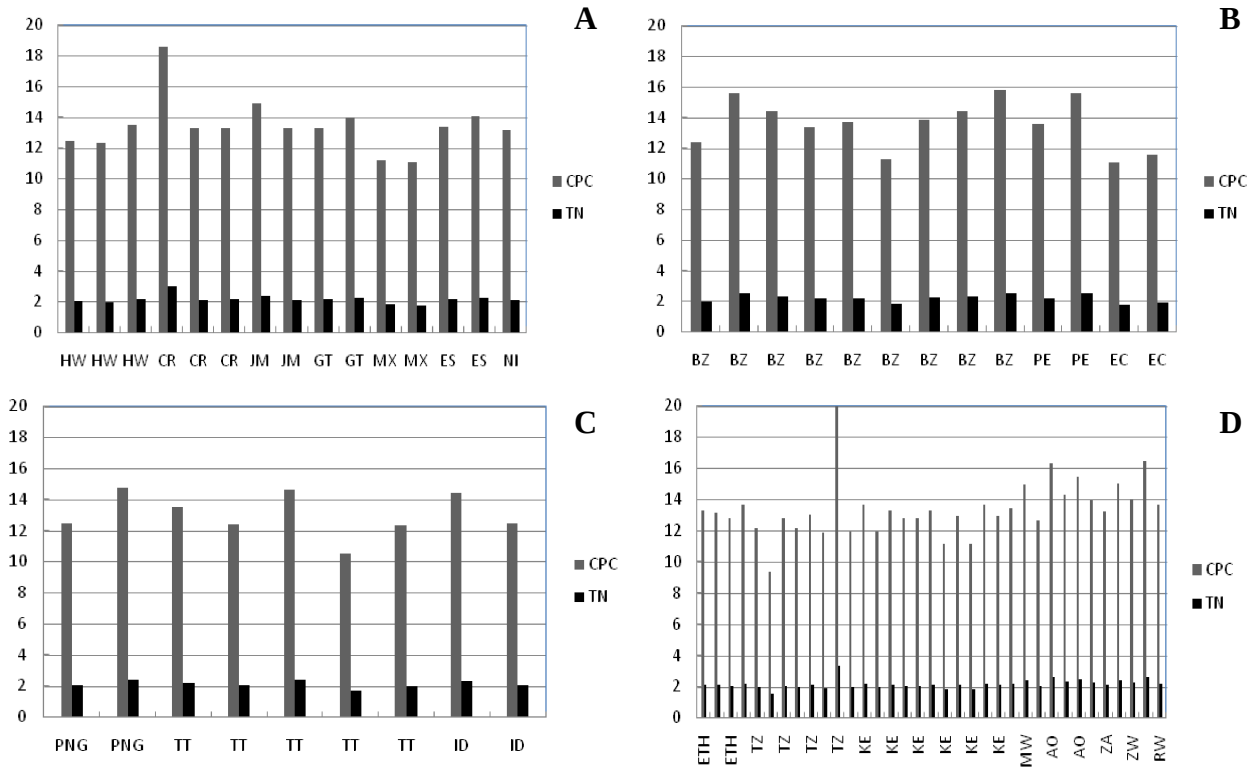

Figure 1 – TN and CPC of green coffees (*Coffea* spp.) from (A) North America (n=15), (B) South America (n=13), (C) Asia (n=9) and (D) Africa (n=35).

On other hand, organic coffees (i.e. Brazil sample number 50 and El Salvador sample number 62; table 3) had TN percentages within the range of values found for other coffees which suggest that organic cultivation programmes may not have impact on final TN and protein amount of the bean. Higher differences in protein content such as between UR Tanzania sample number 8 from Lungi Estate (CPC = 9.3%, w/w) and UR Tanzania sample number 13 from Mount Kilimanjaro (CPC = 20.8%, w/w) may explain differences in aroma profile development of these coffees during roasting process although post-harvest processing also plays an important role on final aroma quality of the coffee (GONZALEZ-RIOS et al., 2007a, 2007b).

Several reports suggest possible contributions from protein or peptides to the formation of aroma and bitter taste in coffee brew (HOMMA, 2001). The principal bean storage protein, representing between 5% and 7% of the coffee bean dry weight, exists *in vivo* as a mature coffee flavour precursor (HOMMA, 2001). Green coffee sugars and amino acids are the main substrates of Maillard and Strecker reactions (BELITZ et al., 2004; YERETZIAN et al., 2002) that occur during roasting process, whose end products are volatile organic compounds. The type and amount of volatile compounds in roasted coffee will depend on roasting time and temperature, type of coffee (Arabica vs. Robusta), geographic origin of the green bean (related with local climate parameters and soil characteristics), plant cultivar, green coffee bean chemical composition (BRADBURY, 2001; HOMMA, 2001; VOSSEN, 2001) and post-harvest processing (GONZALEZ-RIOS et al., 2007a, 2007b). This underlines the importance of knowing TN and CPC of green coffee beans.

In this work, CPC of green coffee bean is determined based on TN of the green bean as has been reported by other authors for plant protein determination (CASAL et al., 2000; Kim et al., 2007). We were concerned about the fact that green coffee TN includes the amount of nitrogen from caffeine which has different

levels depending on the type of coffee (Arabica vs. Robusta). Early published data (RODRIGUES et al., 2007) show that caffeine content in brewed coffee prepared from Arabica coffee can vary from 174 mg/L to 450 mg/L while Robusta brewed coffee caffeine content showed variations between 314 mg/L and 739 mg/L. In spite of this, we do not see such a clear separation between Arabica and Robusta TN and CPC. Robusta green coffees from Angola included in this study show protein content from 12.6 and 16.3% (w/w) (table 3) that is not very different from the values for the Arabica coffees (i.e. UR Tanzania coffees had CPC range from 9.3 to 20.8% (w/w)).

In short, we have shown that organic elemental analysis may be applied to total nitrogen and crude protein content of green coffee. Variations in CPC among the 72 coffees included in this study were observed suggesting that depending on the origin, agricultural practices and post-harvest processing, the TN content will vary and consequently the CPC, and this may have a consequence on the aromatic quality of coffee blends produced from these different coffees.

4 CONCLUSIONS

Elemental analysis is a fast, clean and reproducible analytical tool for total N and crude protein determination in cereals, legumes and foodstuffs. The same approach can be applied to coffee. The elemental analyser is affordable as well as all consumables for the analysis and a number of 300 samples analysed per week is a minimum that can be easily achieved with minor supervision.

Knowing elemental composition of the coffee bean can be important especially in biodiversity studies and when it is important to narrow possible variations in roasted coffee aroma quality.

This work is included in a broader project to access important chemical composition profiles that, correlated with sensory analysis, should make it possible to build a database for identification of different coffee products and improve roasting process and develop new gourmet products. The final database will allow the most accurate calibration.

5 ACKNOWLEDGEMENT

This work was financially supported by Fundação para a Ciência e Tecnologia, through the project PTDC/AGR-AAM/104357/2008 (IsoGeoCoffee).

Carla Rodrigues wish to thank Fundação para a Ciência e a Tecnologia for a grant (SFRH/BD/28354/2006).

6 REFERENCES

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. **Official methods of analysis of AOAC International**: AOAC official method 992.23. Arlington, 2000. v. 2.

BELITZ, H. D. et al. **Food chemistry**. Berlin: Springer-Verlag, 2004.

BRADBURY, A. G. W. Chemistry I: non-volatile compounds. In: CLARKE, R. J.; VITZTHUM, O. G. (Ed.). **Coffee, recent developments**. Oxford: Blackwell Science, 2001.

CASAL, J. A. et al.. A test of two methods for plant protein determination using duckweed. **Aquatic Botany,** Amsterdam, v. 67, p. 61-67, 2000.

GONZALEZ-RIOS, G. et al. Impact of "ecological" post-harvest processing on the volatile fraction of coffee beans: I., green coffee. **Journal of Food Composition and Analysis,** New York, v. 20, p. 289-296, 2007a.

_____. Impact of "ecological" post-harvest processing on the volatile fraction of coffee beans: II., roasted coffee. **Journal of Food Composition and Analysis,** New York, v. 20, p. 297-307, 2007b.

GROSCH, W. Chemistry III: volatile compounds. In: CLARKE, R. J.; VITZTHUM, O. G. (Ed.). **Coffee, recent developments**. Oxford: Blackwell Science, 2001.

HERNANDÉZ, J. A. et al. Analysis of the heat and mass transfer during coffee batch roasting. **Journal of Food Engineering**, London, v. 78, p. 1141-1148, 2007.

HOMMA, S. Chemistry II: non-volatile compounds. In: CLARKE, R. J.; VITZTHUM, O. G. (Ed.). **Coffee, recent developments**. Oxford: Blackwell Science, 2001.

INCLEDON, C. L.; LAM, H. Development and validation of automated methods. In: CHANG, C. et al. (Ed.). **Analytical method validation and instrument performance verification**. New York: Wiley; Interscience, 2004.

INTERNATIONAL CONFERENCE OF HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE. **ICH harmonised tripartite guideline:** validation of analytical procedures: methodology, Q2B. [S.l.], 1996.

KAMIZAKE, N. K. K. et al. Determination of total proteins in cow milk powder samples: a comparative study between the Kjeldahl method and spectrophotometric methods. **Journal of Food Composition and Analysis,** New York, v. 16, p. 507-516, 2003.

KIM, Y. et al. Near-infrared spectroscopic analysis of macronutrients and energy in homogenized meals. **Food Chemistry,** London, v. 105, p. 1248-1255, 2007.

MARCONE, M. F. Compositon and properties of Indonesian palm civet coffee (Kopi Luwak) and Ethiopian civet coffee. **Food Research International,** London, v. 37, p. 901-912, 2004.

MENDES, L. C. et al. Optimization of the roasting of robusta coffee (*C. canephora* conillon) using acceptability tests and RSM. **Food Quality and Preference**, v. 12, p. 153-162, 2001.

RICKETTS, T. H. et al. Economic value of tropical forest to coffee production. **Proceedings of the National Academy of Sciences of the United States of America,** Washington, v. 101, n. 34, p. 12579-12582, 2004.

RODRIGUES, C. I. et al. Application of solid-phase extraction to brewed coffee caffeine and organic acid determination by UV/HPLC. **Journal of Food Composition and Analysis,** New York, v. 20, p. 440-448, 2007.

SCHILLER, B. et al. Health effects and safety considerations. In: CLARKE, R. J.; VITZTHUM, O. G. (Ed.). **Coffee, recent developments**. Oxford: Blackwell Science, 2001.

VOSSEN, H. A. M. van de. Agronomy I: coffee breeding practices. In: CLARKE, R. J.; VITZTHUM, O. G. (Ed.). **Coffee, recent developments**. Oxford: Blackwell Science, 2001.

YERETZIAN, C. et al. From the green bean to the cup: investigating coffee roasting by on-line monitoring of volatiles. **European Food Residue Technology,** v. 214, p. 92-104, 2002.