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Nitrogen/protein and one-step moisture and ash examination in foodstuffs: Validation case analysis using automated combustion and thermogravimetry determination under ISO/IEC 17025 guidelines

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ABSTRACT

Method validation within food science is a not only paramount to assess method certainty and ensure the quality of the results, but a pennant in analytical chemistry. Proximate analysis is an indispensable requirement for food characterization. To improve proximate analysis, automated protein and thermogravimetric methods were validated according to international guidelines (including ISO 17025) and acceptance criteria of results based on certified reference materials and participation within international recognized proficiency schemes. Common food groups (e.g., meat, dairy, and grain products) were included and at the end of validation, we obtained three rugged and accurate methods with adequate z scores ($-2 \geq x \leq 2$) and recoveries (92–105%). During optimization, variables such as gas flows, subsample masses, and temperatures were varied and specific conditions (those that rendered the best results) were selected for each food group. For each validated method, a comparison (technical and economic) among the data obtained and the data extracted for its traditional counterpart were included: assays validated demonstrate to be more cost-effective labor-wise (ca. 9 and 16-fold) than their traditional alternatives. Specifically for combustion assay regression analysis ($y = 0.9361x$, $y = 1.1001x$, and $y = 0.9739x$, for meat, dairy and grain products, respectively) were performed to assess the factor, if any, which must be applied to the results to effectively match those obtained for Kjeldahl method. Finally, in the case of protein, samples can be analyzed under 5 min with no residue and a subsample mass below 400 mg. Moisture and ash analysis can be performed simultaneously using the same subsample. Data herein will also help harmonize and advance food analysis toward more efficient greener methods for proximate analysis.

1. Introduction

The nutritional value of foods is extremely relevant as it is the first step toward characterization of novel or staple food sources; it can be of interest in the food industry for product development, quality control or regulatory purposes (Thangaraj, 2016). Proximate analysis, which refers to the macro quantitative analysis of molecules in food, is included in most of the research considering primary characterization of food (see for example, Suffo Kamela et al., 2016; Kassegn, 2018; Chisomo Chatepa et al., 2018; Aletan and Kwazo, 2019; Dan Ramdath et al., 2020). To this end, a combination of different techniques are used to determine protein, fat, moisture, ash and carbohydrate levels. In this regard, three common assays included in proximate analysis are protein, moisture and ash, which traditionally are determined using Kjeldahl and oven or furnace methods. These standard methodologies, are usually time-consuming, require large amounts of samples, are highly dependent on the accuracy of the operator, and comprise several steps (which may result in low reproducibility) (Torquato et al., 2017). However, green chemistry forward and highly automated specialized laboratory equipment has been developed to assess protein (combustion analyzers), moisture, and ash (thermogravimetric analyzers) and has been slowly replacing traditional methods (Simmone et al., 1997). For example, for 2015 LGC proficiency scheme 226 round 731 (meat products) $n = 3$ participants reported protein using combustion from a total of $n = 42$ participants. Meanwhile, for 2016, scheme 243 round 531 the number of laboratories reporting using combustion analyzer increased in 233%. A similar scenario happens in other proficiency schemes; for FAPAS rounds 2472-2017 and 2492-2021 (porridge oats) 4.9% and 12.3% of

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Table 1
Approved methodologies for combustion analysis in foods.

Method	Matrix
AACC (American Association of Cereal Chemists)	
46–30	Cereal and Cereal Products
AOAC (Association of Official Analytical Chemists)	
990.03	Animal Feed
992.15	Meat/Meat Products and Pet foods
992.23	Cereal Grain and Oil Seed Products
997.09	Beer, Wort, and Brewing Grains
AOCS (American Society of Brewing Chemists)	
BA4E-93	Oil Seeds
BA4F-00	Soybean Meal
ASBC (American Society of Brewing Chemists)	
Combustion	Adjunct materials and cereals, Barley, Beer, Brewers' Grains, Malt, Wort
ISO (International Organization for Standardization)	
14891	Milk and Milk Products
16634	Food Products, Oilseed, and Animal Feed

the participants reported the use of combustion analyzers. This, in contrast with the behavior observed for animal feed where combustion analysis is the primary method (e.g., AAFCO check sample program 202126, where $n = 122$ laboratories against $n = 11$ using Kjeldahl).

A common application of combustion analyzers is the composition determination of organic compounds (see for example, Fadeeva et al., 2008). To date, few methods using combustion automated analyzers have been developed for food analysis *per se* (see for example, Table 1) and those implemented are limited in their scope. For example this technique was recently used to assess nitrogen distribution in a cereal (Bruning et al., 2019). In contrast, feed analysis using combustion, has a single method of its own (Etheridge et al., 1998, Table 1). Some efforts have been conducted to compare among traditional Kjeldahl method versus combustion (Daun and Declercq, 1994; Marcó et al., 2002; Watson and Gallher, 2002). However, for some food matrices, for example, oilseeds, a correction factor has been suggested (Daun and Declercq, 1994). Finally, a recent application of automated determination of CHNS in a discrete samples have been recently reported (e.g., meat, soybean products, and wheat) (Jung et al., 2003; Mihaljev et al., 2015; Lanza et al., 2016; Czaja et al., 2020).

For the case of moisture and ash determination using thermogravimetric analysis, methods developed have just been limited to energy applications (see for example, Tumuluru et al., 2012; Solar et al., 2021). However, thermogravimetric analysis has been used to characterize the proximate analysis of biomass (Torquato et al., 2017). Hereafter, this approach consists in valuable quantitative analytical method because it enables a continuous and fast measurement under controlled temperature conditions, employs a small sample mass, requires minimal operator intervention, and is low risk (Torquato et al., 2017).

Notwithstanding, no standardized method have been reported to be validated for the routine analysis of nitrogen/protein, moisture, or ash in foodstuffs using the techniques aforementioned. Moreover, the necessity for laboratories to use fully validated methods is now universally accepted as a way to obtain reliable results (Raposo and Ibelli-Bianco, 2020).

Hence, we developed validated a method for protein using and automated combustion analyzer and a single-step method for the determination of moisture and ash based on thermogravimetry, according to guidelines established by ISO 17025 and compared these methods to traditional approaches such as Kjeldahl determination and loss on drying using convection or incineration using a furnace muffler. In the case of protein, the validated combustion method allows determining the percentage of nitrogen in dry matrices such as flour, baked goods, sausages, meat (according to 992.15 AOAC) and dairy products such as cheese, condensed milk, and powdered milk with a nitrogen concentration ranging from 0.22 to 100 g/100 g.

We consider the value of this work to be severalfold: i. It would give researchers the opportunity to reconcile standardized guidelines with research in food analysis ii. It will permit to compare traditional and emerging techniques iii. Food analysis lab managers would benefit from validation data that can be useful as a blueprint for their own validations. iv. It can be useful as a teaching example for method validation v. Our data reflects common values for protein, moisture and ash for foodstuffs, thus expanding food characterization data and, finally, vi. Hopefully, it would serve as a scaffold for the future inclusion of combustion and thermogravimetric methods in normalized/official assays for food analysis.

2. Materials and methods

2.1. Reagents and other materials

DL-aspartic acid, D-asparagine and sucrose were acquired from Sigma-Aldrich (catalog numbers A9006, 441597, and S9378, ≥99% purity, St. Louis, MO, USA). Uric acid (SRM 913b, 99.8 ± 0.2 g/100 g) was acquired from National Institute of Standards and Technology (Gaithersburg, MD, USA). CO_2 was used as carrier gas and O_2 were used to achieve a complete oxidation after catalytic post-combustion process and control the atmosphere inside the furnace during the course of an analysis, for protein and moisture/ash determinations, respectively (UHP gas, Praxair Technology, Inc., Uruca, San José, Costa Rica). In the case of protein analysis, the reducing agent (converting nitrogen oxides to molecular nitrogen) was used as recommended by the manufacturer (EAS REDUCTOR® and EAS REGAINER®, Elementar, Lagenselbold, Germany).

2.2. Reference materials

2.2.1. Protein analysis

Validation the proficiency schemes used were as follow: FAPAS MR036, FAPAS 2477, FAPAS 01119, FAPAS TER026RM, FAPAS 2476, FAPAS 2466, FAPAS 25172, FAPAS 25169, FAPAS 25164, FAPAS 2458, FAPAS 2474, FAPAS 2490, FAPAS 2492, FAPAS 01120, LGC 261, PRIDAA 612–2017, PRIDAA 2954-2019, and MUVA MP 218, MUVA MP 219. For all cases, proficiency scheme providers selected were also accredited according to ISO 17043 and validation data was recovered from 2016 to 2021.

2.2.2. Moisture analysis

Validation the proficiency schemes used were as follow: FAPAS T2459QC, FAPAS T2469QC, FAPAS T2458QC, FAPAS 01120, FAPAS T25170QC, FAPAS T25147, FAPAS 2490, FAPAS T24186, LGC 261, MUVA MP 0219, MUVA MP 0214, FAPAS M036, FAPAS TER026RM, PRIDAA 519–2017, 612–2017 PRIDAA, 676–2017 PRIDAA, 863–2017 PRIDAA, 1399–2018 PRIDAA, and 1849-1-2018 PRIDAA.

2.2.3. Ash analysis

Validation the proficiency schemes used were as follow: FAPAS T2459QC, FAPAS T2458QC, LGC 261, FAPAS 1107, FAPAS 1119, FAPAS 1120, FAPAS TER026, FAPAS 2490, PRIDAA 519–2017, PRIDAA 630–2017, PRIDAA 676–2017, PRIDAA 612–2017, PRIDAA 863–2017, PRIDAA 813–2017, PRIDAA 813–2017, PRIDAA 1399–2018, PRIDAA 1849–2018.

2.3. Protein analysis of foodstuffs using combustion analyzer

Nitrogen analysis was performed using a combustion analyzer rapid N exceed® (Elementar). Precombustion and reduction steps were both performed at 600 °C. The combustion of the samples was performed within a high-temperature combustion furnace (960 °C) and nitrogen is subsequently detected using a Thermal Conductivity Detector (TCD). Data analysis and acquisition was executed by means of Software rapid N exceed® V1.1.11 (Elementar). Aspartic acid, asparagine and uric acid

Table 2
Defined test conditions for moisture analysis during the thermogravimetric method, optimized data after validation.

Food matrix	Subsample mass, g	Temperature, °C
Dairy	0.5–1.00	104–110
Fruit firtters	0.20–0.50	80
Wheat meal	0.20–0.50	120–133
Freeze dried fruit	0.20–0.50	80
Baked godos	0.25–0.50	100–110
Bread	0.25–0.50	100
Condiments	0.25–0.50	104
Coffee	0.20–0.50	100–105
Grains and derivates	0.2–0.50	103
Grains and derivates (meals)	0.5–1.0	110
Corn meal	0.20–0.50	100–105
Fresh fruit	0.20–0.50	70–80
Baby foods	0.20–0.50	120
Soybean meal	0.5–0.6	135
Beer	0.20–0.50	71
Fruit Juice	0.5–1.0	110
Meat products	0.5–1.0	100–110

at 10.42, 20.99, 33.27 g N/100 g. Factor conversion from nitrogen to protein used was 6.25 unless otherwise stated; with the exception of the dairy samples, where the majority of proteins have 16 g N/100 g; the 6.38 conversion factor was used. Samples were quartered, milled and sieved using an ultracentrifuge mill (ZM 200 Retsch®, Haan, Germany) to a final 1 mm particle size. A 150–400 mg subsample was accurately measured within tin foils that were immediately placed in the carrousel.

Kjeldahl protein method was conducted according to ISO 17025 accredited AOAC OMA^{SM} methods 920.09, 920.115G, 920.85, 928.08, 930.25, 930.29, 935.39C, 940.25, 945.39C, 945.48, 950.36, 979.09, and 991.20 using a digestion and distillation systems (20-place digestion block Digestor™, Tecator series 2520 and Kjeltec™ 8400 distillation unit, FOSS, Hillerød, Denmark).

2.4. Simultaneous analysis of moisture content and dry ash in foodstuffs using thermogravimetric analysis

A thermogravimetric analyzer (LECO, TGA 801, Saint Joseph, MI, USA) was used for the determination of moisture and ash. Previously dried (at 100 °C for an hour) crucibles and caps were let to dry in a desiccator and cool down to room temperature and then loaded into the sample carousel into ceramic crucibles. An accurately measure form 100 to 2000 mg of previously sieved (see above) sample. The mass change of each sample is sequentially monitored throughout an analysis with percent mass change reported at the end of each step. Data acquisition was performed using TGA v1.46. For moisture analysis, the system was set to a desired temperature (see Table 2) and until constant mass. For ash, oxidative combustion of organic matter was completed until white ash was achieved.

Natural convection or vacuum oven-based moisture was assessed based on AOAC OMA^{SM} 920.116, 925.10, 926.07, 927.05, 935.36, 935.39A, 950.46A, 964.22, 968.11, and 990.19. Meanwhile, ash determinations using a muffler were based on 920.117, 920.153, 920.93, 923.03, 925.11, 925.51, 930.229, 930.30, 935.39B, 940.26, and 950.14. All accredited ISO 17025 methods.

2.5. Statistical analysis

HorRat was based on repeatability, the ratio among the experimental RSD_r and its calculated counterpart. RSD_r and RSD_R are calculated based on mass fraction of the analyte tested using a modified Horwitz equation (e.g., $PRSD_x = 2C^{-0.5}$, Boyer et al., 1985; Horwitz and Albert, 2006). z values were calculated based on standard normal distribution for a 95% confidence level. Then, acceptable z values (i.e., from −2 to 2) were considered as proof of the method acceptable bias, accuracy, and recovery. In this scenario, z values indicate the number of standard deviations from the mean a data point is. Mathematically, $z = (x - \mu)/\sigma$. Then, z values are calculated as follows: robust mean concentration (obtained from the method/analyte performance agreed among several laboratories) subtracted by the result obtained by the laboratory divided by the robust standard deviation. Laboratory scales and direct measurement equipment was calibrated by laboratories also accredited by ISO 17025. Expanded uncertainties are reported with a coverage factor of $k = 2$, which indicates approximate 95.4% confidence. For all statistical analyses, an α of 0.05 was considered a threshold to assess significance. All statistical analyses were performed using SAS JMP 16.1 (Cary, NC, USA). Methods were validated according to performance parameters dictated by AOAC, US FDA, and ICH (AOAC, 2012; US FDA, 2015; Borman and Elder, 2018; Raposo and Ibelli-Bianco, 2020).

Table 3
Analysis of repeatability and reproducibility of various food materials for protein analysis[a].

Food matrix	Mean ± standard deviation, g/100 g	% RSD	HorRat
Repeatibility Coefficient of Variation			
Meat and meat products			
Meat products	1.31 ± 0.017	1.29	0.67
Meat products	2.25 ± 0.071	3.17	1.78
Meat products	3.04 ± 0.038	1.27	0.75
Sausage	1.96 ± 0.062	3.14	1.74
Sausage	2.44 ± 0.056	2.29	1.31
Sausage	2.14 ± 0.030	1.40	0.79
Dairy products			
Milk powder	25.09 ± 0.076	0.30	0.27
Milk powder	3.86 ± 0.042	1.08	0.66
Low fat milk	0.735 ± 0.023	3.14	1.50
Evaporated milk	7.81 ± 0.142	1.82	1.24
Condensed milk	1.34 ± 0.041	3.08	1.61
Sour cream	0.536 ± 0.037	6.97	1.79
Yogurt	1.24 ± 0.037	2.97	1.54
Cheese	3.08 ± 0.082	2.67	1.50
Grain products			
Wheat meal	12.28 ± 0.212	1.73	1.40
Whole wheat pasta	14.65 ± 0.150	1.02	0.85
Oats	1.87 ± 0.023	1.24	1.12
Oats	1.87 ± 0.013	0.69	0.37
Wheat pasta	2.31 ± 0.025	1.07	0.61
Reproducibility Coefficient of Variation			
Milk powder	25.09 ± 0.076	0.30	0.27
Wheat meal	12.28 ± 0.212	1.73	1.40
Whole wheat pasta	14.65 ± 0.150	1.02	0.85
Oats	1.87 ± 0.023	1.24	1.12

[a] At least three independent samples for each food were tested under repeatability or reproducibility conditions.

2.5.1. For protein analysis

During protein analysis ruggedness a two tailed *t*-student assay was used to compare $n = 7$ replicates of each treatment. Determination coefficients were used to assess among the traditional and combustion method for protein, $r^2 \approx 1$ indicates that the regression predictions perfectly fit the data. Protein recovery was assess using $n = 12$ independent experiment all in duplicate using, previously dried, arginine and aspartic acid, the latter is also used as an internal control material of the equipment. Finally, six independent measurements for a nitrogen-free compound (i.e., sucrose) were used to assess limit of detection of the method.

2.5.2. For moisture analysis

Repeatability and reproducibility were performed as described in section 2.51. For ruggedness analysis, three different sample masses (0.2, 0.5, and 1; for butter and coffee samples of 2 g were also tested) and temperatures were assayed using nine independent replicates per treatment/matrix. Temperature ranges, for each food, were based on two criteria (from data that emerge from traditional techniques) i. the temperature recommended by the reference AOAC method and ii. the temperature variability during the oven resistance cycling. Moisture

Table 4
Analysis of ruggedness for protein determination (variation of the subsample target mass and oxygen flow) for three different categories of food materials using certified materials.

Grain products											
		Croutons		Cereal		Wheat meal		Oats		Snack	
Mass. mg	*Flow*	*Concentrations, g/100 g*[a]									
150	Low	2.38[a]	2.57[a]	2.14[a]	–	2.19[a]	–	1.88[a]	1.86[a]	1.20[a]	1.13
	Medium	2.59[a]		2.11[b]		2.15[b]		1.86[a]		1.14[ab]	
	High	2.53[a]		2.10[b]		2.10[c]		1.86[a]		1.13[b]	
250	Low	2.36[a]	2.47[b]	2.18[a]	–	2.10[a]	2.09	1.88[a]	1.87[a]	1.20[a]	1.18[a]
	Medium	2.59[a]		2.08[b]		2.09[a]		1.87[a]		1.12[a]	
	High	2.46[a]		2.06[b]		2.06[b]		1.85[a]		1.08[a]	
400	Low	2.34[a]	2.38[c]	2.17[a]	2.15	–	–	1.89[a]	1.87[a]	1.12[a]	1.11[a]
	Medium	2.56[a]		2.12[a]		–		1.86[a]		1.27[b]	
	High	2.45[a]		2.16[a]		–		1.87[a]		1.11[a]	
Accepted range		2.18–2.51		1.87–2.16		1.91–2.20		1.74–2.01		0.97–1.14	
Canned meat											
150	Low	2.39[a]				2.30[a] ($z = 3.33$)		1.61[a]		1.47[a]	
	Medium	2.27[ab]						1.41[a]			
	High	2.26[b]						1.58[a]			
250	Low	2.17[a]				2.13[b] ($z = -1.02$)		1.41[a]		1.37[a]	
	Medium	2.14[a]						1.27[a]			
	High	2.11[a]						1.24[a]			
400	Low	2.13[a]				2.09[b] ($z = -2.05$)		1.39[a]		1.31[a]	
	Medium	2.08[a]						1.37[a]			
	High	2.08[a]						1.30[a]			
Certified level		2.17 ($\sigma_p = 0.039$)						1.23–1.32			
Dairy products											
		Condensed milk				Pasta and cheese		Evaporated milk		Powdered Milk	
150	Low	1.38[a]	1.36[a]			0.62[a]	0.54[a]	1.24[a]	1.21[a]	5.16[a]	5.48
	Medium	1.36[a]				0.58[a]		1.22[a]		5.50[ab]	
	High	1.35[a]				0.49[b]		1.25[a]		5.77[b]	
250	Low	1.38[a]	1.39[a]			0.50[a]	0.54[a]	1.19[a]	1.19[a]	5.82[a]	5.77
	Medium	1.38[a]				0.62[a]		1.16[a]		5.72[a]	
	High	1.42[a]				0.50[a]		1.13[a]		5.89[a]	
400	Low	1.42[a]	1.48[b]			0.51[a]	0.56[a]	1.21[a]	1.09[a]	5.67[a]	5.65
	Medium	1.47[a]				0.53[a]		1.20[a]		5.64[a]	
	High	1.53[a]				0.67[b]		1.26[a]		5.63[a]	
Accepted range		1.19–1.40				0.47–0.56		1.01–1.19		5.40–5.59	

[a] Dissimilar letters show significant differences ($p < 0.05$) among rows (i.e., among concentrations obtained from different mass treatments or flows).

levels were compared using *t*-student and an ANOVA with a *post-hoc* Tukey-Kramer test to assess mass substitution and temperature ranges, respectively. *t*-student test was also used to assess differences among the values reported by thermogravimetric analysis versus traditional convection oven for a $n = 8$ samples.

2.5.3. For ash analysis

For the case of ruggedness, two different subsample masses (i.e., 0.2 and 1.0 g) were tested in three types of matrices (i.e., powdered milk, cereal, and canned meat). Several experiments were conducted in which ash was determined at four different temperatures (550, 600, 650, and 700 °C) and three levels of oxygen flow (low, medium and high) for milk, meat products and cereals. For the factorial design for coffee, two additional variable levels were tested i.e., subsample masses varying from 0.25 to 1.00 g and the comparison of pure and "torrefacto" roasted coffee (sugar-enriched coffee).

3. Results and discussion

3.1. Protein analysis and validation using combustion

3.1.1. Repeatability and reproducibility

Variations for repeatability and reproducibly ranged from 0.30 to 6.97 and 0.30 to 1.73, respectively (Table 3). HorRat values do not exceed threshold value of two, which is considered suitable as a performance benchmark (Table 3). According to 992.15 SD_r should not exceed 0.15. As such, the laboratory can set is acceptance levels for variation coefficient below a 10%.

Table 5
Veracity and accuracy of the protein method based on certified reference materials and proficiency schemes.

Proficiency scheme results		
Food matrix	*Obtained result, g/100 g*	*z value*
Oat flakes	*1.69*	*−0.16*
Corn meal	*1.32*[a]	*0.92*
Meat and fish pate	*2.03*	*0.88*
Meat or meat products	2.23	1.61
Certified reference materials		
Food matrix	***Obtained result, g/100 g***[a]	***Accepted range***
Meat or meat products	2.94[a]	2.56–2.99
Wheat meal	1.97	1.90–2.19
Wheat meal	2.09	1.91–2.20
Oats	1.87	1.74–2.01
Snack	1.12	0.97–1.14
Pasta and Cheese	0.54	0.47–0.56
Condensed milk	1.21	1.01–1.19
Powdered milk	5.48	5.40–5.59
Condensed milk	1.36	1.19–1.40
Cereal	2.08	1.87–2.16
Meat or meat products	1.27	1.23–1.32
Croutons	2.47	2.18–2.51

[a] Values corrected for a factor obtained from comparing Kjeldahl method versus combustion, K/D = 0.96 (see section 3.1.4).

3.1.2. Ruggedness and bias

Meat and meat products and dairy products seem to be less prone to bias as small modifications or perturbations are introduced within the method (Table 4). In contrast, in grain products $n = 5$ different matrices were tested and some differences arise when conditions (mass and

Table 6
Comparison of Kjeldahl and traditional methods for different food products.

Food Matrix	$N_{Kjeldahl}$	$N_{Combustion}$	$N_{Kjeldahl}/N_{Combustión}$ (K/D ratio)
Meat and meat products			
Ground meat	2.74	2.89	0.97
Pate	1.72	1.71	1.00
Mortadella	1.93	1.82	0.94
Sausage	1.53	1.63	0.88
Sausage	1.75	1.92	0.92
Pork sausage	2.14	2.35	0.92
Canned meat	2.17	2.14	1.01
Canned meat	1.32	1.37	1.04
Dairy products			
Sour cream	0.43	0.51	0.84
Low fat milk	0.52	0.56	0.93
Defatted milk	0.57	0.64	0.90
Whole milk	0.48	0.50	0.96
Milk powder	4.67	4.41	1.06
Fresh cheese	3.47	3.07	1.13
Yogurt	0.67	0.69	0.96
Grain products			
Wheat meal	2.05	2.08	0.99
Croutons	2.35	2.36	1.00
Oats	1.84	1.87	0.98
Wheat meal	1.89	1.97	0.96
Pasta	2.20	2.33	0.94

oxygen flow) are modified. The method seems to be more sensitive toward mass changes (Table 4). Lower masses (i.e., 150 mg or less) will be more likely to suffer from sample heterogeneity, higher masses may overestimate if the detector is saturated (Table 4). However, overall, a medium O_2 flow and 250 mg subsample mass, seem to be enough to gain sample representability, while maintaining accurate values (Table 4).

3.1.3. Veracity and accuracy

Allotted proficiency schemes and certified materials tested within the concentration range reported by the manufacturer or by the supplier (Table 5). This speaks toward a very accurate method for the three most extensive group of foods (i.e., meat and meat, dairy, and grain products).

3.1.4. Direct comparison with Kjeldahl (traditional) method

As stated before, for some food products a correction may be needed to avoid protein overestimation when using combustion analysis. Both Kjeldahl and combustion assays are not equivalent as they use different chemical principles to ascertain nitrogen. The former acknowledges organic protein sources while combustion will definitely will account for other inorganic sources (e.g., nitrate and nitrite used for curing and preserving meats and fish (Majou and Christieans, 2018) or remainder of inorganic or slow release fertilizers in crops). Notwithstanding, our data shows that both methods perform very closely and that the ratio between protein concentrations obtained for the same samples nears one (Table 6). Then, from regression analyses performed r^2 range from 0.95 to 0.99, which indicate that only a small fraction of the cases used cannot be fitted within the model. The regression equation gives a direct association among the fitness between Kjeldahl and combustion methods (Fig. 1). The most significant correction we found was for meat products (slope 0.9361, Fig. 1A), and trivial for dairy and grain products (slopes 1.1001 and 0.9709, respectively) (Fig. 1B and C). In all cases intercepts were insignificant (Fig. 1A–C). Our data is in line with that of Lanza and coworkers (2016).

3.1.5. Recovery

In the case of aspartic acid, mean nitrogen values were 10.52 g/100 g. This is in line with the value reported by the manufacturer i.e., 10.1–10.8 g/100 g. Meanwhile, arginine recoveries, which are also used as quality control for the Kjeldahl method, were 98.94 ± 0.66%. Twice (97.62–100.26%) and thrice (96.96–100.92%) the standard deviation were calculated to define both action and alert thresholds (respectively). Recoveries from 92 to 105, 95–102, and 98–101 for ingredient ranges form 1–100 g/100 g are considered acceptable (González et al., 2010).

3.1.6. Limit of detection and uncertainty

On average, a non-containing nitrogen compound generated a signal

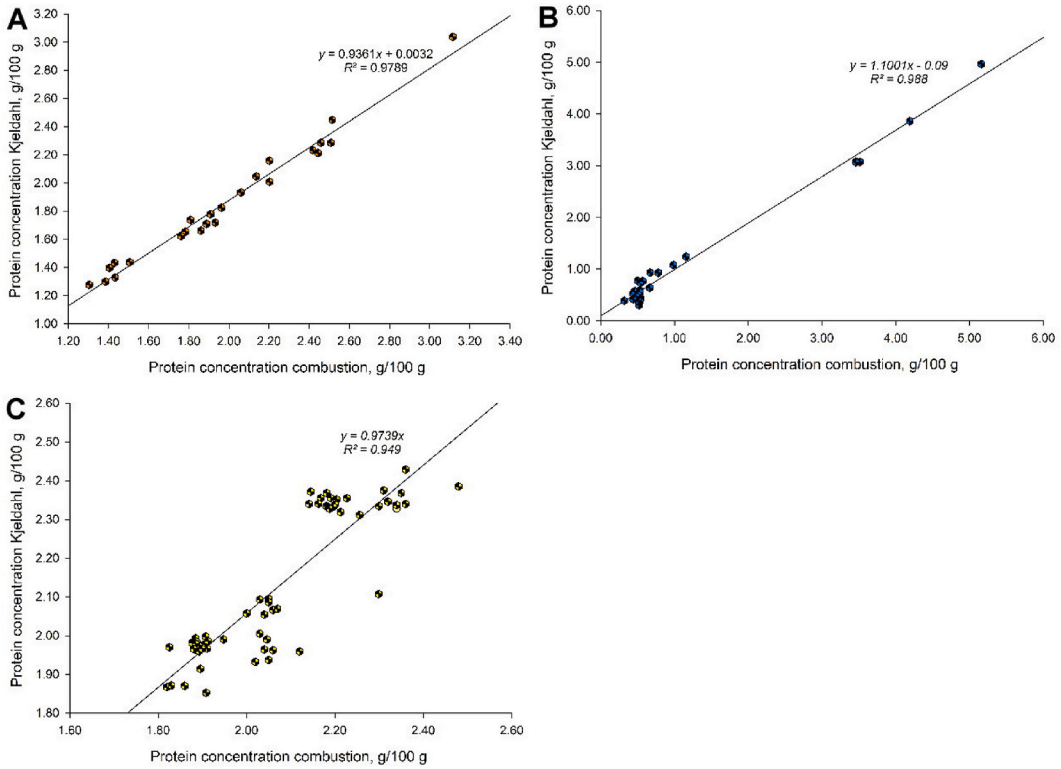

Fig. 1. Association of nitrogen obtained by combustion with the nitrogen obtained by Kjeldahl in A. meat products B. dairy products and C. grain products.

Table 7
Analysis of repeatability and reproducibility of various food materials for moisture analysis[a].

Food matrix	Mean ± standard deviation, g/100 g	% RSD	HorRat
Repeatability Coefficient of Variation			
Meat and meat products			
Meat products	59.70 ± 0.89	1.05	1.08
Dairy products			
Milk powder	3.39 ± 0.07	2.07	1.66
Evaporated milk	74.93 ± 0.19	0.25	1.05
Grain products			
Wheat meal	12.59 ± 0.03	0.21	1.37
Croutons	8.39 ± 0.05	0.54	1.45
Cereal	12.32 ± 0.04	0.35	1.37
Corn meal	10.77 ± 0.25	2.34	1.40
Corn meal	10.66 ± 0.18	1.71	1.40
Other food products			
Chicken stock poder	2.46 ± 0.23	9.54	1.75
Chicken stock poder	2.83 ± 0.07	2.49	1.71
Tomato kétchup	68.22 ± 2.82	4.13	1.06
Reproducibility Coefficient of Variation			
Milk poder	3.48 ± 0.12	3.40	1.66
Wheat meal	12.65 ± 0.09	0.69	1.37
Croutons	8.37 ± 0.07	0.84	1.45
Cereal	12.19 ± 0.10	0.86	1.37

of 0.131 ± 0.009 (i.e., 7.22% RSD) translated to a concentration this represents 0.22 g N/100 g, making the method considerably sensitive. Absolute ($\sqrt{(\Sigma U_i^2)}$), relative (U_x/x) and expanded uncertainty were calculated at 5.51×10^{-3}, 1.03×10^{-2}, and 2.06×10^{-2}, which relatively represents 1.10% of the measurand. Meanwhile, our Kjeldahl method records a 2.85×10^{-2} expanded uncertainty (i.e., 0.89% relative to the measurand). As expected, performance data of analytical methods are the main source of uncertainties (Mołognoni et al., 2019), specifically, in both cases, reproducibility data represent more than 95% of the uncertainty.

3.2. Moisture analysis and validation using thermogravimetric analysis (TGA)

3.2.1. Repeatability

Repeatability and reproducibility coefficient of variation varied from 0.25 to 9.54 (where evaporated milk and chicken stock powder showed the least and most dispersion, respectively) and from 0.69 to 3.40 (with milk powder with the most dispersion among independent replicates), respectively (Table 7). Hence again, laboratory precision can be set below 10% (Table 7).

3.2.2. Ruggedness

Milk powder, pure coffee and corn meal were the most susceptible to increasing temperatures; 5° Δ were sufficient to affect the significantly moisture levels obtained (Table 8). On the contrary, no significant differences were found for sugar-enriched coffee and butter ($p < 0.05$). For coffee and butter, augmenting subsample size to 2 g resulted in diminished moisture levels ($p < 0.05$) (data not shown). In the case of temperature, working ranges (without sacrificing accuracy) were tested for a sample from each food group (Table 8). Additionally, using a certified material standardized subsample of 0.5 g, the modification of ±10 °C does have a significant impact on moisture for grain, milk products or coffee ($p < 0.05$, Table 8). Again, butter moisture values seem to be less affected by variations in temperature.

3.2.3. Comparison between traditional (vacuum/convection oven drying) and thermogravimetric determinations

No significant differences were observed ($p < 0.05$) for canned meat (i.e., 64.95 ± 0.26 and 64.95 ± 0.35 g/100 g), croutons (i.e., 8.87 ± 0.10 and 8.85 ± 0.03 g/100 g), tomato ketchup or milk powder when comparing methods (traditional versus thermogravimetric), in both cases measurements performed at 110 °C (except for croutons where temperature was set 120 °C). Tables 8 and 9 demonstrate that despite the physical (e.g., brittleness and grinding) and chemical differences of roasted pure and sugar-enriched coffee (Andueza et al., 2003; Baggenstoss et al., 2008), moisture can be measured successfully for both matrices. "Torrefacto" coffee is produced by a roasting process in which sugar is added to coffee, normally Robusta (Ludwig et al., 2013). A similar situation arise with powdered and evaporated milk. In fact traditionally, two different AOAC methods, 927.05 and 925.23A, using vacuum oven (≤4 in Hg) or convection oven, respectively on both accounts, to assess moisture in these food products (Martins et al., 2018). Thermogravimetric analysis is able to work with both types of food samples. As a direct measurement of moisture, thermogravimetric analysis additional advantage consist on the fact that the method can be improved if data comparable to Karl Fisher is desired by distinguishing among bound and free water in food (Rückold et al., 2000; Park, 2009; Wang et al., 2017).

3.2.4. Veracity

Acceptable values were obtained when analyzing certified materials and during proficiency scheme tests (Table 9) pointing toward an

Table 8
Analysis of the robustness of moisture at different temperatures for coffee, dry and dairy samples.

Food matrix	**Temperature, °C***			
Meat products	102	106	110	
Croutons	-	120	130	
Wheat meal	110	120	130	
Tomato ketchup	71	104	110	
Baby Food	-	70	120	
Food matrix	**Temperature, °C****			**Accepted value, g/100 g**
	95	100	110	
		Dairy products		
Milk powder	96.87[a]	96.64[b]	96.33[c]	96.11-96.79
Butter	15.04[a]	15.46[a]	15.34[a]	15.42
		Grain products		
Corn meal	9.70[a]	10.02[b]	10.42[c]	9.90
		Coffee		
Coffee (pure)	0.99[a]	1.22[b]	1.47[c]	1.00-1.41
Coffee (Sugar-enriched)	1.09[a]	1.17[a]	1.38[b]	0.79-1.27

*Green colored cells indicate acceptable values for the proficiency test or certified material, whereas orange, values outside specification.
**Dissimilar letters show significant differences ($p < 0.05$) among rows (i.e., among concentrations obtained from temperatures).

Table 9
Accuracy of thermogravimetric moisture analysis.

Proficiency scheme results			
Food matrix	*Obtained value, g/100 g*		*z value*
Evaporated milk	75.15		−0.03
Coffee (Pure)	1.26		−0.70
Coffee (Sugar-enriched)	0.85		−1.20
Wheat meal	10.81		−0.5
Oat	9.31		0.72
Canned meat	66.53		−0.24
Coffee (Pure)	1.22		−0.29
Coffee (Sugar-enriched)	1.17		1.16
Fruit/vegetable puree	83.25		0.00
Certified reference materials			
Food matrix	*Obtained value, g/100 g*	*Experimental values, g/100 g*	*Recovery, %*
Croutons	8.37	7.55–8.49	104.36
Cereal	12.19	11.59–12.94	99.34
Milk powder	3.02	2.93–3.65	91.79
Milk powder	96.26	96,11–96,79	99,80
Milk powder	96.96	96,76–97,56	99,70
Meat product	60.53	60.2–60.8	100.04
Biscuit	2.66	2.05–3.42	97,43
Chocolate	1.95	1.47–2.21	105.16

Table 10
Precision results for ash thermogravimetric determination.

Food Matrix	Mean ± standard deviation, g/100 g	% RSD	HorRat
Repeatability Coefficient of Variation			
Milk poder	5.58 ± 0.06	1.17	1.38
Croutons	2.93 ± 0.06	2.21	1.64
Corn meal	1.35 ± 0.04	2.87	1.72
Coffee (pure)	4.17 ± 0.11	2.54	0.98
Chicken stock powder	53.72 ± 1.63	3.03	0.28
Evaporated milk	1.56 ± 0.09	5.65	1.60
Corn meal	1.37 ± 0.12	8.51	1.71
Coffee (sugar-enriched)	3.14 ± 0.08	2.52	1.13
Coffee (pure)	4.11 ± 0.16	3.94	0.98
Chicken stock poder	61.74 ± 1.14	1.85	0.25
Reproducibility Coefficient of Variation			
Milk poder	5.56 ± 0.08	1.39	1.89
Croutons	2.96 ± 0.05	1.78	1.53

accurate method.

3.2.5. Limit of detection and uncertainty

The minimum value of moisture that can be detected gravimetrically depended on the standard deviation of the repeatability of the analytical balance reported by the manufacturer (i.e., 0.02% RSD). When multiplied by 10 and divided by the minimum mass to be weighed according to the method (0.2 g), corresponds to 0.2 g/100 g. On another hand, absolute, relative, and expanded uncertainty were calculated at 2.07×10^{-3}, 4.54×10^{-2}, and 9.07×10^{-2}, which relatively represents 4.14% of the measurand. Meanwhile, our convection oven method records a 1.15×10^{-1} expanded uncertainty (i.e., 5.23% relative to the measurand). Where the 99% of the uncertainty input was represented by reproducibility.

3.3. Ash analysis and validation using thermogravimetric analysis (TGA)

3.3.1. Precision, repeatability, and reproducibility

For ash, and under repeatability conditions, corn meal throws the most dispersion among the food matrices tested (8.51 %RSD) (Table 10).

Table 11
Ruggedness analysis (effect of oxygen flow and temperature) for ash during thermogravimetry.

Proficiency scheme testing				
Milk powder				
Assay	Temperature, °C	Oxigen flow	Concentration, g/100 g[b]	*z* score
1	550	Low	5.90	0.76
2	600	High	5.88	0.63
3	600	Low	5.89	0.68
4	650	Low	5.87[a]	0.55
5	650	Medium	5.74[b]	0.20
6	650	High	5.70[b]	0.36
7	700	Medium	5.63	0.80
Roasted Coffee (pure)				
Subsample mass, g	**Temperature, °C**	**Oxigen flow**	**Concentration, g/100 g**[b]	***z* score**
0.25	650	Low	4.50^{XY}	.1.45
0.50	550	Low	4.68^{A}	−0.87
	550	Medium	5.15^{B}	0.64
	600	Low	4.59^{BC}	−1.16
	650	Medium	5.20^{A}	0.81
	650	Low	$4.48^{C.X}$	−1.52
1.00	650	Low	4.54^{Y}	−1.33
Roasted Coffee (sugar-enriched)				
0.25	650	Low	3.29	−1.40
0.50	550	Low	3.32	−1.27
	550	Medium	3.52	−0.43
	600	Low	3.20	−1.74
	650	Medium	3.40	−0.92
	650	Low	3.30	−1.35
1.00	650	Low	3.34	−1.17
Meat products				
Assay	**Temperature, °C**	**Oxigen flow**	**Concentration, g/100 g**	
			Experimental	*Accepted range*
1	550	Low	3.00[a]	2.65–3.03
2	600	High	2.91	
3	600	Low	3.02[a]	
4	700	Medium	2.58	
5	650	Low	2.13	1.95–2.25
6	650	Medium	2.08	
7	650	High	2.12	
8	700	Low	2.18	
9	700	Medium	2.11	
10	700	High	2.16	

[a] Samples processed using a 1.00 g subsample.
[b] Dissimilar letters show significant differences ($p < 0.05$) among rows (i.e., among concentrations obtained from temperatures).

This is contrast with milk powder with just 1.17 %RSD (Table 10).

3.3.2. Ruggedness

In the case of cereal and meat, there is a significant difference between both masses tested ($p < 0.05$). Results obtained using 1.0 g sample meet more closely the certificate analysis. In the case of powdered milk, no significant differences were found when the tests are performed using 0.2 or 1.0 g ($p < 0.05$).

In the case of milk powder, when temperature is set to 650 °C and the oxygen flow modified, it is observed that there is a significant difference ($p < 0.05$) at low flows, where the ash value is higher, with respect to those determined for medium and high flows (Table 11). Additionally, the lowest ash values are obtained at 700 °C, there is no difference between 550 and 600 °C, 600 and 650 °C at low flow, or 650 and 700 °C using medium gas flows ($p < 0.05$; Table 11).

Oxygen flow did not significantly affected ($p < 0.05$) ash content in meat products at temperatures of 650 and 700 °C. At 700 °C the values are lower than those reported at 550 or 600 °C, there are no differences between the latter temperatures and the values obtained are in the middle of the range reported by the supplier (Table 11).

For cereals, at 700 °C with medium flow ash values are below

Table 12
Accuracy for ash thermogravimetric method.

Profeciency scheme			
Food matrix		**Obtained result, g/100 g**	***z* score**
Evaporated milk		1.37	−0.83
Roasted coffee (pure)		4.19	−1.40
Roasted coffee (sugar-enriched)		3.65	−0.10
Meat product		3.70	1.90
Pineapple juice		0.48	0.54
Oat		1.73	0.37
Certified reference materials			
Food matrix	**Obtained value, g/100 g**	**Experimental values, g/100 g**	**Recovery, %**
Meat product	4.13	(4.04–4.4)	100.98
Biscuit	1.14	(1.11–1.30)	94.21

acceptable ranges. However, there are no significant differences ($p < 0.05$) between the temperatures of 550 and 600 °C or low and high flows (Table 11).

In the case of pure coffee, it is observed that there are significant differences ($p < 0.05$) between the masses at 650 °C and low flow, and among the other temperatures, the *z* scores closest to 0 are obtained with 0.5 g, 550 °C medium and low flow, as well as at 650 °C and medium flow. With roasted coffee, no significant differences were found between variables and conditions found for pure coffee replicate.

3.3.3. Veracity and comparison with the conventional method

Powdered milk replicates measured by traditional and thermogravimetric methods showed no significant differences ($p < 0.05$, data not shown). In addition, *z* scores ranging from −1.40 to 1.90 were found for ash and a mean recovery of 100.98 for meat products (Table 12), which demonstrate an acceptable method accuracy.

3.3.4. Limit of detection and uncertainty

Dynamic working range was calculated to start at 0.20 g/100 g; similarly as it was analyzed for moisture (see above). On another hand, absolute, relative, and expanded uncertainty were calculated at 9.66×10^{-3}, 2.91×10^{-2}, and 5.80×10^{-2}, which relatively represents 1.93% of the measurand. Meanwhile, our furnace method records a 6.81×10^{-2} expanded uncertainty (i.e., 2.26% relative to the measurand). Where the 99% of the uncertainty input was represented by reproducibility.

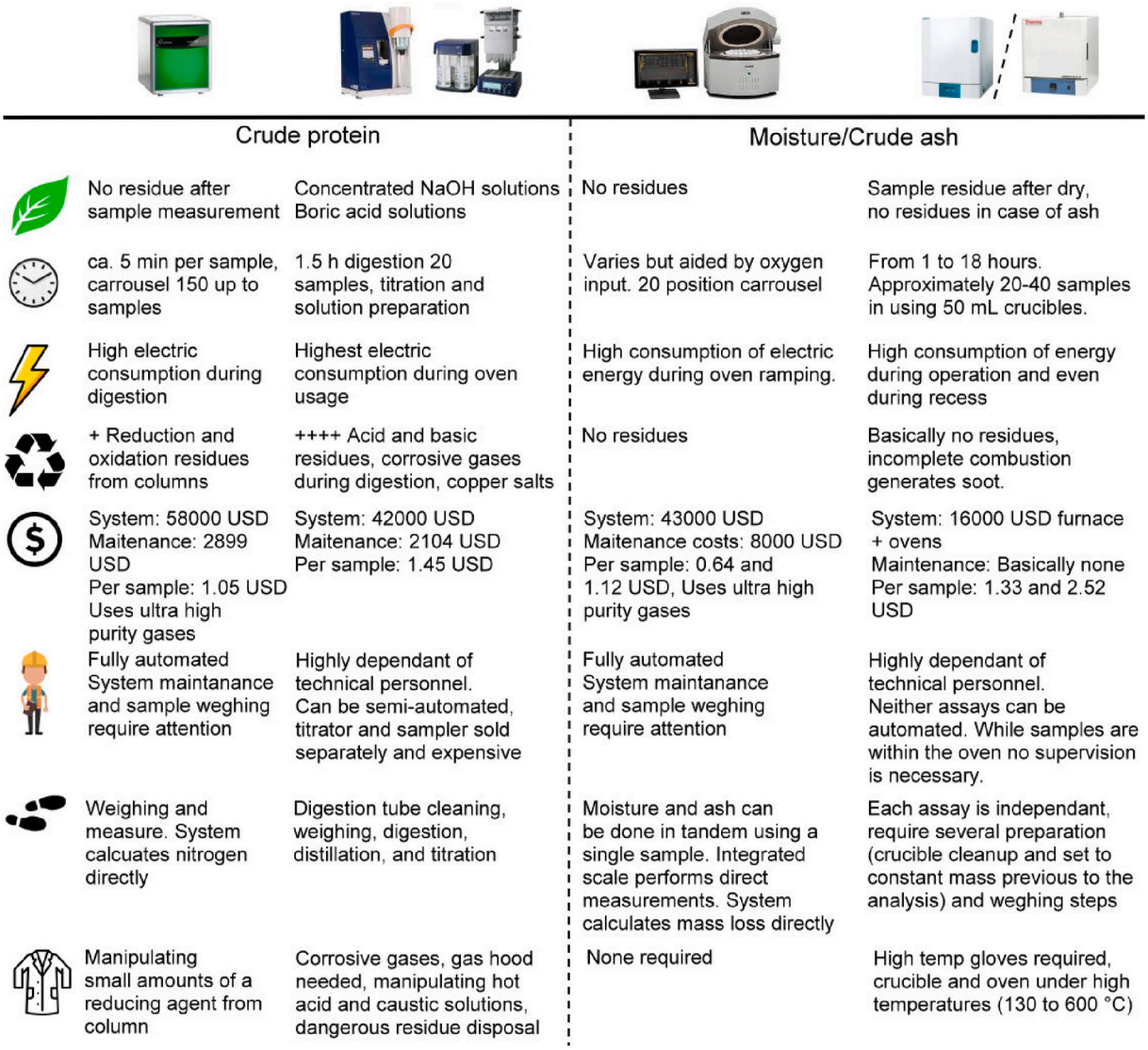

Fig. 2. Diagrammatic comparison of traditional, combustion, and thermogravimetric for the determination of three proximate analysis.

Table 13
Absolute year costs comparison for thermogravimetric versus traditional analysis.

	Moisture analysis			Ash analysis		
Descriptor	Thermogravimertic	Traditional	Difference	Thermogravimertic	Traditional	Difference
Salary, USD	722.4	6305.9	5583.2	444.6	6935.2	6490.9
Hour occupation, %	7.5	64.9	57.5	4.5	71.4	66.9
Energy demand, kWh^{-1}	11369.0	9237.4	2131.6	3598.7	1612.3	1986.3
Energy demand, USD	2956.0	2401.7	554.3	935.6	419.2	516.3

3.4. Costs, environmental impact, and technical requirements of validated methods

An economic analysis for a laboratory with a yearly total demand of 1662 moisture and ash analysis (63.9% of the total were requests for moisture) was performed for the year 2021. For example, thermogravimetry versus the conventional methods demonstrates that energy demands (i.e., 20 and 50% more for moisture and ash, respectively) and maintenance (i.e, parts, consumables, technical support) for thermogravimetric analyses are higher than that for traditional methods (Fig. 2, Table 13). However, the salary demands and operator dependence are extremely low and vastly compensate these costs (Table 13). In the case, of protein analysis, the advantages of using combustion vastly surpass the traditional Kjeldahl method; this includes a migration toward green chemistry analytical methods (Fig. 1) (Evers and Hughes, 2002). For example, for a laboratory performing 2000 protein assays per year, this represents (assuming the digestion block is filled at full capacity) 125 times the Kjeldahl method is run. This spread over the year (i.e., over 52 weeks), implies 2.5 digestions and 50 distillations per week. Both systems necessary for Kjeldahl analysis have an energy demand of 2200 W, which will mean a total consumption of 103 kWh^{-1} per month. On the other hand, a conservative estimate will appraise the time demand per sample at 12.5 min per sample (contemplating digestion, distillation and titration alone (i.e., 3–4 times less than what is required per sample in combustion). Time spent preparing solutions (e.g., boric acid, preparation of acid-base indicators and sodium hydroxide solutions) must be prepended. The same amount of samples can be performed in 150 h using just a third of a 220 cubic feet CO_2 cylinder and no replacement of the reduction column would still be necessary during combustion analysis. Hence, although combustion analysis has in the past been somewhat labeled as expensive and prone to overestimate protein for food analysis (Sáez-Plaza et al., 2013), our data states otherwise. We also contest that a laboratory that can initially afford a Kjeldahl systems like the one described herein, can also afford a combustion system.

4. Conclusions

Both combustion and thermogravimetric analyses can replace the often slow, labor-intensive, traditional manual digestion or gravimetric techniques that require multiple sample weighing and transfer steps and involves numerous equipment (e.g., digestors, distillation units, digestion tubes, desiccators, vacuum and convection ovens). Flexible method settings, automation, and hardware capabilities deliver an automated analysis process while requiring only the manual measurement of the initial sample mass, which translates in productivity. In the case of thermogravimetric analysis, the chemical principle involving the measurement are equivalent to those traditional methods. Hence, the migration of the latter to their automated versions (and in the specific case of protein to a more proficient one) is a less laborious task validation-wise, and more cost effective (especially for high throughput food analysis or research laboratories). Small samples used in either method may force to improve in sample pretreatments (milling and sieving using $\leq$ 1 mm particle size) to ensure representability. As combustion analysis is, in its core, a gas separation technique, which is able to detect NO_x gases after an oxidation step using a TCD, this analysis is highly selective.

CRediT authorship contribution statement

Carolina Cortés-Herrera: Conceptualization, Methodology, Validation, Resources, Data curation, Writing – review & editing, Supervision. **Silvia Quirós-Fallas:** Formal analysis, Validation. **Eduardo Calderón-Calvo:** Formal analysis, Validation. **Randall Cordero-Madrigal:** Formal analysis, Validation. **Laura Jiménez:** Formal analysis, Validation. **Fabio Granados-Chinchilla:** Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Graciela Artavia:** Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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