



# Quality assuring of wheat flour as a reference material for protein measurements

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## Abstract

Certified reference materials (CRM) are essential tools for calibration of measuring instruments, validation of analytical methods and assuring quality of measurement results. However, in the case of the mass fractions protein and moisture in wheat flour, which is used to determine the quality and the market ability of flour, there is a lack of matrix reference materials. CRM for protein and moisture in wheat flour has been measured by the National Institute of Standards, Egypt. This article describes the value assignment of the mass fractions nitrogen and moisture in wheat flour by using Kjeldahl and dry oven; then, measuring the samples by inframatic analyzer system, the results were statistically analyzed and weighted mean; the results were found to be  $10.33 \% \pm 0.36 \% (k = 2)$  for protein and  $16.98 \% \pm 0.30 \% (k = 2)$  for moisture (as mass fractions). Finally, with performance test program for the samples used from wheat flour our lab reported satisfactory results.

**Keywords** Protein · Wheat flour · Calibration · Kjeldahl · PT · Quality assuring

## Introduction

In view of the lack of a reference material, whether to calibrate inframatic devices or conduct proficiency testing according to metrological requirements to estimate protein and moisture values in flour samples and the associated uncertainty, that it can provide suitable certified reference materials (CRMs) which had been characterized by means of highly reliable methods [1, 2].

Wheat flour is the main ingredient used in the bakery industry, being the basis of products, such as bread, muffins, crackers and cookies [3]. The importance of wheat flour in world nutrition has resulted in a massive literature on the subject of wheat and wheat flour. Much of this literature refers to flour in its relationship to bread where the influence of fats and sugars in the dough is minimal [4]. Wheat flour has a chemical characteristic, namely moisture, starch, cellulose, fat, protein and ash contents [5]. The reference method for the determination of the protein content of wheat flour as an indirect measurement of nitrogen and subsequent conversion to protein, or interference from other chemical

substances [6] which is based on measurement of total nitrogen by Kjeldahl analyzer system then, multiplied nitrogen (as mass fraction nitrogen) by a factor, which is typically 6.38, to express the results of total protein (as mass fraction total protein) [7, 8] according to standard method [7, 9] to determine the nitrogen content (as mass fraction) in wheat flour, and also by using this official method for analysis [4] in both organic and inorganic samples, which present in foods and drinks, meat, feeds, cereals, forages, waste waters [10], soils.

In this study, the protein and moisture content (as mass fraction) of a wheat flour sample were determined by the Kjeldahl device and drying oven methods, respectively; then, this value was used to calibrate the inframatic analysis system after calculating the associated uncertainty. The preparation process described in this paper includes steps of material processing, homogeneity testing, stability study, characterization and value assignment [11, 12].

## Materials and methods

### Materials and standards

All experimental work was carried at ambient conditions of temperature and relative humidity. With chemicals of

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sulfuric acid (99.98 %), copper sulfate, potassium sulfate, sodium hydroxide (NaOH)<sup>1</sup> with certified value  $\pm 40$  %, hydrochloric acid (HCl)<sup>2</sup> with certified value  $(0.250 \pm 0.002) \text{ mol L}^{-1}$ , boric acid (H<sub>3</sub>BO<sub>3</sub>)<sup>3</sup> with certified (as mass fraction)  $\pm 4$  %, homogenized wheat flour (WF-cal.)<sup>4</sup> and ammonium dihydrogen phosphate [NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>] (ADP) [5] with certified value  $12.130 \% \pm 0.047$  %. All chemicals used were purchased from (Corner Lab) Chemical Co. Ltd. and Tiba Scientific (Giza-Egypt).

## Devises and instruments

### Kjeldahl analyzer system

This analyzer system includes nitrogen distill later of model DNP 3000 and manufactured by Raypa, ASPIN. The instrument was optimized daily as recommended by the manufacturer to achieve optimum sensitivity and stability with certificate of calibration No. 290/41/2017 ( $U = 0.16$  %,  $k = 2$  to provide a level of confidence 95 %).

### Electronic digital balance

Electric balance model CP2244S is manufactured by Sartorial-Germany [Certificate No.16/13/2017] with ( $U = 0.4$  g,  $k = 2$  to provide a level of confidence 95 %).

### Dry oven with digital indicator

Dry oven with digital indicator manufactured by Germany, Model: TARKO with Certificates<sup>5</sup> [Calibration No. 2383/32/2018] with ( $U = 1.0$  °C.,  $k = 2$  to provide a level of confidence 95 %)

## Procedure

The procedure and performance characteristics of the Kjeldahl method for determination of total nitrogen content (as mass fraction) of wheat flour are described in approved method [13]. To describe the method for protein content (as mass fraction) in wheat flour, and so it can be calculated

<sup>1</sup> Sodium hydroxide (NaOH) 40% with certificate of analysis associated by Panreac—SPAIN with code 171220, Batch No. 0000463126.

<sup>2</sup> Hydrochloric acid (HCl) 0.25 mol/l with certificate of analysis associated by Panreac—SPAIN with code 182318, Batch No 0000370059.

<sup>3</sup> Boric acid (H<sub>3</sub>BO<sub>3</sub>) 4% with certificate of analysis issued by Panreac—SPAIN with case No. 10043-35-3, EC-No.: 233-139-2.

<sup>4</sup> WF-cal. Means wheat flour (CRM) which used in calibration process with certificate with NIS [273/41/2018].

<sup>5</sup> These certificates traceable to National Institute of Standard (NIS)—Egypt.

**Table 1** Values of ANOVA-one-way analysis

Item	<i>F</i> value	<i>F</i> crit.	<i>P</i> value
Protein content mass fraction	0.0017	4.9646	0.9679
Moisture content mass fraction	0.0092	4.7472	0.9251

protein content (as mass fraction) in wheat flour by nitrogen (as mass fraction) measured by calibrated Kjeldahl system occurs according to ASTM D2216-10 [14].

## Results and discussion

### Assessment of wheat flour material for calibration

#### Homogeneity assessment

The homogeneity [15] of wheat flour material (WF-cal.) was stabilized by bottling and storing the samples at room temperature (24 °C); twenty bottles were chosen using sample scheme. A homogeneity test was performed on twenty bottles with two subsamples taken from each bottle. The sample size taken for homogeneity testing was approximately 0.2 g, no significant difference in the between and within bottles variance was found, and the uncertainty of the material variability was estimated by using one-way analysis of variance ANOVA [16–18]. Table 1 shows the obtained values :

The above data show that  $F$  value  $< F$  crit. and  $P$  value  $> 0.05$ , so we conclude that both samples were homogeneous, where  $P$  value is the probability of observing test statistic value,  $F$  is Fisher Snedecor distribution and  $F$  critical is a function of the degrees of freedom of the numerator and the de-numerator and significance level [ISO 3534-1:2006] [19, 20].

#### Stability assessment

The stability assessment was performed via measuring samples every three months with repeating time scheme for a year, then calculate the stability criterion accordance with (ISO 13528:2015) and apply Eq. (1):

$$|X_{\text{initial}} - X_{\text{final}}| \leq 0.3 (\delta_{\text{final}})^2 \quad (1)$$

where  $X_{\text{initial}}$ ,  $X_{\text{final}}$  are the test results before and after each repeating time scheme for both protein and moisture content (as mass fractions) and the value of  $\delta_{\text{final}}$  which represents the standard deviation for final results that appear in Table 2.

From Table 2, the values achieve the uncertainty calculated from Eq. (1), for protein equal ( $U = 10.33 \% \pm 0.36$  %)

**Table 2** Results of stability assessment performed for the material tested for the current time scheme (initial–final)

Item	$X_{initial}$	$X_{final}$	$ X_{initial} - X_{final} $	$0.3 (\delta_{final})^2$	Stability criterion
Protein mass fraction	10.33	10.31	0.02	0.025	Fulfilled
Moisture mass fraction	16.98	17.01	0.03	0.027	Fulfilled

and moisture content (as mass fraction) with uncertainty value equal ( $U = 16.98 \% \pm 0.30 \%$ ) in the wheat flour used, uncertainty calculated with the coverage factor ( $k = 2$ ), according to standard method [1, 21, 22] for the calibrated flour sample refers to NIS certificate with NIS [273/41/2018] which was measured by *Kjeldahl Analyzer system* and *Dry oven* with digital indicator used to prepare reference material

**Method and traceability of calibration for Inframatic system**

The instruments have been calibrated using homogenized wheat flour material (WF-cal.) (CRM) according to standard methods [4]; Table 3 shows the results obtained.

**Wheat flour material for proficiency testing (PT)**

The purpose of the evaluation of performance methods determination of protein and moisture content is to provide a normalized performance evaluation so that all PT results are comparable and the performance of each participant can be measured. In testing PT schemes, the performance of the participants is evaluated usually by measuring the deviation of the participant’s results from the assigned value using evaluation criterion. There are

seven participants with inframatic analysis system; all serial numbers for these appear in Table 4, our lab code 5 in 2017 and code 4 in 2018

**ζ-score performance statistics**

The ζ-score measures the deviation of reference and participants laboratories result depending on standard uncertainty of the reference value and participated laboratory as appeared in Eq. 2 [22, 23] by equation:

$$\zeta = \frac{x - X}{\sqrt{u^2(\text{lab}) + u^2(\text{ref})}} \tag{2}$$

where

- $X$  and  $x$  are the reference value and laboratory result, respectively.
  - $u(\text{lab})$  and  $u(\text{ref})$  are the standard uncertainty of the reference value and participated laboratory, respectively.
- ζ-score (as absolute value) is interpreted as follows:

**Table 4** Serial number of all inframatic analysis system in 2017 and 2018

2017		2018	
Serial number	Lab code	Serial number	Lab code
<i>Inframatic analysis system</i>			
3639	2	2939	2
3216	3	3639	3
2852	4	2832	4*
2832	5*	2637	5
2939	6	2852	6
2997	7	2997	7
2637	8	3216	8

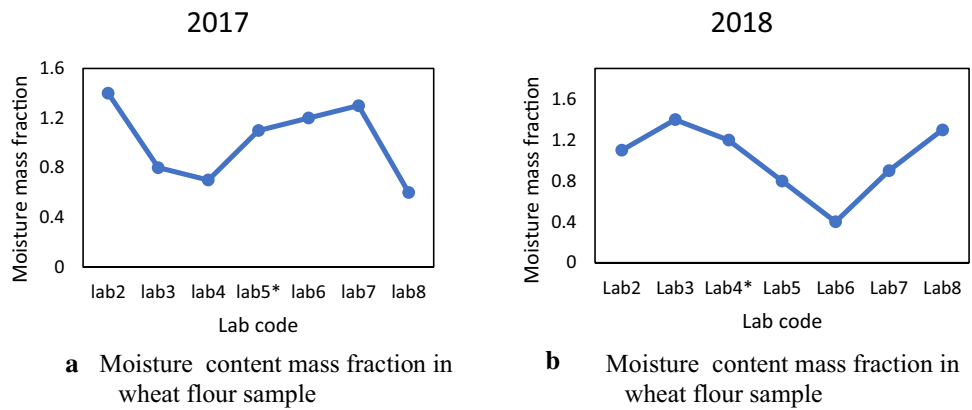
\*Our lab code 5 in 2017 and code 4 in 2018

**Table 3** Uncertainty values for all inframatic analysis system used in this PT

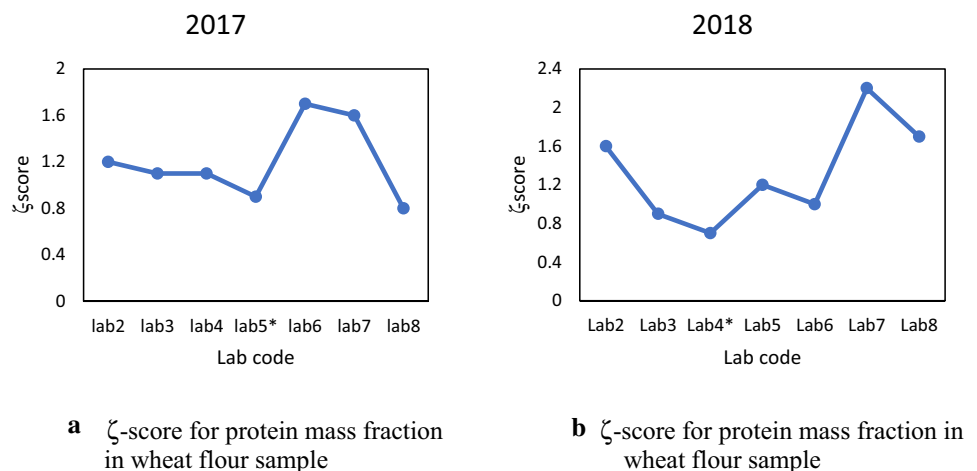
Serial number	Nominal value of protein mass fraction	Protein mass fraction (%)	Nominal value of moisture mass fraction	Moisture mass fraction (%)
<i>Inframatic analysis system</i>				
3639	10.33 ± 0.36	10.42 ± 0.38	16.98 ± 0.30	16.86 ± 0.40
3216		10.24 ± 0.37		17.2 ± 0.50
2852		10.24 ± 0.39		16.89 ± 0.40
2832*		10.38 ± 0.40		16.82 ± 0.40
2939		10.39 ± 0.39		17.00 ± 0.50
2997		9.97 ± 0.45		16.79 ± 0.60
2637		10.21 ± 0.38		16.96 ± 0.50
2962		10.11 ± 0.39		16.88 ± 0.40

\*Inframatic system of our laboratory with serial no.

**Fig. 1 a** Moisture content mass fraction in wheat flour sample. **b** Moisture content mass fraction in wheat flour sample



**Fig. 2 a**  $\zeta$ -score for protein mass fraction in wheat flour sample. **b**  $\zeta$ -score for protein mass fraction in wheat flour sample



$|\zeta|$ -score  $\leq 2.0$  Satisfactory performance;  $2.0 < |\zeta$ -score  $< 3.0$  Questionable performance;  $|\zeta|$ -score  $\geq 3.0$  Unsatisfactory performance

Figure 1a, b represents all participant laboratories with  $\zeta$ -score for moisture content (as mass fraction) in proficiency testing (2017, 2018), respectively. We note all participated laboratory in the satisfactory performance

From Fig. 2a, b, all participated laboratories have satisfactory results of protein with  $\zeta$ -score in proficiency testing (2017, 2018), respectively, except lab of code 7 has unsatisfactory result for protein measurement of Proficiency Testing (PT) in 2018.

## Conclusion

The importance of using wheat flour as a reference material with known protein and moisture content (as mass fractions) due to the shortage in the market and using it as a reference material for calibration of informatics devices as well in

technical competence tests to ensure the quality of measurement results, and track results according to the international system of SI units. In order to ensure its accuracy, reliability and comparability in accordance with ISO Guides 30–35, all devices and chemicals have accredited certifications.

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