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Influence of Altitude on the indirect Analysis of α-amylase Content on Wheat Flours

Luiz Cláudio Garcia[1*], Felipe Augusto Rogrigues Vaurof[1], Alisson Fogaça[1], Pedro Henrique Weirich Neto[1], Carlos Hugo Rocha[1], Jaime Alberti Gomes[1], Ivo Mottin Demiate[1], Polyana Elvira Tobias Pinto Christmann[1], Janaine Ritter[1], Sérgio Roberto Piaskowski[2], Evandra Fátima Webber[2]

[1] Department of Soil Science and Agricultural Engineering, State University of Ponta Grossa, Campus Uvaranas - Av. General Carlos Cavalcanti, 4748 – CEP 84030-900, Ponta Grossa (PR) Brazil. E-mail: lcgarcia@uepg.br, felipevaurof@gmail.com, alifogaca@hotmail.com, lama1@uepg.br, chrocha@uepg.br, jagmtp@gmail.com, demiate@yahoo.com, polyanaelvira@gmail.com, janaineritter@hotmail.com.

[2] CONAB - Companhia Nacional de Abastecimento, Rod BR-376, km 510, Colônia Dona Luíza - CEP: 84046-000, Ponta Grossa, PR, Brazil. srp@netpar.com.br; evandra.webber@conab.gov.br.

***Abstract*— *The objective of this study was to verify the influence of altitude on the indirect analysis of α-amylase content on wheat flours. The experimental designused was completely randomized, with eight treatments and three repetitions. The treatments consisted of the analysis of the falling number from flours of four wheat classes (basic, domestic, bread and improver) on the elevations zero, 412, 540, 761, 934, 975, 1,040 and 1,095 meters. After the trial results, under the correction of the averages above 600 meters of elevation, it was verified that there was a significant difference between the results of distinct altitudes, for the four wheat classes. When a polynomial regression is applied, for the values without correction, it was obtained that aquadratic regression equation correlates the falling number values with altitude; however, the coefficient of determination was very low, highlighting the major influence of the different equipments that were used to measure the falling number instead of the different altitudes.*

***Keywords*— falling number, food analysis, food composition, flours, wheat classes.**

I. INTRODUCTION

Wheat (*Triticum aestivum*) is a major cereal cropworldwide. Wheat flour is the basic ingredient to produce many foods, including breads, pasta, biscuits/cookies, cakes, among others. Even though,with the estimated production of 4.6 millionmetric tons of wheat(2018harvest season), Brazil is unable to supply its demand, standing as a huge importer country (CONAB, 2018).

Wheat flour is qualified by its physical, chemical, rheological, and nutritional characteristics, considering its large use inbakeries. The exigence on technological attributes has been increasingly consideredby the consumer markets when buying wheat and wheat flour. Therefore, reaching the required quality is a key-factor for the success of planting, commercializing and processing wheat (Pinnow et al., 2013; Finck et al., 2015).

In Brazil, the classification of wheat is ruled by the Normative Instruction n.º 38, from November 30,2010. The wheat group II(destined to milling, and other ends) is divided into five classes according to the values obtained of gluten strength, stability and falling number. To be classified in the "Improver", "Bread", "Domestic", "Basic, and Others Uses classes the falling number must present the minimum values of 250, 220, 220 and 200 s, respectively. For the "Other Uses" class, the falling number minimum value is not established (BRASIL, 2010).

Falling number is based on the α-amylase capacity to hydrolyze the starch gel. The intensity of the activity of the α-amylase enzyme in the wheat grain and in the wheat flour is estimated indirectly using the equipment called Falling Number®, which measures the starchypaste (like a porridge) liquefaction of the grinded wheat grain suspension warmed in a boiling water bath, with the result being expressed in seconds (AACC, 2010; Mohler et al., 2014; Ral et al., 2015).

Starting from the assumption that at low atmospheric pressures the boiling temperature in the water bath will decrease, Lorenz and Wolt (1981) proved that the falling number determination method suffers from influence of altitude. Thus, the falling number values increase with the elevation of altitude; because of a lower temperature, the sample in the test tube will be cooler and the activity of the α-amylase enzyme will be lower, therefore, increasing the falling number.

As the determination equipment of the falling number reproduces the method, the same suffers influence of the altitude. Thus, when the analysis is done from 600 m above the sea level, the operator programs the equipment to automatically perform the correction of the result (PERTEN INSTRUMENTS, 2016).

Examining the precision level of the replication of the procedure to determine the falling number, under controlled laboratory conditions, Delwiche et al. (2014) analyzed 24 wheat samples and found falling number values ranging from 168 to 404 s. The authors concluded that there was a variation of the values up to 16 s, between elevation at sea level and 800 m, with linear regression as best adjustment of data.

Due to the scarcity in the literature of experiments on altitude interference in the falling number, the objective of the present study was to verify the influence of altitude on the indirect analysis of α-amylase content on wheat flours.

II. MATERIALS AND METHODS

The experimental design utilized was completely random, with eight treatments and three repetitions. The treatments consisted of the analysis of the falling number on different altitudes related to the sea level, inselectedBrazilian cities: Fortaleza –CE, sea level; Medianeira – PR, 412 meters; Campinas – SP, 540 meters; Campo Mourão – PR, 761 meters; Curitiba – PR, 934 meters; Ponta Grossa – PR, 975 meters; Castro – PR, 1,040 meters; and Guarapuava – PR, 1,095 meters. The analyses were carried out on different equipments from Perten®models FN 1500, FN 1700 and FN 1900. All the equipmentswere certified and were used for wheat flour analysis in public agencies and private companies.

Samples from four wheat classes were considered: “Basic”, “Domestic”, “Bread” and “Improver”. The wheat samples were granted by CONAB, Ponta Grosse – PR unity and classified according to the Normative Instruction nº 38, from November 30, 2010. They were used on the experiment with available samples that had falling number values that were the most discrepant.

The procedure to prepare the sample and to determine the falling numberwas the method ICC nº 107/1 – Approved in 1968 / Revised in 1995; and AACC nº 56-81B – Approved in 1972 / Revised in 1999 (AACC, 2010). The wheat was milled, and then collected 300 g to make the three repetitions on all the localsabove mentioned.

The samples were codified to keep the experiment secrecy, and then, sent the samples properly milled and sealed in plastic bags,avoiding external moisture in the bags. After the delivery of the samples to their proper research sitesthey stayed stored in a cool dry placeand protected from light, until the analysis date. All analyses were carried on the same day.

To perform the analyses, 7.0 g of each sample was put into the properFN test tube and 25 mL of distilled water was added, then the tube was sealed with a stopper and shaken vigorously until a homogeneous mix was achieved. The stopperwas removed, and replaced by aclean and dry viscometer stirrerthat was plunged into the tube. After this process the viscometer stirrer and the tube connected tothe equipment to start the analysis. All the procedures, from stirring up until putting the tubes in the equipment, has not takenmore than 60 seconds.

All laboratories were oriented to manipulate the equipment and prepare the samples the same way, in order to avoid possible differences on the results. In accordance with the manufacturer’s recommendation of the equipment, it was applied a correction to values above 600 meters altitude using the the following formula:

$Log10\ (NQnm) = 1.0 \times \log 10\ (NQalt)$, wherein

NQ represents the falling number, nm represents the sea level and alt represents altitude.

The corrected results from altitude were submitted to Hartley tests, to verify the homoscedasticity of variances, and Kolmogorov-Smirnov to examine the normality of the data. The analysis of variance used the F test, andScott-Knott test was used for mean comparison, with a confidence interval above 95% of probability. Thus, it was intended to verify the formula of correction between the results on the different altitudes.

Without correcting data with the formulafor altitudes above 600 meters, it was applied the Hartley test, Kolmogorov-Smirnov, ANOVA, and polynomial regression. With the above tests,we aimed to verify the influence of altitude on the determination of the falling number, with defined equations, both linear or quadratic regression.

III. RESULTS AND DISCUSSION

All the analyses had homoscedasticity and normality of variance, dispensing the transformation of data. When the wheat flours of different classes were analyzed, and the FN values were corrected for altitude above 600 meters, significant differences were found, with confidence interval above 95% of probability (Table 1).

Therefore, Lorenz and Wolt (1981) observations were confirmed, that altitude influences the falling number analysis. However, the correction factor was not sufficient to correct the different results with the altitudes, contradicting the instructions of PERTEN INSTRUMENTS (2016).

Therefore, it is possible to observe that wheat of the"Basic" class has not presented statistic difference between altitude zero and 540 m. This tendency, however,did not repeat on the others wheat classes. On all the wheat analyses there was a significant difference between altitudes, however it was not established as a standard error for altitude.

When the falling number factor is analyzed by itself, through the Normative Instruction n.º 38 (BRASIL, 2010), the "Domestic", "Bread" and "Improver" wheat classes fit on the "Improver" class on all altitudes. This demonstrates that, even though the significant discrepancy of the results and lack of standard error, the classification of wheat should not be affected by the variable under analysis.

However, it stands that the difference between values of altitudes were always higher than the parameters established to distinguish the wheat classes, which is the maximum of 30 seconds. The discrepancies of classes obtained were: "Basic" with 43, "Domestic" with 103, "Bread" with 166 and "Improver", distancing 148 seconds between the lowest and highest values. The values obtained in the presentstudy have more than doubledthose determined by Delwiche et al. (2014), between the altitudes on the sea level and 800 m.

Considering the analyses performed without correction, the data obtained by the equation of polynomial regression demonstrated that the different altitudes have interfered on the falling number, for the distinct wheat flour classes. In the case of the "Basic", "Domestic", "Bread" and "Improver" wheat classes, the polynomial regression was significant, with confidence interval above 95% of probability, for the quadratic equation. Thus, it is confirmed that the recommendations of PERTEN INSTRUMENTS (2016) and Lorenz & Wolt (1981), that the altitude significantly influences the falling number analysis. However, the results are not in conformity with the formula purposed by PERTEN INSTRUMENTS (2016) and the linear regression indicated by Delwiche et al. (2014) to explain the relation between the falling number and altitude.

Nevertheless, the dispersion of data that varies in function of altitude had the phenomenon explained by 44% of the curve adjustmentfor the "Basic" wheat class (Fig. 1), 29%for the "Domestic" wheat class (Fig. 2), only 10% for the "Bread" wheat class (Fig. 3), and 31% for the "Improver" wheat class (Fig. 4). Therefore, due to the lack of a standard error, it is not advisable to use the equation of curves found on each class to correlate the influence of altitude on the falling number.

Lorenz and Wolt (1981) used the equipment FN® 1400, and the method AACC 56-81A (AACC 1969), both outdated. However, equipment models FN® 1500, FN® 1700, FN® 1800, FN® 1900, and method AACC 56-81B (AACC 2010) were used in the present study. Different equipments may be the cause of results variation.

However, Delwiche et al. (2014) utilized the same method to define the falling number (AACC, 2010), and the equipment (FN® 1700) that was used in this experiment; with discrepant results. That fact deserves attention to the equipment being certified and used on the commercial analysis of wheat flour.

IV. CONCLUSIONS

After performing all the analyses, with the correction of the average values above 600 meters of altitude, it was verified that there was a significant difference between the results of distinct altitudes; for the four wheat classes.

When applied the polynomial regression, to the values without correction, it was obtained that the quadratic equations correlated the falling number values with altitude; however, the coefficient of determination was very low, emphasizing the major influence of different equipment utilized to determine the falling number than the distinct altitudes.

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Table.1: Falling number (seconds) on different altitudes of wheat classes[1] (Triticumaestivum), with correlation values above 600 meters[2].

Altitude (m)	Wheat classes			
	Basic	Domestic	Bread	Improver
Zero	214 b[3]	276 c	461 b	517 b
412	217 b	354 a	526 a	545 a
540	212 b	304 b	437 d	517 b
761	203 c	282 b	453 c	489 c
934	197 d	269 c	386 f	416 e
975	231 a	251 d	360 g	369 f
1,040	206 c	280 b	426 e	471 d
1,095	188 d	272 c	439 d	408 e
CV (%)	1.2	1.4	1.1	1.6

(1) Classified according to Normative Instruction n.º 38, from November 30 of 2010.

(2) Log10 (NQnm) = 1.0 x log10 (NQalt).

(3) Means with the same letter in the column do not differ significativly by Scott-Knott (p>0.05).

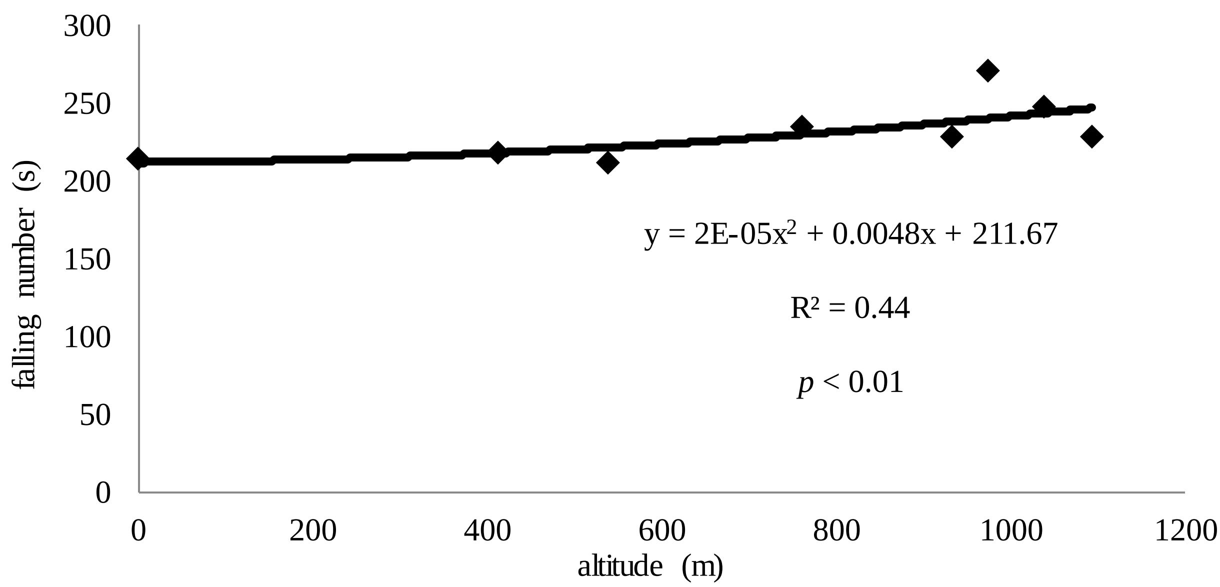

Fig.1: Falling number under different altitudes, without correction ("Basic" class wheat sample).

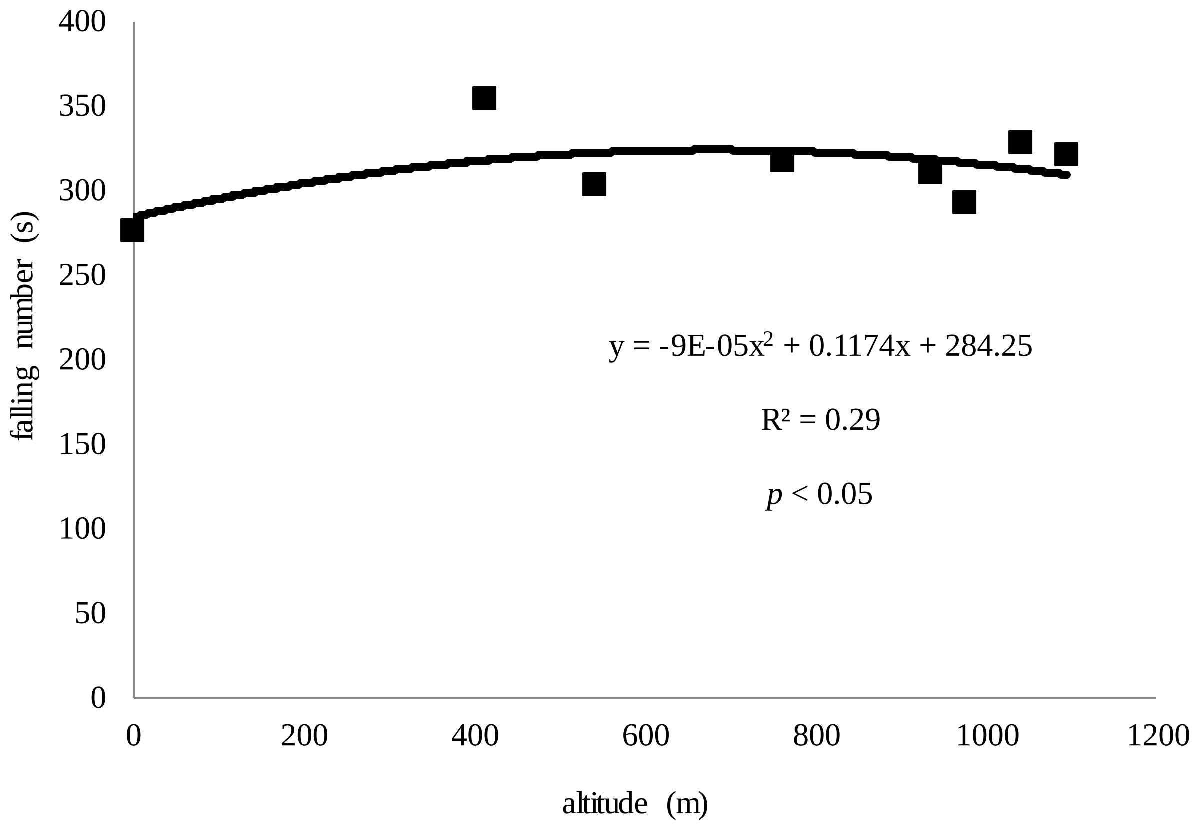

Fig. 2: Falling number under different altitudes, without correction ("Domestic" class wheat sample).

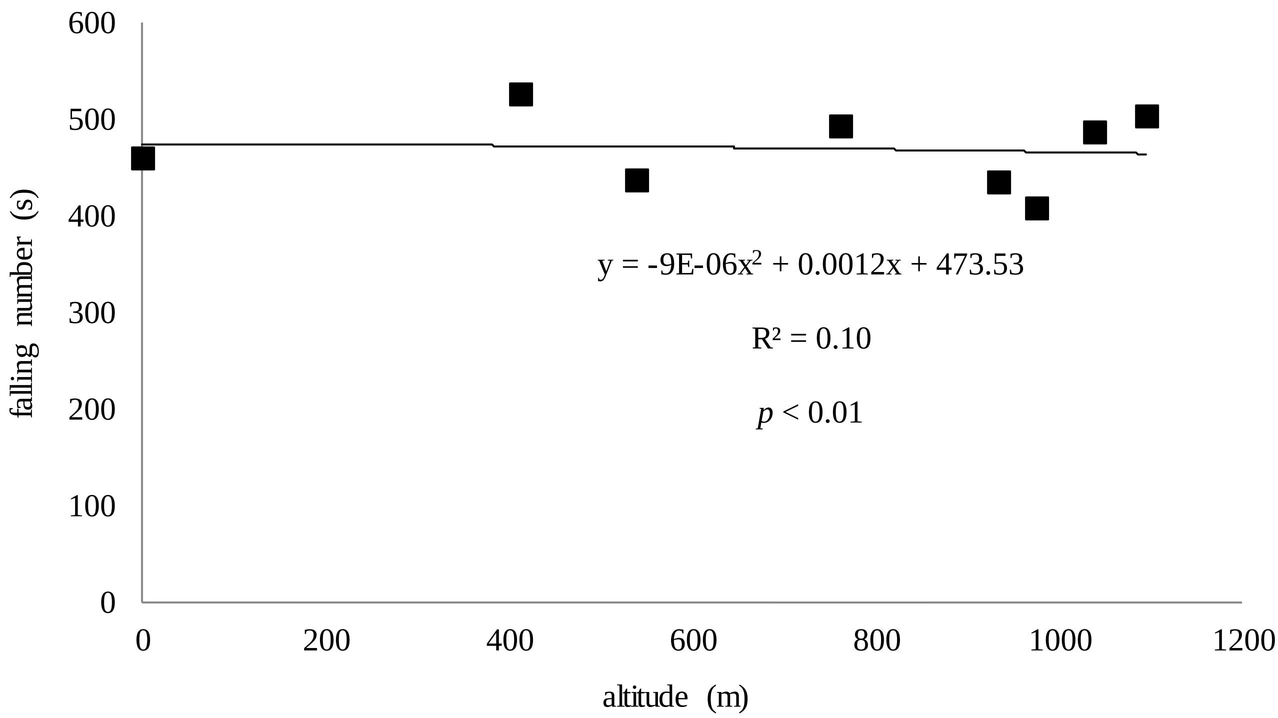

Fig. 3: Falling number under different altitudes, without correction ("Bread" class wheat sample).

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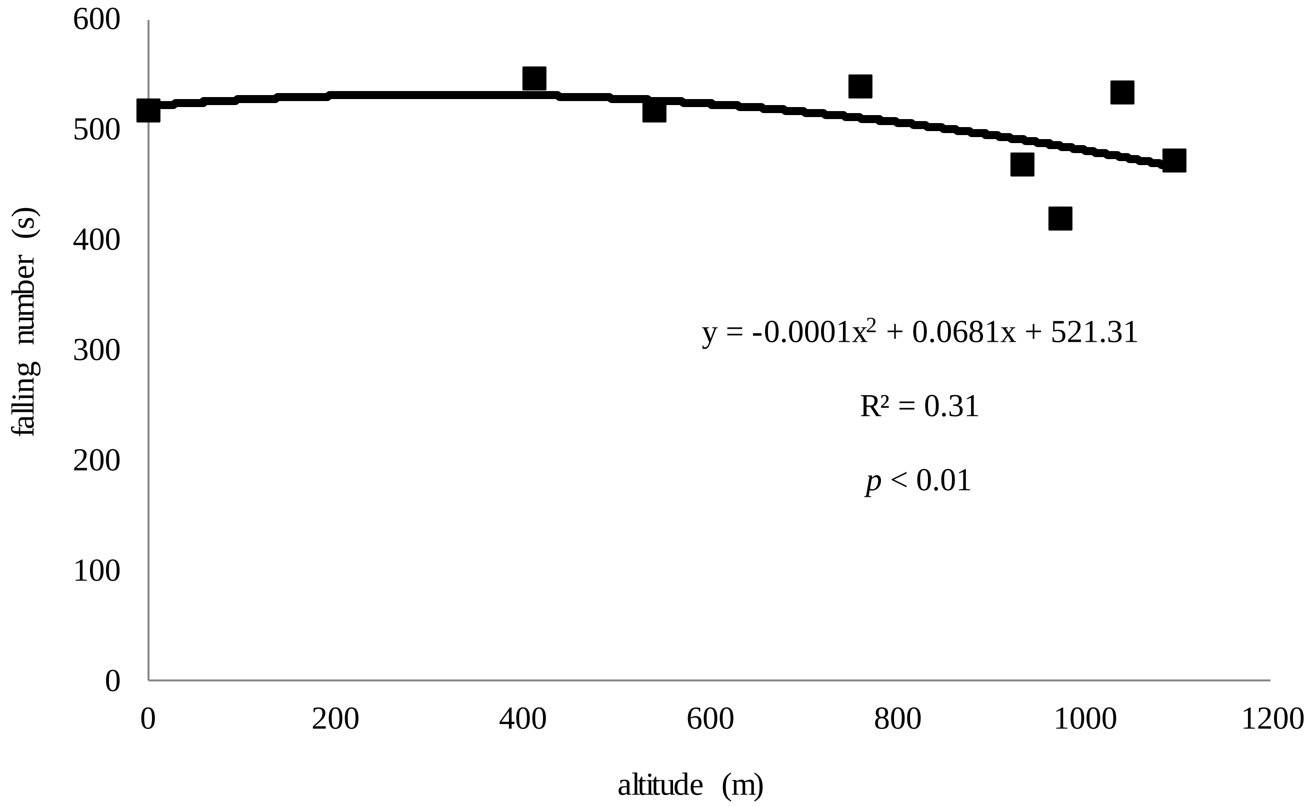

Fig. 4: Falling number under different altitudes, without correction ("Improver" class wheat sample).