






## Article

# Application of the Falling Number Method in the Evaluation of the $\alpha$ -Amylase Activity of Malt Flour

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**Featured Application:** The modified method provides a viable and practical tool for evaluating  $\alpha$ -amylase activity in malt flour and has the potential to become an important tool in the brewing and distilling industry to facilitate daily monitoring of the malting process.

**Abstract:** The Falling Number (FN) test is not suitable for the determination of  $\alpha$ -amylase activity in malt flour because the amylolytic activity is too high to be determined by the FN instrument. The aim of this study was to modify the FN method to allow quantification of high  $\alpha$ -amylase activity in malt flour. The modifications were performed in two ways: using different amounts of malt flour (0.05, 0.1, 0.2, and 0.3 g) or by using malt extracts (0.5, 1, 2, 3, 4, 5, and 6 mL). In both cases, 6 g of standard wheat starch was used as substrate. The results of the modified methods were compared with the  $\alpha$ -amylase activity determined by the standard Ceralpha method. Linear and nonlinear exponential regression was used to calculate the predicted amylase activity, and Bland-Altman analysis was used to measure the agreement between standard and modified methods. The modifications of the FN method using 0.1 g of malt flour showed that the modified method was able to accurately measure high levels of  $\alpha$ -amylase activity in malt flour, and the results obtained by the modified method were in agreement with the results obtained by the standard Ceralpha method.

**Keywords:**  $\alpha$ -amylase activity; malt flour; modified Falling Number method; Ceralpha method



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## 1. Introduction

The Falling Number (FN) test, a widely used method for evaluating  $\alpha$ -amylase activity in flours of various cereals (mainly wheat and barley flours), has been performed for decades according to a more or less unchanged procedure [1]. It has been approved by all relevant international organizations, such as the International Organization for Standardization (ISO), the American Association of Cereal Chemists (AACC), the International Association for Cereal Science and Technology (ICC), the Association of Official Analytical Chemists (AOAC), and the American Society of Brewing Chemists (ASBC) [2–6].

This method is very simple, and it is based on the viscosity change of a hot flour paste and involves mixing 7 g of flour with 25 mL of water in a test tube, which is then immersed in a boiling water bath and mixed with the stirrer in an up-down direction for 55 s. During this time, rapid gelatinization of starch occurs. After mixing, the stirrer is released to drop to the bottom of the test tube. The total time of mixing and the time taken for the stirrer to “fall” to the bottom of the test tube is defined as FN and expressed in seconds. Increased  $\alpha$ -amylase activity leads to faster liquefaction of the paste, and thus, a shorter fall time, i.e., a smaller FN. The values of FN represent an indirect measure of  $\alpha$ -amylase activity and they are inversely proportional [2,7,8].

The FN test is recognized worldwide because of its simplicity and the relatively short duration of the procedure. It is now considered the standard method for evaluating grain quality in the context of the market and trade in grain, as well as for evaluating flour and adjusting flour blends for bakery production. The FN test is very successful in detecting increased levels of  $\alpha$ -amylase in mature grains caused by preharvest sprouting (PHS) or late maturity amylase (LMA) [9–12]. Wheat grains with FN values below 250 s are considered inferior grains and usually fetch a lower price in trade [2,13]. However, these increased levels of  $\alpha$ -amylase induced by PHS and LMA are still relatively low compared to the levels of  $\alpha$ -amylase in malted grain.

The FN test is not suitable for the determination of  $\alpha$ -amylase activity in malt flour because the amylolytic activity is too high to be determined with the FN instrument. This is because the liquefaction of the hot starch paste in malt flour samples is very intense and the viscosity decreases so much that the stirrer of the FN instrument immediately falls through the liquid starch paste and the instrument records a value of 61 s. This is the time required for the stirrer to mix the flour-water suspension and freely fall through the completely liquefied paste [14]. According to some previous studies, the FN value of 61 s corresponds to  $\alpha$ -amylase activity of about 5 Ceralpha Units per gram (CU/g), and this is the maximum  $\alpha$ -amylase activity of flour that can be measured by the standard FN method [15]. Considering that the activity in malt flour is much higher and can exceed 300 CU/g, depending on the length of germination period in the malting process and the origin of the malt [15–17], it is clear that the standard method for determining FN cannot be used for malt flour samples.

For this reason, several other methods are used to determine amylolytic activity in malt flour. Determination of the total amylolytic activity in malt and malt flour is usually carried out by the method of determination of diastatic power. Diastatic power is influenced by all amylolytic enzymes present ( $\alpha$ - and  $\beta$ -amylase, limit dextrinase, and  $\alpha$ -glucosidase), but mainly by  $\beta$ -amylase [18,19]. If only  $\alpha$ -amylase activity is to be determined, other specific methods must be used, which can be divided into several groups: (a) the saccharogenic method or the spectrophotometric determination of the reducing sugars formed by the action of  $\alpha$ -amylase on the substrate (starch), using different reagents, of which the 3,5-dinitrosalicylic acid (DNS method) is the most common; (b) amylolytic methods to determine the reduction in the intensity of the blue coloration of the starch-iodine complex (e.g., Farrand and Wohlgemuth method); (c) nephelometric methods based on the reduction of turbidity of the reaction mixture of the tested starch sample; (d) ELISA test; (e) spectrophotometric methods using synthetic substrates that release color during the reaction, such as nitrophenyl derivatives of maltooligosaccharides (Ceralpha method) [20,21].

All of the abovementioned methods are generally time-consuming and/or relatively expensive. Therefore, the possible adaptation of established rheological techniques commonly used in grain analysis, such as the FN method, in order to evaluate amylolytic activity in malt flour represents a viable alternative. So far, to our knowledge, there has been only one study aimed at adapting the FN method to estimate amylolytic activity in malt flour. In this study, the mixture of malted sorghum flour (0.2 g) with different amylolytic activities and corn starch (5.8 g) was used in FN assay and the results were compared with the diastatic power of the malt flour samples [14].

The aim of this study was to modify the FN method, normally reserved for the assay of flour samples with low amylolytic activity, to allow the quantification of high  $\alpha$ -amylase activity in malt flour. The modifications were performed in two ways: with different amounts of malted barley and wheat flour or by using malt extracts. In both cases, standard wheat starch was used as substrate and the results were compared with  $\alpha$ -amylase activity determined by the standard Ceralpha method.

## 2. Materials and Methods

### 2.1. Materials

Three winter barley cultivars obtained from Agricultural Institute Osijek (Croatia) were used in this study: malting barley *Tristan*, feed barley *Zlatko*, and hullless barley *Osvit*. One cultivar of winter wheat *Lorena* (Bc Institute, Zagreb, Croatia) was also used. All cultivars were collected in the 2021 growing season. Wheat starch (Denes Natura Kft., Pecs, Hungary) was used as substrate. All reagents required for the determination of  $\alpha$ -amylase activity were included in the enzyme assay kit (Megazyme International, Bray, Ireland).

### 2.2. Methods

#### 2.2.1. Malting Procedure

Grain samples were malted in a micro-malting Automated Joe White Malting Systems Unit (Perth, Australia) as follows: (a) steeping by the combined wet-dry method for 37 h at 16 °C (5 h submerged, 12 h dry, 6 h submerged, 12 h dry, 2 h submerged); (b) germination for 96 h (4 days) at 17 °C with turning every 2 h; (c) the part of the sample was removed after steeping and after each day of germination and dried for 20 h (5 h at 60 °C, 12 h at 65 °C, 2 h at 70 °C, 1 h at 80 °C) to approximately 4–6% moisture content. In this way, 20 samples with different  $\alpha$ -amylase activity were obtained. The dried samples were placed in a laboratory vibrating shaker Analysette 3 (Fritsch, Germany) using a sieve with a mesh size of 1 mm. Three rubber balls were placed on the sieve and malted grains were shaken for 5 min at an amplitude of 2 mm to remove the rootless. Malt milling was performed on an IKA MF-10 laboratory mill (IKA-Werke GmbH & Co., Staufen, Germany). The malt flour samples were stored in plastic bags in a cool place until further analysis.

#### 2.2.2. Enzyme Extraction from Malt Flour

The extraction was similarly performed as described in the AACC International Method 22-02.01 (Ceralpha Method) [22]. An amount of 7.5 g of each malt flour sample was added to 150 mL of extraction buffer solution (pH 5.4) containing 1 M sodium malate, 1 M sodium chloride, 40 mM calcium chloride, and 0.1% sodium azide. The extraction was performed in a glass beaker on a magnetic stirrer for 30 min at 40 °C. After extraction, the solution was centrifuged ( $1000\times g$ ) for 10 min and the supernatant (malt extract 5% *w/v*) was stored in the plastic tubes in the refrigerator until further analysis.

#### 2.2.3. Determination of $\alpha$ -Amylase Activity

The  $\alpha$ -amylase activity in all 20 malt flour samples was determined according to the AACC International Standard Method 22-02.01 (Ceralpha Method) using the enzyme assay kit (Megazyme International, Bray, Ireland) [22]. One Ceralpha Unit of  $\alpha$ -amylase activity is defined as the quantity of  $\alpha$ -amylase required to hydrolyze one  $\mu\text{mol}$  of p-nitrophenol from blocked p-nitrophenyl maltoheptaoside (BPNPG7) in one minute in the presence of excess thermostable  $\alpha$ -glucosidase. All analyses were performed in triplicate.

#### 2.2.4. Modification of the Falling Number (FN) Method

The modifications of the FN method (AACC International Method 56-81.03) [4] were performed on a Perten FN 1500 instrument (Perten Instruments AB, Stockholm, Sweden) according to the two different procedures. In the first procedure, part of the wheat starch was substituted with the small amount (0.05, 0.1, 0.2 and 0.3 g) of malt flour. Previously, it was established that 6 g of wheat starch (14% moisture basis) has an FN value of exactly 350 s (Table 1). In the second procedure, part of the water was replaced with the aliquot of 0.5, 1, 2, 3, 4, 5, and 6 mL of 5% *w/v* malt extract sample (Table 1). All analyses were performed in triplicate. During the study, it was found that the amounts of more than 0.3 g of malt flour and 6 mL of malt extract were too large to measure FN in all samples, as the values reached the detection limit of the device of 61 s.

**Table 1.** Adaptation procedures of the FN method.

Procedure 1 (Malt Flour)			Procedure 2 (Malt Extract 5% w/v)		
Wheat Starch <sup>1</sup> (g)	Malt Flour <sup>1</sup> (g)	Water (mL)	Wheat Starch <sup>1</sup> (g)	Malt Extract (mL)	Water (mL)
5.95	0.05	25	6.00	0.5	24.5
5.90	0.1	25	6.00	1.0	24.0
5.80	0.2	25	6.00	2.0	23.0
5.70	0.3	25	6.00	3.0	22.0
			6.00	4.0	21.0
			6.00	5.0	20.0 <sup>1</sup>

<sup>1</sup> 14% moisture basis.

Considering that the relationship between the FN and the  $\alpha$ -amylase activity is curvilinear, linearization can be achieved by converting the FN to the liquefaction number (LN) according to the following equation:

$$LN = \frac{6000}{FN - 50} \quad (1)$$

where 6000 is a constant and 50 is the time in seconds required, approximately, for the starch in the sample to become gelatinized and susceptible to amyolytic hydrolysis [7].

### 2.2.5. Statistical Analysis

The FN values were transformed to LN to obtain a linear relationship with amylase activity. The linear regression was used to obtain an equation to calculate the predicted amylase activity of the samples. Second, nonlinear exponential regression ( $Y = a \times \exp(b \times X)$ ) was used to calculate the predicted amylase activity directly from the FN values. The results of the Ceralpha method were used as the gold standard. The calculated repeatability coefficient for the Ceralpha method was 2.56 CU/g. The Pearson correlation coefficient was calculated between the amylase activity estimated by the Ceralpha method and the amylase activity estimated by modified methods.

The Bland-Altman repeated-measures method was used to measure the agreement between standard and modified methods [23–25]. The bias was calculated as the mean difference of the mean values between the Ceralpha standard method and the modified methods. Precision was assessed by calculating standard errors and 95% confidence intervals for bias. Repeatability was estimated as within-subject standard deviation for each method. The obtained regression equations were used to calculate the upper limit of quantification (ULOQ) using 61 s as the lowest measurable FN (FN of 61 s corresponds to LN of 545.45 s<sup>-1</sup>). Statistical analysis was performed using XLSTAT software ver. 2019.2.2 (Addinsoft, New York, NY, USA).

## 3. Results and Discussion

The standard FN is limited in its ability to quantify high levels of  $\alpha$ -amylase activity in malt flour. To overcome this limitation, the study aimed to modify the FN method to quantify high  $\alpha$ -amylase activity in malt flour. The modification of the method was carried out in two different ways: (1) using small amounts of malted barley and wheat flour and (2) using malt extracts. In both procedures, standard wheat starch was used as substrate, and the results were compared with  $\alpha$ -amylase activity as determined by the standard Ceralpha method. Twenty malt samples with different  $\alpha$ -amylase activity were prepared by micromalting the three winter barley varieties and one winter wheat variety.

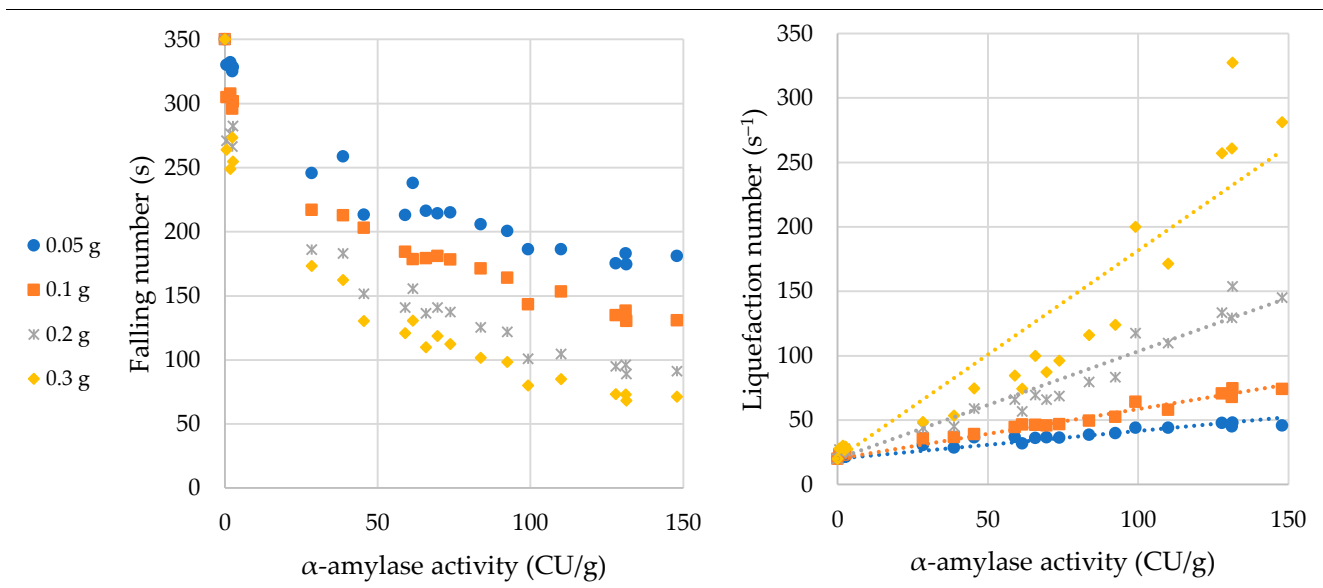
From the results presented in Table 2, the range of  $\alpha$ -amylase activity measured was from 0.6 CU/g (wheat malt *Lorena* after steeping) to 147.9 CU/g (barley malt *Tristan* after the fourth day of germination). It can be noted that  $\alpha$ -amylase activity significantly increased after the first day of germination during the malting process. According to preliminary

studies (not published), it was not possible to determine FN using the standard method after the first day of germination because the  $\alpha$ -amylase activity of the samples was too high and the instrument reached its detection limit at 61 s, which according to some studies is about 5 CU/g [15].

**Table 2.** The  $\alpha$ -amylase activity of malt flour samples determined by the Ceralpha method.

Sample Produced after	Barley Malt Zlatko (CU/g)	Barley Malt Osvit (CU/g)	Barley Malt Tristan (CU/g)	Wheat Malt Lorena (CU/g)
Steeping	2.5 ± 0.26	1.8 ± 0.20	2.7 ± 0.23	0.6 ± 0.26
1st day of germination	73.8 ± 0.38	38.7 ± 3.77	61.5 ± 3.33	28.4 ± 1.61
2nd day of germination	99.2 ± 1.32	69.6 ± 1.80	110.0 ± 0.28	45.5 ± 0.93
3rd day of germination	127.9 ± 1.48	83.7 ± 0.42	131.2 ± 5.69	59.0 ± 2.10
4th day of germination	131.4 ± 0.81	92.5 ± 2.19	147.9 ± 3.29	65.8 ± 1.43

The results presented in Figure 1 show the effects of adding different amounts of malt flour on the FN and LN. It is evident that FN decreased when higher amounts of malt flour were used in the analysis. Moreover, FN decreased below 200 s when more than 0.1 g of malt flour was used, even for samples with  $\alpha$ -amylase activity of 28.4 CU/g. This means that when high amounts of malt flour (0.2 and 0.3 g) are used, the detection range is significantly reduced and all samples analyzed fall in the 61–200 s range, which may make it difficult to distinguish samples according to their  $\alpha$ -amylase activity.

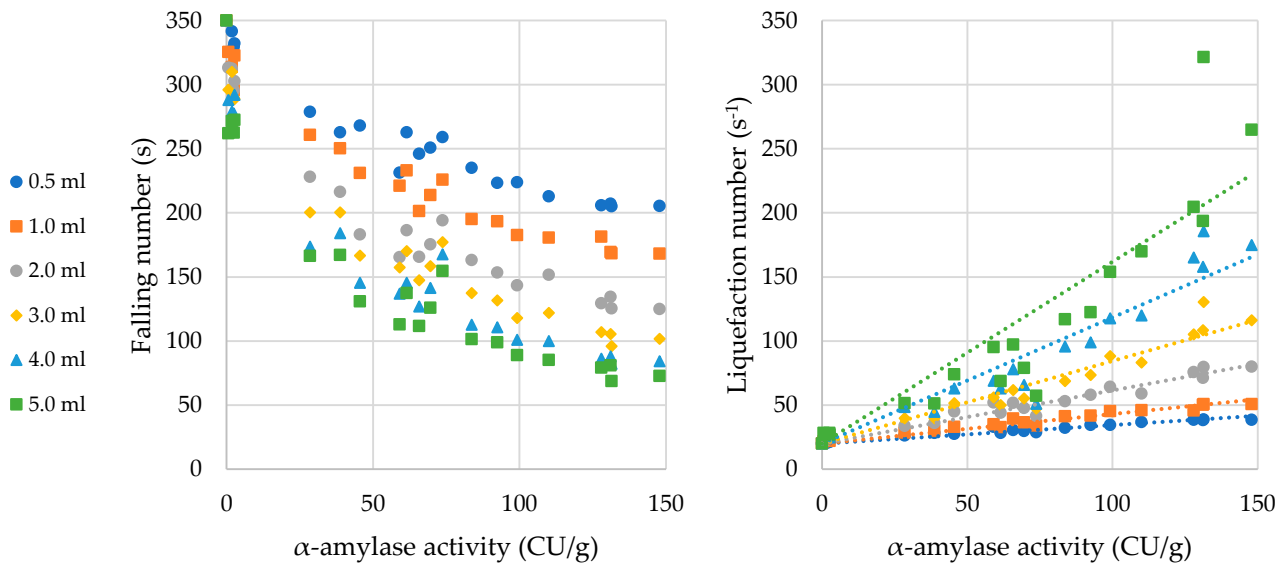


**Figure 1.** Influence of the addition of different amounts of malt flour on the Falling Number and Liquefaction Number (lines indicate a linear trend).

Considering that the relationship between FN and  $\alpha$ -amylase activity is curvilinear, the FN values can be transformed into LN to ensure a linear relationship with  $\alpha$ -amylase activity, since the LN values are proportional to  $\alpha$ -amylase activity [7,26]. By transforming the FN values into LN values, it becomes possible to use linear regression analysis to establish a linear relationship between LN and  $\alpha$ -amylase activity, providing a simpler method for estimating  $\alpha$ -amylase activity in a sample. In this way, the values from LN can be used to create a standard curve for quantitative analysis, further increasing the precision of the analysis. As shown in Figure 1, the slope of the curve increased with the increasing amount of malt flour in the analysis, confirming the reduction of the detection

range. The intercept was set at  $20 \text{ s}^{-1}$ , which corresponds to the FN value of 350 s obtained for standard wheat starch without  $\alpha$ -amylase activity.

Similar to the results obtained by using malt flour, the use of the different amounts of malt extract (5% *w/v*) also resulted in a nonlinear relationship between FN and  $\alpha$ -amylase activity (Figure 2). FN decreased below 200 s when more than 3 mL of malt flour was used for samples with  $\alpha$ -amylase activity higher than 40 CU/g. As with the use of large amounts of malt flour, this also means that the detection range is significantly reduced when using large aliquots of malt extract (>3 mL).



**Figure 2.** Influence of the addition of different amounts of malt extract on the Falling Number and Liquefaction Number (lines indicate a linear trend).

To determine the agreement between the standard Ceralpha method and the modified FN methods, a Bland-Altman analysis was performed. The results are shown in Tables 3 and 4. The results presented in Table 3 refer to the use of LN to estimate  $\alpha$ -amylase activity in the malt samples. The standard method and the modified methods were highly correlated for all quantities of malt flour and extracts analyzed, as the correlation coefficients were greater than 0.900. However, it should be noted that the correlation coefficient decreased as the amounts of malt flour and malt extract increased. The highest correlation was found between the standard method and the modified method when 0.1 g of malt flour was used ( $r = 0.984$ ), and between the standard method and the modified method when 2 mL of malt extract was used ( $r = 0.977$ ). However, the presence of a high correlation between two methods does not necessarily indicate a good agreement between the results obtained by these methods. In other words, a high correlation does not necessarily mean that the results are in close agreement. It is important to consider this fact because it indicates that a strong relationship between two methods does not necessarily imply accurate results. This may be the case when the differences between the results are consistent, implying that the error is systematic rather than random. In such cases, the correlation remains high even if the results do not agree with each other [27,28]. In view of this, the Bland-Altman analysis was also used to investigate the agreement between the methods.

**Table 3.** Agreement between standard Ceralpha method and modified Falling Number method (linear relationship between Liquefaction Number and  $\alpha$ -amylase activity).

	Equation <sup>1</sup>	<i>r</i>	Bias (CU/g)	SE <sub>B</sub> (CU/g)	CI <sub>B</sub> (95%) (CU/g)	ULOQ (CU/g)	Repeatability (CU/g)
Malt flour (g)							
0.05	AA = (LN – 20)/0.215	0.956	3.99	12.18	–1.55–9.54	2445.1	8.55
0.1	AA = (LN – 20)/0.386	0.984	2.41	7.64	–1.07–5.89	1363.0	6.42
0.2	AA = (LN – 20)/0.834	0.973	–0.65	11.61	–5.94–4.63	629.4	4.69
0.3	AA = (LN – 20)/1.616	0.926	–4.35	21.47	–14.12 ± 5.42	325.2	4.69
Malt extract (mL)							
0.5	AA = (LN – 20)/0.1452	0.970	2.93	10.63	–1.91–7.77	3618.8	7.67
1.0	AA = (LN – 20)/0.2304	0.974	3.38	9.35	–0.88–7.63	2280.6	5.56
2.0	AA = (LN – 20)/0.4154	0.977	1.89	9.66	–2.50–6.29	1264.9	4.31
3.0	AA = (LN – 20)/0.6445	0.965	–0.91	13.41	–7.01–5.20	815.3	4.91
4.0	AA = (LN – 20)/0.9832	0.954	–2.40	15.95	–9.66–4.86	534.4	3.00
5.0	AA = (LN – 20)/1.4179	0.907	–3.87	24.50	–15.03–7.28	370.6	3.14

<sup>1</sup> AA— $\alpha$ -amylase activity (CU/g); LN—Liquefaction Number (s<sup>–1</sup>); *r*—Pearson correlation coefficient; SE<sub>B</sub>—Standard Error of Bias; CI<sub>B</sub>—95% Confidence Interval of Bias; ULOQ—Upper Limit of Quantification.

**Table 4.** Agreement between standard Ceralpha method and modified Falling Number method (nonlinear relationship between Falling Number and  $\alpha$ -amylase activity).

	Equation <sup>1</sup>	<i>r</i>	Bias (CU/g)	SE <sub>B</sub> (CU/g)	CI <sub>B</sub> (95%) (CU/g)	ULOQ (CU/g)	Repeatability (CU/g)
Malt flour (g)							
0.05	AA = 3645.0 × exp(–0.019 × FN)	0.973	0.86	11.07	–4.18–5.90	1167.5	11.31
0.1	AA = 1114.4 × exp(–0.016 × FN)	0.987	1.24	7.64	–2.24–4.72	425.4	6.70
0.2	AA = 546.5 × exp(–0.015 × FN)	0.987	3.10	7.44	–0.28–6.49	222.6	3.86
0.3	AA = 401.4 × exp(–0.016 × FN)	0.989	0.68	7.07	–2.53–3.90	155.5	2.46
Malt extract (mL)							
0.5	AA = 6089.6 × exp(–0.019 × FN)	0.974	1.20	10.97	–3.79–6.20	1961.8	8.61
1.0	AA = 2335.6 × exp(–0.017 × FN)	0.979	1.18	9.76	–3.26–5.62	839.5	6.16
2.0	AA = 962.9 × exp(–0.015 × FN)	0.978	0.93	9.99	–3.61–5.48	377.8	4.23
3.0	AA = 536.3 × exp(–0.014 × FN)	0.981	1.11	9.37	–3.15–5.37	235.0	3.92
4.0	AA = 442.9 × exp(–0.014 × FN)	0.978	0.68	9.99	–3.87–5.22	187.1	2.19
5.0	AA = 391.7 × exp(–0.015 × FN)	0.973	0.59	11.17	–4.50–5.67	160.3	1.81

<sup>1</sup> AA— $\alpha$ -amylase activity (CU/g); LN—Liquefaction Number (s<sup>–1</sup>); *r*—Pearson correlation coefficient; SE<sub>B</sub>—Standard Error of Bias; CI<sub>B</sub>—95% Confidence Interval of Bias; ULOQ—Upper Limit of Quantification.

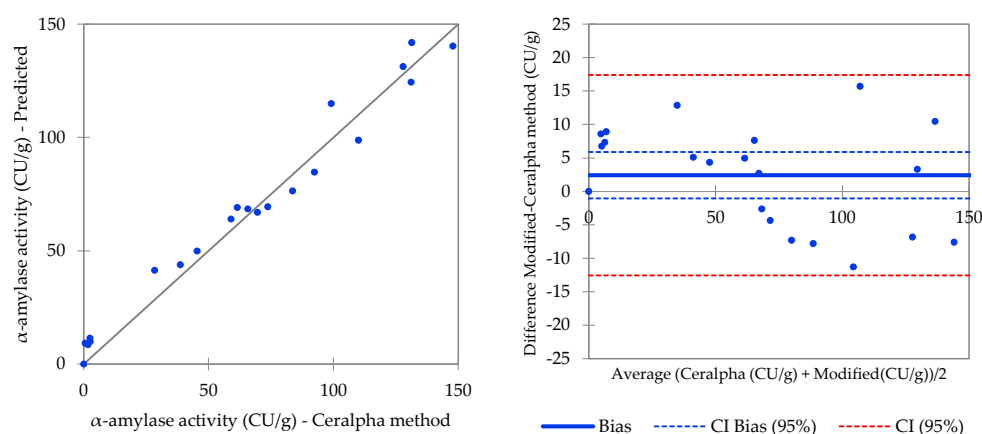
The bias moved from positive to negative values with increasing amounts of added malt flour and malt extract. A tendency to underestimate  $\alpha$ -amylase activity was observed at higher  $\alpha$ -amylase levels. This phenomenon can be attributed to the reverse flow effect that occurs when the stirrer approaches the bottom of the test tube. This effect is more pronounced in low viscosity paste due to a higher upward flow velocity. For samples where a high  $\alpha$ -amylase content leads to a lower final viscosity of the paste, the stirrer slows down, resulting in higher FN readings, i.e., an underestimation of the  $\alpha$ -amylase activity [29,30]. The smallest absolute value of bias was calculated when 0.2 g of malt flour (–0.65 CU/g) and 3.0 mL of malt extract (–0.91 CU/g) were used. The standard error of bias and 95% confidence interval of bias were the smallest for the method using 0.1 g of malt flour (SE<sub>B</sub> = 7.64; CI [–1.07, 5.89]) and when using 1 mL of malt extract (SE<sub>B</sub> = 9.35; CI [–1.07, 5.89]). The calculated upper limit of quantification (ULOQ), which was defined as the maximum  $\alpha$ -amylase activity that can be measured by the method, gradually decreased with increasing amounts of added malt flour and malt extract. The smallest ULOQ was determined for the method with 0.3 g of added malt flour (325.2 CU/g). It can be concluded that, using this amount of malt flour for analysis, the  $\alpha$ -amylase activity cannot be accurately estimated in malts with higher activity. The repeatability of the

modified methods also decreased with increasing amounts of added malt flour and malt extract. This was to be expected, since it is known that the relative repeatability coefficient is significantly lower for samples with small FN than for samples with higher FN (as indicated in the standard method ISO 3093) [3].

To improve the accuracy of the estimate, nonlinear regression was also used to fit the FN data (Table 4). As expected, the correlation coefficients were higher than when linear regression and LN were used for estimation. The smallest coefficient ( $r = 0.973$ ) was found when 0.01 g of malt flour and 5 mL of malt extract were used. In contrast to the linear estimation of LN, all calculated biases were positive but lower; between 0.68 and 3.10 CU/g when malt flour was used and between 0.59 and 1.20 CU/g when malt extract was used. The modified method using 0.3 g of malt flour or 3 mL of malt extract showed the lowest  $SE_B$  and the narrowest 95% CI, with  $SE_B$  values of 7.07 and 9.37 and CIs  $[-2.53, 3.90]$  and  $[-3.15-5.37]$ , respectively. However, the ULOQs were too low to consider these amounts of malt flour and malt extract as suitable for performing the modified procedure, since some highly active malts will not fall within the measurable range. Furthermore, the repeatability of the modified methods was observed to decrease with increasing amounts of malt flour and malt extract.

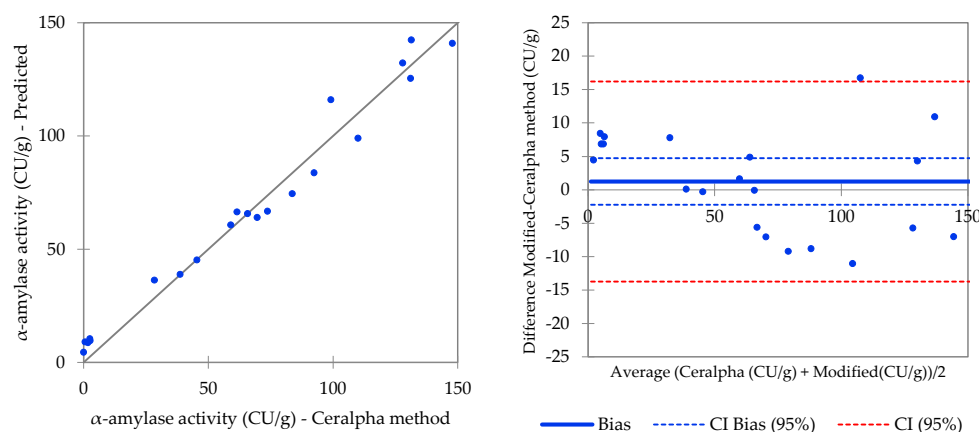
Considering the combination of the small bias ( $<2$  CU/g), low  $SE_B$  ( $<10$  CU/g), narrow 95% CI, high ULOQ ( $>400$  CU/g), and relatively acceptable repeatability ( $<10$  CU/g), the best agreement between the standard Ceralpha method and the modified method was found for the modified method with 0.1 g of added malt flour or 2 mL of added malt extract. Since there is not much difference between the agreement of the methods when malt extract is used instead of malt flour, it can be concluded that the modified method does not require an extraction step and in this way the time required for the analysis can be reduced.

Thus, to estimate  $\alpha$ -amylase activity in malt samples, the proposed modification of the FN method is to use 0.1 g of malt flour and standard starch in an amount equivalent to the FN value of 350 s. In our study, 6 g of wheat starch was used, but if a different starch is to be used, the exact amount must be adjusted beforehand to obtain the FN value of 350 s. The graphical representation of the Bland-Altman analysis of the agreement between the Ceralpha method and the proposed modified FN methods are shown in Figures 3 and 4. Both the linear estimation of LN data and the nonlinear estimation of FN data can be used. However, future studies are needed, especially interlaboratory analyzes, to confirm the results of this study and to determine the reproducibility of the modified method.



**Figure 3.** The Bland-Altman analysis of the agreement between the Ceralpha method and the proposed modified FN method using 0.1 g of malt flour (Linear estimation of LN).





**Figure 4.** The Bland-Altman analysis of the agreement between the Ceralpha method and the proposed modified FN method using 0.1 g of malt flour (Nonlinear estimation of FN).

#### 4. Conclusions

The modifications of the FN method with the use of 0.1 g of malt flour showed that the modified method was able to accurately measure high levels of  $\alpha$ -amylase activity in malt flour. The results obtained by the modified method were in agreement with the results obtained by the standard Ceralpha method. This indicates that the modified FN method is a reliable and practical tool for evaluating  $\alpha$ -amylase activity in malt flour.

The modified method provides a feasible and practical tool for evaluating  $\alpha$ -amylase activity in malt flour and offers a cost-effective alternative to the conventional methods of  $\alpha$ -amylase determination. The modified FN method has the potential to become an important tool in the field of grain and flour analysis, especially in the brewing and distilling industries where high values of  $\alpha$ -amylase activity are essential and the modified FN method can facilitate daily monitoring of the malting process.

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