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Estimation of Dietary Gluten Content using Total Protein in Relation to Gold Standard Testing in a Variety of Foods

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Abstract

Objective: To compare the accuracy of the Osborne calculation for estimating gluten content in food in relation a laboratory (ELISA) based method.

Methods: We evaluated 25 commonly consumed gluten-containing food products for ELISA testing of gluten to determine analyzed gluten content. This was compared with calculated gluten content (using the Osborne method) which was determined as 80% of the plant protein content of each food item using nutrition information. Correlation coefficient (r), along with a 95% confidence interval (CI) and Bland Altman plots were used to estimate the level of agreement between calculated and analyzed gluten.

Results: A reasonable overall correlation coefficient of r = 0.46 a 95% CI (0.08 – 0.73, R^2 = 0.22) was seen. We observed that variability in the Osborne (calculated) and analyzed gluten increased as the average gluten content increased and the average difference was not constant over the range of gluten measurements. In addition, the calculated gluten measure tended to be higher than analyzed and thus overestimated gluten content (net overestimation was 3.3 g (95% CI -4.0 to 10). Stronger correlations were observed in foods with a gluten content that was lower than the total protein content (N=18, r=0.70, 95% CI=0.35 to 0.88, R^2 = 0.49).

Conclusions: These findings indicate that the Osborne (calculated) to analyzed gluten shows a reasonable correlation in foods with lower gluten content (less than 5 g gluten), and that the Osborne method is a practical way to estimate gluten content.

Keywords: Celiac disease; Gluten; Gluten estimation; ELISA

Abbreviations: CD: Celiac Disease; ELISA: Enzyme Linked Immunoabsorbent Assay

Introduction

Celiac Disease (CD) is an autoimmune enteropathy characterized by damage to the small intestine [1]. It is triggered by the ingestion of gluten [1] of particular grains and cereals. Gluten, a storage protein, is a general term given to the prolamines fractions of wheat (gliadin), rye (secaline), barley (hordein) and oats (avenin). The current management of CD is a gluten-free diet for life with strict avoidance of wheat, rye, barley, oats, triticale and their cross bread varieties [2,3].

It is appreciated that consuming a diet completely devoid of gluten may be difficult to achieve and that trace amounts of gluten are found in both natural gluten-free, and labeled gluten-free products [4-6]. Studies have quantified a select number of products consumed by individuals with CD to be tested for gluten consumption under controlled settings using enzyme linked immunoabsorbent assays (ELISA) [5,7] which is the gold standard test for gluten detection and quantification. While reliable, this methodology is not a practical measure for quantification of dietary gluten for evaluation of gluten consumption in individuals or populations. Having an accurate estimation of gluten content in foods is important in the study of CD presentation, symptomotology or risk of CD development.

A simplified scheme to estimate gluten consumption was developed in the Netherlands where gluten consumption patterns of the general population were compared to that of first degree relatives of individuals with CD to explain CD presentation and prevalence in the latter group [8]. The calculation of gluten in foods was adopted from Osborne's definition and classification for the protein fractions in wheat, rye, barley and oats [8]. Based on this classification, the gluten content was calculated at 80% of the plant protein content of a given food item known to be an overt source of gluten (wheat, rye, barley and oats). This

method was also used in another study conducted in the Netherlands to assess the gluten consumption in infants and children [9,10] and used to quantify gluten against serology to assess for compliance and quality of life in young adults and adolescents in Italy [11].

To the best of our knowledge, the Osborne equation has not been applied in North America with application to a variety of commonly consumed foods. The purpose of this pilot study was to evaluate the accuracy of the Osborne equation for estimating gluten content through conducting a comparative test of this calculation on a variety of commonly consumed foods in relation to ELISA as the gold standard in the detection and quantification of gluten content.

Methods

Food samples & handling

A sample of 25 commonly consumed and readily available gluten-containing products were purchased in October, 2012. The products selected for testing included overt gluten sources of ready to eat and non ready to eat items (Table 1). To avoid the possibility of any external contamination, using a disposable spatula, approximately 50 g of each food sample was placed in a sterile plastic sample bag, and

Food Item	Calculated Gluten (g)	Analyzed Gluten (g)	Gluten as a Percentage of Total protein (%)
All Bran Flakes ®	9.41	0.109	0.9
*All Purpose Flour	10.67	44.82	336
Chicken Noodle Soup	0.96	0.5178	43
Chocolate Chip Cookies	2.7586	2.3817	70
Couscous	9.68	1.1531	9
Dry Penne Pasta	8.89	1.9696	18
*Egg Quesadilla	2.97	3.8244	102
Fish Sticks	2.24	0.6773	24
Lean Cuisine Chicken/Pasta Frozen Dinner ®	1.49	0.716	38
*Mini Muffin	3.478	4.5667	105
Mini Ravioli	1.887	1.6562	72
Multigrain Bagel	8.727	5.6472	52
Nutrigrain Bar ®	4.324	1.3303	25
Orzo Pasta	10.353	0.7013	5
Pizza Pops ™	4.8	3.4085	57
Pot Barley	20	20.565	82
**Quick Oats	10	0.005	0.0004
*Rye Bread	5.33	13.77	206
*Rye Flour	9.1429	26.591	233
Shreddies ®	6.4	4.5022	56
Spelt Bread	6.956	5.6007	64
Vegetable Barley Soup	0.64	0.7915	99
Wheat Crackers	8.4	6.5077	62
*White Bread	6.76	14.883	176
*Whole Grain Bread	8.89	29.704	267

**Quick Oats < 5 ppm
*Analyzed gluten> 100% of total protein
Table 1: Food Items studied with Calculated and Analyzed Gluten content per 100 g; and analysed gluten expressed as a percentage of Total protein per 100 g food items.

homogenized. The grain products provided for analysis were powdered in a mortar with a pestle. The soup cans were shaken well, opened and the required amounts were obtained using sterile pipettes.

ELISA Gluten extraction & analysis

0.25 g of each sample was placed in a sterile disposable 15 ml centrifuge tube, and 2.5 ml cocktail solution (r-Biopharm #R7006) was added and incubated at 50°C for 40 minutes. After incubation, the tubes were cooled to room temperature; 7.5 ml of 80% ethanol solution was added to each tube and shaken for 1 hour. Subsequently the samples were centrifuged at 3000 RPM for 10 minutes and the supernatant was used for the test. The dilution factor of the sample extract at this point was 1/500. Another factor of 2 was applied to convert the detected gliadin value to gluten value because it is assumed that the glaidins and glutelins (which together make up the glutens) are roughly in equal proportion. If the results were higher than 80 ppm gluten in the 1st testing, additional dilutions were conducted (1/10, 1/100, 1/1000 and 1/10000) and the test (using 80% ethanol extraction for Gliadin) was repeated with each dilution. Optical Density was used with dilutions falling within the 5 ppm to 80 ppm range to calculate the final result.

All testing was completed at the Food Science Technology Center (FSTC), in Mississauga, ON, Canada. A minimum of 100 g/sample was required for analysis. Gluten content was analyzed by enzyme-linked immunosorbent assay (ELISA) (Ridascreen Gliadin; R-Biopharm AG, Darmstadt, Germany) with a sensitivity limit of 5 parts per million (ppm) and a linear measure up to 80 ppm (i.e. sensitive for samples between the ranges 5-80 ppm gluten). If the results were higher than 80

ppm, further dilutions were carried out, and the tests were repeated to calculate the final result.

Results from the ELISA analysis were provided in ppm gluten, and converted to grams of gluten per 100 grams of food item.

Calculated Gluten content

Using the nutrition information available on the packaging of the 25 food items, the protein content as per the Nutrition Facts table was converted to 100 g of the food item. The gluten content was calculated at 80% of the plant protein content of that food item.

In North America the Canadian Nutrient File, USDA National Nutrient Database and food labels declare the total protein in food but do not specify the source (i.e. plant versus animal). As such, in foods that were considered 'mixed foods' (foods falling into two or more food groups, as defined by Eating Well with Canada's Food Guide and USDA's MyPlate), the amount of plant protein was estimated based on the approximate number of grain and starches servings available in that item using the Canadian Diabetes Association exchange system [12], with the assumption that 15 grams of available carbohydrate is equal to 1 grain and starch serving, and contains 3 grams of plant protein.

Statistical analysis

The estimated correlation coefficient (r), along with a 95% confidence interval (CI), is reported for each analysis (SAS). R squared values and regression coefficient estimates, where calculated gluten was used to predict analyzed gluten were conducted. R squared values represent the strength of the linear relationship between analyzed and calculated gluten. Bland Altman Plots were used to assess the consistency of the difference between calculated and analyzed gluten over all values of average gluten content [(calculated+analyzed)/2] [13,14].

Results

Analysis of analyzed food items

25 foods of various types are included in the analysis. Calculated gluten using the Osborne classification as well as measured gluten using ELISA are shown in Table 1. The results show an overall correlation coefficient between calculated and analyzed gluten of r = 0.46 a 95% confidence intervals (0.08 – 0.73, R^2 =0.22). This is illustrated in Figure 1.
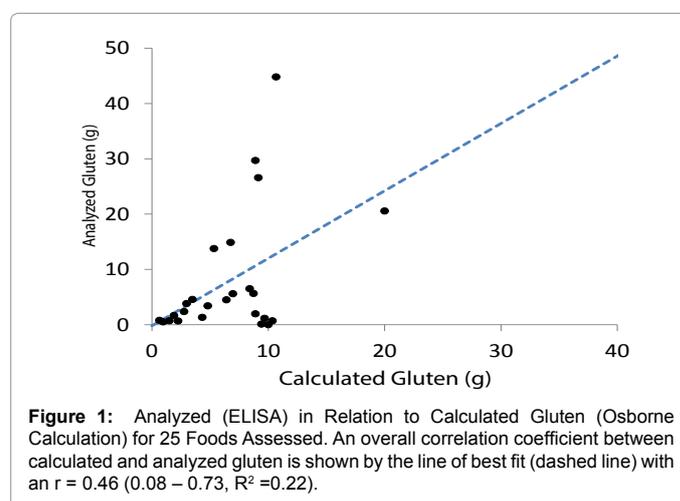

Figure 1: Analyzed (ELISA) in Relation to Calculated Gluten (Osborne Calculation) for 25 Foods Assessed. An overall correlation coefficient between calculated and analyzed gluten is shown by the line of best fit (dashed line) with an r = 0.46 (0.08 – 0.73, R^2 =0.22).

A comparison of calculated and measured gluten was also evaluated using Bland-Altman plots (Figure 2). It was observed that variability in the difference between the calculated and analyzed gluten increased as the average gluten content increased. Furthermore, the average difference was not constant over the range of gluten measurements.

On the basis of this variability, we refined the range to include an average difference between calculated and analyzed gluten in categories with a lower gluten content ranging from 0 – 5 g, 0 – 7.5 g, and 0 – 10 g. It was revealed that with increasing calculated gluten values there was a subsequent drop in correlation and wider limits of agreement occurred as evidenced by the R^2 and 95 % CI from 0 – 5 g range being 0.42 and -1.8 to 2.9 g to 0 – 10 g range being 0.14, and -15.2 to 14.6 respectively. These results indicate that the strength of the correlation was strongest in the category of calculated gluten products ranging from 0 – 5 g with a tighter limit of agreement (Table 2).

Analysis of foods with high gluten protein content

Using the rationale that the calculation of gluten is a fixed percentage of total plant protein, in instances where the calculated gluten content exceeded protein content (per 100 g of sample) the calculation was rendered less valid.

Therefore, we limited our comparison to foods with a gluten content that was lower than the total protein content (Table 1). In making this choice, 18 foods were included in the reanalysis. The estimated correlation coefficient for analyzed to calculated gluten was significantly higher (r=0.70, 95% CI 0.35 to 0.88, R^2 = 0.49).

Discussion

The objective of this pilot study was to evaluate the accuracy of the Osborne calculation of gluten content with the gold standard ELISA

Figure 2: Bland-Altman Plot of Analyzed (ELISA) in Relation to Calculated Gluten (Osborne Calculation) for 25 Foods Assessed. This figure illustrates the variability in the difference between the calculated and analyzed gluten; this variability increased as the average gluten content increased.

test. Overall, the results indicate that calculated to analyzed gluten shows a reasonable correlation, suggesting that this is a practical, "real world" method of estimating gluten content. Across the range of gluten content the calculated measure tended to be higher than the analyzed gluten measure and thus overestimated gluten content of the tested food products. This overestimation was 3.3 g (SD 3.7, 95% CI -4.0 to 10.6).

We selected 25 foods with variable protein (animal and vegetable) and gluten contents to represent a variety of foods as would typically be purchased by consumers. In this analysis, a significant variability between the analyzed and calculated gluten was observed and the variability of this relationship was not consistent across the range of gluten content; such that the correlation was much weaker in foods with a higher gluten content. To delineate these relationships, we further limited the assessment to foods less than 10 g of calculated gluten and found a decreasing correlation between these foods as the calculated gluten content increased.

The assessment of gluten content has dual clinical and research utility. Overbeek et al. were the first group to use the Osborne equation to investigate the pattern of gluten consumption in two Dutch populations and concluded that gluten intake did not explain the presentation and prevalence of CD in first degree relatives of individuals with CD [8]. An estimation of gluten content has also been used to develop and validate a food-frequency questionnaire (FQ-gluten) assessing gluten consumption in infants and subsequently children [9,10]. The potential of this tool was identified as a standardized method to provide better gluten consumption comparisons in populations around the world. Compliance with the gluten free diet was also evaluated with teenagers and young adults with CD [11]. The Osborne calculation was used to estimate the dietary gluten content and was subsequently applied to correlate intake to tissue transglutaminase antibodies. Further, it has been used to measure degree of compliance with the gluten-free diet.

However, use of the Osborne calculation does require some qualification as to the utility in types of foods tested as well as methodological concerns. In this study, vegetable proteins of mixed foods were estimated as North American sources (The Canadian Nutrient File, USDA National Nutrient Database and Nutrition Facts tables) report the total amount of proteins of given foods and do not make the distinction between animal and plant proteins. This is in contrast to studies that have utilized the formula for gluten quantification that used The Dutch Food Composition Database (NEVO) which reports both animal and plant proteins in foods [15].

This study comparing a measured estimation of gluten content with a gold standard test (ELISA) also highlights methodologic limitations. In order to calculate gluten content as per the Osborne method, gluten is taken as a fixed percentage of total vegetable protein. When the calculated gluten content exceeds protein content (per 100 g of sample) the calculation is rendered less valid. This was evidenced by the stronger correlations we observed by restricting our comparison to
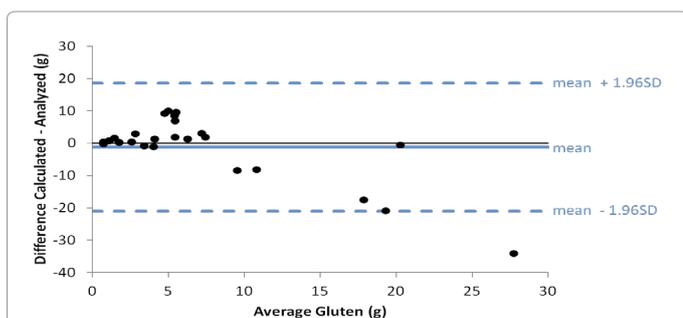
Calculated Gluten (Range)	Number of Samples	R^2	Average Difference	95% Limits of Agreement	
				(Calculated Analyzed), g (95% CI)	95% Limits of Agreement (95% CI)
0 to 5 grams	10	0.42	0.6	-1.8 (-3.3,-0.3)	2.9 (1.4,4.4)
0 to 7.5 grams	14	-0.5	-0.5	-7.3 (-10.8,-3.9)	6.2 (2.8,9.7)
0 to 10 grams	22	-0.3	-0.3	-15.2 (-21.0,-9.3)	14.6 (8.8, 20.5)

Table 2: Average difference between calculated and analyzed gluten, with 95% limits of agreement, for different calculated gluten categories illustrating greatest correlation of estimated gluten for foods with lower gluten content.

foods with gluten content that was lower than the total protein content. An additional methodological concern relates to the accuracy of the ELISA assay at high levels of gluten above the upper threshold detection limit which requires additional dilution steps and renders the ELISA to be less accurate.

In summary, we found that estimating gluten content using the Osborne calculation is a reasonable method with a stronger correlation as average gluten content decreases (< 5 g), and that the calculation tended to overestimate gluten content. Additional foods should be tested to evaluate the accuracy of this formula at large. The application of this formula may provide unique opportunities to describe practical gluten consumption in the context of diverse eating patterns in various populations. In addition it may allow for clinical assessment of gluten content with regards to the study of: CD development, occurrence and symptomology in individuals and across various populations.

Acknowledgements

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