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Characterizing the Distribution of Ppm Gluten in Gluten Free Oatmeal Servings Contaminated with a Barley Kernel

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Abstract

Oats are regularly contaminated with gluten-containing grains like wheat, barley and rye. For producers of gluten free oatmeal, contamination potential makes it prudent to understand the consequences in terms of gluten dosing, as labeling requirements specify a gluten maximum. To do this, statistical simulation has been used to produce virtual oat servings (40g) contaminated with either two row or six row spring barley. The results are probability distributions for 'actual' ppm gluten (free of measurement influences) and 'as measured' via R5 ELISA. Findings show 'actual' ppm gluten to be normally distributed with 57 ppm and 41 ppm gluten averages with 14 and 12 ppm standard deviations (stdevs) for two and six row barley, respectively. 'As measured' ppm gluten results are lognormally distributed with 61 and 44 ppm gluten averages with 63 and 47 stdevs for two and six row barley, respectively, employing an 80% analytical recovery rate and a multiplier of 1. These analyses show that 'as measured' results possess false negative probabilities (relative to a < 20 ppm gluten regulatory requirement) from 0.14 to 0.34 depending on recovery rate and barley type. This work highlights the need for non-homogenous grinding issues to be addressed in whole grain gluten assessment, for analytical recovery rates to be defined for gliadin in oats, and for appropriate conversion factors to be determined in order to attain capable measurement of gluten in oats due to barley kernel contamination.

Keywords: oats, barley, ELISA, gluten, kernel-based gluten contamination, gluten analysis, gluten-free

1. Introduction

Oats are commonly contaminated with gluten containing grains (GCG's) such as wheat, barley and rye (Hernando et al, 2008; Koerner et al, 2011; Thompson, Lee & Grace, 2010). 'Kernel based' contamination like this has recently been found to complicate determination of whether oats labeled gluten free (GF) end up as such on store shelves (Fritz, Chen & Contreras, 2017; Fritz & Chen, 2017a). Due to oversights in this regard, much of industry has been mis-diagnosing their GF labeled oat offerings, concluding they are pure when in fact one in every few dozen servings contains a GCG (Fritz, Chen & Contreras, 2017; Chen, Fritz & Ferrini, 2017; Fritz & Chen, 2019). False negative dispositions in this regard affect celiac disease (CD) patients who rely on a GF diet to avoid adverse effects (Leonard, Sapone, Catassi et al., 2017).

Due to the prevalence of contamination and inherent difficulties in assessing oats as GF, it is believed prudent to understand the consequences of GCG contaminated servings. This is addressed here by characterizing the distribution of ppm gluten in servings of pure oats containing a single barley kernel. This is a corollary study to that carried out by the authors where wheat kernels were the GCG of focus (Fritz & Chen, 2017b). A 'divide and conquer' approach has been taken regarding these two GCG contaminates due to inherent differences in these grains along with the dissimilarities in how gluten is assessed due to them.

The approach used here is statistical simulation. With this we first create a virtual barley kernel based on published weight, protein and gluten distributions, and then determine the ppm gluten with this kernel present in a 40g serving of otherwise pure oats. Doing this 25,000 times, provides a distributional assessment of 'actual' gluten in a barley contaminated serving, providing insight into true contamination unencumbered by measurement influences.

As a second analysis, we add influences of measurement, obtaining an 'as measured' assessment by simulating the use of R-Biopharm R5 ELISA testing. Doing this 25,000 times provides a distributional assessment of gluten

in a barley contaminated serving as it would be perceived through the lens of measurement.

These two simulated distributions provide new insights into gluten dosing when a barley kernel exists in a serving of oats. Comparison of them provides insights into how measurement affects assessment and what the requirements are in this regard.

Figure 1 shows a flow chart of the approach taken herein.

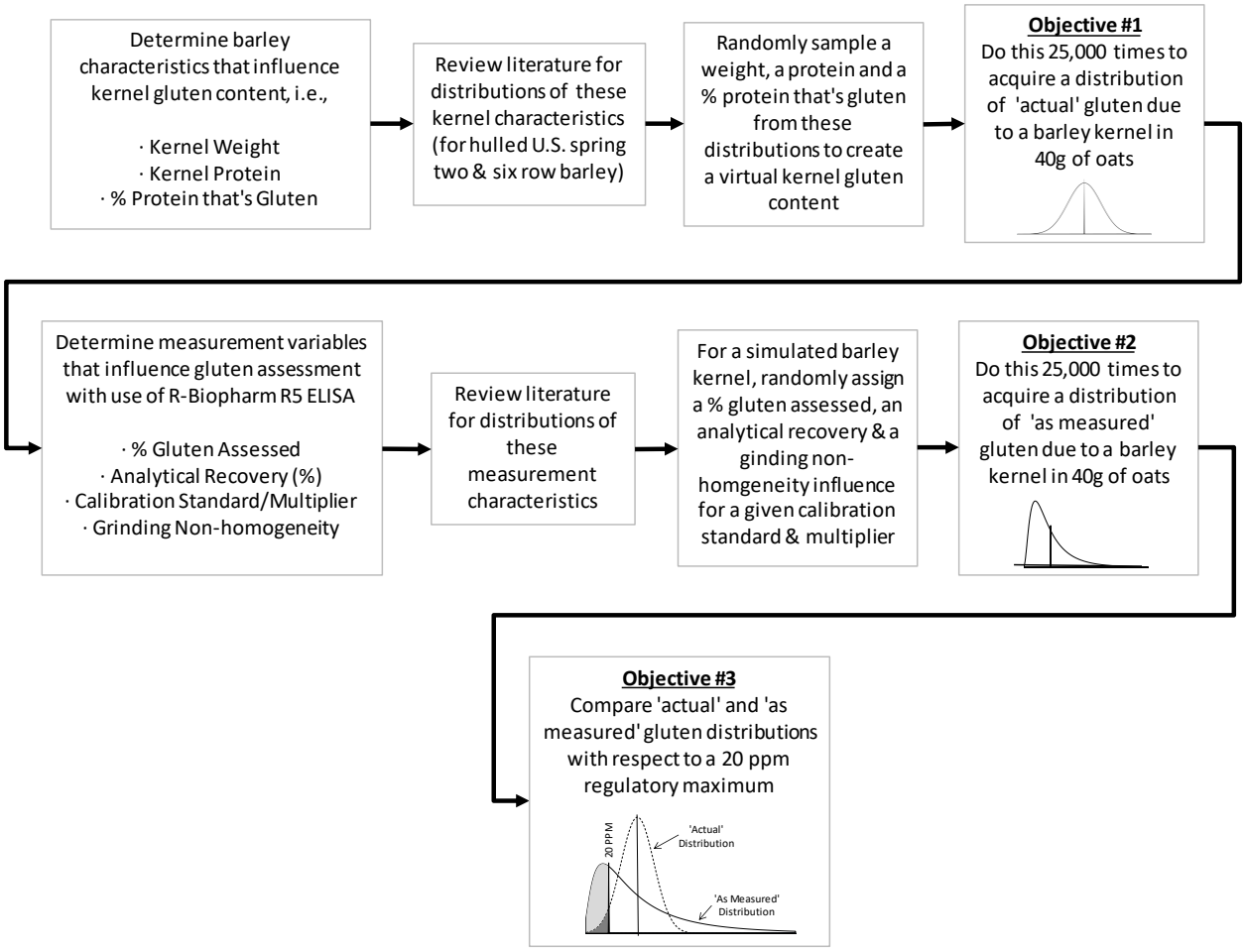


Figure 1. Research approach flowchart

2. Materials and Methods

2.1 Methods – Kernel Variability

It has been widely published that gluten resides in the endosperm of barley in the form of storage proteins called hordeins (Kirkman et al, 1982; Shewry et al, 1985). Given this, there are three variables, believed to directly influence the gluten content of a barley kernel. They are 1) kernel weight (dry basis), 2) % protein in the kernel on a dry weight basis, and 3) % gluten (i.e., hordeins) in the protein. Following a literature search, estimates of distribution parameters for the above-mentioned factors have been shown in Table 1. This has been done for U.S. Two Row and Six Row spring barley types.

Table 1. Approximate distributional parameters for individual barley kernels based on row classification for three key variables affecting individual kernel gluten content

	Kernel Weight (g) (Dry basis)		**Kernel % Protein**		**% Protein as Gluten*** (Hordein - g, B, C & D)	
Assumed Distribution Type	**Normal**		**Normal**		**Normal**	
	~ Avg.	~ Stdev	~ Avg.	~ Stdev	~ Avg.	~ Stdev
Hulled U.S. Spring Two Row Barley	0.0400	0.0047	12.75%	2.42%	45.0%	5.0%
Hulled U.S. Spring Six Row Barley	0.0347	0.0039	10.49%	2.43%		
Data Source	Nair, 2010		Nair, 2010	Nair, 2010 & Fox, 2011**	Kirkman, 1982	

* - As per Kirkman et al '82, hordein content tends to vary from 35% - 55% so 45% used as an average with stdev to vary roughly across the 35% - 55% range.

** - Stdev's obtained via Monte Carlo simulation combining variation in 'line to line' averages with 'kernel to kernel' variability from Fox '11 (for Gairdner cultivar used here as representative of other cultivars)

Averages and stdevs shown in Table 1 are parameter estimates reflecting ‘kernel to kernel’ variability across varietal lines for each of the two barley classification types. The stdev for ‘kernel % protein’ was not directly found in the literature but derived by combining a ‘line to line average’ variance component (Nair et al, 2010) with a ‘kernel to kernel’ variance component (for the Gairdner variety used as a surrogate for other lines) (Fox, 2011). This approach is outlined in Table 2.

Table 2. Estimation of % Protein Stdev incorporating both ‘Kernel to Kernel’ variability (Fox, ’10) and ‘Line to Line’ Average Protein variability variance components

Hulled U.S. Spring Barley Line's Average Protein (Nair et al, '10 except as noted)						
Line Name	**Row Type**	**SKCS HI***	**Average Protein (%)**	**Proten (%) Stdev Across Line's Averages**	**Kernel to Kernel Protein (%) Stdev****	**Combined Line & Kernel to Kernel Protein (%) Stdev*****
04AB092-34	2	30.1	12.3%	0.87%	2.30%	2.42%
03WA-124.1		60.1	12.9%			
04WA-104.39		74.0	11.9%			
04WA-119.2		90.6	13.9%			
FEG125-68	6	52.0	12.9%	1.20%		2.43%
UT04B2074-846		54.1	11.4%			
FEG147-14		63.0	11.4%			
UT04B2092-1297		66.1	11.0%			
UT04B2041-42		79.2	10.0%			
04AB059-51		91.2	9.5%			

* - Single kernel characterizaion system hardness index

** - For the Gairdner barley variety used here as a surrogate for other lines (Fox, 2011).

*** -Obtained by sampling across lines for a given row type using Fox '11 kernel to kernel % Protein findings.

The three variables shown in Table 1 are assumed herein to be independent of each other. Research into such relationships was unable to be found in the literature except for protein content relative to dry kernel weight for wheat (Delwiche, 1995), where no relationship was apparent.

2.2 Methods – Measurement Variability

Four measurement variables have been utilized in characterizing the ppm gluten in a barley contaminated serving of otherwise gluten-free oatmeal. This was to determine gluten content ‘as measured’ relative to estimation of ‘actual true gluten’ exclusive of measurement. These variables are 1) % of overall gluten protein actually assessed, 2) % analytical recovery of the fraction of hordeins assessed, 3) test kit calibration standard and multiplier used, and 4) effects of grinding non-homogeneity (of a barley kernel in oats).

Percentage (%) of overall gluten protein actually assessed has been included, since the R-Biopharn ELISA test kit only measures three of the four barley hordeins, namely the γ, B & C (and not D) components (Shewry et al, ’85, Tanner et al, ’19). It is assumed to be normally distributed.

Also, percentage (%) analytical recovery of the fraction of hordeins assessed has been included, since no

validation for gluten in oats is believed to have been performed, as there's no record of this published by Biopharm up till now (R-Biopharm, 2012). It is also possible that this may vary somewhat 'test to test' as well. It has been assumed to be normally distributed as well.

'Test kit calibration standard and multiplier' is included since this obviously has a profound effect on estimated ppm gluten.

Finally, 'effects of grinding non-homogeneity' is included since this has been shown to inflate gluten ppm test outcome variability, producing a lognormal distribution of gluten test results (Fritz, 2017b.)

Following a literature search, estimates of distribution parameters for the above have been shown in Table 3. Note that the stdev of 'analytical recovery' was unable to be found in the literature and has been 'guesstimated' here. Sensitivity analysis around this 'guesstimate' has been performed and will be reported in the results section of this paper.

Table 3. Approximate distributional parameters for measurement related variables that affect gluten content assessments of gluten-free oatmeal contaminated with a single barley kernel

	% Gluten Protein that's assessed* (Hordein's g, B & C only)		**Analytical Recovery (%)** (gliadin)		**Calibration Standard & Multiplier** (for Biopharm ELISA)		**Grinding Non-Homogeneity**	
Assumed	**Normal**		**Normal**				**Log-Normal**	
Distribution Type	~ Avg.	~ Stdev	~ Avg.	~ Stdev	Calibration Standard	Gliadin Multiplier	Avg. of Stdev of Ln values	Stdev of Stdev of Ln Values
Barley	97.0%	0.50%	80.0%	8.5%	Gliadins (wheat prolamins)	1	0.793	0.082
Data Source	Shewry, 1985		R-BioPharm, 2012 (for gliadin recovery in corn)	Guesstimate	Hernando, 2008		Fritz, 2017b (values for Barley unpublished)	

* - As per Shewry et al, '85, hordeins g, B & C % content of total hordeins tend to vary from 96% - 98% so 97% used as an average here with stdev to vary roughly across the 96% - 98% range.

2.3 Methods – Simulation

These seven variables, three being barley characteristics (Table 1) and four measurement variables (Table 3), have been used to simulate servings of gluten-free oatmeal (of 40g size), where each is contaminated with a single barley kernel. These contaminated barley kernels are 'created' based on random selection from the distributions for the three key kernel variables defined in Table 1. Since these kernel characteristics are considered independent of each other, the approach is simply to first 'create' a kernel of a certain dry weight by randomly selecting a weight from the 'dry weight' probability distribution. Then to this '% protein' and '% protein that's gluten' values are applied, with these values being randomly selected from their respective distributions.

So, for a 'true' ppm gluten assessment (exclusive of any measurement influences), the following equation defines each simulated serving's ppm gluten:

$$\text{PPM gluten}_{\text{actual}} = [(\text{dry weight in g}) \times (\text{'\% Protein'}/100) \times (\text{'\% Protein that's Gluten'}/100)]/40\text{g} \quad (1)$$

For an 'as measured' assessment of ppm gluten due to barley, there are two steps. The first is to get what we refer to as a 'pre-grind' assessment of gluten and this is then used to obtain the final 'post grind' ppm denoted herein as the 'as measured' value. By 'pre-grind' we mean prior to incorporating variability due to the inability to achieve a homogenous grind of the kernel based gluten in a serving of oats (Fritz et al., 2017b).

Obtaining the 'pre-grind' assessment is done by randomly selecting measurement values for '% protein that will be assessed' and 'analytical recovery' from their respective probability distributions, and then choosing a calibration standard and multiplier to use. Herein we have opted to use a wheat calibration with a multiplier of 1. This is recognized as appropriate practice for barley related gluten in oats (Hernando et al, 2008), although the best multiplier is under debate. Use of a different multiplier would simply convert results by that amount so a

conversion factor of 1.0 has been selected here for simplicity.

The equation for ppm gluten 'pre-grind' is then:

$$\text{PPM gluten}_{\text{pre-grind}} = (\text{PPM gluten}_{\text{actual}}) \times 1.0 \times (\text{'\% Gluten that's Gliadin'})/100) \times (\text{'\% Analytical Recovery'}/100) \quad (2)$$

The 1.0 in equation 2 simply denotes the conversion factor added here for reference.

For the final 'as measured' value, or 'post-grind' ppm value for a serving, the 'PPM gluten $_{\text{pre-grind}}$' outcome is used as the gluten ppm average of a log-normally distributed collection of 0.25g test results in a 40g serving (Fritz, 2017b.) The natural log of this value is considered the average of a normally distributed random variable whose stdev is then randomly selected from a normal distribution whose parameters are shown in Table 3, namely 0.793 average and 0.082 stdev. These values come from studies conducted previously (Fritz et al., 2017b) although the barley results were not published at that time (as the focus in the previous publication was wheat contamination of oats).

So the final 'as measured' ppm gluten for a serving, denoted as 'PPM gluten $_{\text{post-grind}}$', is then the anti-log of this log transformed value, defined by:

$$\text{PPM gluten}_{\text{post-grind}} = e^{\ln(\text{PPM gluten post-grind transformed})} \quad (3)$$

So the above is done for 25,000 simulated barley kernels, each in 40g servings of otherwise pure oats. The resultant 25,000 simulated ppm gluten values are then used to characterize the 'as measured' distribution of ppm gluten contamination of a 40g serving of pure oats contaminated with a single kernel of barley. This has been done for each of the two U.S. spring barley types, namely two and 6 row barley.

2.4 Data Analysis

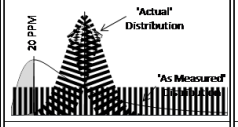
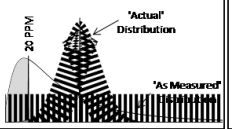
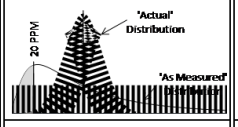
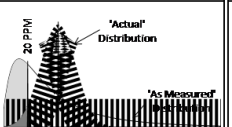
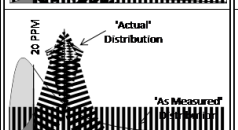
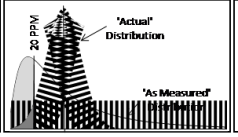
Simulation of servings was performed in custom routines created in Excel, Microsoft Office 2013.

3. Results

Table 4 shows the outcomes obtained for 25,000 simulated 40g servings of oats each contaminated with a single 'virtual' barley kernel. The seven barley and measurement variable settings for each simulation are shown on the left-hand side with resultant simulation findings shown on the right-hand side. Rows 1-4 of the table are for two row spring barley and 5-8 for six row cultivars. Rows 1 and 5 are simulated 'actual' outcomes which are estimates of 'true' ppm gluten in 40g contaminated servings. Rows 2-4 and 6-8 then show 'as measured' outcomes for various analytical recoveries. 70%, 80% and 90% analytical recovery simulations have been conducted due to the uncertainty of this variable's average value.

The simulation findings part of the table includes resultant distribution type, parameters for that distribution, % of servings < 20 ppm (a common regulatory maximum for GF labeling) and comparative distribution plots which overlay 'actual' and 'as measured' distributional outcomes.

Table 4. Simulated outcomes of ppm gluten in gluten-free oatmeal servings (40g) contaminated with a single barley kernel for Hulled U.S. Spring Two Row and Six Row Barley with and without the influence of measurement. (n = 25,000 simulated servings.)

				Barley Kernel Variable Settings						Measurement Variable Settings						Simulation Findings					
	Simulation		Theor-etical PPM Calc. (Dry Basis)	Kernel Weight (g) (dry basis)		Kernel % Protein		% Protein as Gluten (Hordeins)		Hordein % Measured (γ, B & C)		Analytical Recovery (% Gliadin)		ELISA Calibra-tion	Grinding Non-Homogeneity		Dist. Shape	Avg. PPM Gluten	Stdev of PPM Gluten	% Servings Found Compliant (<20 ppm)	Distribution Plots 'Actual' vs. 'As Measured' False Negative Rate shown in light gray (ppm gluten)	Calibration Multiplier to get same % Compliant as 'Actual'
Row				Avg.	Stdev	Avg.	Stdev	Avg.	Stdev	Avg.	Stdev	Avg.	Stdev		Avg. of Stdev of 'In' values	Stdev of Stdev of 'In' Values					
1	Hulled U.S. Spring 2 Row Barley in 40g Oats	'Actual' (absent of msmt. influence)	57 (using wheat with 1x multiplier)	0.040	0.0047	12.75%	2.42%	45.0%	5.0%	NA		NA		NA	NA		Normal	57	14	0.08%		7x Avg. = 427 Stdev = 441
2		'As Measured'								97%	0.50%	80%	8.5% *	Wheat, 1x multiplier	0.793	0.082	Log-Normal	61	63	17.86% (17.78% false negative rate)		
3												70%						54	58	22.94% (22.86% false negative rate)		8.25x Avg. = 446 Stdev = 478
4												90%						69	72	14.47% (14.39% false negative rate)		6.7x Avg. = 460 Stdev = 491
5	Hulled U.S. Spring 6 Row Barley in 40g Oats	'Actual' (absent of msmt. influence)	41 (using wheat with 1x multiplier)	0.0347	0.0039	10.49%	2.42%	45.0%	5.0%	NA		NA		NA	NA		Normal	41	12	2.25%		3.75x Avg. = 165 stdev = 175
6		'As Measured'								97%	0.50%	80%	8.5% *	Wheat, 1x multiplier	0.793	0.082	Log-Normal	44	47	30.64% (28.39% false negative rate)		
7												70%						39	41	36.5% (34.25% false negative rate)		4.3x Avg. = 167 Stdev = 177
8												90%						49	52	26.2% (23.95% false negative rate)		3.25x Avg. = 160 Stdev = 168

* - Varying this from 1% to 8.5% resulted in no change in average ppm gluten & <= 3 ppm change in ppm gluten stdev for either two row and six row barley.

NOTE: For each of these 8 simulations, ten replicates of the same 'settings' were performed. This was to evaluate the stability or lack thereof of the resultant simulation outcomes. Outcomes were found repeatable with less than +/- 1 ppm difference in averages obtained and less than +/- 3 ppm differences in standard deviations obtained across all eight simulations.

4. Discussion

4.1 'Actual' PPM Gluten Outcomes

Simulated 'actual' outcomes are shown in rows 1 and 5 in Table 4. These distributions provide insight regarding gluten dosing potential as unencumbered by the influence of measurement. Resultant distributions are normally distributed averaging 57 ppm and 41 ppm gluten with 14 and 12 ppm stdevs for two and six row barley respectively. For both barley type classifications, a single barley kernel in 40g of otherwise pure oats appears likely to produce a non-compliant serving (prob. < 20 ppm being 0.0008 and 0.0225 respectively).

With two row barley averaging 39% higher gluten than six row, we can also infer that different barley classifications can produce significantly different gluten contamination potential. Within both barley classifications we have also observed substantial variability as well, with 95% control limits for these two types spanning 29 to 85 ppm and 33 to 81 ppm gluten respectively. This suggests cultivar to cultivar differences may also exist.

There is a lack of kernel level characterization of weight, protein and hordein fraction research at present, preventing more detailed analyses beyond the broad categories dealt with here. Looking into sub-classifications

and more commonly used common cultivars in this way would enable better understanding of barley related gluten contamination of oats.

4.2 'As Measured' PPM Gluten Outcomes

Simulated 'as measured' outcomes are shown in rows 2-4 and 6-8 in Table 4. These ppm gluten distributions are intended to reflect what one would 'view' through the lens of measurement. Again, all simulations assume assessment via ELISA R5 using a wheat calibration and 1x multiplier. Resultant distributions are lognormally distributed due to the prevalence of non-homogenous grinding shown to create this circumstance (Fritz, Chen & Contreras, 2017).

Using an 80% average analytical recovery rate, average ppm gluten outcomes obtained are 61 & 44 for two and six row barley respectively. With 70% and 90% recovery rates, these change from 54 to 69 and 39 to 49 ppm gluten for two and six row respectively. Consequently, and as expected, analytical recovery rate is a significant influencer on resultant gluten obtained. Since regulatory dispositions are made regarding ppm gluten content in oats, it is hoped recovery rates for RIDASCREEN Gliadin in oats will be determined to aid assessment accuracy.

The stdev for analytical recovery was a 'guesstimate' as well. This was varied from 1% to 8.5% in simulations (results not shown) to test outcome sensitivity due to this potential variability. Doing so resulted in no change in average ppm gluten and less than 3 ppm change in ppm gluten stdevs across barley types and analytical recoveries evaluated.

4.3 'Actual' vs. 'As Measured' PPM Gluten Discussion

Referring to Table 4, simulated 'as measured' average ppm gluten values are comparable to simulated 'actual' average ppm gluten values, this being the case for both two row and 6 row barley types. It appears a recovery rate of around 75% would produce the most similar results for both barley types given a calibration conversion of 1x as assumed here.

Despite being comparable in average though, dissimilarity in distributional shapes, i.e., normally distributed 'actual' outcomes vs. lognormally distributed 'as measured' ones, produce a significant difference in resultant probability of diagnosing contaminated servings as 'compliant'. As mentioned earlier, employing the common < 20 ppm regulatory requirement as a standard (Sharma, Pereira & Williams, 2015), two row and six row barley types are simulated to possess 0.0008 and 0.00225 probabilities of a compliant 40g serving when containing one of their kernels. In contrast though, their 'as measured' counterparts provide probabilities of ~ 0.18 and 0.31 for two and six row barley respectively (using an 80% recovery rate). Assuming the 'actual' simulation results reflect true contamination potential, this equates to false negative rates of ~ 0.18 and 0.28 'as measured'.

Since 'as measured' results are obviously what are used to disposition compliance, false negative potential like this is problematic. This is driven by the inherent inability to homogenously grind gluten in these grains which causes the lognormal distribution of gluten test outcomes. Mitigating this has been addressed though (Chen et al, 2018) and use of a method such as this is encouraged.

Finally, in Table 4, in the rightmost column, calibration mulitpliers needed to provide 'as measured' probabilities of being < 20 ppm equal to those obtained in the 'actual' simulations are provided. These range from 3.75x to 7.0x based on barley type and analytical recovery rate.

5. Conclusion

Statistical simulation has been used to characterize the ppm gluten in otherwise pure servings of oats. This provides insight regarding potential gluten dosing due to barley contamination. This has also shown how measurement can adversely affect assessment, highlighting the need for inventive methods (Chen et al, 2018) to overcome non-homogeneity in grinds, for analytical recovery studies for gliadin in oats to be conducted, and for studies to better ascertain proper conversion factors for gluten in oats due to barley to be pursued.

Disclosure of Competing Interests

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References

Chen, Y., Fritz, R. D., Kock, L., Garg, D., Davis, R. M., & Kasturi, P. (2018). A stepwise, 'test-all-positives' methodology to assess gluten-kernel contamination at the serving-size level in gluten-free (GF) oat production. *Food Chemistry, 240*(39), 1-5. https://doi.org/10.1016/j.foodchem.2017.07.153

Diaz-Amigo, C., & Popping, B. (2013). Accuracy of ELISA Detection Methods for Gluten and Reference Materials: A Realistic Assessment. *J. Agric. Food Chem, 61*, 5681-5688. https://doi.org/10.1021/jf3046736

Delwiche, S. R. (1995). Single Wheat Kernel Analysis by Near-Infrared Transmittance: Protein Content. *Cereal Chemistry, 72*(1), 11-16. Retrieved from https://www.cerealsgrains.org/publications/cc/backissues/1995/Documents/72_11.pdf

Fox, G. P. (2011). Development of a Single Kernel NIR Barley Protein Calibration and Assessment of Variation in Protein on Grain Quality. *J. Inst. Brew, 117*(4), 582-586. https://doi.org/10.1002/j.2050-0416.2011.tb00507.x

Fritz, R. D., & Chen, Y. (2017a). Kernel-based gluten contamination of gluten-free oatmeal complicates gluten assessment as it causes binary-like test outcomes. *International Journal of Food Science & Technology, 52*(2), 359-365. https://doi.org/10.1111/ijfs.13288

Fritz, R. D., Chen, Y., & Contreras, V. (2017). Gluten-containing grains skew gluten assessment in oats due to sample grind non-homogeneity. *Food Chemistry, 216*, 170-175. https://doi.org/10.1016/j.foodchem.2016.08.031

Fritz, R. D., & Chen, Y. (2017a). Kernel-based gluten contamination of gluten-free oatmeal complicates gluten assessment as it causes binary-like test outcomes. *International Journal of Food Science & Technology, 52*(2), 359-365. https://doi.org/10.1111/ijfs.13288

Chen, Y., Fritz, R., & Ferrini, L. (2017). Compliance assessment of US gluten-free labeled oatmeal produced under a "Purity Protocol" indicates "Serving Level Compliance" not attained. *Cereal Chem., 2018(00)*, 1-2. https://doi.org/10.1002/cche.10008

Fritz, R. D., & Chen, Y. (2019). Oat Consumption by Celiac Disease Patients: Outcomes Range from Harmful to Beneficial, Depending on the Purity of the Oats. *Ann. Gastroenterol Dig. Dis., 2*(1), 003-005. Retrieved from https://www.somatopublications.com/oat-consumption-by-celiac-disease-patients-outcomes-range-from-harmful-to-beneficial-depending-on-the-purity-of-the-oats.pdf

Fritz, R. D., & Chen, Y. (2017b). Characterizing the Distribution of Ppm Gluten in Gluten Free Oatmeal Servings Contaminated with a Wheat Kernel. *Jour. Food Res., 6*(5), 92-98. https://doi.org/10.5539/jfr.v6n5p92

Haraszi, R., Chassaigne, H., Maquet, A., & Ulberth, F. (2011). Analytical Methods for Detection of Gluten in Food - Method Developments in Support of Food Labeling Legislation. *Journal of AOAC International, 94*(4), 1006-1025. https://doi.org/10.1093/jaoac/94.4.1006

Hernando, A., Mujico, J. R., Mena, M. C., Lombardia, M., & Mendez, E. (2008). Measurement of wheat gluten and barley hordeins in contaminated oats from Europe, the United States and Canada by Sandwich R5 ELISA. *Eur J Gastroenterol Hepatol, 20*(6), 545-554. https://doi.org/10.1097/MEG.0b013e3282f46597

Kirkman, M. A., Shewry, P. R., & Miflin, B. J. (1982). The effect of nitrogen nutrition on the lysine content and protein compostition of barley seeds. *J. Sci. Food Agric., 33*, 115-127. https://doi.org/10.1002/jsfa.2740330203

Koerner, T. B., Cleroux, C., Poirier, C., Cantin, I., Alimkulov, A., & Elamparo, H. (2011). Gluten contamination in the Canadian commercial oat supply. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 28*(6), 705-710. https://doi.org/10.1080/19440049.2011.579626

Leonard, M. M., Sapone, A., Catassi, C. et al., (2017). Celiac Disease and Nonceliac Gluten Sensitivity: A Review. *Jour Amer Med Assoc., 318*(7), 647-656. https://doi.org/10.1001/jama.2017.9730

Nair, S., Hayes, P., (2010). Variation in Kernel Hardness and Associated Traits in U.S. Barley Breeding Lines. *Cereal Chemistry, 87*(5), 461-466.

Olsson, U. (2005). Confidence Intervals for the Mean of a Log-Normal Distribution. *Jour. Stat. Ed. 1*. https://doi.org/10.1080/10691898.2005.11910638

R-Biopharm, A. G. (2012). Ridascreen Gliadin Art. No. R7001 Validation Report. https://doi.org/10.1080/10691898.2005.11910638

Rallabhandi, P., Sharma, G. M., Pereira, M., & Williams, K. M. (2015). Immunological Characterization of the Gluten Fractions and Their Hydrolysates from Wheat, Rye and Barley. *J. Agric. Food Chem., 63*, 1825-1832. https://doi.org/10.1021/jf505716p

Sharma, G. M., Pereira, M., & Williams, K. M. (2015). Gluten detection in foods available in the United States – A market survey. *Food Chemistry, 169*, 120-126. https://doi.org/10.1016/j.foodchem.2014.07.134

Shewry, P. R., Kreis, S., Parmar, E. J., Lew, L., & Kasarda, D. D. (1985). Identification of γ-type hordeins in barley. *Fed. Of Euro. Biochem. Soc., 190*(1), 61-64. https://doi.org/10.1016/0014-5793(85)80427-0

Tanner, G. J., Colgrave, M. L, Blundell, M. J., Howitt, C. A., & Bacic, A. (2019). Hordein Accumulation in Developing Barley Grains. *Front. Plant Sci., 10*(1), 1-10. https://doi.org/10.3389/fpls.2019.00649

Thompson, T., Lee, A. R., & Grace, T. (2010). Gluten contamination of grains, seeds, and flours in the United States: a pilot study. *J Am Diet Assoc, 110*(6), 937-940. https://doi.org/10.1016/j.jada.2010.03.014

Weiser, H., & Koehler, P. (2009). Is the calculation of the gluten content by multiplying the prolamin content by a factor of 2 valid?, *Euro. Food Res. & Tech., 229*(1), 9-13. https://doi.org/10.1007/s00217-009-1020-5