The Accuracy of Food Labels in Canada

by

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Abstract

The objective of this experiment is to test the hypothesis which is in Canada, the label on foods displays an accurate measurement of the food contents. This is done by using the commercially available version of the Bradford method to obtain the actual protein concentration of tested sample A, B and C which are 2% milk, protein drink and rice milk. 7 experimental concentration of each sample are used to compare with its theoretical value statistically by using one sample t-test. The result is that the t-value of sample A, B and C are 32.3470, 15.3361and 7.5365 respectively. They are all greater to the t-value which is 0.896when p is equal to 0.2 with freedom degree of 7. Therefore, it can be said that the hypothesis is rejected. In other words, in Canada, the label on foods does not display an accurate measurement of the food contents.

L'objectif de cette expérience est de tester l'hypothèse qui est au Canada, l'étiquette sur les aliments affiche une mesure précise de la teneur en produits alimentaires. Cela se fait en utilisant la version disponible dans le commerce de la méthode de Bradford pour obtenir la concentration réelle en protéine de l'échantillon testé A, B et C, qui sont de 2% de lait, des boissons et des protéines du lait de riz. La concentration de chaque échantillon expérimental est utilisée pour le comparer à sa valeur théorique statistiquement en utilisant un t-test de l'échantillon. Le résultat est que la valeur t de l'échantillon A, B et C sont 32,3470, 15.3361and 7,5365 respectivement. Elles sont toutes supérieures à la valeur t lorsque p est égal à 0,2 qui est 0,896. Par conséquent, on peut dire que l'hypothèse est rejetée. En d'autres termes, au Canada, l'étiquette sur les denrées alimentaires ne présente pas une mesure précise de la teneur de denrées alimentaires.

Introduction

Protein is a very important and benefit nutrition for human health. For example, a diet with proteins can reduce the chance of having chronic diseases such as cardiovascular disease, hypertension and osteoporosis (Health Canada 2013). Many aliments such as milk, rice drinks and yogurt contain proteins. Labeling on foods helps Canadian to know the amount of protein they consume every day.

In order to evaluate the accuracy of the food label, Canadian Food Inspection Agency (CFIA) use its own test called the Nutrition Labeling Compliant Test. The test uses different methods to find out the actual concentration of different nutrition and compares it to the value on the label. For evaluating protein concentration, the test uses Dumas method (Canadian Food Inspection Agency 2014). However, there are many other scientific methods to quantify protein in foods.

Biuret Protein Assay and Bradford Protein Assay are spectrophotometric methods that allow researchers to quantify the protein in a solution or a product. They are mostly based on the effect of chromophore of amino acids or bonds in the protein. Like many other organic molecules, based on their chemical structure, proteins can absorb some wavelengths and reflect other wavelengths of visible light, which means some wavelengths of visible light can transmit the protein but some cannot (McNaught and Wilkinson 1997).

Beer-Lambert Law states that the relationship between the concentration of the protein in a solution and its absorbance of a specific wavelength of visible light is a linear related (Robinson 1996).

Beer-Lambert Law: $A=\varepsilon lc$

A: absorbance (no unit)

ε: the molar absorbtivity with units of L mol^{-1} cm^{-1}

l: the path length of the sample - that is, the path length of the cuvette in which the sample is contained

c: the concentration of the compound in solution, expressed in mol L^{-1}

ε and b can become constant if the same protein is tested within the same cuvette. As the result, if ε and b are constant, the absorbance of the wavelength of light increases as the concentration of the protein or molecules increases (Robinson 1996).

The hypothesis of this experiment is that in Canada, the label on foods displays an accurate measurement of the food contents. Therefore, as a prediction, the experimental concentration of protein of each tested sample is not significant different to its protein concentration displaying on the label. According to CFIA, there is a 20% tolerance between the experimental value and the labeling value (Canadian Food Inspection Agency 2013). Therefore, being not significant difference in this lab means the experimental value must be statistically within +/- 20% of the labeling protein value of the tested sample.

In order to test the hypothesis, we have to find out the actual concentration of protein of three different tested samples which are rice milk, 2% milk and protein drink by using the commercially available version of the Bradford method. This method uses Coomassie Brilliant Blue G-250 dye to measure the absorbance of a protein. This dye is red under acidic condition, green under neutral condition, and blue when it binds to proteins. The procedure is that Coomassie Brilliant Blue G-250 dye is initially under an acidic condition. When it binds to protein, it becomes blue form. Different amount of protein it binds to, it has different blue form. The absorbance of the amount of protein can be measured by detecting this blue form at 610 nm in the assay by using a colorimeter. Unfortunately, in order to obtain a more precise value, the Bradford method can only measure concentrations between 0 μg/mL to 2000 μg/mL. As the result, each testing sample has to be diluted first. By measuring the absorbance of several solutions with known protein concentration, one can make a standard curve to estimate the protein concentration from the absorbance of unknown solution. Generally, the method measures the absorbance of pure known bovine serum albumin (BSA) concentration to make the standard curve (Bradford 1976).

Procedure

At the beginning, 6 different BSA standard concentrations have to be prepared as indicated in Table 1 below in 6 large and labelled test tubes (1s to 6s). These standard concentrations help to create a standard curve dilutions after.

Table 1. Standard BSA concentration

Microtube unmber	Standard BSA volume (ul)	Source of BSA standard	Diluent (H2O) volume (ul)
1s	625	1.44mg/l stock	375
2s	486	1.44mg/l stock	514
3s	347	1.44mg/l stock	653
4s	243	1.44mg/l stock	757
5s	193	1.44mg/l stock	861
6s	0	1.44mg/l stock	1000

Within other 9 labelled test tubes (1 to 6 and A to C), standard protein solutions and tested sample solutions are mixed with the diluted dye reagent by following the amount in Table 2 below. The dilution factor of each tested sample must be recorded after in order to calculate the original concentration of the tested samples.

Table 2. Reaction tubes

Test tube label	Standard protein solution (ul)	Unkown sample (ul)	Diluted dye reagent (ml)
1	100 ul from microtube 1s		5
2	100 ul from microtube 2s		5
3	100 ul from microtube 3s		5
4	100 ul from microtube 1s		5

5	100 ul from microtube 5s		5
6	100 ul from microtube 6s		5
A		100 ul from tested sample A (2% milk)	5
B		100 ul from tested sample B (Protein drink)	5
C		100 ul from tested sample C (rice milk)	5

After finishing the preparation, all reaction tubes incubate at room temperature for 5 minutes. During these 5 minutes, one can connect the colorimeter to iPad and calibrate the calorimeter sensor with distilled 5 mL of water. When 5 minutes are gone, the content of each test tube is transferred to colorimeter cuvettes which inserts into the colorimeter after. By using iPad, the absorbance of each cuvette must be measured for 10 seconds at 610 nm, and the average of absorbance values measured in 10 seconds of each cuvette has to be recorded. After the calibration ends, one puts the average absorbance value of all test tubes in Excel and uses the values of tubes 1 to 6 to create a figure of absorbance versus protein concentration with a linear trendline (standard curve) displaying its equation and value of R^2. For having a better accuracy, the value of R^2 has to be greater than 0.9. (A R^2 greater than 0.9 means the two variables have a strong linear relationship). In addition, a regression test must be used to test if there is a dependent relationship between the absorbance and concentration of BSA. The p-value of regression test must be smaller than 0.05. If one of the condition is not satisfied, the estimation of the protein concentration of the samples is not accurate, and the results are rejected. If both condition are satisfied, the protein concentration of each tested sample can be estimated by using the equation of the trendline on the figure. Since each tested sample is diluted, the protein concentration of tested samples obtained from the equation have to be multiplied to their dilution factor to get back to the original concentration. In order to do a suitable statistic analyze, 7 groups of scientists do the same experiment with same samples at the same time. At the end, the 7 sets of data are going to be analyzed and used to compare with the

concentration on the label by using one sample t-test to see if they are significant different or not. Since there is an 20% tolerance for the experimental concentration, in order to support the hypothesis, t-value must be greater than 0.896 (the t-value when p=0.2 with degree of freedom of 7).

Result

- Each group has a trendline with a R^2 that is above 0.9 and a p-value obtained from regression test smaller than 0.05. (As example, the figure of "***Concentration of BSA** vs **Absorbance***" and the regression test of group 1 are present in Appendix)

- the estimation of BSA concentration of the tested samples for each group are also present in Appendix.

Table 3. The summary of the data analyzed of the estimated concentration BSA of the tested samples for each group

Descriptive statistics			
	Protein concentration of sample A	Protein concentration of sample B	Protein concentration of sample C
Number of Observations	7	7	7
Mean (mg/mL)	0.2576	15.2594	54.5507
Standard Deviation	0.1098	3.5782	8.9341
Standard Error of the Mean	0.0415	1.3524	3.3768
95% confidence Intervals	0.1015	3.3092	8.2626

Note: the estimating protein concentration of each sample of each group has already been multiplied to its dilution factor before applying for the descriptive statistics.

Standard deviation, standard error of the mean and 95% confidence intervals become larger and larger from sample A to C.

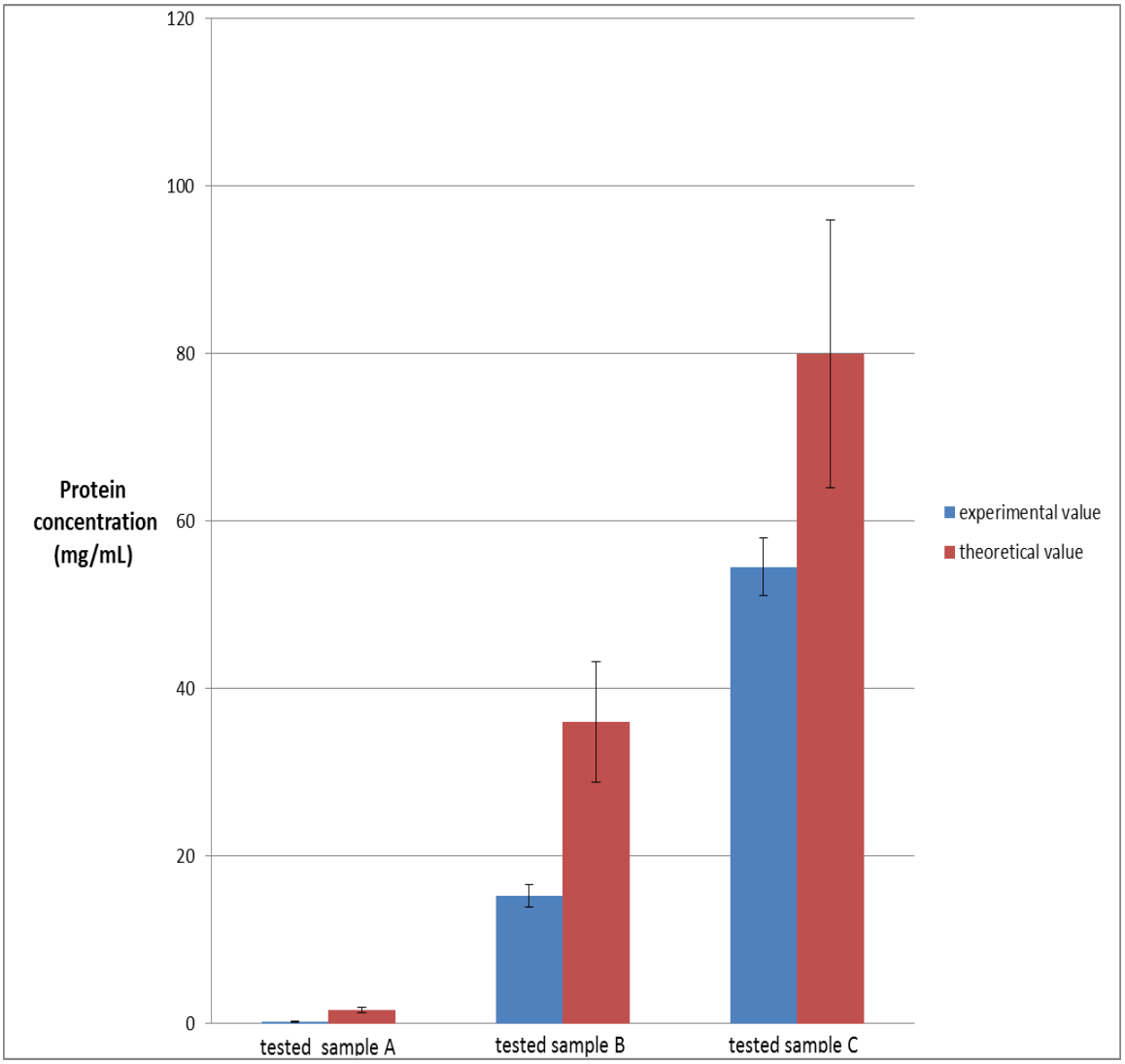
In this table, standard deviation, standard error of the mean and 95% confidence intervals of each sample are all smaller than the mean of the sample. That means the estimating protein concentration of each sample of each group is quite close to an expected value which is the mean.

Table 4. Result of one sample t-test

	Sample A (rice milk)	Sample B (2% milk)	sample C (protein drink)
Protein concentration on the label	1.6 mg/mL	36 mg/mL	80 mg/mL
Experimental protein concentration	0.2576 mg/mL	15.2594 mg/mL	54.5507 mg/mL
SEM	0.0415	1.3524	3.3768
t-value	32.3470	15.3361	7.5365

Table 4 shows that for each sample, there is a significant difference between the protein concentration on the label and the experimental protein concentration because their t-values are all greater than 0.896 (the t-value when p=0.2 with degree of freedom of 7).

Figure 1. Expermental result vs Theoretical value



Note: the error bars of experimental results are their standard error, and the error bars of theoretical values are 20% of the value.

Figure 1 shows clearly that the error bars of experimental value of each tested sample do not overlap with its error bars of the theoretical value of the.

Discussion

Result explication

The objective of this experiment is to compare the experimental and the theoretical protein concentration of three tested samples which are rice milk, 2% milk and protein drink. We hypothesize in Canada, the label on foods displays an accurate measurement of the food contents. As prediction, there is not a significant difference between the obtained experimental protein concentration of each tested sample and its value displaying on the label.

By referring *table 4. Result of one sample t-test*, the t-value of rice milk is 32.3470 which is greater than 0.896. The t-value of 2% milk is 15.3361 which is also greater than 0.896. The t-value of protein drink is 7.5365 which is greater than 0.896 too. That means in a statistical way, the experimental protein concentration of each tested sample is significant different to its protein concentration on the label. In addition, by referring *figure 1. Expermental result vs Theoretical value*, the error bars of experimental value of tested sample A do not overlap with the errors bars of its theoretical value. And it is also the same for tested sample B and C. This means no matter how many times the experiment repeats, it will never get a result of protein concentration within the range of +/20% of the labelled value of each tested sample. In other words, the experimental concentration of each tested sample is not within +/- 20% of the concentration on the label. Therefore, the hypothesis of this experiment which is in Canada, the label on foods displays an accurate measurement of the contents is rejected which means the label does not display accurately the measurement of the contents.

Reasons

From the first glance, the significant difference between the experimental and theoretical value is due to the irresponsibility of the company. However, there are also other factors beside this one that can cause the difference.

As the introduction mentions, the Canadian Food Inspection Agency uses the Nutrition Labeling Compliant Test to find out the experimental concentration of the

nutrition of foods. This test analyzes statistically the nutrition contents based from a lot. (A lot means a collection (minimum 12) of identically labelled[1] products produced under conditions as nearly uniform[2] as possible and available for inspection at one time). Moreover, for quantifying protein in food, the CFIA uses Dumas method (Canadian Food Inspection Agency 2014).

In Dumas method, a tested sample of known mass is combusted in high temperature vessel in the presence of oxygen. As the result, the sample releases carbon dioxide, water and nitrogen. The released nitrogen gas is then determined volumetrically either by gas chromatography, thermal conductivity or even chemiluminescence. After, the determined nitrogen concentration of the sample is converted to the crude protein content by using conversion factors which depend on the particular amino acid sequence of the measured protein (Fox & McSweeney 2003).

It is evident that the commercially available version of the Bradford method using in this experiment and the Dumas method used by CFIA are different. The Bradford method tries to estimate the protein concentration, and Dumas method tries to find out the mass of protein. Therefore, it is reasonable to obtain two different protein concentrations from the same product. Moreover, the requirements for CFIA's test and the requirements for this experiment are not the same. The CFIA analyzes a collection of the same products which are under conditions nearly uniform possible in order to get the most accurate value possible of the same food label. However, in this lab, we evaluate respectively the accuracy of three different food label from 7 tested samples of each three products. As the result, this also leads to have a different protein concentration. This is due to the chance for one product to have contaminated concentration of contents is usually higher than the chance for a collection of same products does.

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[1] Identically labelled means the label is uniform in every detail, including, but not limited to, brand name, common name, net quantity, lot coding (if present), best before date (if present), legal agent name and address, in addition to nutrition labelling and claims. That the products are identically labelled implies that the products themselves are identical within the terms stated above.

[2] As nearly uniform as possible includes, but is not restricted to, a continuous eight-hour production period. Some flexibility is necessary to allow for sampling where no lot codes are present, or where the production period is not clearly defined or is not continuous.

Another reason may be the inaccuracy of Beer-Lambert Law. Theoretically, the standard curve of Beer-Lambert Law is linear. However in reality, the standard curve becomes more and more curved as the protein concentration increases. Therefore, the estimation gets more and more accurate as the absorbance value of tested sample gets closer and closer to the lowest value of the standard curve (Robinson 1996). However in this experiment, the value of absorbance of each tested samples is far away from the lowest point on the standard curve. By referring *table 3 The summary of the data analyzed of the estimated concentration BSA of the samples for each group*, standard deviation, standard error of the mean and 95% confidence intervals become larger and larger from sample A to C. This is because the absorbance value of sample C (in Appendix) is the farthest value from the lowest point on the standard, and sample A has the closest value from the lowest point.

Suggestion and new question

In order to evaluate the accuracy of the food label, it is better to use the same method with same requirement as CFIA. It can give a more accurate results when we are trying to comparing with what CFIA gets because the experiments run under the same conditions.

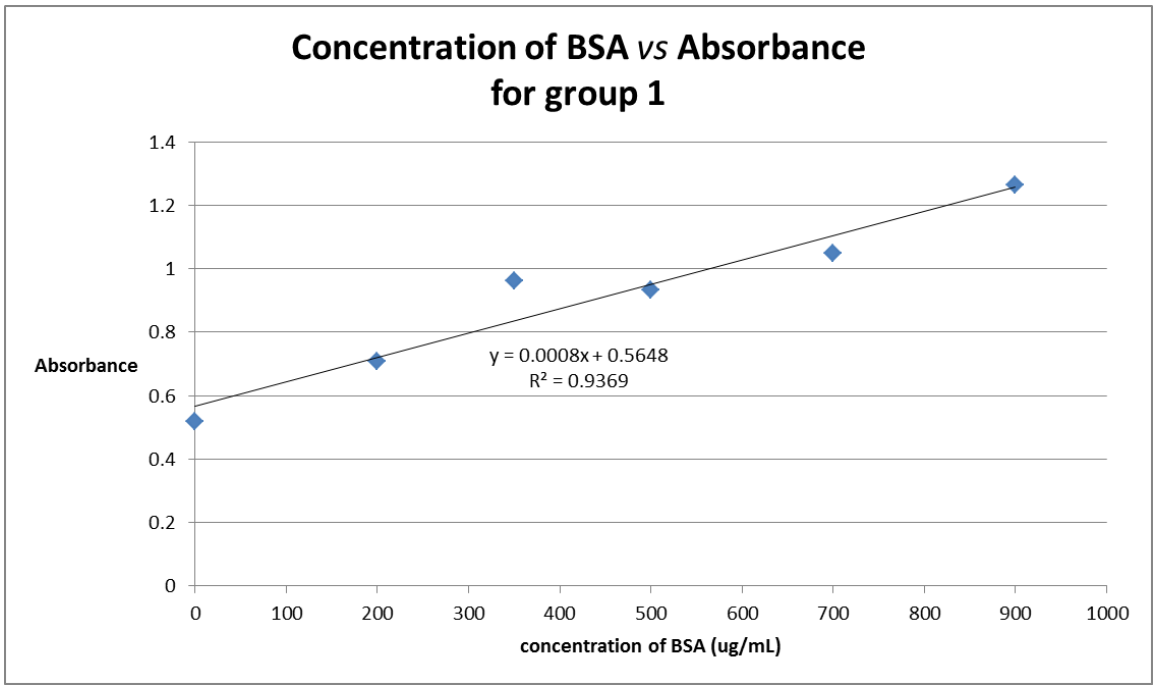
At the end, we come up with a new hypothesis. Since the Bradford method builds a standard curve by measuring the pure known BSA concentration, we ask is the estimation only accurate for pure protein solution? Therefore, we hypothesize the presence of other substances has an effect on the absorbance of the protein.

Appendix

Table 5. the absorbance value of test tube 1 to 6 for group 1

test tube	concentration (ug/ml)	absorbance
1	900	1.2645
2	700	1.05
3	500	0.9317
4	350	0.9607
5	200	0.70789
6	0	0.51659

Figure 2. Concentration of BSA *vs* Absorbance for group 1



t-test for group 1

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.96794713
R Square	0.936921647
Adjusted R Square	0.921152059
Standard Error	0.073630873

Observations	6

ANOVA

	df	*SS*	*MS*	*F*
Regression	1	0.322108971	0.322109	59.41319659
Residual	4	0.021686022	0.005422	
Total	5	0.343794993		

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	0.564772421	0.053427713	10.57078	0.000453156
concentration of BSA (ug/mL)	0.000770847	0.000100006	7.707996	0.001524614

Significance F
0.001524614

Lower 95%	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
0.416433308	0.713111535	0.416433308	0.713111535
0.000493186	0.001048509	0.000493186	0.001048509

Table 6. Results for tested samples for group 1

Tested sample	Absorbance at 610 nm	Concentration (ug/mL)	Dilution Factor	Original concentration
A	0.945	475.25	1X	0.47525
B	0.993	535.25	40X	21.41
C	1.294	911.5	80X	72.92

Table 7. The concentration of tested sample of each group

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
concentration of A (mg/mL)	0.48	0.14	0.30	0.21	0.20	0.28	0.20
concentration of B (mg/mL)	21.41	17.49	14.45	15.56	10.75	11.66	15.49
concentration of C (mg/mL)	72.92	57.25	53.77	50.91	47.69	46.10	53.22

Reference

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