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Laboratory Experiment

Quick and Cheap Colorimetric Quantification of Proteins Using 96-Well-Plate Images

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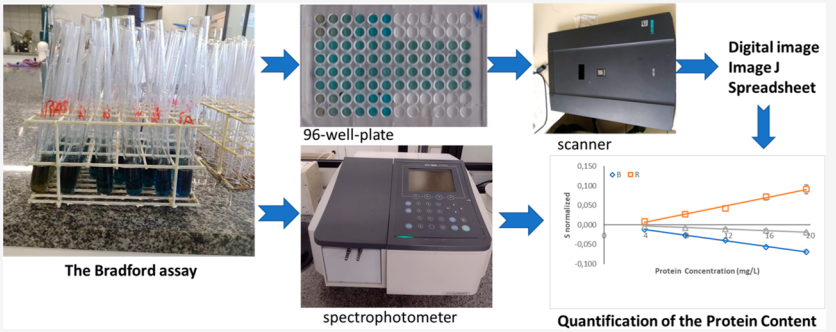

ABSTRACT: Students quantified the protein content in beer, milk, powdered milk, and whey protein using the Bradford assay. The assay was carried out using absorbance measured at 595 nm (standard method) and 96-well-plate images (proposed method). They built analytical curves using bovine serum albumin (BSA) and casein and determined that protein type affected the Bradford assay. They also determined figures of merit such as the limit of detection (LOD), the limit of quantification (LOQ), linear working range, sensitivity, precision (standard deviation, F-test), and accuracy (percent error, *t* test). In addition, an interference test was carried out using nitrogen-rich compounds, organic bases, and amino acids, and it was shown that the Bradford assay was not affected by these compounds. Student learning outcomes were assessed by a final test using Microsoft Forms. As a learning model, students can determine the protein content in various food samples using just sample dilution, without the need for intricate sample preparation procedures.

KEYWORDS: *Second-Year Undergraduate, Analytical Chemistry, Computer-Based Learning, Instrumental Methods, UV−vis Spectroscopy, Hands-On Learning/Manipulatives, Food Science, Calibration*

INTRODUCTION

Reliable quantitative methods for determining the total protein content of foods are essential to ensure their quality, safety, and trade. The lack of such methods directly led to the adulteration of foods with nitrogen-rich compounds.[1] In this context, colorimetric protein assays are widely used to determine the protein content of foods. One of the most used protein colorimetric assays is the Bradford assay.[2]

The Bradford assay is based on the chromic shift that occurs when Coomassie Brilliant Blue G-250 dye binds to proteins. In the acidic environment of the reagent, the protein binds to the Coomassie dye. This results in a spectral shift from the reddish/brown form of the dye (λ_{max} = 465 nm) to the blue form of the dye (λ_{max} = 610 nm). The difference between the two forms of the dye is greatest at 595 nm, so that is the optimal wavelength to measure the blue color from the Coomassie dye-protein complex.[3,4]

Colorimetry is the science and technology used to quantify and describe physically the human color perception.[5] Normally, it is carried out using photometers, spectrophotometers, and 96-well-plate readers. Nowadays, simple, and inexpensive electronic instruments, such as smartphones, digital cameras, and desktop scanners, have been used for colorimetric measurements.[6] Several laboratory practices describing quantitative colorimetry using electronic instruments were published in this Journal. These publications were reviewed by Kovarik et al.,[7] and, since that, some laboratory experiments have been published such as starch quantification in ripe banana,[8] determination of biodiesel in diesel blends,[9] analysis of salivary amylase using starch from food as a substrate,[10] iron(III) determination in water,[11,12] quantification of colored household products,[13] determination of ciprofloxacin,[14] and acetylsalicylic acid in pharmaceuticals,[15] exploration of chemical Kinetic of the bleaching reaction of RD40,[16] building a Visible Spectrophotometer,[17] exploration of

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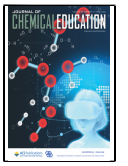

fluorescence using homemade equipment,[18] finding the pK_a values of thymol blue,[19] quantification of Allura Red in maraschino cherry juice,[20] construction of adsorption isotherms,[21] determination of glucose,[22] and quantification of Cu^{2+} in solution.[23]

Digital images acquired using smartphones, digital cameras, webcams, and desktop scanners were also used in academic research, and their uses can be found in recent reviews.[5,24−31]

The Kjeldahl assay is the authoritative reference method for total protein quantification in foods, and it was developed in 1883.[1] The Brazilian official methods for total protein quantification in foods (037/IV and 036/IV) use the Kjeldahl assay.[32]

The Kjeldahl assay is a simple four-step protocol: it is based on the digestion of the raw sample in a strong acid, the liberation of ammonia, the capture of the released ammonia by a weak acid solution, and back-titration of the acid residue.[33]

The Kjeldahl assay is prone to mistakes as it can measure nitrogen present due to other compounds. Several water-soluble nitrogen compounds, such as melamine, ammonium sulfate, and urea, produce the same analytical signal as proteins using the Kjeldahl assay, and these compounds can be used as food adulterants.[34]

In 2007, pet food sold in the United States and Canada was adulterated with melamine.[35] In 2008, the most famous case of food adulterations occurred; it was the milk adulteration with melamine that resulted in the injury and death of infants.[1] The fraudulent seller of milk knew that milk quality control was carried out using the Kjedhal assay. They increased the volume of milk delivered to the market by adding water, as a result, the concentration of milk proteins fell below accepted standards and melamine was added to correct the apparent milk protein content.[34,36]

Colorimetric protein assay responses were not affected by nitrogen-rich compounds, and they have been used together with the Kjedhal assay to detect food adulteration.[34] Thus, interfering tests using nitrogen-rich compounds were also carried out in this laboratory experiment.

Here, students built analytical curves using bovine serum albumin (BSA) and casein standards. Then, they quantified the protein content in beer, milk, powdered milk, and whey proteins using absorbance measured at 595 nm (standard method) and 96-well-plate images (proposed method).

STUDENTS' LEARNING GOALS

UV−vis absorption spectroscopy is one of the most accessible instrumental techniques at the higher educational level. It is highly versatile, applicable in many fields of chemistry, simple, and low-cost.[37−39] The laboratory experiment may be carried out using UV−vis absorption spectroscopy and digital images obtained using a desktop scanner.

Students used scanning spectrophotometry to identify the λ_{max} for the Bradford assay. They constructed analytical curves using casein and BSA. They determined the protein content in beer, milk, powdered milk, and whey protein samples. Finally, they did an interfering test, observing that the Bradford assay has few interferents.

After completing the laboratory class, students should be able to

- understand principles involved in the Bradford assay
- identify compounds which interfere in the Bradford assay
- understand that each sample needs an adequate standard, which represents the principal protein in the sample
- construct analytical curves and determine the protein content in real samples
- calculate the limit of detection (LOD) and limit of quantification (LOQ)

EXPERIMENTAL OVERVIEW

In 2021, this laboratory experiment was performed four times, in a 200 min laboratory class. It was realized in analytical chemistry class, but it may be also implemented in biochemistry classes.

Ten students attended each laboratory class, they were second-year chemical engineering, food engineering, and biomedicine students, they worked alone due to the COVID-19 pandemic,[40] but this laboratory experiment can be done with students working in pairs.

The dye reagent was prepared exactly as reported in literature,[2,41] and it must be filtered before use. The dye reagent and solutions were prepared before the class by a technician or the professor.

This work offers an addition to already-existing laboratory experiments dealing with quantitative analysis using digital images,[7] beer and brewing,[42−44] and protein quantification.[45−47]

In addition to our previous works,[48−54] students could quantify the protein content in a series of real-world samples, choose the right standard for each sample, determine LOD and LOQ using different methods, and do an interfering test.

MATERIALS AND METHODS

Equipment

Deionized water was obtained using a Permutation deionization system from E. J. Kringer & Cia LTDA (Curitiba, Paraná, Brazil). All solutions were prepared using deionized water. Absorbances were measured using a Shimadzu (model 1800) UV−Vis spectrophotometer. Digital images were obtained using a Canon desktop scanner (model LIDE 120).

Reagents

NaOH, H_3PO_4, casein, and tris(hydroxymethyl)aminomethane (tris base) were purchased from Dinâmica (Diadema, São Paulo). Glucose, glycine, ammonium sulfate, and urea were purchased from Vetec (Duque de Caxias, Rio de Janeiro). Coomassie Brilliant Blue G and BSA were purchased from Sigma-Aldrich.

Bradford Assay

The Bradford assay was carried out exactly as reported in the literature.[2,41] The dye reagent is a mixture of 0.01% Coomassie Brilliant Blue G-250 (w/v), 4.7% (w/v) ethanol, and 8.5% (w/v) phosphoric acid.[2,41]

Acquiring Images

The 96-well-plate images were acquired using a Canon LIDE 120 scanner; images were recorded in PNG (Portable Graphics Format) format with 600 dpi (dots per inch).

Extraction of RGB-Values from 96 Microwell Plate Digital Images Using ReadPlate

After absorbance measurements, the standard and samples were placed in a 96-well-plate and a digital image was obtained using a desktop scanner. The ImageJ plugin ReadPlate extracts R-, G-, and B-values from all wells of the 96-well-plate image at the same time. Then, all data collected were imported to a Microsoft Excel spreadsheet as shown in our previous works.[48−52]

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R-, G-, B-values were transformed into analytical signals using eq 1 and eq 2.

$$S = -\log_{10}(I/I_0) \quad (1)$$

$$S_{nor} = -\log_{10}(\overline{I}/\overline{I_0}) \quad (2)$$

In eq 1, I corresponds to the R-, G-, and B-value of each sample or standard solution and I_0 corresponds to the R-, G-, and B-value for the dye reagent

In eq 2, $\overline{I}$ corresponds to the R-, G-, and B-normalized value of each sample or standard solution and I_0 corresponds to the R-, G-, and B-normalized value for the dye reagent.

Spreadsheets (see the Supporting Information) organize the data taken from the ReadPlate and convert it into an analytical signal (eq 2). It also plots the analytical curves and calculates sample concentration.[48−52]

Analytical Curves

Analytical curves were prepared by mixing 20, 40, 60 80, and 100 μL of a 1 g/L protein (casein or BSA) standard solution with 5 mL of the dye reagent providing a 4 to 20 mg/L protein concentration range. The blank was 100 μL deionized water plus 5 mL of the dye reagent.[2,41] Absorbance measured at 595 nm ($A_{595\ nm}$), S (eq 1), and S_{nor} (eq 2) were plotted against protein concentration (mg/L). Analytical curves followed a straight line (eq 3) in which "a" is the slope and "b" is the y-intercept.[55,56]

$$y = ax + b \quad (3)$$

Limit of Detection (LOD) and Limits of Quantification (LOQ)

LOD is the lowest concentration of an analyte in a sample which can be detected but not quantified as an exact value. LOQ is the lowest concentration of analyte that can be quantified as an exact value. Several approaches for determining LOD and LOQ are possible.[57,58] Here, LOD and LOQ are defined in eq 4 and eq 5, respectively, where "s" was the standard deviation of the lowest concentration point on the analytical curves or the blank standard deviation ($n = 6$) and "a" is the slope (eq 3).[59]

$$\mathrm{LOD} = \frac{3.3s}{a} \quad (4)$$

$$\mathrm{LOQ} = \frac{10s}{a} \quad (5)$$

Hypothesis Tests

Variances were compared using the F-test, and average values were compared using the t test. Hypothesis tests were carried out using spreadsheets as described in our previous papers.[49,60]

Wavelength vs Absorbance Plots

The dye reagent and a standard casein solution had their absorbances recorded at different wavelengths (400 to 800 nm range) against deionized water. The casein standard solution is 5 mL of the dye reagent plus 100 μL of 1 g/L casein.

Percent Error and Relative Standard Deviation

Percent Error (% E) was calculated using eq 6, where C_{exp} is the experimental concentration found and C_T is the theoretical concentration.[61] Percent error was carried out using casein in three different concentrations.

$$\%E = \frac{(C_{exp} - C_T)}{C_T}100 \quad (6)$$

The relative standard deviation (RSD) was determined in eq 7, where "m" was the average value and "s" was the standard deviation. Acceptable RSD values were calculated using the Horwitz equation (eq 8),[62] where "C" is the concentration.

$$RSD = \frac{m}{s} \times 100 \quad (7)$$

$$2^{(1-0.5\log C)} \quad (8)$$

Interferents Test

The interfering compounds tested were urea, $(NH_4)_2SO_4$, tris (base), glycine, glucose, and EDTA. Interfering compounds concentrations were 5000 mg/L, with the exception of EDTA, which was 3000 mg/L. The casein standard solution was 1000 mg/L. Interfering compounds solutions were prepared in deionized water, with the exception of EDTA that was prepared in 0.1 mol/L NaOH.

The effect of interfering compounds in the blank was carried out by mixing 50 μL of interfering compounds, 50 μL of deionized water, and 5 mL of the dye reagent. The effect of interfering compounds in the response of proteins to the dye reagent was carried out by mixing 50 μL of interfering compounds, 50 μL of 1000 mg/L casein solution, and 5 mL of the dye reagent. The control for the dye reagent was prepared by mixing 100 μL of deionized and 5 mL of the dye reagent. The control for casein was prepared by mixing 50 μL deionized water, 50 μL of 1000 mg/L casein solution, and 5 mL of the dye reagent.

Samples

All samples were purchased in the Blumenau (Santa Catarina, Brazil) retail market.

Beer Sample Analyses

Four beer samples were analyzed (samples A−D). Beer was degassed by gently stirring with a magnetic stirrer at low speed (20 mim). Then, 100 μL of degassed beer was mixed with 5 mL of the dye reagent. The protein content in beer was determined using BSA as a standard.

Whey Protein Sample Analyses

Two whey protein samples were analyzed (samples D and F). The protein content claimed in labels for samples D and F were 81.48% and 80%, respectively. 250 mg of whey protein samples were diluted in 250 mL of 0.1 mol/L NaOH. Then, 100 μL of the diluted sample was mixed with 5 mL of the dye reagent. The protein content in whey protein samples was determined using the casein as a standard.

Milk Samples Analyses

One UHT milk sample was analyzed (sample G). One portion of sample G was extracted in hexane (sample H) to remove its fat. The portioning in hexane was realized by mixing 50 mL of milk with 50 mL of hexane in a separatory funnel. Then, the mixture was shaken, and the lower phase was collected after 15 min.

Samples G and H were diluted (100 μL of sample diluted to 10 mL of NaOH 0.1 mol/L). After sample dilution, 100 μL of sample was mixed with 5 mL of the dye reagent. The protein content in milk samples was determined using casein as the standard.

Powdered Milk Sample

The protein content claimed in the label of powdered milk (sample I) was 24.61%. 500 mg of the sample I was diluted in

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250 mL of 0.1 mol/L NaOH. Then, 100 μL of the diluted sample was mixed with 5 mL of the dye reagent. The protein content in powdered milk samples was determined using casein as the standard.

HAZARDS

Lab coats, gloves, and safety glasses must be worn throughout this laboratory session. The dye reagents can damage skin and eyes and will stain skin and clothes.[4,36] This reagent must be handled with care. The dye reagent is acidic; it may be neutralized appropriately before disposal down the drain with excess water.[4,36]

RESULTS AND DISCUSSION

Bradford Assay

The Bradford assay is based on the chromic shift that occurs when Coomassie Brilliant Blue G-250 dye binds to proteins.[2] Students did a λ versus absorbance plot for the blank and a dye-casein solution (5 mL of the dye reagent plus 100 μL of 1 g/L casein solution) and observed that the λ_{max} for the blank is 465 nm, and it changes to λ_{max} = 595 nm due to its interactions with casein (Figure 1). It also identified that absorption must be measured at 595 nm, due to the maximum absorption of dye-casein and the lowest interference of the blank.

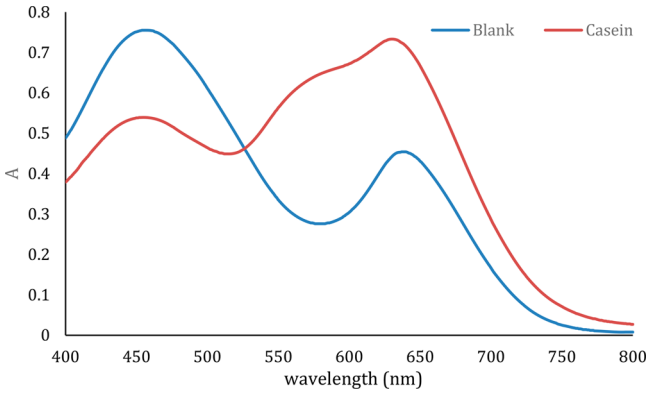

Figure 1. Absorbance vs wavelength for the blank and a casein standard solution. The blank was 100 μL deionized water mixed with 5 mL of the dye reagent.

Plotting Analytical Curves using R-, G-, B-Values Extracted from Digital Images

In the proposed method, analytical curves were built plotting R-, G-, and B-values against casein concentration (Figure 2). The *R*-values showed evident changes corresponding to the casein concentration, whereas the G-values and B-values showed negligible variations in their intensities with casein concentration.

R- and B-normalized values showed evident changes corresponding to the casein concentration (Figure 3). The G-normalized values showed negligible variations in their intensities with casein concentration. R-normalized values decrease while casein concentration increases, and B-normalized values increase while casein concentration increases.

Analytical curves built using unnormalized RGB-values (eq 1) had larger standard deviations than analytical curves built using normalized RGB-values (eq 2). Analytical curves built using B-normalized values (Figure 3) had lower standard deviations than the analytical curve obtained using R-normalized values. Thus,

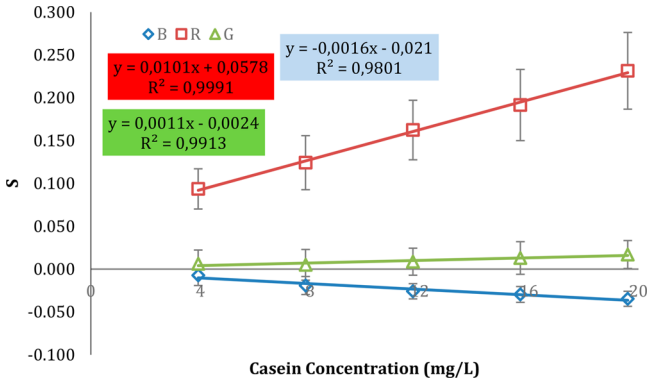

Figure 2. Analytical curves obtained plotting R-, G-, and B-values against casein concentration.

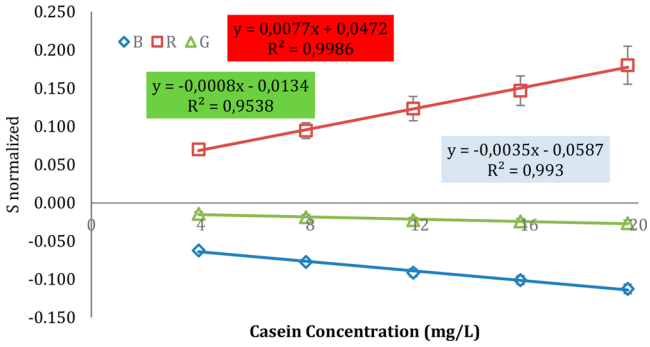

Figure 3. Analytical curves obtained plotting R-, G-, and B-normalized values against casein concentration.

in the proposed method, quantitative analysis was carried out using B-normalized values.

Analytical Curves Using Casein and BSA as Standards

Analytical curves were built using casein and BSA standards. There is some degree of variation in the efficiency of dye binding to various proteins and students observed that the Bradford assay presents different responses for each protein, and the Bradford assay is more sensitive for BSA than for casein. Figure 4 shows BSA and casein analytical curves using the standard method, and Figure 5 shows BSA and casein analytical curves using the proposed method.

Albumins are the most abundant form of protein in beer;[63] casein is the most abundant form of protein in milk, powdered

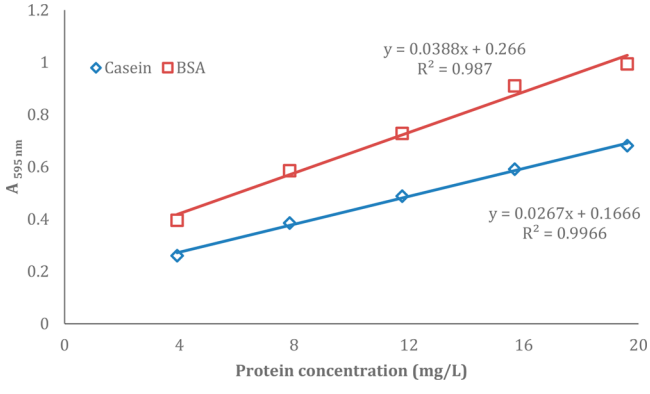

Figure 4. Analytical curves built using the standard method.

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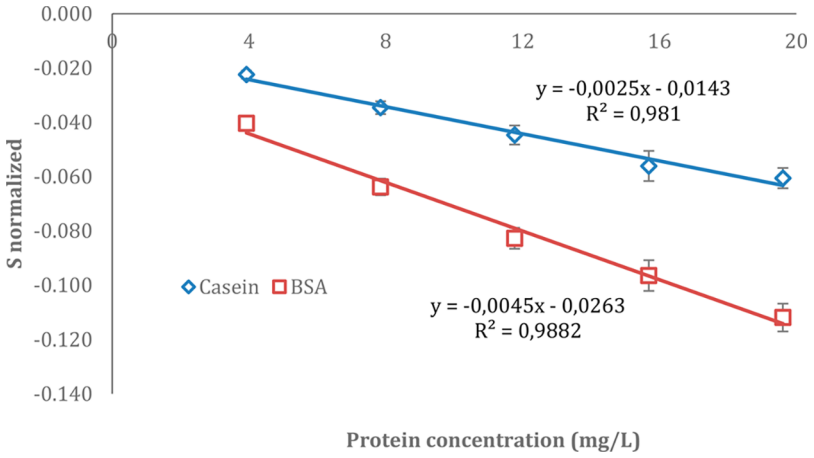

Figure 5. Analytical curves built using the proposed method.

milk, and whey protein.[64] Thus, the right standard must be used in each specific sample.

Analytical Performance

The proposed method showed a linear regression at 4 to 20 mg/L casein concentration range. LOD and LOQ were calculated using the standard deviation of the blank (n = 6) and the standard deviation of the lowest concentration point on the analytical curve (Table 1). Both approaches provided close results. The analytical curve obtained using R-normalized values had a higher slope than the analytical curve obtained using B-normalized values, but both curves provided equivalent LOD and LOQ.

Table 1. LOD and LOQ for the Proposed Method

	B-normalized		R-normalized	
	LOD[a]	LOD[b]	LOQ[b]	LOQ[b]
blank[c]	1.14	3.46	0.89	2.71
lowest[d]	1.33	4.04	1.52	4.62

[a]Calculated using eq 4. [b]Calculated using eq 5. [c]Calculated using "s" as the standard deviation of the blank. [d]Calculated using "s" as the standard deviation of the lowest concentration point on the analytical curve.

Accuracy and Precision Evaluation

Students determined the RSD and %E of the casein standard solutions (Table 2). RSD values were lower than 16%. In accordance with the Horwitz equation, for mg/L concentrations, 16% RSD is acceptable, for example, using the Horwitz equation (eq 8), for a 1 mg/L concentration (10^{-6}), 0.5 $\log_{10}(10^{-6}) = -3$, $2^{(1+3)} = 16\%$.

Table 2. Percent Error (%E) Obtained for Casein Standard Solutions Using the Proposed Method

concentration			
theoretical C_T	experimental[a] C_{exp} (N = 6)	RSD (N = 6)	%E[b] (N = 6)
5.9	7.8	13.0	32.1
9.8	10.5	2.4	7.5
17.6	20.2	10.0	14.7

[a]Concentration determined using the proposed method. [b]Calculated using eq 6.

Determination of Protein Content in Beer

Beer is the oldest and most consumed alcoholic beverage in the world, which is rich in carbohydrates, amino acids, minerals, vitamins, and phenolic compounds.[65,66]

Four beer samples, popular in Brazil, were analyzed using the standard and the proposed method (Table 3). In three samples the protein content was lower than the LOD, and in one sample, it was lower than the LOQ. The Bradford assay does not detect proteins or peptides smaller than 5 kDa.[67] Therefore, it shows that the molecular weight of proteins in these beers was smaller than 5 kDa.

Table 3. Protein Content in Beer

beer	type	label (g/L)[b]	found
A	larger	–[a]	<LOD
B	larger	3.1	<LOD
C	stout	3.4	<LOD
D	stout	5.7	<LOQ

[a]Information not provided by supplier. [b]Protein content claimed in the label.

Determination of Protein Content in Whey Protein

In two separate classes, students measured the protein content in two whey protein samples (E and F) using the standard and the proposed method (Table 4). Protein percentages found in samples E and F for those classes were close to each other.

In class 1, comparing variances using the F-test, the standard and the proposed method had equivalent variances for sample E and nonequivalent variances for sample F. In class 2, both methods provided equivalent variances.

In class 1, comparing means using the t test, the standard and proposed method had nonequivalent means for sample E and equivalent means for sample F. In class 2, comparing means using the t test, the standard and proposed method had equivalent means for sample E and nonequivalent means for sample F.

Determination of Protein Content in Milk

Two milk samples (G and H) were analyzed using the standard method and the proposed method, sample H was the same as sample G, but its fat content was removed by portioning in hexane (Table 5). Comparing the standard method with the proposed method, standard deviations were nonequivalent for both samples. Sample G had equivalent means with both

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Table 4. Quantification of the Protein Percentage in Whey Protein Samples Using the Proposed Method and the Standard Method

class	1				2			
sample	E		F		E		F	
method	UV−vis[a]	DI[b]	UV−vis[a]	DI[b]	UV−vis[a]	DI[b]	UV−vis[a]	DI[b]
average (N = 6)	89.1	96.9	92.5	96.9	89.6	92.6	89.2	91.6
RSD (N = 6)	0.96	7.94	1.88	1.65	2.34	4.41	1.53	3.20
%E[c]	9.4	18.9	15.7	21.1	9.9	13.6	11.5	14.6
F calculated[d]	3.47		19.52		3.79		4.59	
t calculated[e,f]	4.63		1.49		1.59		1.86	

[a]Standard method. [b]Proposed method. [c]Calculated using eq 6. [d]The F critical value 5.05. [e]The t critical value for the one-tailed test 1.81. [f]The t critical value for the two-tailed test 2.23.

Table 5. Quantification of the Protein Content in Milk Samples Using the Standard Method and the Proposed Method

sample	G		H	
method	UV−vis[a]	DI[b]	UV−vis[a]	DI[b]
average (N = 6)	37.41	38.42	37.44	36.01
RSD (N = 6)	0.77	2.08	1.48	3.61
F calculated	52.77[c]		5.50[c]	
T calculated	1.18[d]		2.49[e,f]	

[a]Standard method. [b]Proposed method. [c]The F critical value = 5.05. [d]The t critical value for the one-tailed test = 1.81. [e]The t critical value for the one-tailed test = 1.89. [f]The t critical value for the two-tailed test = 2.36.

methods. Sample H had higher average values with the standard method than with the proposed method ($t_{calculate} > t_{critical\ two\text{-}tailed}$).

In comparing sample G with sample H using the standard method, equivalent standard deviations ($F_{calculated} = 3.73 < F_{critical} = 5.05$) and average values ($t_{calculated} = 0.13 < t_{critical\ one\text{-}tailed} = 1.81$) were observed. It shows that fats do not affect the Bradford assay, and milk can be analyzed using only sample dilution as reported by Kamizake et al.[68]

Comparing sample G and H using the proposed method, equivalent standard deviations ($F_{calculated} = 4.32 < F_{critical} = 5.05$) and nonequivalent average values ($t_{calculated} = 2.41 > t_{critical\ one\text{-}tailed} = 1.81$) were observed.

Results obtained for both samples using the standard method and the proposed method were numerically close (Table 5), but hypothesis tests showed nonequivalent results due to the small standard deviations obtained using both methods.

Determination of Protein Content in Powdered Milk

The protein percentage in powdered milk was determined using the standard method and the proposed method (Table 6). The proposed and the standard method provided close results to those claimed in the label. The hypothesis tests showed that methods had nonequivalence variances and nonequivalent mean values, and it was due to the small standard deviation observed in both methods.

Table 6. Quantification of the Protein Content in Powdered Milk Using the Standard Method and the Proposed Method

method	standard method	proposed method
average (N = 6)	25.7	22.1
RSD (N = 6)	2.5	11.2
%E[a]	4.39	−10.02
F calculated[b]	15.4	
t calculated[c]	3.4	

[a]Calculated using eq 6. [b]The F critical value = 5.05. [c]The t critical value for the one-tailed test = 1.94.

Interfering Compounds Effect on Colorimetric Assays

The Bradford assay is relatively free from interference by nitrogen-rich compounds, reducing compounds, and organic bases. However, few compounds may significantly alter the absorbance of the reagent blank or modify the response of proteins to the dye. The compounds that are most likely to interfere in this assay are detergents and ampholytes.[41,69]

Students measured the absorbance of interfering compounds in the Bradford assay (Table 7), observing that the Bradford assay was unaffected by nitrogen rich-compounds, amino acids, sugars, and organic bases.

Table 7. Effects of Common Reagents on the Bradford Assay

interferent[a]	blank	casein
control	0.452[b]	0.834[c]
$(NH_4)_2SO_4$	0.441[d]	0.749[e]
Glycine	0.437[d]	0.797[e]
urea	0.433[d]	0.820[e]
Tris base	0.433[d]	0.820[e]
EDTA	0.436[d]	0.822[e]
glucose	0.428[d]	0.838[e]

[a]Interfering compounds were 5000 mg/L, with the exception of EDTA, which was 3000 mg/L. [b]100 μL deionized water mixed with 5 mL of the dye reagent. [c]50 μL of deionized water mixed with 50 μL of 1000 mg/L casein solution and 5 mL of the dye reagent. [d]50 μL of deionized water mixed with 50 μL of interfering compounds and 5 mL of the dye reagent. [e]50 μL of interfering compound mixed with 50 μL of 1000 mg/L casein solution and 5 mL of the dye reagent.

Description of Assessment of Learning Outcomes

After the laboratory class, students were evaluated using a questionnaire (Table 8) using Microsoft Forms.[70] Questions involved data obtained by students during laboratory classes (Table 9 and Figure 6). Each question (Q1−Q8) is worth 1.25 points. Q1 evaluated abilities related to obtaining the analytical curve using a spreadsheet. Q2 and Q3 evaluate abilities related to understanding the analytical curve and how the choice of the right standard may affect the results. Q4 evaluated abilities related to calculating LOD and LOQ. Q5 assessed whether the students understood the principles of the Bradford assay and how food was adulterated. Q6, Q7, and Q8 evaluated whether they could choose the right standard for each sample, use the

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Table 8. Questionnaire for Student Evaluation

question	expected response
Q1: Students measured absorbances of casein and BSA standard solutions using the Bradford assay (Table8). Plot standard curves and obtain linear equations relating protein concentrations with absorbance.	Figure 6.
Q2: BSA is the most abundant protein in beer. Someone uses casein as a standard for quantification of the total protein content in beer. How does it affect the result?	The beer protein content was overestimated. The BSA analytical curve (Figure 6) had a higher angular coefficient than the casein standard curve.
Q3: Casein is the most abundant protein in milk. In the determination of the protein content in milk, someone uses BSA as a standard. How does it affect the result?	The protein content was underestimated (Figure 6).
Q4: Using the proposed method, the standard deviation of six casein standards at the lowest calibration point of the analytical curve was 0.00174806. Calculate LOD and LOQ. The analytical curve was $y = 0.0061x + 0.0194$.	0.94 mg/L and 2.86 mg/L, respectively.
Q5: Is it possible to use Kjeldahl and the Bradford assays together to identify food adulteration with nitrogen-rich compounds?	Yes, acceptable protein content obtained using the Kjeldahl assay and unacceptable protein content obtained using the Bradford assay represents sample adulteration using nitrogen-rich compounds.
Q6: Use your answer in Q1. 0.1 mL of beer was mixed with 5 mL of the dye reagent, and the solution presented an absorbance of 0.356. What was the percentage of protein in the beer?	Students must use the BSA analytical curve and calculate sample concentration using it. $C = (0.356-0.2522)/0.0518 = 2$ mg/L. Then, they use the dilution factor to find the protein content in beer $[(2\ mg/L) \times (5.1/0.1)] = 102.2$ mg/L
Q7: Use your answer in Q1. 0.1 mL of milk was diluted to 10 mL in a volumetric flask with NaOH 0.1 mol/L, 0.1 mL of the diluted solution was mixed with 5 mL of the dye reagent, the solution presented an absorbance of 0.425. What was the protein content in milk?	Students must use the casein analytical curve and calculate sample concentration using it. $C = (0.425-0.182)/0.0377 = 6.45$ mg/L. Then, they use the dilution factor to find the protein content in milk $[(6.45\ mg/L) \times (5.1/0.1) \times (10/0.1)] = 32.9$ g/L.
Q8: Use your answer in Q1. 124 mg of whey protein was dissolved in 100 mL of NaOH 0.1 mol/L, 1 mL of the solution was mixed with 1 mL of deionized water. Then, 0.1 mL of the diluted solution was mixed with 5 mL of the dye reagent. The solution presented an absorbance of 0.401. What was the percentage of protein in the whey protein?	Students must use the casein analytical curve and calculate sample concentration using it. $C = (0.401-0.182)/0.0377 = 5.81$ mg/L. Then, they use the dilution factor to find the concentration of the undiluted sample $[(5.81\ mg/L) \times (5.1/0.1)*2] = 592.5$ mg/L. The protein % in the whey protein was $[(592.5\ mg/L/1240\ mg/L) \times 100] = 47.8\%$.
Q9: Did you enjoy this laboratory practice?	Students rate the experiment from one to five stars, where five stars is the highest grade.

Table 9. Absorbances Measured for Casein and BSA Standard Solutions Using Bradford Assay

concentration (mg/L)	casein	BSA
1.82	0.245	0.331
2.73	0.288	0.402
3.64	0.32	0.446
4.55	0.364	0.498
5.45	0.380	0.533
6.36	0.420	0.581
7.27	0.457	0.622

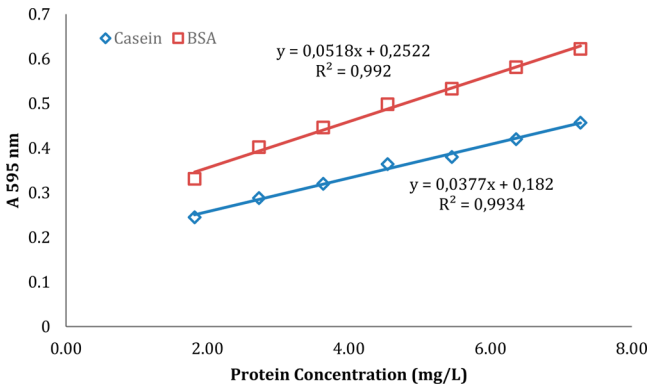

Figure 6. Standard curves obtained for BSA and casein.

analytical curve to calculate the sample concentration, and calculate the sample concentration using dilution factors.

CONCLUSION

This manuscript described a simple method for the determination of proteins in real-world samples (beer, milk, powdered milk, and whey protein). The method is easy, quick, and cheap and can be easily carried out by students in laboratories. In addition, the Bradford assay may determine the protein content in real-world samples using only sample dilution, which makes the experiment simple and fast.

The standard method and the proposed method are applicable for educational purposes. Figures of merit (such as LOD, LOQ, linear range, and percent error) were exploited during the experiment. To get identical results between both methods, normalization of B-values was mandatory. The effect of the protein type and some compounds in the Bradford assay were also investigated.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.1c00756.

Spreadsheets used to organize data taken from ReadPlate, plot analytical curves, and to calculate sample concentrations (ZIP)

Student laboratory handout for the experiment and hazards (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Moore, J. C.; DeVries, J. W.; Lipp, M.; Griffiths, J. C.; Abernethy, D. R. Total Protein Methods and Their Potential Utility to Reduce the Risk of Food Protein Adulteration. *Comprehensive Reviews in Food Science and Food Safety* **2010**, *9* (4), 330−357.

(2) Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72* (1−2), 248−254.

(3) Chemistry of Protein Assays | Thermo Fisher Scientific - BR https://www.thermofisher.com/br/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/chemistry-protein-assays.html (accessed 2019 −10 −29).

(4) Gee, C. T.; Kehoe, E.; Pomerantz, W. C. K.; Penn, R. L. Quantifying Protein Concentrations Using Smartphone Colorimetry: A New Method for an Established Test. *J. Chem. Educ.* **2017**, *94* (7), 941−945.

(5) Fan, Y.; Li, J.; Guo, Y.; Xie, L.; Zhang, G. Digital Image Colorimetry on Smartphone for Chemical Analysis: A Review. *Measurement* **2021**, *171*, 108829.

(6) Oliveira, K. A.; Cardoso, T. M. G.; Oliveira, H. F.; Fioravanti, M. C. S.; Coltro, W. K. T. Rapid and Inexpensive Colorimetric Detection of Total Serum Protein Using Microzone Plates Wax-Printed on Polyester Films. *Article J. Braz. Chem. Soc.* **2021**, *32* (2), 311−319.

(7) Kovarik, M. L.; Clapis, J. R.; Romano-Pringle, K. A. Review of Student-Built Spectroscopy Instrumentation Projects. *J. Chem. Educ.* 2020, DOI: 10.1021/acs.jchemed.0c00404.

(8) Doughan, S.; Shahmuradyan, A. At-Home Real-Life Sample Preparation and Colorimetric-Based Analysis: A Practical Experience Outside the Laboratory. *J. Chem. Educ.* 2021, DOI: 10.1021/acs.jchemed.0c01299.

(9) Cavalcante Dos Santos, R.; Cabral Cavalcanti, J. N.; Werneck Do Carmo, E. C.; de Souza, F. C.; Soares, W. G.; Gimenes De Souza, C.; França De Andrade, D.; D'Avila, L. A. Approaching Diesel Fuel Quality in Chemistry Lab Classes: Undergraduate Student's Achievements on Determination of Biodiesel Content in Diesel Oil Applying Solvatochromic Effect. *J. Chem. Educ.* **2020**, *97* (12), 4462−4468.

(10) Maqsood, S.; Kilpatrick, S. M.; Truong, C. D.; Lefler, S. R. Analysis of Amylase in the Kitchen: An At-Home Biochemistry Experiment for the COVID-19 Pandemic. *J. Chem. Educ.* **2021**, *98* (3), 858−865.

(11) Armenta, S.; Esteve-Turrillas, F. A.; Herrero-Martínez, J. M. Development and Evaluation of Paper-Based Devices for Iron(III) Determination in an Advanced Undergraduate Laboratory. *J. Chem. Educ.* **2020**, *97* (10), 3852−3857.

(12) Meelapsom, R.; Rattanakaroonjit, W.; Prakobkij, A.; Malahom, N.; Supasorn, S.; Ruangchai, S.; Jarujamrus, P. Smartphone-Assisted Colorimetric Determination of Iron Ions in Water by Using Anthocyanin from Ruellia Tuberosa L. as a Green Indicator and Application for Hands-on Experiment Kit. *J. Chem. Educ.* **2022**, DOI: 10.1021/acs.jchemed.1c01120.

(13) Destino, J. F.; Cunningham, K. At-Home Colorimetric and Absorbance-Based Analyses: An Opportunity for Inquiry-Based, Laboratory-Style Learning. *J. Chem. Educ.* **2020**, *97* (9), 2960−2966.

(14) Bliese, S. L.; O'Donnell, D.; Weaver, A. A.; Lieberman, M. Paper Millifluidics Lab: Using a Library of Color Tests to Find Adulterated Antibiotics. *J. Chem. Educ.* **2020**, *97* (3), 786−792.

(15) Oskolok, K. v.; Monogarova, O. v.; Garmay, A. v. Molecular Optical Analyzers Based on Smartphones for High School and Universities. *J. Chem. Educ.* **2021**, *98* (6), 1937−1945.

(16) Madriz, L.; Cabrerizo, F. M.; Vargas, R. Exploring Chemical Kinetics at Home in Times of Pandemic: Following the Bleaching of Food Dye Allura Red Using a Smartphone. *J. Chem. Educ.* **2021**, *98* (6), 2117−2121.

(17) Pap, L. G. An Inexpensive 3D-Printable Do-It-Yourself Visible Spectrophotometer for Online, Hybrid, and Classroom-Based Learning. *J. Chem. Educ.* **2021**, *98* (8), 2584−2591.

(18) Hamer, M.; Beraldi, A. M.; Gomez, S. G. J.; Ortega, F.; Onna, D.; Hamer, M. Glowing-in-the-Screen: Teaching Fluorescence with a Homemade Accessible Setup. *J. Chem. Educ.* **2021**, *98* (8), 2625−2631.

(19) Yimkosol, W.; Dangkulwanich, M. Finding the PKa Values of a Double-Range Indicator Thymol Blue in a Remote Learning Activity. *J. Chem. Educ.* **2021**, *98* (12), 3930−3934.

(20) Ambruso, K.; Riley, K. R. At-Home Laboratory Experiments for the Analytical Chemistry Curriculum. *J. Chem. Educ.* **2022**, *99* (2), 1125−1131.

(21) Al-Soufi, W.; Carrazana-Garcia, J.; Novo, M. When the Kitchen Turns into a Physical Chemistry Lab. *J. Chem. Educ.* **2020**, *97* (9), 3090−3096.

(22) Schmuck, V. D. E.; Romine, I. C.; Sisley, T. A.; Immoos, C. E.; Scott, G. E.; Zigler, D. F.; Martinez, A. W. At-Home Microscale Paper-Based Quantitative Analysis Activity with External Standards. *J. Chem. Educ.* **2022**, *99* (2), 1081−1086.

(23) Zhu, P.; Ling, Y. Amount of Copper(II) Ions in a Solution Cannot Be Determined Using the Hue Values. *J. Chem. Educ.* 2022, DOI: 10.1021/acs.jchemed.1c01074.

(24) Rezazadeh, M.; Seidi, S.; Lid, M.; Pedersen-Bjergaard, S.; Yamini, Y. The Modern Role of Smartphones in Analytical Chemistry. *TrAC Trends in Analytical Chemistry* **2019**, *118*, 548−555.

(25) Roda, A.; Michelini, E.; Zangheri, M.; di Fusco, M.; Calabria, D.; Simoni, P. Smartphone-Based Biosensors: A Critical Review and Perspectives. *TrAC Trends in Analytical Chemistry* **2016**, *79*, 317−325.

(26) Dutta, S. Point of Care Sensing and Biosensing Using Ambient Light Sensor of Smartphone: Critical Review. *TrAC Trends in Analytical Chemistry* **2019**, *110*, 393−400.

(27) Pohanka, M. Colorimetric Hand-Held Sensors and Biosensors with a Small Digital Camera as Signal Recorder, a Review. *Reviews in Analytical Chemistry* **2020**, *39* (1), 20−30.

(28) Capitán-Vallvey, L. F.; López-Ruiz, N.; Martínez-Olmos, A.; Erenas, M. M.; Palma, A. J. Recent Developments in Computer Vision-Based Analytical Chemistry: A Tutorial Review. *Anal. Chim. Acta* **2015**, *899*, 23−56, DOI: 10.1016/j.aca.2015.10.009.

(29) Fernandes, G. M.; Silva, W. R.; Barreto, D. N.; Lamarca, R. S.; Lima Gomes, P. C. F.; Flávio da S Petruci, J.; Batista, A. D. Novel Approaches for Colorimetric Measurements in Analytical Chemistry − A Review. *Anal. Chim. Acta* **2020**, *1135*, 187−203.

(30) Christodouleas, D. C.; Nemiroski, A.; Kumar, A. A.; Whitesides, G. M. Broadly Available Imaging Devices Enable High-Quality Low-Cost Photometry. *Anal. Chem.* **2015**, *87* (18), 9170−9178.

(31) Meenu, M.; Kurade, C.; Neelapu, B. C.; Kalra, S.; Ramaswamy, H. S.; Yu, Y. A Concise Review on Food Quality Assessment Using Digital Image Processing. *Trends in Food Science & Technology* **2021**, *118*, 106−124.

(32) Zenebon, O.; Pascuet, N. S.; Tiglea, P. *Métodos Físico-Químicos Para Análise de Alimentos - Secretaria Da Saúde - Governo Do Estado de São Paulo, IV.*; Zenebon, O., Pascuet, N. S., Tiglea, P., Eds.; Instituto Adolfo Lutz: São Paulo, 2008.
(33) Urban, P. L. Name Concepts in Analytical Science. *J. Chem. Educ.* **2014**, *91* (11), 1753−1756.
(34) Finete, V. de L. M.; Gouvêa, M. M.; Marques, F. F. de C.; Netto, A. D. P. Is It Possible to Screen for Milk or Whey Protein Adulteration with Melamine, Urea and Ammonium Sulphate, Combining Kjeldahl and Classical Spectrophotometric Methods? *Food Chem.* **2013**, *141* (4), 3649−3655.
(35) Kimbrough, D. R.; Jensen, A. C. Using the Melamine Contamination of Foods to Enhance the Chemistry Classroom. *J. Chem. Educ.* **2010**, *87* (5), 496−499.
(36) Astrof, N. S.; Horowitz, G. Protein Colorimetry Experiments That Incorporate Intentional Discrepancies and Historical Narratives. *J. Chem. Educ.* **2018**, *95* (7), 1198−1204.
(37) Jurinovich, S.; Domenici, V. Digital Tool for the Analysis of UV−Vis Spectra of Olive Oils and Educational Activities with High School and Undergraduate Students. *J. Chem. Educ.* 2022, DOI: 10.1021/acs.jchemed.1c01015.
(38) Czegan, D. A. C.; Hoover, D. K. UV−Visible Spectrometers: Versatile Instruments across the Chemistry Curriculum. *J. Chem. Educ.* **2012**, *89* (3), 304−309.
(39) González-Jiménez, M.; Arenas-Valgañón, J.; Céspedes-Camacho, I. F.; García-Prieto, J. C.; Calle, E.; Casado, J. Detection of Nitrite in Water Using Minoxidil as a Reagent. *J. Chem. Educ.* **2013**, *90* (8), 1053−1056.
(40) Soares, R.; de Mello, M. C. S.; da Silva, C. M.; Machado, W.; Arbilla, G. Online Chemistry Education Challenges for Rio de Janeiro Students during the COVID-19 Pandemic. *J. Chem. Educ.* **2020**, *97*, 3396.
(41) *The Protein Protocols Handbook*; Walker, J. M., Ed.; Springer Protocols Handbooks; Humana Press: Totowa, NJ, 2009, DOI: 10.1007/978-1-59745-198-7.
(42) Hunter, R. A.; Dompkowski, E. J. Quantifying Beer Bitterness: An Investigation of the Impact of Sample Preparation. *J. Chem. Educ.* **2018**, *95* (11), 2009−2012.
(43) McDermott, M. L. Lowering Barriers to Undergraduate Research through Collaboration with Local Craft Breweries. *J. Chem. Educ.* **2016**, *93* (9), 1543−1548.
(44) Khalafi, L.; Doolittle, P.; Wright, J. Speciation and Determination of Low Concentration of Iron in Beer Samples by Cloud Point Extraction. *J. Chem. Educ.* **2018**, *95* (3), 463−467.
(45) Ramos, C. v.; Samelo, J.; Martins, P. A. T.; Moreno, M. J. Protein Quantification in Complex Matrices. *J. Chem. Educ.* 2022, DOI: 10.1021/acs.jchemed.2c00109.
(46) Pirinelli, A. L.; Trinidad, J. C.; Pohl, N. L. B. Introducing Students to Protein Analysis Techniques: Separation and Comparative Analysis of Gluten Proteins in Various Wheat Strains. *J. Chem. Educ.* **2016**, *93* (2), 330−334.
(47) Schwarz, G.; Ickert, S.; Wegner, N.; Nehring, A.; Beck, S.; Tiemann, R.; Linscheid, M. W. Protein Quantification by Elemental Mass Spectrometry: An Experiment for Graduate Students. *J. Chem. Educ.* **2014**, *91* (12), 2167−2170.
(48) Filgueiras, M. F.; Jesus, P. C. de; Borges, E. M. Quantification of Nitrite in Food and Water Samples Using the Griess Assay and Digital Images Acquired Using a Desktop Scanner. *J. Chem. Educ.* 2021, DOI: 10.1021/acs.jchemed.0c01392.
(49) Ledesma, C. M.; Krepsky, L. M.; Borges, E. M. Using a Flatbed Scanner and Automated Digital Image Analysis To Determine the Total Phenolic Content in Beer. *J. Chem. Educ.* **2019**, *96*, 2315.
(50) da Silva, R. S.; Borges, E. M. Quantitative Analysis Using a Flatbed Scanner: Aspirin Quantification in Pharmaceutical Tablets. *J. Chem. Educ.* **2019**, *96* (7), 1519−1526.
(51) Curbani, L.; Gelinski, J. M. L. N.; Borges, E. M. Determination of Ethanol in Beers Using a Flatbed Scanner and Automated Digital Image Analysis. *Food Analytical Methods* **2020**, *13* (1), 249−259, DOI: 10.1007/s12161-019-01611-7.
(52) Volmer, D. A.; Curbani, L.; Parker, T. A.; Garcia, J.; Schultz, L. D.; Borges, E. M. Determination of Titratable Acidity in Wine Using Potentiometric, Conductometric, and Photometric Methods. *J. Chem. Educ.* **2017**, *94* (9), 1296−1302.
(53) Filgueiras, M. F.; de Oliveira Lima, B.; Borges, E. M. A High-Throughput, Cheap, and Green Method for Determination of Ethanol in Cachaça and Vodka Using 96-Well-Plate Images. *Talanta* **2022**, *241*, 123229.
(54) Colzani, H.; Rodrigues, Q. E. A. G.; Fogaça, C.; Gelinski, J. M. L. N.; Pereira-Filho, E. R.; Borges, E. M. DETERMINAÇÃO DE FOSFATO EM REFRIGERANTES UTILIZANDO UM SCANNER DE MESA E ANÁLISE AUTOMATIZADA DE DADOS: UM EXEMPLO DIDÁTICO PARA ENSINO DE QUÍMICA. *Química Nova* **2017**, *40* (7), 833−839.
(55) Mathews, K. R.; Landmark, J. D.; Stickle, D. F. Quantitative Assay for Starch by Colorimetry Using a Desktop Scanner. *J. Chem. Educ.* **2004**, *81* (5), 702−704.
(56) Angarita-Rivera, P. A.; Gabbard, D. B.; Main, K. A.; Timmermann, K. M.; Kinkade, K. B.; Wilson, K. J.; Doçi, C. L. Vitamin C as a Model for a Novel and Approachable Experimental Framework for Investigating Spectrophotometry. *J. Chem. Educ.* **2019**, *96*, 2578.
(57) Thompson, M.; Ellison, S. L. R.; Wood, R. Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis (IUPAC Technical Report). *Pure Appl. Chem.* **2002**, *74* (5), 835−855.
(58) Peters, F. T.; Drummer, O. H.; Musshoff, F. Validation of New Methods. *Forensic Science International* **2007**, *165* (2−3), 216−224.
(59) DOQ-CGCRE-008. Coordenação Geral de Acreditação Orientação Sobre Validação De Métodos Analíticos. http://www.inmetro.gov.br/Sidoq/Arquivos/CGCRE/DOQ/DOQ-CGCRE-8_02.pdf (accessed 2021−07−21).
(60) Sidou, L. F.; Borges, E. M. Teaching Principal Component Analysis Using a Free and Open Source Software Program and Exercises Applying PCA to Real-World Examples. *J. Chem. Educ.* **2020**, *97* (6), 1666−1676.
(61) Sedwick, V.; Leal, A.; Turner, D.; Kanu, A. B. Quantitative Determination of Aluminum in Deodorant Brands: A Guided Inquiry Learning Experience in Quantitative Analysis Laboratory. *J. Chem. Educ.* **2018**, *95* (3), 451−455.
(62) Horwitz, W. Protocol for the Design, Conduct and Interpretation of Method-Performance Studies. *Pure Appl. Chem.* **1995**, *67* (2), 331−343.
(63) Silva, F.; Ferreira, I. M. P. L. V. O.; Teixeira, N. Polypeptides and Proteins That Influence Beer Foam Quality and Analytical Methods Used in Their Study. *Quimica Nova. Sociedade Brasileira de Quimica* **2006**, *29*, 1326−1331.
(64) Got Protein? Kit | Educação em Ciências da Vida | Bio-Rad https://www.bio-rad.com/pt-br/product/got-protein-kit?ID=4dad28a9-0b07-49f2-b399-9e5b55643b04 (accessed 2020−08−04).
(65) Kawa-Rygielska, J.; Adamenko, K.; Kucharska, A. Z.; Prorok, P.; Piórecki, N. Physicochemical and Antioxidative Properties of Cornelian Cherry Beer. *Food Chem.* **2019**, *281*, 147−153.
(66) Korolija, J. N.; Plavsic, J. v.; Marinkovic, D.; Mandic, L. M. Beer as a Teaching Aid in the Classroom and Laboratory. *J. Chem. Educ.* **2012**, *89* (5), 605−609.
(67) Evans, D. E.; Sheehan, M. C. Don't Be Fobbed Off: The Substance of Beer Foam—A Review1. *J. Am. Soc. Brew. Chem.* **2018**, *60* (2), 47−57, DOI: 10.1094/ASBCJ-60-0047.
(68) Kamizake, N. K. K.; Gonçalves, M. M.; Zaia, C. T. B. V.; Zaia, D. A. M. Determination of Total Proteins in Cow Milk Powder Samples: A Comparative Study between the Kjeldahl Method and Spectrophotometric Methods. *Journal of Food Composition and Analysis* **2003**, *16* (4), 507−516.
(69) Kruger, N. J. *Bradford Method For Protein Quantitation* **2009**, 17−24.
(70) Quick and Cheap Colorimetric Quantification of Proteins Using 96 microwell plate images. (Visualização) Microsoft Forms https://forms.office.com/Pages/ShareFormPage.aspx?id=KiItDNrscEuWCqzvbO0wUjE2u3GUvStJhVEt_N4T7BBUOUQ2N1cyOEQ4WFZYRVhCTFhRNVlBTUpM

Qy4u&sharetoken=UL9Ewj6ovQ0J32yBUYL5 (accessed 2022−01−15).

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