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Original Article

Determination of total proteins in cow milk powder samples: a comparative study between the Kjeldahl method and spectrophotometric methods

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Abstract

In the present paper, a comparative study between the Kjeldahl method and several spectrophotometric methods was carried out for the determination of total proteins in a range of milk powder samples (skim milk powder, whole milk powder, whey protein powder, buttermilk powder). The most important finding of this paper was that the Bradford method could be used for the determination of total proteins in skim milk powder and whole milk powder samples (without extraction of lipids) instead of the Kjeldahl method. The Bradford method showed the highest sensitivity of the spectrophotometric methods. Using casein and BSA as standard proteins, the Lowry method showed the lowest variation of specific absorbance indicating either casein or BSA could be used as a standard. The UV-220 nm method with previous extraction of lipids showed the best results for the determination of total proteins in all the samples; all the results were not statistically different ($P > 0.05$) from those obtained by the total protein nitrogen (TPN) without extraction of the lipids. The Bradford method (without extraction of lipids) showed the best results for the determination of total proteins in all the samples whose results were not statistically different ($P > 0.05$) from those obtained for total nitrogen by the Kjeldahl method. However, when these results were compared to TPN they were statistically different from each other ($P < 0.05$) for the buttermilk powder and whey protein powder samples. This means that the high sensitivity of the Bradford method was enough to overcome the turbidity of solutions due to the fat material in the samples. The determination of total proteins using the Bradford method for the whole milk powder and whey protein powder samples with and without the extraction of lipids was not statistically different ($P < 0.05$) from each other.
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Keywords: Protein analysis; Kjeldahl method; Bradford method; Spectrophotometric methods

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1. Introduction

Milk is a mixture of several substances (lactose, lipids, proteins, amino acids, urea, creatinine, etc.) and its composition depends on several factors such as genetic breeding programs, feeding schemes and climate conditions, among others. In the last few years, an increase in cheese consumption occurred, so the determination of protein content of milk is an important factor for the price paid for by the industry (Bruhn & Franke, 1979; Depeters & Ferguson, 1992; Baker, Ferguson, & Chalupa, 1995; Fox, 1997; Coulon, Hurtaud, Remond, & Verite, 1998; Ferguson, 2000).

Determination of total proteins using spectrophotometric methods is commonly used in several areas such as clinical analysis, biochemistry, physiology, medical research as well as many other areas. Although there are two main problems with the Kjeldahl method (Helrich, 1990), namely, the long period of time needed to carry out the whole assay and the necessity to carry out two analyses to determine the difference between non-protein nitrogen (NPN) and total protein nitrogen (TPN), it is widely used in food science and technology and is the officially recognized standard reference method.

As pointed out by Zaia, Zaia, and Lichtig (1998) there are many studies of interfering substances in spectrophotometric methods for the determination of protein, however, there are not many comparative studies among spectrophotometric methods or between spectrophotometric methods and others, such as the Kjeldahl method.

In the present paper, a comparative study between the Kjeldahl method and several spectrophotometric methods (UV-280 and 220 nm (Stoscheck, 1990), biuret-340 and 550 nm (Gornall, Bardawill, & David, 1949), Bradford (Bradford, 1976), Lowry (Lowry, Rosebrough, Farr, & Randall, 1951), *p*-chloranil (Zaia, Verri, & Zaia, 1999)) was carried out to determine total proteins in cow milk powder samples (skim milk powder, whole milk powder, whey protein powder, buttermilk powder).

These methods were chosen because they are easy to carry out and they are based on different reactions. In the UV-220 nm (Stoscheck, 1990) and biuret (Gornall et al., 1949) methods, the absorbances are due to electronic transitions of peptide bond and electronic transitions of the complex copper/peptide bond, respectively, so in both methods peptides are measured. The absorbance in the UV-280 nm method (Stoscheck, 1990) is due to electronic transitions of a few amino acids. In the *p*-chloranil method (Birks & Slifikin, 1963; Zaia et al., 1999) the absorbance is due to a charge transfer complex amino acid/quinone. Hence, in both methods (UV-280 nm and *p*-chloranil), amino acids are measured. The absorbance of the samples in the Lowry method (Lowry et al., 1951; Chou & Goldstein, 1960; Legler, Müller-Platz, Meniges-Hetikamp, Pflieger, & Jülich, 1985) depends on the concentration of some amino acids and also on the amount of tetra peptides bonds because both are responsible for the reduction of the Folin–Ciocalteau reagent. Bradford method is based on the interaction between the dye BG-250 and proteins, small peptides or amino acids do not show any reaction with dye BG-250 (Bradford, 1976; Snyder & Desborough, 1978; Wei, Li, & Tong, 1997). Thus, it should be pointed out that among the spectrophotometric methods of this study, Bradford method is the only one that measured proteins. The *p*-chloranil was tested, because it was recently proposed for the determination of protein and as far as we know there is only one comparative study with it (Zaia, Verri, & Zaia, 2000). The Lowry method was chosen because among all the spectrophotometric methods, it is the most widely used and studied until today.

2. Materials and methods

Ultraviolet and visible spectrophotometries were carried out on spectrophotometer Shimadzu UV-1203.

2.1. Milk samples and solutions

2.1.1. Milk samples

The samples of skim milk powder, whole milk powder, whey protein powder and buttermilk powder were a gift from the Confepar Company of Londrina-PR, Brazil.

2.1.2. Milk solutions

All the milk samples (skim milk powder, whole milk powder, whey protein powder and buttermilk powder) were prepared in the following concentrations: 1.25 g/l for the UV-280 nm, 0.125 g/l for the UV-220, 10.00 g/l for the biuret, 0.2000 g/l for the Bradford, 1.000 g/l for the Lowry, and 10.0 g/l for the *p*-chloranil methods.

2.1.3. Extractor solution of chloroform and methanol

A solution of chloroform–methanol (2:1 v/v) was prepared and used for extraction of lipids in milk samples.

2.2. Standard solutions

2.2.1. Bovine serum albumin-fraction V (BSA)

BSA (Sigma) solutions were prepared in NaOH 0.10 M and used as standard in all assays in the following concentrations: 1.25 g/l for the UV-280 nm, 0.125 g/l for the UV-220, 10.0 g/l for the biuret, 0.2000 g/l for the Bradford, 1.000 g/l for the Lowry, and 6.0 g/l for the *p*-chloranil methods.

2.2.2. Casein

Casein (Riedel) solutions were prepared in NaOH 0.10 M and used as standard in all assays in the following concentrations: 1.25 g/l for the UV-280 nm, 0.125 g/l for the UV-220, 10.0 g/l for the biuret, 0.2000 g/l for the Bradford, 1.000 g/l for the Lowry, and 6.0 g/l for the *p*-chloranil methods.

2.3. Methods

2.3.1. Extraction of lipids

A measure of 2 ($\pm$0.01) g of samples of whole milk powder, whey protein powder and buttermilk powder were weighed into suitable tubes and 18.0 ml of the solution of chloroform–methanol were added. The tubes were sealed and shaken vigorously for 5 min. The solutions were filtered using a quantitative filter paper and the organic phase was discarded. Then, 6.0 ml of chloroform and 6.0 ml of water were added to the solid samples and they were shaken for 5 min and filtered. For all samples the biuret test in the aqueous/chloroform phase was negative. The solid samples were dried and used for the determination of total proteins.

2.3.2. Kjeldahl method

The Kjeldahl method was used as described by AOAC (Helrich, 1990).

2.3.3. Spectrophotometric methods

The spectrophotometric methods of ultraviolet-280 and 220 nm, biuret-340 and 550 nm, Bradford, Lowry, and *p*-chloranil were used as described by Stoscheck (1990), Gornall et al. (1949), Bradford (1976), Lowry et al. (1951), and Zaia et al. (1999), respectively.

2.3.4. Statistical analysis

Comparisons between means were assessed using Student's *t*-test at a significance level of $P < 0.05$.

3. Results and discussion

Table 1 shows straight-line equations, range of casein and BSA concentrations of work, and relative specific absorbance (RSA) [$RSA^{\#}$ (specific absorbance of casein/specific absorbance of BSA) and RSA^{t} (specific absorbance of casein or BSA for the **X** method/specific absorbance of casein or BSA for the biuret-550 nm method)]. The correlation coefficients for all the straight lines showed in Table 1 were at least 0.98. As shown in Table 1, the Bradford method showed the highest sensitivity for proteins (range of concentration of protein of work 1.0–5.0 μg/ml and RSA^{t} 1559.7 for casein and 1127.5 for BSA). On the other hand, the biuret-550 nm method showed the lowest sensitivity for proteins (range of concentration of protein of work 2000–10 000 μg/ml and RSA^{t} 1.0 for casein and 1.0 for BSA). The UV-220 nm and Lowry methods showed almost the same sensitivity for proteins (Table 1). For the proteins casein and BSA, the Bradford and Lowry methods showed the best results of $RSA^{\#}$ (1.08 and 1.06, respectively; Table 1). The biuret-340 nm method showed the worst result of $RSA^{\#}$ (0.57). The $RSA^{\#}$ values of UV-220 and 280 nm methods were close to each other (1.14 and 1.19, respectively), as were the biuret-550 nm and *p*-chloranil methods (0.78 and 0.75). A good agreement for specific absorbance between casein and BSA for the Bradford and Lowry methods was expected. The development of the color in the Bradford method depends strongly on the molecular weight of proteins. Casein and BSA are not proteins of low molecular weight as are the urinary proteins where this method should be used only with urinary proteins as calibrator (Marshall & Williams, 2000). As proposed by Chou and Goldstein (1960) and Legler et al. (1985) in the Lowry method, the reduction of Folin–Ciocalteau reagent is not done only by the amino acids tyrosine, tryptophan, cysteine, asparagine and histidine, but also by each tetra unit of peptides in the proteins. Thus, this could explain why the Lowry method showed a uniform response to casein and BSA. For the UV-280 nm method, $RSA^{\#}$ was 1.19 because the amino acid composition (phenylalanine, cysteine, methionine, tryptophan, histidine, tyrosine) of casein and BSA are different from each other (Haschemeyer & Haschemeyer, 1973). Despite the principle involved in biuret-550 nm method (planar square complex between the copper and the peptide bond), the specific absorbance should not depend on the amino acid composition of the protein, although the $RSA^{\#}$ for the biuret-550 nm method (0.78) showed that this reaction depends on the composition of the amino acids. In agreement with the biuret method, Gornall et al. (1949) showed that casein has lower specific absorbance

Table 1
Straight line, number of experiments (n), range of work of protein concentration, and relative specific absorbance (RSA^a and RSA^b) for the following methods: UV-280 and 220 nm (Stoscheck, 1990), biuret-340 and 550 nm (Gornall et al., 1949), Bradford (Bradford, 1976), Lowry (Lowry et al., 1951) and p-chloranil (Zaia et al., 1999)

Method	Straight-line equation Y (absorbance) $= mX$ (μg/ml) $+ b$	n	Range of concentration (μg/ml)	$RSA^{\#}$	RSB^t
UV-280 nm	Casein $Y = 8.0607 \times 10^{-4}X + 2.2 \times 10^{-2}$	4	200–1000	1.19	26.9
	BSA $Y = 6.7834 \times 10^{-4}X - 1.6 \times 10^{-2}$	3	200–1000		17.6
UV-220 nm	Casein $Y = 9.4200 \times 10^{-3}X + 1.5 \times 10^{-2}$	7	9.0–40	1.14	314.0
	BSA $Y = 8.2900 \times 10^{-3}X + 0.6 \times 10^{-2}$	6	9.0–40		216.4
Biuret-340 nm	Casein $Y = 0.6010 \times 10^{-4}X + 2.5 \times 10^{-2}$	3	2000–10 000	0.57	2.0
	BSA $Y = 1.0510 \times 10^{-4}X + 4.5 \times 10^{-2}$	3	2000–10 000		2.7
Biuret-550 nm	Casein $Y = 3.0000 \times 10^{-5}X + 0.4 \times 10^{-2}$	3	2000–10 000	0.78	1.0
	BSA $Y = 3.8300 \times 10^{-5}X + 1.1 \times 10^{-2}$	3	2000–10 000		1.0
Bradford	Casein $Y = 4.6790 \times 10^{-2}X + 5.8 \times 10^{-2}$	11	1.0–5.0	1.08	1559.7
	BSA $Y = 4.3520 \times 10^{-2}X + 5.3 \times 10^{-2}$	11	1.0–5.0		1136.3
Lowry	Casein $Y = 1.0450 \times 10^{-2}X + 4.2 \times 10^{-2}$	8	20–60	1.06	348.3
	BSA $Y = 0.9890 \times 10^{-2}X + 5.5 \times 10^{-2}$	8	20–60		258.2
p-chloranil	Casein $Y = 1.3933 \times 10^{-3}X - 1.6 \times 10^{-2}$	3	30–120	0.75	46.4
	BSA $Y = 1.8500 \times 10^{-3}X + 0.3 \times 10^{-2}$	3	30–120		48.3

The specific absorbance from straight line was used to calculated $RSA^{\#}$ and RSB^t, using the following equations: $RSA^{\#}$ = [specific absorbance of casein]/[specific absorbance of BSA] and RSB^t = [specific absorbance of **X** method]/[specific absorbance of biuret-550 nm method].

than albumin and globulin. The $RSA^{\#}$ for the p-chloranil method (0.75) showed that this reaction depends on the amino acid composition and the same value was obtained by Zaia, Barreto, Santos, and Endo (1993) with p-benzoquinone. $RSA^{\#}$ for biuret-340 nm (0.57) was different from $RSA^{\#}$ for biuret-550 nm (0.78) methods (Table 1) because the band at 550 nm is a d–d transition and the band at 340 nm is a charge transfer complex, so there are a different phenomena involved (Huheey, Kecter, & Kecter, 1993).

Table 2 shows the concentration of total proteins in whole milk powder, whey protein powder and buttermilk powder, after extraction of lipids for UV-280 and 220 nm, biuret-340 and 550 nm, Bradford, Lowry, and p-chloranil methods, and without extraction of lipids for the Kjeldahl method. Table 2 also shows the determination of total nitrogen (TN) and non-protein nitrogen (NPN) using Kjeldahl method and the TPN by the difference between TN and NPN. To overcome the problem of turbidity of solutions due to the presence of the lipids, besides the method described in the methodology, we also tried to centrifuge the samples at 4000 rpm at 4°C, and tried to minimize the turbidity of solutions by using triton X-100 or sodium dodecyl sulfate (SDS). However, the procedure described in the methodology (extraction of lipids) showed the best results. Using casein as standard protein, the UV-220 nm method showed the best results for

Table 2
Concentration of total proteins in whole milk powder, whey protein powder and buttermilk powder, after extraction of lipids as measured by different protein assay

Method	Standard curve	Whole milk powder (% w/w)	Whey protein powder (% w/w)	Buttermilk powder (% w/w)
Kjeldahl (TN)[a,b]	—	25.4±0.7 (3)	11.3±0.6 (3)	30.7±0.9 (3)
Kjeldahl (NPN)[c,b]	—	1.3±0.1 (3)	1.3±0.1 (3)	2.0±0.1 (3)
TPN[d] = TN−NPN	—	24.1	10.0	28.7
UV-280 nm	Casein	68.7±10.4[e,f] (5)	9.1±1.7[g] (4)	39.1±8.0[g] (4)
	BSA	84.4±2.7[e,f] (4)	23.4±2.2[e,f] (4)	59.6±2.3[e,f] (4)
UV-220 nm	Casein	27.5±2.1[g] (7)	9.5±2.0 (3)	35.4±2.6[g] (6)
	BSA	36.3±1.1[e,f] (6)	11.1±1.4 (5)	43.9±1.8[e,f] (6)
Biuret-340 nm[h]	Casein	48.5±0.4[e,g,f] (3)	25.7±0.5[e,g,f] (3)	50.2±1.7[e,g,f] (3)
	BSA	26.3±0.8 (3)	14.1±0.6[e,f] (3)	27.3±1.4 (3)
Biuret-550 nm[h]	Casein	36.5±0.4[e,g,f] (3)	10.7±0.6[g] (3)	35.5±0.5[e,g,f] (3)
	BSA	27.4±0.7[f] (3)	8.1±0.6[e] (3)	26.7±0.7[e] (3)
Bradford	Casein	21.3±0.4[e,g,f](4)	11.8±0.5[f,g](4)	26.7±0.6[e,f,g](4)
	BSA	26.0±0.7 (4)	16.6±0.5[e,f](4)	31.4±0.8[f](4)
Lowry	Casein	27.6±0.8[f](4)	10.7±0.8(4)	31.3±1.1(4)
	BSA	27.3±0.7[f](4)	9.4±0.6[e](4)	31.1±0.2[f](4)
p-chloranil	Casein	41.4±0.7[e,g,f](3)	9.4±0.3[g](3)	40.9±1.1[e,g,f](3)
	BSA	26.2±1.1(3)	5.4±0.1[e,f](3)	24.6±1.0[e,f](3)

The numbers of assays are given in parentheses. The results are presented as mean±SEM.
[a] TN—total nitrogen.
[b] Kjeldahl method without the extraction of lipids.
[c] NPN—non-protein nitrogen.
[d] TPN—total protein nitrogen.
[e] For comparison between Kjeldahl (TN) and the spectrophotometric methods ($P<0.05$).
[f] For comparison between TPN and spectrophotometric methods ($P<0.05$).
[g] For comparison between casein and BSA as standard protein for each method ($P<0.05$).
[h] After extraction of lipids the samples were spun for 2 min at 2000 rpm.

the concentration of total proteins in the samples; all these results were not statistically different ($P<0.05$) from those of TN obtained by the Kjeldahl method or TPN. The biuret-340 nm showed the worst results, because all concentrations of total proteins were statistically different ($P<0.05$) from TN or TPN. For all methods showed in Table 2, with the exception of the Bradford method, the concentration of total proteins in the sample of whole milk powder was higher than that of TN obtained by the Kjeldahl method as well as TPN. Probably, this was because the extraction of lipids was not enough to defat the sample, and fat material increased the turbidity of solutions, hence the higher values of total proteins obtained. The biuret-340 and 550 nm methods could also be affected by the lactose, because of its reducing properties on copper, which gives a positive

interference as great as 15% in human milk (Verheul, Bosch, & Cornelissen, 1986). The high sensitivity of the Bradford method (Table 1) overcomes the turbidity problem (Table 2). The sensitivity of the Lowry and UV-220 nm methods (Table 1) was enough to minimize this problem, because the concentration of total protein was very close to TPN (Table 2). For the Bradford method, the concentration of total proteins in the samples of whole milk powder and buttermilk powder was lower and statistically different ($P<0.05$) from that of TN obtained by the Kjeldahl method (Table 2); for samples of whole milk powder, whey protein powder, buttermilk powder the results of total proteins were compared to the TPN and were statistically different ($P<0.05$) from each other. In the samples of whole milk powder and buttermilk, the concentration of proteins obtained with the Bradford method was smaller than TPN. Proteins could be lost in phase aqueous/chloroform, when the lipid extraction was carried out, but the biuret test for all samples in this phase was negative. The concentration of proteins in whey protein powder obtained with the Bradford method is higher than TPN, probably because most of the whey proteins have higher molecular weight than casein used as standard and specific absorbance of BCG-250/protein is dependent on the molecular weight of proteins (Fox, 1997; Marshall & Williams, 2000). The concentration of total proteins obtained with the Lowry method (Table 2), using casein or BSA as standard protein, was not statistically different ($P<0.05$) from each other. This was expected because the specific absorbances for both proteins are very close (Table 1). However, biuret-340 and 550 nm, Bradford, and *p*-chloranil methods showed that concentration of total proteins in all the samples using casein or BSA as standard proteins were statistically different ($P<0.05$) from each other. This was expected because the specific absorbances for both proteins are different (Table 1).

Table 3 shows the results of determination of total proteins in skim milk powder for the UV-280 and 220 nm, biuret-340 and 550 nm methods as well as the results of total proteins in the skim milk powder, whole milk powder, whey protein powder, and buttermilk powder samples using Kjeldahl for TN, Kjeldahl for NPN determinations, Bradford, Lowry, and *p*-chloranil methods, without extraction of lipids. Table 3 also shows the difference between TN and NPN that is TPN. Using the casein as standard protein, the Bradford method showed the best results for the concentration of total proteins in the samples, all these results were not statistically different ($P<0.05$) from those of TN obtained by the Kjeldahl method. This means that the high sensitivity of the Bradford method was enough to overcome the turbidity of solutions, due to the fat material in the samples (Table 3). However, when these results were compared to TPN only the skim milk and whole milk samples were not statistically different ($P<0.05$) as shown in Table 3. The concentrations of total proteins using the Bradford method for the milk powder and whey samples with and without the extraction of lipids were not statistically different from each other. However, for the buttermilk powder samples the results were statistically different ($P<0.01$). For Lowry and *p*-chloranil methods, in all samples, and for the biuret-340 and 550 nm in skim milk samples, the concentrations of total proteins were statistically different ($P<0.05$) from those of TN obtained by the Kjeldahl method or TPN. So, those methods were not sensitive enough (Table 1) to overcome the problem of turbidity of the samples, due to the fat material. The concentration of total proteins in skim milk samples using UV-280 and 220 nm methods was not statistically different from that obtained by the Kjeldahl method (TN), because the SEM in both methods were very high. As shown in Table 3 using the casein or BSA as standard protein, the values for total proteins in the skim milk samples for the methods UV-280 and 220 nm and in the

Table 3
Concentration of total proteins in skim milk powder, whole milk powder, whey protein powder and buttermilk powder, without extraction of lipids as measured by different protein assay

Method	Standard Curve	Skim milk powder (% w/w)	Whole milk powder (% w/w)	Whey protein powder (% w/w)	Buttermilk powder (% w/w)
Kjeldahl (TN)[a]	—	34.2±1.9 (3)	25.4±0.7 (3)	11.3±0.6 (3)	30.7±0.9 (3)
Kjeldahl (NPN)[b]	—	1.5±0.1 (3)	1.3±0.1 (3)	1.3±0.1 (3)	2.0±0.1 (3)
TPN[c] = TN−NPN	—	32.7	24.1	10.0	28.7
UV-280 nm	Casein	45.6±5.8 (5)	—	—	—
	BSA	58.1±9.1 (4)	—	—	—
UV-220 nm	Casein	47.2±4.0[d] (7)	—	—	—
	BSA	58.8±4.0[e,d] (7)	—	—	—
Biuret-340 nm	Casein	57.4±0.8[e,f,d] (3)	—	—	—
	BSA	31.1±0.7 (3)	—	—	—
Biuret-550 nm	Casein	41.6±0.9[e,f,d] (3)	—	—	—
	BSA	31.1±1.1(3)	—	—	—
Bradford	Casein	33.7±0.9[f] (7)	22.4±0.9[f] (7)	12.8±1.0[d,f] (7)	33.5±0.9[d,f] (7)
	BSA	39.1±0.6[e,d] (7)	26.7±0.6[d] (7)	17.3±0.5[e,d] (7)	37.3±0.8[e,d] (7)
Lowry	Casein	50.5±0.6[e,d,f] (4)	38.9±1.2[e,d] (4)	17.3±0.6[e,d,f] (4)	42.6±1.6[e,d] (4)
	BSA	47.9±0.2[e,d] (4)	36.1±0.9[e,d] (4)	14.1±0.4[e,d] (4)	40.2±1.5[e,d] (4)
p-chloranil	Casein	49.0±1.0[e,d,f] (3)	86.6±2.8[e,d,f] (3)	17.3±0.4[e,d,f] (3)	57.1±1.0[e,d,f](3)
	BSA	30.7±1.4 (4)	57.6±2.2[e,d] (3)	10.5±0.1 (3)	36.6±0.7[e,d] (3)

The numbers of assays are given in parentheses. The results are presented as mean±SEM.
[a] TN—total nitrogen.
[b] NPN—non protein nitrogen.
[c] TPN—total protein nitrogen.
[d] For comparison between TPN and spectrophotometric methods ($P<0.05$).
[e] For comparison between Kjeldahl (TN) and spectrophotometric methods ($P<0.05$).
[f] For comparison between casein and BSA as standard protein for each method ($P<0.05$).

samples of whole milk powder and buttermilk for the Lowry method, were not statistically different from each other. On the other hand, for all samples studied with the biuret-340 and 550 nm, Bradford, and *p*-chloranil methods showed concentration values of total proteins statistically different ($P<0.05$) from each other.

4. Conclusion

Among the studied methods, the Bradford method showed the highest sensitivity for proteins and the Lowry method showed the least variation of specific absorbance for casein and BSA. After the extraction of lipids, UV-220 nm could be used for the determination of total proteins because the values obtained were not statistically different from the TPN ones. The most important achievement of this paper was that the Bradford method could be used for the determination of total proteins in whole milk and skim milk samples (without extraction of lipids) instead of the Kjeldahl method. Several advantages characterize the Bradford: higher sensitivity for proteins, determination of only protein nitrogen, simpler execution, and shorter period of time for the whole assay to be carried out.

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