Determination of True Proteins in Dairy Products: A Comparative study between Kjeldahl and Sprint Protein Analyzer

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Abstract

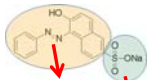
True proteins (TrP) in milk play a major role in yield, structure-function, and nutritional value of dairy products and products containing dairy proteins. It is standard practice in milk procurement for milk processing plants to pay premium for protein content of milk. In the cheese industry, TrP is of greater economic value than total protein (TP) content hence the need for rapid determination of TrP is of significant interest. In this study, the Sprint Rapid Protein analyzer, SPA (CEM Corporation, Matthews, NC) was compared with the standard Kjeldahl method to measure the TP in selected dairy products. The SPA is based on the Orange G dye binding method that measures the TrP directly unlike Kjeldahl in which TrP is calculated as the difference between total nitrogen and non-protein nitrogen multiplied by 6.38. Milk, cheeses and milk protein powders such as whey protein concentrate 80 (WPC 80), whole milk powder (WMP) and nonfat dry milk (NFDM) were analyzed for TrP contents by both the methods and the results were compared. TrP values measured by both methods were not statistically different (p-value > 0.05) for milk, Cheddar cheese, Mozzarella cheese and the milk protein powders analyzed. The repeatability within the samples for milk, Cheddar cheese, mozzarella cheese and milk powders were similar in both methods (standard deviation 0.02–0.42 for Kjeldahl; and 0.04–0.49 for SPA). The relative standard deviation for all the products measured in both the methods was always less than 2%. In the preliminary studies the precision and accuracy in measuring TP by SPA in all the samples tested was comparable to values obtained using the Kjeldahl method.

Introduction

True proteins (TrP) in milk play a major role in yield, structure-function, and nutritional value of dairy products and products containing dairy proteins. It is standard practice in milk procurement for milk processing plants to pay premium for protein content of milk. In the cheese industry, TrP is of greater economic value than total protein content hence the need for rapid determination of true proteins is of significant interest. Similarly, in the milk powder industry, the protein content is used commercially to distinguish powder type. Milk protein content is traditionally determined by Kjeldahl N × 6.38. In addition to the Kjeldahl N determination being cumbersome and time-consuming, it also measures non-protein nitrogen which has no economic significance. Hence, solutions for rapid determination of TrP by combining chemical and instrumental methods is sought without compromising accuracy. CEM Corporation introduced a new instrument, Sprint Rapid Protein Analyzer (SPA), which is based on the Orange G dye binding method to measure TrP in less than five minutes. The purpose of this study is to comparable TrP analyzed by SPA with the Kjeldahl method for different dairy products (e.g., cheese and various milk protein powders).

Working Principle of SPA

iTag Molecule



The aromatic group that absorbs light and appears orange

Acid

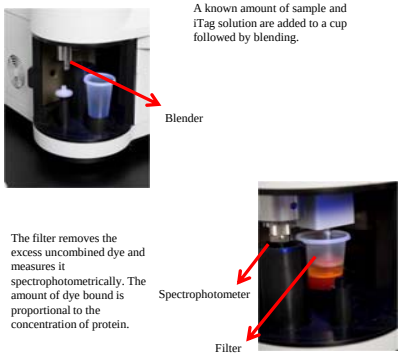
The Sprint analyzer consist of iTag solution which consist of anionic Orange G dye with as acid coupled to it. The pH of this solution is 2 at which it combines with the positively charged amino acids, lysine, arginine and histidine and precipitates out the proteins.

Table 1. The amount of Basic Amino Acids in Bovine Milk

Amino Acids	Bovine Casein	Bovine Whey Protein
Lysine	518 mg/g TN	704 mg/g TN
Histidine	186 mg/g TN	144 mg/g TN
Arginine	239 mg/g TN	175 mg/g TN

Amount given as mg amino acid per g of total Nitrogen (Fox and McSweeney, 2003)

SPA Analyzer



A known amount of sample and iTag solution are added to a cup followed by blending.

Blender

The filter removes the excess uncombined dye and measures it spectrophotometrically. The amount of dye bound is proportional to the concentration of protein.

Spectrophotometer

Filter

Materials and Methods

Samples

Dairy products analyzed are listed in Table 2. Literature values for TP contents (Kjeldahl TN × 6.38) in products range from 3 to 85%. Different quantities of each dairy product were obtained. Each of the products was analyzed in triplicate by both the Kjeldahl and Sprint Rapid Protein Analyzer methods.

Table 2. Dairy products analyzed in this study

Dairy Products	% Protein (TN × 6.38)
Milk	3.0 ~ 3.8
Cheddar Cheese (CC)	21 ~ 24
Mozzarella Cheese (MC)	21 ~ 28
Nonfat Dry Milk (NFDM)	32 ~ 36
Butter Milk Powder (BMP)	32 ~ 35
Milk Powder Isolate (MPI)	80 ~ 85
Whey Protein Concentrate 80 (WPC 80)	80 ~ 82

Kjeldahl method

Cheese

Total nitrogen, TN (%) was measured by AOAC method 920.123. There is negligible amount of NPN in cheese. Hence percent TrP in cheese was calculated as %TN × 6.38.

Milk powders

Milk protein powders were reconstituted in water prior to determination of TN by AOAC method 930.29 and NPN by AOAC method 991.21. Percent TrP = (%TN - % NPN) × 6.38

Milk

Total nitrogen in milk was measured by AOAC method 991.20 and % NPN was measured by AOAC method 991.21. Percent TrP was calculated as (%TN - % NPN) × 6.38

Results and Discussion

In order to determine whether SPA can be used as an alternative method to Kjeldahl analysis for TrP in dairy plants, both methods were compared for accuracy, precision and other performance characteristics (sample throughput, automation capabilities, environmental acceptance and cost).)

Table 3. Comparison of true protein in various dairy products by Kjeldahl and SPA methods

	Milk	Cheese			Powder		
	Milk	Cheddar	Mozzarella	NFDM	BMP	MPI	WPC80
Test/No. of samples	8	7	6	4	2	5	3
Kjeldahl	3.359±0.069	23.332±0.254	23.18±0.464	32.827±0.664	32.828±0.273	81.618±0.664	78.918±0.829
SPA	3.357±0.063	23.261±0.388	23.392±0.507	32.300±0.700	33.240±0.725	81.403±0.655	79.635±0.834
Difference (%)	0.002	0.072	-0.212	0.527	-0.413	0.215	-0.717
p-value	0.925	0.715	0.126	0.210	0.529	0.538	0.085

The results are presented as mean ± SEM. Each sample was done in triplicate. NFDM = nonfat dry milk; BMP = butter milk powder; MPI = milk powder isolate; WPC = whey protein concentrate.

Accuracy

Comparison was made of the results obtained applying both of the methods to several dairy products covering a wide range of TrP (milk, cheeses and powders). The results are summarized in Table 3. The application of the paired t-test (95% confidence level) demonstrated that there is no significant differences between the mean values of the results obtained from Kjeldahl method and SPA (all the p-value > 0.05). Moreover, tryptophan was used as standard to check the efficiency of the digestion procedure of Kjeldahl method – giving higher than 97% nitrogen recovery.

Precision

To compare the precision of both methods, triplicate analyses of the same sample was performed and the standard deviation (S.D.) and relative standard deviation (R.S.D) were calculated (Table 4). By applying the paired t-test to the S.D., p-values > 0.05 suggest there is no significant difference between the two methods for TrP analysis. Hence, it is concluded that both methods resulted in similar TrP measurements.

Table 4. Comparison of the precision of the Kjeldahl and SPA methods for true protein analysis

	Kjeldahl Method		SPA Method	
	Mean S.D.	Mean R.S.D	Mean S.D.	Mean R.S.D.
Milk	0.04	1.07	0.01	0.39
Cheddar	0.31	1.37	0.18	0.79
Mozzarella	0.17	0.77	0.36	1.59
NFDM	1.09	3.36	0.73	2.29
BMP	0.26	0.79	0.58	1.74
MPI	0.96	1.18	0.75	0.93
WPC80	0.90	1.13	0.32	0.40

S.D. = standard deviation in repeatability conditions; R.S.D. = relative standard deviation indicating repeatability of methods. NFDM = nonfat dry milk; BMP = buttermilk powder; MPI = milk powder isolate; WPC = whey protein concentrate.

Statistical Analysis

TrP contents were analyzed using paired t-test in Minitab version 15.1. 20. p-values > 0.05 were obtained for Cheddar cheese, mozzarella cheese and milk protein powders. The mean , S.D. and R.S.D. were calculated using Microsoft Excel.

Other Analytical Performances

	Kjeldahl Method	SPA
Analysis time	5~7 hours	1 min to prepare sample, 2~3 min for analyzing
Automation	Most of work have to be done manually	High throughput applications
Cost per test	$5.39	$3.43
User-friendliness	Training and practicing are needed	Comes with preprogrammed methods
Safety and environmental acceptance	Hazardous reagents and heavy metals are used	Disposable sample cups and filters are used, no dangerous agents
Application	Standard method for nitrogen determination	Only detect true protein. If protein is broken down to amino acid, cannot give right results.

References

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