

## Correlation between acidic ninhydrin and HPLC methods to evaluate fraudulent addition of whey in milk

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**Abstract** – The acidic ninhydrin spectrophotometric method (ANSM) for quantitative determination of free and bound sialic acid of milk glycoprotein has been proved to be fast and efficient for routine detection of fraudulent addition of rennet whey to fluid milk. In this research the ANSM was compared with the high performance liquid chromatography (HPLC) method, internationally recommended for caseinomacropetide (CMP) determination, which besides its high accuracy is more sophisticated and requires trained personnel. For several sample conditions (raw milk and milk with variable added amounts of rennet cheese whey), the methods showed an excellent linear correlation, with  $r = 0.981$  when milk was deproteinized with a  $120 \text{ g}\cdot\text{L}^{-1}$  final concentration of trichloroacetic acid (TCA) concentration. The best correlations could be seen with final concentrations of  $100 \text{ g}\cdot\text{L}^{-1}$  and  $80 \text{ g}\cdot\text{L}^{-1}$  TCA; respectively,  $r = 0.992$  and  $0.993$ .

**Milk / adulteration / whey / addition / acidic ninhydrin / HPLC**

**Résumé** – Détection de l'addition frauduleuse de lactosérum au lait de consommation : comparaison de la méthode à la ninhydrine acide (détermination de l'acide sialique) avec la méthode HPLC de détermination du CMP. La méthode spectrophotométrique à la ninhydrine acide (ANSM : acidic ninhydrin spectrophotometric method) pour la détermination des formes libres et liées de l'acide sialique s'est avérée être rapide et performante pour la détection en routine de l'addition frauduleuse de lactosérum doux dans le lait de consommation. Dans l'étude réalisée, l'ANSM a été comparée avec la méthode recommandée au niveau international, à savoir la méthode de détermination du caséinomacropetide (CMP) par HPLC qui est certes très précise mais requiert un équipement sophistiqué et du personnel hautement qualifié. Une excellente corrélation linéaire a été trouvée entre les deux méthodes lors de l'analyse de filtrats TCA (acide trichloroacétique) issus d'échantillons de lait cru additionnés de quantités variables de lactosérum doux soumis préalablement à un traitement thermique. Selon la concentration finale en TCA :  $120 \text{ g}\cdot\text{L}^{-1}$ ,  $100 \text{ g}\cdot\text{L}^{-1}$ ,  $80 \text{ g}\cdot\text{L}^{-1}$ , les valeurs de corrélation linéaire atteignaient respectivement  $0,981$ ,  $0,992$  et  $0,993$ .

**Lait / fraude / lactosérum / addition / ninhydrine acide / HPLC**

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## 1. INTRODUCTION

The  $\kappa$ -casein, one of the casein fractions of milk, is found in the casein micelles, which are spherical complex structures containing  $920 \text{ g}\cdot\text{kg}^{-1}$  of protein and  $80 \text{ g}\cdot\text{kg}^{-1}$  of inorganic salts, mainly calcium phosphate, with a porous structure and linked by calcium phosphocaseinate. Currently, one accepted micellar model states that micelles consist of smaller submicelles and that the content of  $\kappa$ -casein in the submicelles varies, as the submicelles lacking in  $\kappa$ -casein are located inside of the micelles while the submicelles rich in  $\kappa$ -casein are concentrated on the surface, giving the micelles a layer rich in  $\kappa$ -casein. This model proposes that the hydrophilic region C-terminal of the  $\kappa$ -casein is projected toward the surface, giving the micelles a hairy appearance. This hairy layer is responsible for the stability of the micelle and once it is removed, the colloidal stability of the micelles is destroyed and milk coagulates or precipitates. The caseins are very susceptible to proteolysis because they contain random structures with little space organization, represented by a small amount of secondary structures of helical type [2, 9, 10].

The  $\kappa$ -casein, with a minimum molecular weight of  $19\,000 \text{ g}\cdot\text{mol}^{-1}$  and 169 residues of amino acids, is a phosphoprotein containing  $60\text{--}100 \text{ g}\cdot\text{kg}^{-1}$  of carbohydrates approximately, consisting of N-acetyl-Neuraminic Acid (NANA or sialic acid), galactosyl and N-acetylgalactosamine [16,17]. It is the fraction of the caseins not sensitive to calcium and, in normal conditions, carries out an important role in the stability of the micelle, preventing the precipitation of the  $\alpha_s$ - and  $\beta$ -casein, when this ion is present [18]. This stabilizing property is lost during enzymatic rennet milk coagulation when only the  $\kappa$ -casein is hydrolyzed specifically in the cleavage of the amino acids Phe<sub>105</sub>-Met<sub>106</sub>. This bond site is much more susceptible to hydrolysis by acid proteinases, such as the commercial rennets, than those of the proteic system of the milk [3].

In the initial cleavage by the rennin's action, there is the formation of two peptides, one composed of the residues of amino acids 1 to 105, named para- $\kappa$ -casein, and another by the residues 106 to 169, that contains all the sugars of the  $\kappa$ -casein, named glycomacropeptide or GMP. The  $\kappa$ -casein is heterogeneous and contains 2 genetic variants, A and B. The content of carbohydrates in the molecule varies from 0 to 15% and there is the possibility of obtaining fragments with the absence of carbohydrate. In this case, the terminal peptide is named macropeptide or caseinomacropeptide (CMP), and both (CMP and GMP) remain soluble on the whey and are separated from the curd during cheese production [8,18].

One of the methods of monitoring coagulation action based on  $\kappa$ -casein is the determination of sialic acid content in cheese whey, a specific indicator of the liberation of GMP [10].

Van Hooydonk and Olieman [19] described High Performance Liquid Chromatography (HPLC) as a fast and sensitive method to monitor the action of the chymosin in milk. In 1983, Olieman and van den Beden [14] used this method to detect and estimate, in skimmed powdered milk, the presence of solids of serum of milk obtained by the action of the rennin.

The composition variability in the glycosidic fraction is responsible for CMP solubility variations in TCA. The macropeptide soluble in  $20 \text{ g}\cdot\text{L}^{-1}$  of TCA refers to CMP that includes all the glycosidic forms and, eventually, also the non-glycosylated forms, if present, while the macropeptide soluble in  $120 \text{ g}\cdot\text{L}^{-1}$  of TCA refers to those that contain the total carbohydrate of the  $\kappa$ -casein named GMP [11].

Macropeptide evaluation made by HPLC or Fast Performance Liquid Chromatography (FPLC) depends on the removal of the other proteins using TCA [11]. For van Hooydonk and Olieman [19], the best final concentration, to prevent interfering factors in the determination of GMP through the chromatography methodology, is  $80 \text{ g}\cdot\text{L}^{-1}$  of

TCA, in which the cheese whey proteins are virtually absent. Low concentrations of TCA result in insufficient removal of the whey proteins, while high concentrations cause precipitation of larger amounts of GMP.

The European Union, concerned with the fraudulent addition of whey to the skimmed powder milk destined for public stockpiling, made official through the Regulation of the EU Commission n° 213/1981 the HPLC method for GMP determination and the calorimetric method with resorcinol reagent for sialic acid detection as methods for the identification of this economic adulteration, methodologies that are still accepted today [1].

In Brazil, the Ministry of Agriculture established, in 1991 [12], two official analytical methods of rennet whey detection in milk, a quantitative method for the determination of GMP through the technique of HPLC, and a qualitative method, based on the research of sialic acid adapted by Wolf-schoon-Pombo and Pinto, using Ehrlich reagent [20].

In 1994, Fukuda [4] adapted the application to milk of a technique described by Yao et al. [21] for the determination of the concentration of sialic acid in sialoglycoprotein in ascitic liquid formed from abdominal tumors, using acidic ninhydrin as chromophor (Gaitonde Reagent 2).

In that same year, Fukuda et al. [5] proposed the standardization of the spectrophotometric (470 nm) acidic ninhydrin method for quantitative determination of sialic acid (N-acetyl-Neuraminic Acid – NANA); free, or linked to the glycoprotein in fluid milk and rennet whey. The method was shown to be precise, accurate and sensitive to variations around 2%. Besides these characteristics, the method could be performed easily and with good possibility of use in routine application, since it did not require sophisticated equipment or qualified personnel, and also required less time to execute when compared with other methodologies.

The determination of GMP by the acidic ninhydrin reaction standardized by Fukuda et al. [5] used a 120 g·L<sup>-1</sup> TCA final concentration, with the objective of working only with the glycosylated forms and free from possible interfering factors. On the other hand, the HPLC technique uses an 80 g·L<sup>-1</sup> TCA final concentration in order to search for all the forms of CMP [19].

Based on the results obtained, Fukuda et al. [6] proposed the use of this method as a screening test of the milk received by the dairy plants to detect possible milk adulteration by whey addition. This proposal classified the milk as negative (absorbance at 470 nm  $\leq$  0.300 or  $\leq$  5.14  $\mu$ g free sialic acid·mL<sup>-1</sup> of milk), suspect (absorbance at 470 nm between 0.300 and 0.600 or between 5.14 and 10.64  $\mu$ g free sialic acid·mL<sup>-1</sup> of milk) or positive (absorbance at 470 nm  $>$  0.600 or  $>$  10.64  $\mu$ g free sialic acid·mL<sup>-1</sup> of milk).

Recently, Furlanetti and Prata [7] studied the variability of the free and total GMP content (after milk sample coagulation with commercial rennet) in bovine milk during lactation, using the method of the acidic ninhydrin reaction standardized by Fukuda et al. [5], and concluded that free and total GMP vary according to the period of lactation and the sanitary conditions of the udder and that it is inversely proportional to the cow's milk production. The free GMP content is higher when close to the colostrum period and during the end of the lactation period, while GMP liberated by the rennin shows a similar behavior; however, the content is ten times higher than free GMP.

Prata [15], working with a larger number of samples, confirmed the acidic ninhydrin reaction method as being efficient in fraud detection of whey addition to milk, and suggested altering the values proposed by Fukuda et al. [6] to:  $A_{470\text{nm}} \leq 0.300$  or  $\leq 5.14$  mg free sialic acid·L<sup>-1</sup> of milk for the negative category;  $0.300 < A_{470\text{nm}} < 0.450$  or 5.14 to 7.89 mg free sialic acid·L<sup>-1</sup> of milk for the suspect category and  $A_{470\text{nm}} \geq 0.451$  or  $> 7.89$  mg free sialic acid·L<sup>-1</sup> of milk for the positive category.

In April 2003, the technique of acidic ninhydrin reaction for the quantitative determination of sialic acid was approved by the Brazilian Ministry of Agriculture as an official method, published through the Normative Instruction n° 22 [13].

The purpose of this work was to verify the correlation of the acidic ninhydrin and the HPLC methods in the detection of rennet whey added to milk using raw milk samples and raw milk with intentionally added rennet whey samples, in different proportions, and also the influence of the use of TCA concentrations of 80, 100 and 120 g·L<sup>-1</sup>.

## 2. MATERIALS AND METHODS

Raw milk samples of known origins were obtained over 2 months in dairy plants of the District of Barretos, Sao Paulo State, Brazil. They represented a pool of several producers. Rennet cheese whey from cheese plants from this same region was used, which was heated at 80 °C for 15 min in a water-bath after curd separation, for enzymatic inactivation, and after this, it was chilled in ice. All of the milk and whey samples were stored in thermal boxes with ice until arriving at the laboratory. The whey not used on the day of production was frozen.

Adulterated milk samples were prepared by addition of 25 to 600 mL·L<sup>-1</sup> rennet whey to raw milk. Raw milk samples and adulterated milk samples were frozen (-14 °C) until they were submitted to chromatography. During all the determinations samples of raw milk or of adulterated raw milk were performed in duplicate.

To obtain equidistant points that allowed comparisons at a large interval of variations, the addition of different amounts of rennet whey to milk samples was necessary, with the content of GMP previously determined through the acidic ninhydrin method. Six series of experiments were performed.

All the acidic ninhydrin method determinations were realized with 80, 100 and 120 g·L<sup>-1</sup> TCA final concentrations, and

for the HPLC an 80 g·L<sup>-1</sup> TCA final concentration was used.

The results were submitted to linear regression, to obtain the equation of the straight line and the values of the correlation coefficient (*r*) and of determination (*r*<sup>2</sup>).

### 2.1. Analytical methodologies

#### 2.1.1. The acidic ninhydrin method

Ten mL of milk were mixed with 10 mL of a 240 g·L<sup>-1</sup> TCA solution. After the mixture had been standing for 30 min, the precipitate was filtered off. Then, 10 mL of filtrate were placed in centrifuge tubes, and 1 mL of a 200 g·L<sup>-1</sup> phosphotungstic acid solution was added. After centrifugation (3500 rpm for 10 min), the supernatant was gently discarded and the sediment dispersed with 6 mL of 950 mL·L<sup>-1</sup> ethyl alcohol. Another centrifugation was carried out for 10 min and the supernatant was gently discarded again. Then 2 mL of glacial acetic acid and 1 mL of acidic ninhydrin reagent (1g of ninhydrin, 16 mL of hydrochloridric acid P.A. and 24 mL of glacial acetic acid) were added to the residue. After homogenization, the mixture was heated in a strong water-bath for exactly 10 min. There was a color development varying from yellow to brown-yellowish. The solution was chilled in cold water and immediately after it was submitted to a spectrophotometric reading in the wavelength of 470 nm, against the white of the reagents. The readings can be transformed in sialic acid concentration (µg·mL<sup>-1</sup> of milk), comparing them with a standard curve prepared with sialic acid [4, 5]. For the described method, the final dilution of TCA was 120 g·L<sup>-1</sup>.

To achieve the final concentration of 80 g·L<sup>-1</sup>, 20 mL of milk and 10 mL of TCA 240 g·L<sup>-1</sup> were mixed, and for 100 g·L<sup>-1</sup>, 14 mL of milk and 10 mL of TCA 240 g·L<sup>-1</sup> were used; the other procedures were the same as those already described.

### 2.1.2. HPLC method

To each 10 mL of fluid milk 5 mL of TCA 240 g·L<sup>-1</sup> was added in drips, under constant stirring. After the mixture had been standing for 60 min, the precipitate was filtered off, discarding the first drops. Fifteen to 30 µL of the filtrate were submitted to a chromatography reading in HPLC equipment (Hewlett-Packard Co., USA), with manual injection and looping of 100 µL, a Zorbax Bio Series GF-250 (DuPont Company) column with 9.4 mm of internal diameter by 25 cm of length and an integrator-recorder. The flow was 1.0 mL per minute and the detection happened in the band of the UV-VIS, in the wavelength of 205 nm. The eluent used in the separation was a pH 6 phosphate standard solution with 21.41 g·L<sup>-1</sup> of sodium sulfate added. This solution was filtered through a membrane of 0.45 µm and degassed. The standard curve was elaborated using milk of known origin and also through the same milk with 50, 100, 150 and 200 g·L<sup>-1</sup> of added cheese whey [13, 19].

## 3. RESULTS AND DISCUSSION

The results obtained with the acidic ninhydrin method with 80, 100 and 120 g·L<sup>-1</sup> TCA final concentrations are presented in Table I. The results obtained with the HPLC method and for the 80 g·L<sup>-1</sup> TCA final concentration are shown in Table II. Results were considered in the acidic ninhydrin method as the value of the absorbance at 470 nm and for the HPLC, the height of the peaks of the HPLC chromatograms, with a retention time of around 6.8 min.

The variation coefficients of the duplicates in each experiment were satisfactory, always under 10%. The medium value of 0.162 of absorbance to 470 nm obtained for raw milk (without whey addition) obtained by the acidic ninhydrin method with 120 g·L<sup>-1</sup> of TCA (Tab. II) is within the range of values obtained by Fukuda [4] for authentic raw milk of 0.248 ± 58.8%.

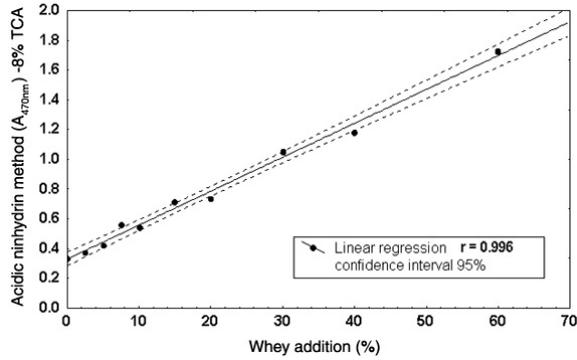
The percentages of rennet whey added to the milk were correlated with the results obtained by the acidic ninhydrin methodology for each one of the TCA final concentrations used (Figs. 1, 2 and 3). The linear regressions obtained, for a confidence interval of 95%, resulted in values of *r* of 0.996, 0.995 and 0.985, respectively, for the TCA final concentrations of 80, 100 and 120 g·L<sup>-1</sup>. As can be observed in Figures 1, 2 and 3, the experimental data obtained was in agreement with the Beer's law, where the value of the absorbance for a certain substance is proportional to its concentration, confirming the viability of this methodology for the spectrophotometric determination of the addition of cheese whey to milk.

The *r* values obtained for ANSM with the three different TCA final concentrations showed a small difference between them. The use of the 80 g·L<sup>-1</sup> TCA concentration presented the best result and represents a reagent economy, rendering a cheaper methodology; it is also the same one recommended by van Hooydonk and Olieman [19], who found it to be the most suitable for the HPLC method. The concentration of 120 g·L<sup>-1</sup> of TCA as described by Fukuda et al. [6] presented a smaller value for *r*.

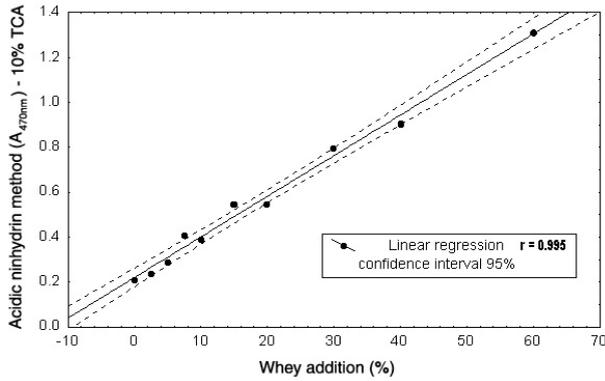
Figure 4 shows the correlation of the whey addition to the milk with the results obtained by the HPLC method, confirming the proof precision for a *r* value of 0.999 for a confidence interval of 95%.

The chromatographic analysis (Fig. 5) presented the best correlation index for the four cases; however, the differences between them were small, mainly when comparing HPLC and ANSM with 80 g·L<sup>-1</sup> TCA: respectively, 0.999 and 0.996.

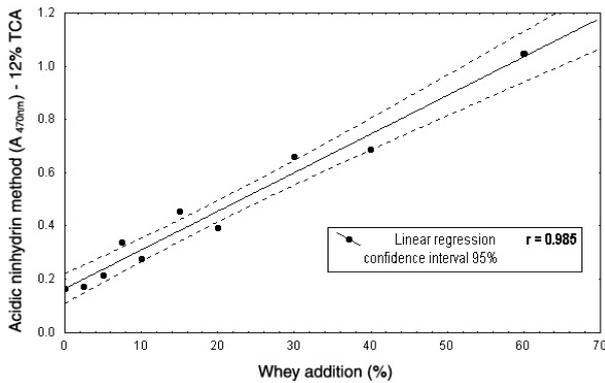
The values obtained with the spectrophotometric methodology with acidic ninhydrin were correlated with the values obtained with the HPLC methodology (Figs. 6, 7 and 8). The *r* values found, 0.993, 0.992 and 0.981, respectively, for the TCA final concentrations of 80, 100 and 120 g·L<sup>-1</sup>, were highly satisfactory, indicating that the first one presented a slightly superior result.



**Figure 1.** Correlation between rennet whey addition to milk and the results obtained by the acidic ninhydrin method (spectrophotometric determination at 470 nm) with the 80 g·L<sup>-1</sup> TCA final concentration.



**Figure 2.** Correlation between rennet whey addition to milk and the results obtained by the acidic ninhydrin method (spectrophotometric determination at 470 nm) with the 100 g·L<sup>-1</sup> TCA final concentration.

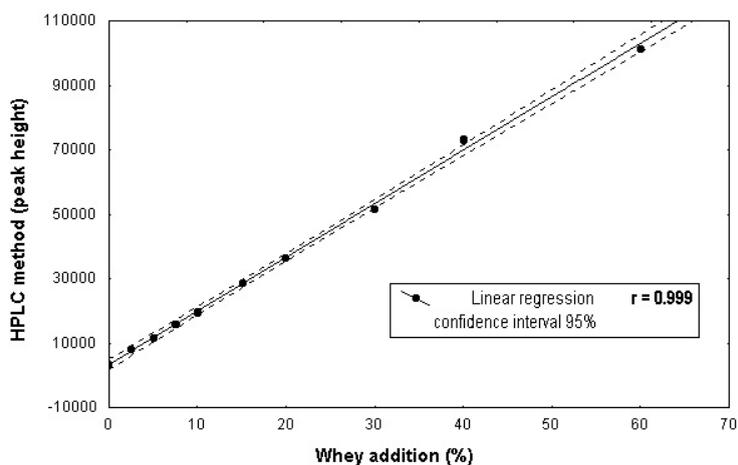


**Figure 3.** Correlation between rennet whey addition to milk and the results obtained by the acidic ninhydrin method (spectrophotometric determination at 470 nm) with the 120 g·L<sup>-1</sup> TCA final concentration.

**Table I.** Values obtained with acidic ninhydrin method (absorbance at 470 nm) in raw milk samples and rennet whey milk added samples with 80, 100 and 120 g·L<sup>-1</sup> trichloroacetic acid (TCA) final concentration.

TCA g·L <sup>-1</sup>	Whey addition g·L <sup>-1</sup>	Absorbance at 470 nm*						Average	Relative s.d.** %
		Experiments							
		I	II	III	IV	V	VI		
80	0 Raw Milk	0.386	0.254	0.316	0.307	0.355	0.353	<b>0.328</b>	± 14.1
	25	0.439	0.312					<b>0.375</b>	± 23.9
	50	0.475	0.336	0.435	0.428			<b>0.418</b>	± 14.1
	75					0.543	0.575	<b>0.559</b>	± 4.0
	100	0.543	0.458	0.571	0.579			<b>0.538</b>	± 10.3
	150					0.762	0.664	<b>0.713</b>	± 9.7
	200	0.738	0.652	0.796	0.760			<b>0.736</b>	± 8.3
	300					1.127	0.974	<b>1.050</b>	± 10.3
	400			1.245	1.116			<b>1.180</b>	± 7.7
100	600					1.875	1.576	<b>1.725</b>	± 12.2
	0 Raw Milk	0.235	0.153	0.202	0.188	0.233	0.233	<b>0.207</b>	± 15.9
	25	0.272	0.205					<b>0.238</b>	± 19.9
	50	0.327	0.234	0.301	0.297			<b>0.290</b>	± 13.6
	75					0.405	0.411	<b>0.408</b>	± 1.0
	100	0.397	0.313	0.437	0.411			<b>0.389</b>	± 13.8
	150					0.552	0.549	<b>0.550</b>	± 0.4
	200	0.540	0.462	0.613	0.566			<b>0.545</b>	± 11.6
	300					0.834	0.756	<b>0.795</b>	± 6.9
120	400			0.917	0.891			<b>0.904</b>	± 2.0
	600					1.384	1.239	<b>1.311</b>	± 7.8
	0 Raw Milk	0.183	0.112	0.151	0.164	0.169	0.192	<b>0.162</b>	± 17.5
	25	0.204	0.140					<b>0.172</b>	± 26.3
	50	0.240	0.164	0.229	0.237			<b>0.217</b>	± 16.6
	75					0.347	0.330	<b>0.338</b>	± 3.5
	100	0.266	0.220	0.292	0.324			<b>0.275</b>	± 16.0
	150					0.439	0.466	<b>0.452</b>	± 4.2
	200	0.366	0.316	0.450	0.437			<b>0.392</b>	± 16.1
120	300					0.709	0.608	<b>0.658</b>	± 10.8
	400			0.685	0.695			<b>0.690</b>	± 1.0
	600					1.103	0.996	<b>1.049</b>	± 7.8

\* Average of duplicates; \*\* standard deviation.



**Figure 4.** Correlation between rennet whey addition to milk and the results obtained by the HPLC method (height of peaks determination) with the  $80 \text{ g}\cdot\text{L}^{-1}$  TCA final concentration.

**Table II.** Values obtained with high performance liquid chromatography (HPLC) method in raw milk samples and rennet whey milk added samples with  $80 \text{ g}\cdot\text{L}^{-1}$  trichloroacetic acid (TCA) final concentration.

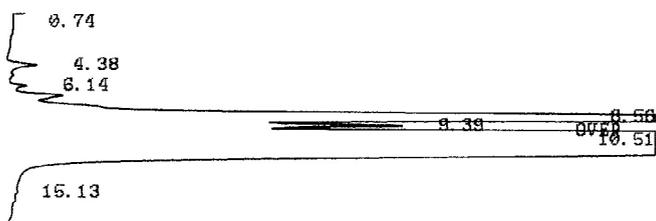
TCA $\text{g}\cdot\text{L}^{-1}$	Whey addition $\text{g}\cdot\text{L}^{-1}$	Absorbance at 470 nm*						Average	Relative s.d.** %
		Experiments							
		I	II	III	IV	V	VI		
80	0 Raw Milk	4767	2817	2925	3179	2774	3696	<b>3 359</b>	$\pm 22.9$
	25	8266	7608					<b>7 937</b>	$\pm 5.9$
	50	11175	12229	11686	11026			<b>11 529</b>	$\pm 4.7$
	75					15400	16377	<b>15 889</b>	$\pm 4.3$
	100	17107	20998	20917	19311			<b>19 583</b>	$\pm 9.3$
	150					26809	30276	<b>28 542</b>	$\pm 8.6$
	200	30249	40384	39167	35909			<b>36 427</b>	$\pm 12.4$
	300					50837	52401	<b>51 619</b>	$\pm 2.1$
	400			76569	70217			<b>73 393</b>	$\pm 6.1$
	600					105094	102095	<b>103 594</b>	$\pm 2.0$

\* Average of duplicates; \*\* standard deviation.

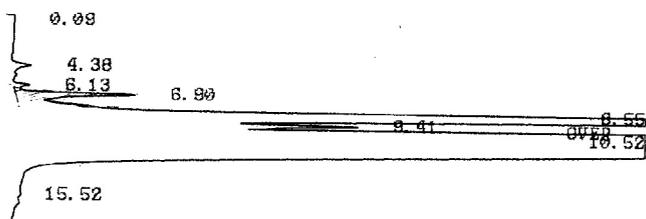
This means that all of the variations in the CMP contents detected by the HPLC had a correspondence of high confiability with the content of sialic acid obtained by spec-

trophotometry, even considering the results with a smaller correlation index ( $120 \text{ g}\cdot\text{L}^{-1}$  TCA). It was verified that the determination coefficient between the techniques was 96%

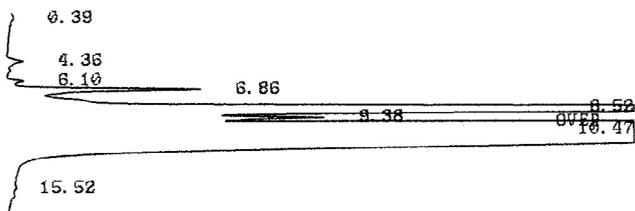
a) Milk



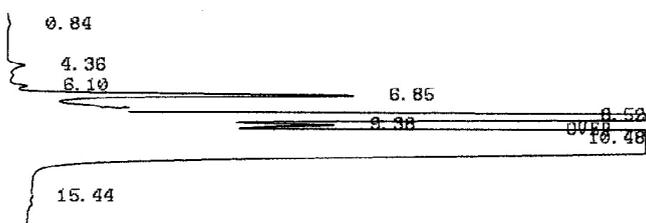
b) Milk and 5% of whey



c) Milk and 10% of whey



d) Milk and 20% of whey

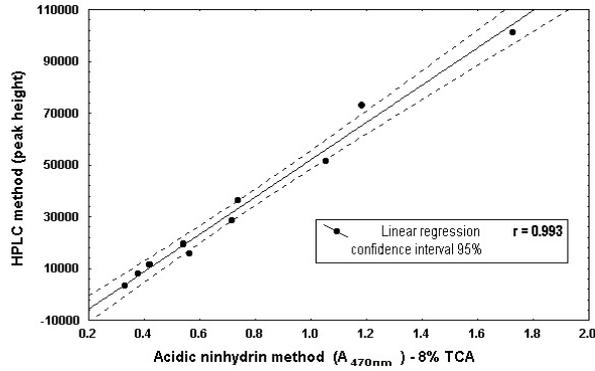


**Figure 5.** Detection of GMP by HPLC of normal milk and with several levels of whey added to milk.

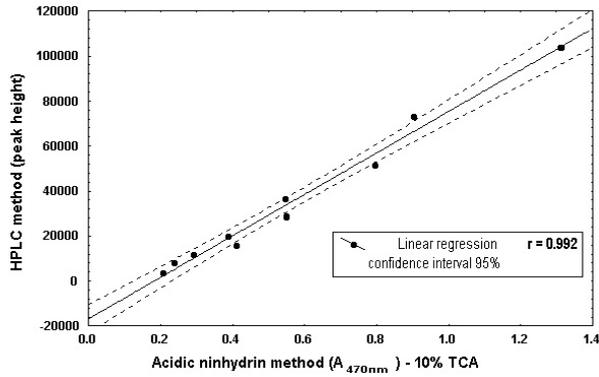
of the results. The equations for the straight line obtained were  $HPLC = -20478.9 + 73033.2 \times A_{470 \text{ nm}}$ ,  $HPLC = -16687.3 + 92024.6 \times A_{470 \text{ nm}}$  and  $HPLC = -14152.2 +$

$112007.2 \times A_{470 \text{ nm}}$ , respectively, for 80, 100 and 120  $\text{g}\cdot\text{L}^{-1}$  final TCA concentrations.

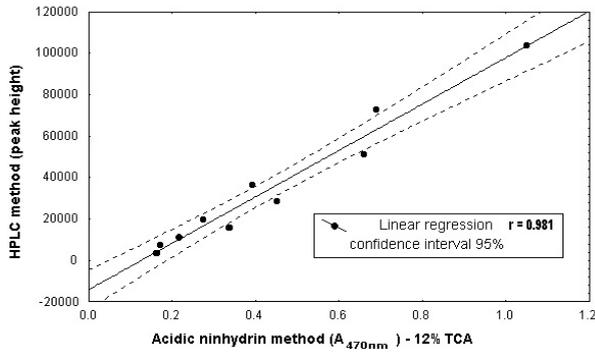
As milks from different places were used in the many experiments, the correlation



**Figure 6.** Correlation between the acidic ninhydrin method using the 80 g·L<sup>-1</sup> TCA final concentration and HPLC in the evaluation of rennet whey addition to milk.



**Figure 7.** Correlation between the acidic ninhydrin method using the 100 g·L<sup>-1</sup> TCA final concentration and HPLC in the evaluation of rennet whey addition to milk.



**Figure 8.** Correlation between the acidic ninhydrin method using the 120 g·L<sup>-1</sup> TCA final concentration and HPLC in the evaluation of rennet whey addition to milk.

between the two methodologies can be considered quite reliable, even considering the observations of Guinee and Wilkinson [8] concerning the heterogeneity of the  $\kappa$ -casein, its 2 genetic variants, A and B, and the content of carbohydrates in the molecule, that can vary by up to 15%.

For developing countries of continental dimensions, where sample remittance to the government network of laboratories is difficult, and where there are not always enough laboratories to meet the demand, the acidic ninhydrin methodology can be used as "screening" methodology by the government's milk industry control services. Other methods, such as HPLC and imported kits from other countries for determination of sialic acid, are either expensive and/or require specialized personnel to set up and perform such methods.

#### 4. CONCLUSION

The results obtained in this experiment allow us to conclude that the acidic ninhydrin method can be considered either as a screening methodology or as a substitute for HPLC methodology for the detection of whey addition to milk, leaving the HPLC, an onerous process that demands qualified personnel, for the doubtful cases or double-checking proofs.

The results obtained with the spectrophotometric methodology also allow us to suggest the modification of the Fukuda [4] methodology for a final TCA concentration of 80 g·L<sup>-1</sup>.

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