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COMPARATIVE STUDIES ON SOME ANALYTICAL METHODS
Thermal decomposition of powder milk

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Abstract

This work present comparative results on powder milk storage quality, obtained from analytical methods. Protein content was determined conventional (Kjeldahl) and colorimetric with biuret reagent at 540 nm and integral quality by thermogravimetric and biological methods. A method was developed for the protein separation of powder milk. Powder milk was submitted to degradation processes at 45, 60 and 80°C for 20 days. The results indicated that protein content values were inconsistent if determinations by Kjeldahl and colorimetric methods and biological tests were compared. There is evidence of thermal decomposition of powder milk as detected by biological and thermogravimetric methods.

Keywords: analytical methods, kinetics, powder milk, temperature

Introduction

Foodstuffs analysis is realized by conventional methods which are generic and may not detect adulteration and decomposition of the product, leading to low quality of food products. Food thermal processing that produce structural chemical changes can modify the functionality, nutritional quality or safety of the food.

Depending on temperature, the different constituents of milk can undergo decomposition reactions during processing and storage [1–3]. The paper presents experimental results comparison between analytical, spectrophotometric, Kjeldahl, thermogravimetric and biological methods in the determination of whole powder milk submitted to the thermal decomposition.

Experimental

Whole powder milk was acquired in the retail shops in the city of João Pessoa, Paraíba, Brazil, and were produced recently. Reagents and solvents of analytical grade were used and the other products were commercial grade.

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Protein separation

A method of protein separation from other milk constituents was developed, using an aliquot of 0.25 g of the whole powder milk which it was transferred to a centrifuge tube and solubilized with 3.0 mL of 0.1 *N* sodium hydroxide solution. It was stirring mechanically at 1800 rpm for 15 s. To this solution was added with stirring 1.0 mL of 1.0 *M* citric acid solution followed by the addition of 4.0 mL distilled water and stirred for 15 s at 1800 rpm and centrifuged for 5 min at 7000 rpm. The precipitate was isolated and solubilized with 4.0 mL of 0.1 *N* sodium hydroxide with mechanical stirring. The fats were extracted by the addition of 2.0 mL of ethyl ether and the ethereal phase separated for centrifugation 7000 rpm for 5 min and collected with an eye dropper. The aqueous fraction was transferred to a 25.0 mL volumetric flask and its volume made up with 0.1 *N* sodium hydroxide. The final solution was filtered.

Thermal decomposition of protein

Powder milk samples weighing exactly 0.2500 g were stored in a penicillin type flask, wrapped in aluminum paper. The samples were distributed in three different incubators with temperatures 45, 60 and 80°C and collected after 1, 2, 4, 6, 8, 10, 15 and 20 days of exposure under different degradation conditions.

Powder milk analysis

The proteins of milk were separated by the above mentioned method and analysed by spectrophotometry in the Spekol 11 of Carl Zeiss-Jena using colorimetric reagent biuret at 540 nm [4].

The samples submitted to zero time and 20 days of exposition in the temperatures of 45, 60 and 80°C were analysed by Kjeldahl Method [5].

Thermogravimetric curves were obtained with a SHIMADZU thermobalance, model TGA-50, operating with air flow of 50 mL min^{-1} in the temperature interval of 25–900°C and a heating ratio of 10°C min^{-1}. Isothermal TG were conducted at 45, 60 and 80°C during 60 min.

The rate constants were determined by the isothermal method in the temperatures of 45, 60 and 80°C, using Arrhenius equations.

The biological assay [6] was realized employing Wistar rat, young after weaning, utilizing ration containing 90% of whole powder milk without thermal treatment (control) and with exposure to temperatures of 45, 60 and 80°C for 20 days (tests). The other 10% was completed with corn starch. The rats were weighed after experimental nourishment for 28 days.

Results and discussions

The results obtained with various analytical methods showed significant differences between the four methods.

Spectrophotometric and Kjeldahl methods

The whole powder milk analysed by spectrophotometry using biuret colorimetric reagent biuret is presented in Table 1 showing very small variations in the protein contents for the temperatures of 45 and 60°C during 20 days. At the temperature of 80°C however the apparent amount of protein increases significantly between the first and eighth day, stabilizing in the following days. The increase in the protein contents at 80°C could be explained by decomposition of the secondary and tertiary structures [7] of protein leading to dipeptide bond free to form the complex dipeptide-Cu(II) and increasing the absorbance in 540 nm characteristic of the Cu-complex. In the subsequent days primary structure breaks will occur decreasing the quantity of complex formed.

Table 1 Protein contents in the whole powder milk submitted to heat by spectrophotometric and Kjeldahl methods

Time/days	Protein contents/%					
	Spectrophotometry			Kjeldahl		
	45°C	60°C	80°C	45°C	60°C	80°C
0	28.7	28.7	28.7	24.3	24.3	24.3
1	27.4	26.6	34.3			
2	27.3	26.3	43.2			
4	28.0	28.1	85.0			
6	27.4	27.8	31.7			
8	28.8	29.8	18.5			
10	28.8	29.7	23.9			
15	29.1	30.8	23.9			
20	30.1	32.6	25.8	24.1	24.4	24.2

The samples analysed by the Kjeldahl method show similar protein contents for whole powder milk irrespective of whether if had been submitted to thermal decomposition (Table 1).

Thermogravimetric method

The thermogravimetric curve shows fundamental data that can be used for the analysis of whole powder milk subjected to heating. Figure 1 illustrates the thermoanalytical profile of decomposition of the powder milk. In the first step is attributed water loss and the other steps to reactions of milk constituents: protein, carbohydrates, lipids and microconstituents. The moisture and ash contents determined by thermogravimetric curve were, respectively, 3.40 and 5.22%.

The rate constants were calculated by equations:

first order reaction:

$$\Delta[P]/\Delta(t) = K_1[P] \quad (1)$$

second order reaction

$$-\Delta[P]/\Delta(t) = K_2[P]^2 \tag{2}$$

where P is the concentration in mg and t the time in min.

The kinetic studies using the isothermal method at temperatures of 45, 60 and 80°C show three reactive processes of protein denaturation that occur with time, resulting in K_1, K_2 and K_3 for each temperature (Table 2). The first step, K_1, log$m$ *vs.* t shows linear dependence revealing a first order reaction. The second and third steps, K_2 and K_3, show linear dependence of $1/m$ *vs.* t typical for a reaction with second order kinetics. These results are in agreement with data obtained for denaturation studies in DSC of serum albumin [8] that showed three thermal processes.

The residual milk contents were determined by the following equations:

first order reaction

$$\log[P] = -K_1/2.3t + \log[P]_o \tag{3}$$

second order reaction

$$1/[P] = K_2t + 1/[P]_o \tag{4}$$

where $[P]$ is final concentration (mg); K is reaction rate constant; t is the time in min and $[P]_o$ is the initial concentration.

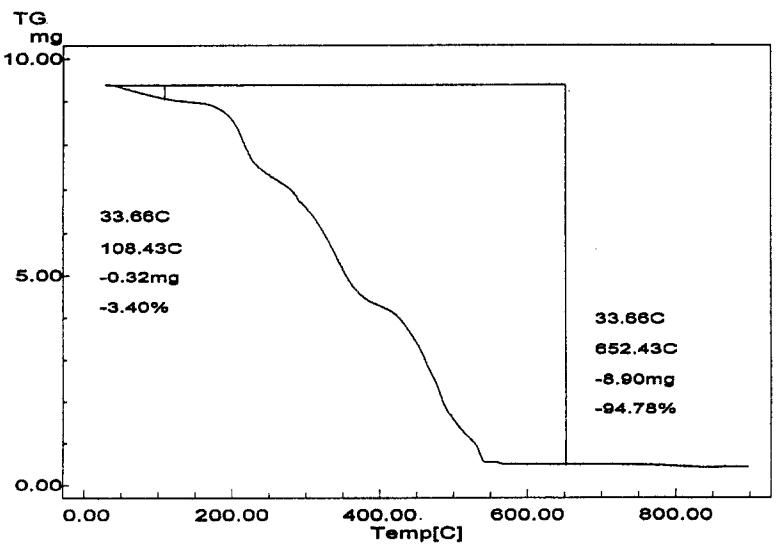

Fig. 1 TG curves of the thermal decomposition of the whole powder milk. Measurements were performed using Shimadzu thermobalance, Model TGA-50. Samples (9–11 mg) were heated at a rate of 10°C min^{-1} in a dynamic air atmosphere

Table 2 Reaction rate constants of thermodecomposition isothermal and protein residual contents of whole powder milk submitted to different temperatures

T/	Rate constants			Milk residual/
°C	K_1	K_2	K_3	%
45	0.5513E-05	0.6040E-06	0.1654E-07	85.3
60	2.3920E-05	1.3321E-06	1.2278E-07	50.2
80	6.8980E-05	2.2900E-06	3.7079E-07	13.7

Biological method

The rats submitted to experimental nourishment for 28 days with rations containing whole powder milk treated at temperatures of 45, 60 and 80°C for 20 days showed a growth rate in weight of 135.6, −15.1 and −28.7%, respectively (Fig. 2) which is lower than the value obtained with the rats on a control ration, 226.2%. These findings reveal that the thermodecomposition of whole powder milk in the temperatures of 45, 60 and 80°C produces chemical deterioration which is probably caused by Maillard reaction [9] with consequent loss of nutritional quality due: a) destruction of the essential aminoacids, b) digestibility diminution, and c) anti-nutritional and toxics compounds production [7].

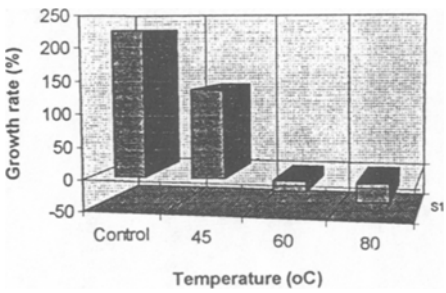

Fig. 2 Effects of thermal decomposition of the whole powder milk on the growth rate of Rattus wistar

The utilization of the rate constants, Table 2, for the calculation of protein not denaturated present results coherent with those obtained by the biological method. At temperature of 45°C the reaction occurs slowly, leading to intact residual mass of 85.5% after 20 days of the exposition in same temperature. At 60 and 80°C the degradative process is fast, resulting in formation anti-nutritional and toxics products. This can explain the development of the rats submitted to the experimental nourishment with ration containing whole powder milk in the following order control >45>60>80°C.

The comparison of the results of chemical analysis protein in samples by spectrophotometric and Kjeldahl methods with data of the biological method show to be incoherent. The results of thermogravimetric and biological methods were compared presenting coherence between rate constant of thermodecomposition of whole

powder milk and nutritional value. The residual milk contents and growth rate of the rats confirm the relation between the last two methods.

The data analyse reveal that spectrophotometric and Kjeldahl methods are not satisfactory to determine quantitatively protein content of whole powder milk submitted to thermal decomposition process, while thermogravimetric and biological methods were coherent and could be correlated which shows their viability for studies of thermal decomposition whole powder milk.

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