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Spectrophotometric assay using *o*-phthaldialdehyde for determination of reactive lysine in dairy products

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The applicability of a simple and rapid spectrophotometric assay using *o*-phthaldialdehyde (OPA) to determine reactive lysine in dairy products was evaluated. For this purpose the values of reactive lysine of model systems and dairy products obtained by this procedure were compared with those obtained by the 1-fluoro-2,4-dinitrobenzene (FDNB) method in dialyzed and non-dialyzed samples. As a reference the latter method was applied to dialyzed samples to avoid interference of sugars. The results obtained showed that the OPA procedure is reliable for use even in the presence of high levels of sugars and can also be used to evaluate lysine damage after Maillard-type reactions.

INTRODUCTION

The results obtained with most chemical methods for reactive lysine analysis are seriously affected by the presence of high levels of carbohydrates (Posati *et al.*, 1972; Hall *et al.* 1973; Hall & Henderson, 1979). Furthermore, only a few methods give acceptable estimates when reactive lysine is inactivated by Maillard-type reactions in food proteins.

The FDNB procedure is considered useful for monitoring reactive lysine and sometimes has been selected as a standard reference method (Hurrell & Carpenter, 1974, 1981; Erbersdobler & Anderson, 1983). However, it is fairly complicated, taking about 20 hours per assay and requires special precautions. Dialysis of carbohydrate-rich samples is recommended since it avoids the uncertainty inherent in applying correction factors for reaction interference, but then each assay requires two or three days more (Tomarelli *et al.*, 1985). So it is of interest to find a new method that is rapid and simple enough to be used routinely without sacrificing reliability and is applicable to carbohydrate-rich foods.

Goodno *et al.* (1981) devised a fluorometric assay, using *o*-phthaldialdehyde (OPA) to estimate reactive lysine in proteins, which was used by Swaisgood and Catignani (1985) in modified milk proteins. This method applied the fluorescence reaction for amino acids developed by Roth (1971). The same reaction was applied by Svedas *et al.* (1980) for the development of a spectrophotometric assay for the evaluation of amino acids, and by Church *et al.* (1983) to determine proteolysis in milk proteins.

The aim of this work was to show that the OPA spectrophotometric method could be used to estimate reactive lysine in a variety of dairy carbohydrate-rich products, before and after 'Maillard-type' reactions. Though the fluorometric assay is more sensitive than the spectrophotometric method, the latter was chosen because of the effect of the fluorescence quenching from peptide bonds (Church *et al.*, 1983) and because fluorimeters, unlike spectrophotometers, are less readily available laboratory equipment.

MATERIALS AND METHODS

Sample preparation

A model system containing 5 g each of casein, lactose and sucrose and 2·5 g of pH 6·5 phosphate buffer solution was used to prepare four samples which were packed in airtight glass containers and heated under the following conditions: sample 1 (unheated), sample 2 (60°C for 20 h), sample 3 (100°C for 1 h) and sample 4 (120°C for 45 min).

Pasteurized milk, sweetened condensed milk and milk powder were purchased at the local market. 'Dulce de leche', an Argentine confectionery, was prepared by heating a mixture of 100 ml of reconstituted milk powder and 20 g of sucrose in an open vessel with constant stirring at 121±2°C for 2 h, in an oil bath. One millilitre of 10% $NaHCO_3$ solution was added to avoid protein coagulation.

Part of each sample was dialyzed against water at 4°C for 48 h (dialysis tubing, 12 000 MW), and immediately analysed.

Nitrogen determination

Total nitrogen in samples before and after dialysis were determined by the macro-Kjeldahl method (AOAC, 1980).

FDNB reactive lysine determination

The procedure of Carpenter (1960), as revised by Booth (1971), was the method employed for comparison with the OPA method.

OPA reactive lysine determination

Samples were dissolved in the following solutions: sample 1 in pH 9·0 sodium tetraborate buffer solution; samples 2 and 3 in 1% sodium dodecylsulfate (SDS) solution; sample 4 in 2% SDS; liquid milk, sweetened condensed milk and milk powder in 10% SDS; and 'dulce de leche' in 15% SDS.

The OPA reagent was prepared essentially as described by Goodno *et al.* (1981). The following compounds were diluted to 100 ml with water: 80 mg OPA (dissolved in 2 ml 95% ethanol); 50 ml of 0·1 M sodium tetraborate buffer solution, pH 9·7–10·0; 5 ml of 20% SDS; and 0·2 ml of 2-mercaptoethanol. This reagent was prepared daily. To determine reactive lysine, 50 μl of sample solution, containing 50–500 μg protein were added to 2·0 ml of OPA reagent; the solution was stirred briefly and incubated for 2 min at 25°C. The absorbance was measured at 340 nm with a Carl Zeiss PM QII spectrophotometer and plotted against a standard curve that was prepared from purified casein dissolved in pH 9·0 sodium tetraborate buffer solution to give a range of concentration from 1·0–10·0 mg ml^{-1}.

The presence of small peptides, amino acids and amines was tested for by analysis of the supernatant before and after treatment with 10% tricholoroacetic acid (TCA) solution (Goodno *et al.*, 1981). For this purpose all samples were dissolved in pH 9·0 sodium tetraborate buffer solution.

Statistical analysis

The standard curve data were analysed by linear regression according to the method of least squares. FDNB determinations were performed in triplicate, OPA determinations were analysed in nine replicates, and coefficients of variation for each sample were calculated. The analytical values of OPA and FDNB determinations were submitted to analysis of variance (ANOVA). Fisher's least significant differences were calculated to determine differences between means.

RESULTS AND DISCUSSION

The standard curve data were linear over the range used for determination (r = 0·9997). The mean coefficient of variation for the OPA method was calculated as 2·8%, based on data of the 16 samples analysed (nine replicates). This showed that the proposed method was quite reproducible for measuring reactive lysine in dairy samples. The heating conditions of four model samples and the dairy products were chosen to obtain different grades of reactive lysine damage.

The analytical results are summarized in Table 1. The values of reactive lysine obtained by applying the FDNB method to dialyzed samples were taken as reference. From the values obtained using the FDNB method, it can be seen that those before dialysis were significantly (p >0·05) lower than after dialysis. This loss of reactive lysine in non-dialyzed samples (9–22%)

Table 1. Reactive lysine values (g per 16 g N) by OPA and FDNB methods in a model system and dairy foods with and without dialysis

	Non-dialyzed samples		Dialyzed samples	
	OPA (SD)[a]	FDNB (SD)	OPA (SD)	FDNB (SD)
Sample 1	8·2 (0·16)	7·3 (0·09)	8·2 (0·10)	8·4 (0·12)
Sample 2	7·0 (0·21)	6·5 (0·15)	7·1 (0·15)	7·1 (0·08)
Sample 3	6·2 (0·17)	5·0 (0·09)	6·3 (0·18)	6·1 (0·05)
Sample 4	4·6 (0·11)	4·0 (0·04)	4·5 (0·03)	4·6 (0·03)
Pasteurized milk	8·9 (0·19)	6·9 (0·04)	8·8 (0·19)	8·5 (0·16)
Milk powder	8·0 (0·09)	6·5 (0·07)	8·0 (0·15)	7·7 (0·04)
Condensed sweetened milk	7·4 (0·12)	6·4 (0·13)	7·3 (0·18)	7·1 (0·05)
'Dulce de leche'	6·0 (0·13)	4·7 (0·11)	5·9 (0·15)	6·1 (0·02)

[a] Standard deviation of means.

could be attributed to the presence of high levels of sugars. Therefore, the reactive lysine is underestimated by the FDNB method when sugars are present. The results obtained with the OPA method in samples with and without dialysis were not significantly different (p <0·05). Thus sugars, even present in high levels, do not seem to be a problem with the OPA method.

The TCA test detected negligible quantities of small peptides, whose α-amino groups could provide a considerable error in the estimation of reactive lysine if they were present at appreciable levels.

Comparison of the OPA method before dialysis and the FDNB method after dialysis showed no significant differences (p <0·05). Thus, the OPA method, which is a simple and rapid procedure, can be applied even in the presence of high levels of sugars, with similar results to those obtained by the FDNB method in samples free of carbohydrates.

Furthermore, the samples in which early Maillard compounds would be expected to be present—sample 2, sweetened condensed milk and milk powder—and samples in which advanced Maillard reactions had clearly taken place—sample 3, sample 4 and 'dulce de leche'—showed good agreement between the results by FDNB after dialysis and the OPA method (p <0·05). Thus the latter could also be considered to be a reliable method for evaluating lysine damage after 'Maillard-type' reactions.

The OPA method for estimating reactive lysine in dairy products has several advantages compared with other chemical procedures. Sugars do not interfere, small quantities of sample are needed, the manipulations are simple, there is no severe treatment (hydrolysis of the protein, heating or solvent extraction) and it can be performed in short time. It is shown to be reproducible and as accurate as the FDNB method after dialysis in dairy products. On the other hand, because the protein must be soluble and absorbance from α-amino groups cannot be higher than 10% of total absorbance, the application of the OPA method to other food products must be carefully considered.

REFERENCES

AOAC (1980). *Official Methods of Analysis* (13th edn). Association of Official Analytical Chemists, Washington, DC.

Booth, V. H. (1971). Problems in the determination of FDNB-available lysine. *J. Sci. Food Agric.* **22**, 658–66.

Carpenter, K.J. (1960). The estimation of the available lysine in animal protein foods. *Biochem. J.*, **77**, 604–10.

Church, F. C., Swaisgood, H. E., Porter, D. H. & Catignani, G. L. (1983). Spectrophotometric assay using *o*-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins. *J. Dairy Sci.*, **66**, 1219–27.

Erbersdobler, H. F. & Anderson, T. R. (1983). Determination of available lysine by various procedures in Maillard-type products. *ACS Symp. Ser. 215 (Maillard React. Foods Nutr.)*, pp. 419–27.

Goodno, C. C., Swaisgood, H. E. & Catignani, G. L. (1981). A fluorimetric assay for available lysine in proteins. *Anal. Biochem.*, **115**, 203–11.

Hall, R. J. & Henderson, K. (1979). An improvement in the determination of available lysine in carbohydrate-rich samples. *Analyst*, **104**, 1097–1100.

Hall, R. J., Trinder, N. & Givens, D. I. (1973). Observations on the use of 2,4,6-trinitrobenzene sulfonic acid for the determination of available lysine in animal protein concentrates, *Analyst*, **98**, 673–86.

Hurrell, R. F. & Carpenter, K. J. (1974). Mechanisms of heat damage in proteins: 4. The reactive lysine content of heat damaged material as measured in different ways. *Br. J. Nutr.* **32**, 589–604.

Hurrell, R. F. & Carpenter, K. J. (1981). The estimation of available lysine in foodstuffs after Maillard reactions. *Progr. Food Nutr. Sci.*, **5**, 159–76.

Posati, L. P., Holsinger, V. H., De Vilbiss, E. D. & Pallansch, M. J. (1972). Factors affecting the determination of available lysine in whey with 2,4,6-trinitrobenzene sulfonic acid. *J. Dairy Sci.*, **55**, 1660–5.

Roth, M. (1971). Fluorescence reaction for amino acids. *Anal Chem*, **43**, 880–2.

Svedas, V. J. K., Galaev, I. J., Borisov, I. L. & Berezin, I. (1980). The interaction of amino acids with *o*-phthaldialdehyde: a kinetic study and spectrophotometric assay of the reaction product. *Anal. Biochem.*, **101**, 188–95.

Swaisgood, H. E. & Catignani, G. L. (1985). Digestibility of modified milk proteins: nutritional implications. *J. Dairy Sci.*, **68**, 2782–90.

Tomarelli, R. M., Yuhas, R. J., Fisher, A. & Weaber, J. R. (1985). An HPLC method for the determination of reactive (available) lysine in milk and infant formulas. *J. Agric. Food Chem.*, **33**, 316–18.