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Characterisation of milk samples with various whey protein contents by pyrolysis–mass spectrometry (Py–MS)

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Abstract

Different milk samples and their 2, 5 and 10% mixtures with 'artificial' or natural whey protein were analysed by means of pyrolysis–mass spectrometry (Py–MS). Py–MS followed by multivariate analysis of the resulting mass spectra enabled the determination of the whey protein addition in milk samples. Obtained results showed that this determination seems not to be influenced by the fat content and freezing of the samples, as well as a dilution of the samples.

Keywords: Milk; Whey protein; Pyrolysis mass spectrometry; Multivariate analysis

1. Introduction

For a long time milk fat was regarded as the most valuable milk constituent and it has been common practice to standardise its content in milk. By the time interest in milk protein has increased due to its high nutritional quality and physico-chemical and functional properties (Rattray & Jelen, 1996).

Bovine milk contains 3–3.5% protein, about 80% of casein and about 20% of whey protein. The casein fraction can be divided in α_{S1}-, α_{S2}- , β- and κ-casein and it is present as a complex 'micelle' in 4:1:4:1 ratio. β-lactoglobulin and α-lactalbumin are present in a ratio of approximately 3:1 and form the main part of the whey protein (Belitz & Grosch, 1992; Kiermaier & Lechner, 1973).

A number of different methods for the determination of the protein content (Holtzhauer, 1996; Ribadeau-Dumas & Grappin, 1989) in dairy products already exist; electrophoresis (Kim & Jimenez-Flores, 1994; Kunz & Lonnerdal, 1989, 1990), ultraviolet spectroscopy (Clark & Hester, 1986; Demchenko, 1981), high performance liquid chromatography (HPLC) (Diossady, Bergen, & Harwalkar, 1980; Nollet, 1992), polarography (Lechner, 1990), etc. However, analytical problems with processed food stuffs (due to heat denaturation or fermentation) and matrix effects (Nollet, 1992; Reimerdes, 1980) lead to uncertainty of the results and may even hinder the analysis altogether.

Presently, standardisation of the protein content of drinking milk is not allowed (EG Richtlinie,1992; Codex Alimentarius Commission) VO (EWG), 1971, but there is a continuously growing interest in standardisation knowing that the price of milk depends on its protein content, which varies with the season. From the economic point of view it is attractive to substitute milk powder by whey protein (the leftover from cheese production) up to a few per cent (Glaeser, 1994; Hessing, 1994; Weindlmaier, 1997).

In this paper an approach is presented for the application of Py–MS for the direct determination of the whey protein addition in milk samples. Multivariate data analysis is applied for evaluation of the results.

2. Materials and methods

2.1. Samples

A number of different milk samples (fresh milks and UHT milks with fat contents from 0.2 up to 3.6%) from Northern Italy were analysed. All milk samples were purchased from local supermarkets. Two series of each milk sample with 2, 5 and 10% of added whey protein were prepared. For the first series a whey protein

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was composed of a mixture (4:9) of α-lactalbumin and β-lactoglobulin (Sigma/Aldrich). This 'artifical' whey protein was then added in appropriate quantities to each milk. The second series was prepared by mixing each milk with a certain quantity of natural whey protein concentrate (lactalbumin 70 SHG from MILEI). The concentrate contained about 70% of whey protein while the remaining 30% consisted of lactose, water, acids and minerals. The mixtures obtained with the 'artificial' whey protein were used to test the suitability of the method in determining the addition of whey protein in milk and to see the influence—if there is any—of the dilution effect on it. The second series was used to check whether fat content, sample homogeneity or freezing the sample are affecting the results of determination of the addition of whey protein in milk.

2.2. Pyrolysis–mass spectrometry (Py–MS)

Py–MS was performed on a Rapyd-400 (Horizon Instruments Ltd., Heathfield, East Sussex, UK) based on a quadrupole mass analyser and employs Curie-point pyrolysis. One microlitre of milk samples was carefuly applied onto iron–nickel foils (Horizon Instruments) to give a uniform surface coating. The foils were subsequently inserted into small glass tubes (Horizon Instruments) and dried in an oven at 60°C for 10 min. The foils were pushed into the tube using a stainless-steel depth gauge so as to lie 10 mm from the mouth of the tube. Finally, Viton 'O'-rings (Horizon Instruments) were placed on the tubes. Three replicates of each sample were analysed.

The pyrolysis temperature (530°C) was held for 3 s and the resulting pyrolysate was ionised at 25 eV. The mass range was scanned between m/z 50 and 300. Expansion chamber temperature was 160°C while the temperature of the ion source was kept at 200°C.

2.2.1. Data handling

The data from Py–MS are displayed as quantitative pyrolysis mass spectra where the abscissa represents the m/z ratio and the ordinate gives the total ion count for any particular m/z value ranging from 50 to 300.

The normalised data were processed using the GENSTAT package (Nelder, 1991) supplied by the manufacturer of the instrument. The first step was the 'characteristicity' selection. The aim is to choose those peaks in the spectra, masses, which best serve to discriminate between groups. Characteristicity could be defined as the ratio of the between-groups variance to the within-group variance. The calculation is done by first calculating the average value of each 'mass' within each group, and second calculating the variance between the group means. For each mass the within-group variance is averaged across all groups. The between-groups variance is found in a similar way. Once the masses have all been calculated they are all sorted in order of characteristicity, i.e. the ratio of the within-group varaince and the between-group variance. After this, the Py–MS data were subjected to combined principal components/canonical variates analysis (PCCV). First the data will be subjected to principal component analysis (PCA), which is a technique to reduce multivariate data down to a smaller set of variates whilst preserving most of the variance. Only those principal components (PCs) contributing more than 0.1% to the total variance were retained. Details, as well as numerous applications of this method can be found in the literature (Kowalski, 1984; Martens & Naes, 1989; Wolds, 1987). The principal components matrix was then subjected to canonical variates analysis (CVA) (Mac Fie, Gutteridge, & Norris, 1978), also known as discriminant analysis, which seeks to separate the objects (samples) into groups on the basis of the retained PCs and the a priori knowledge of the appropriate number of groupings. The a priori groups here are the known triplicate pyrolysis mass spectra and so do not bias the analysis in any way. The purpose of CVA is to maximise the ratio of the between-group to within-group variance, to clearly display the discrimination.

3. Results and discussion

Pure milk samples and milk samples with added 'artificial' whey protein were analysed by means of Py–MS applying the combined canonical variates analysis (CVA) and principal component analysis (PCA) on the spectra. Fig. 1 represents the scores of the first three principal components (PCs) calculated from the signals of 20 significant masses as determined by CVA. Pure milk (A) was well separated from mixtures of milk with added whey protein (B) as it is shown in Fig. 1.

Eight pure milk samples, produced by different manufacturers, are labelled as 'A'. The samples labelled as 'B' consist of 24 mixtures (2, 5 and 10%) of pure milk with 'artificial' whey protein. The pure milk samples are showing a rather compact cloud while the concentration range of added whey protein within group B causes a dispersion of these samples in Fig. 1.

Knowing that group B (in Fig. 1) contained milk samples with three different concentrations of added whey protein, the possibility of further discrimination inside this group was investigated. Fig. 2 represents the scores of the first three PCs calculated from the signals of 12 significant masses as determined by CVA.

Pure milk samples are again labelled as A while the mixtures were divided into three groups: the samples with 2% of added whey protein were labelled as B, the samples with 5% of added whey protein were labelled as C and the samples with 10% of added whey protein were labelled as D.

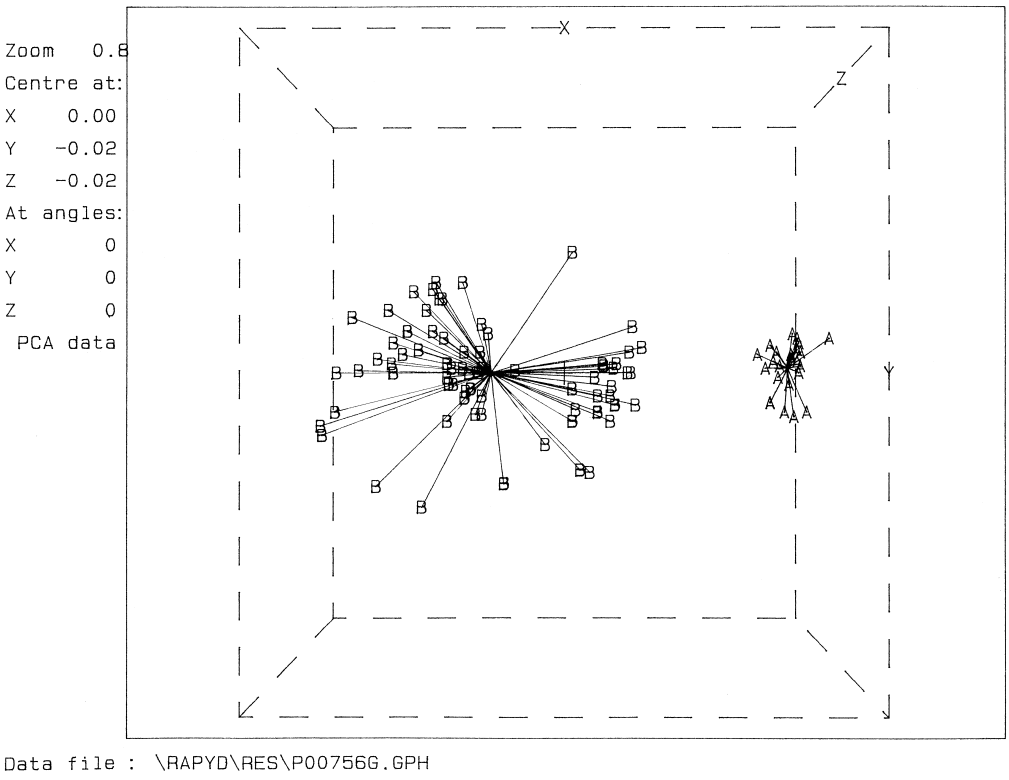

Fig. 1. Py–MS graph showing two groups of analysed milk samples; A: pure milk and B: 2, 5 and 10% mixtures of pure milk with whey protein. Three replicates of each sample were analysed. 3D graph of the score vectors was obtained by the PCA based on the signals of 20 masses.

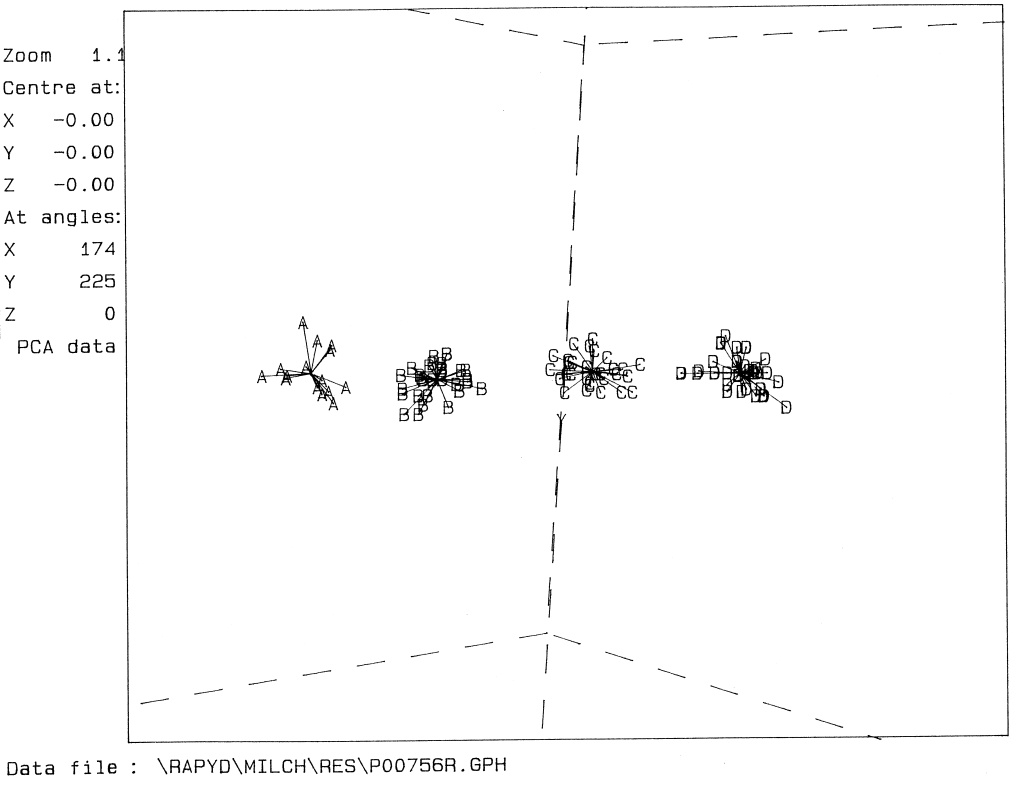

Fig. 2. Py–MS graph showing four groups of analysed milk samples; A: pure milk, B: 2% mixtures, C: 5% mixtures and D: 10% mixtures of pure milk with whey protein. Three replicates of each sample were analysed. 3D graph of the score vectors was obtained by the PCA based on the signals of 12 masses.

Four well separated groups forming four rather compact clouds are visible in Fig. 2. The results given in Fig. 3 indicate, that a further differentiation of the samples regarding the per cent of added whey protein by Py–MS is possible. Moreover, this experiment seems to prove as well that a heat treatment of the milk is not effecting the separation, because all groups consist of samples being prepared from fresh and UHT milk.

The influence of different fat contents on the determination of the concentration of whey protein in milk was investigated. Therefore, milk samples containing fat in a range from 0.2 to 3.6% were analysed. The same amounts of whey protein as above (2, 5 and 10%) were added to all milk samples. Fig. 3 represents the scores of the first three PCs calculated from the signals of 12 significant masses as determined by CVA. Pure milk samples are labelled as A, the 2% mixtures are labelled as B, the 5% mixtures are labelled as C and the 10% mixtures are labelled as D.

The results given in Fig. 3 indicate that a differentiation of the milk samples regarding the per cent of added whey protein is possible even if the fat content in milk is varying.

In order to investigate the influence of storing a sample in the freezer (−20°C) on the determination of the concentration of whey protein in milk, milk samples were kept in a freezer for 1 month. Subsequently the unfrozen samples were analysed by means of Py–MS. Separation, as in Fig. 3, was obtained, showing that this storage temperature does not seem to influence the separation of the milk samples according to different concentrations of whey protein.

In order to determine the influence of the total protein concentration, diluted milk samples and mixtures with whey protein were analysed. Dilutions (1:10 v/v) of three pure milk samples (two full cream and one medium fat milk) as well as of the same milks with added whey protein were prepared and analysed together with the original undiluted samples. For the dilutions pure destillated water was used. Fig. 4 represents the scores of the first three PCs calculated from the signals of 12 significant masses as determined by CVA. Pure milk samples (diluted and undiluted) are labelled as A, while undiluted and diluted 2% mixtures of milk with the whey protein are labelled as B, undiluted and diluted 5 and 10% mixtures as C and D, respectively.

The milk samples are separated in four groups according to the quantity of added whey protein and it seems that the dilution effect has no influence on this separation. Thus it seems that the separation of the samples is due to the added whey protein and not because of the increasing total protein content.

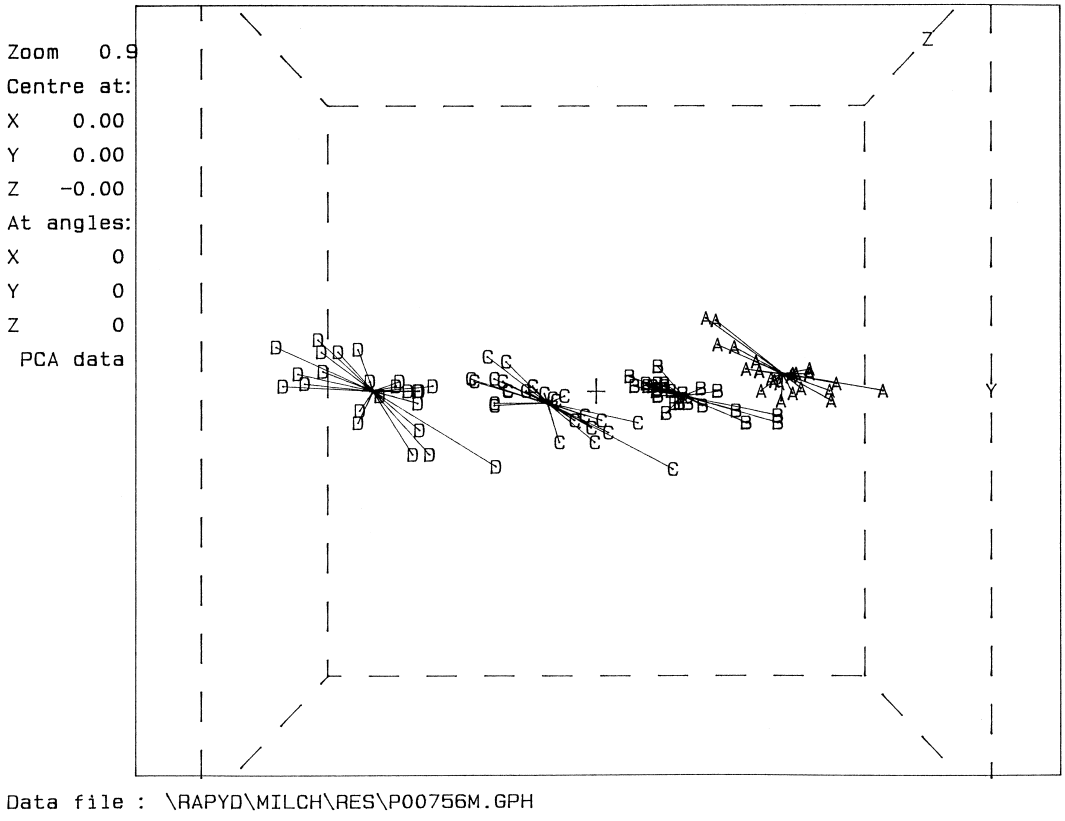

Fig. 3. Py–MS graph of eight milk samples with different fat content; A: pure milk, B: milk with 2% added whey protein, C: milk with 5% added whey protein and D: milk with 10% added whey protein. Three replicates of each sample were analysed. 3D graph of the score vectors was obtained by the PCA based on the signals of 12 masses.

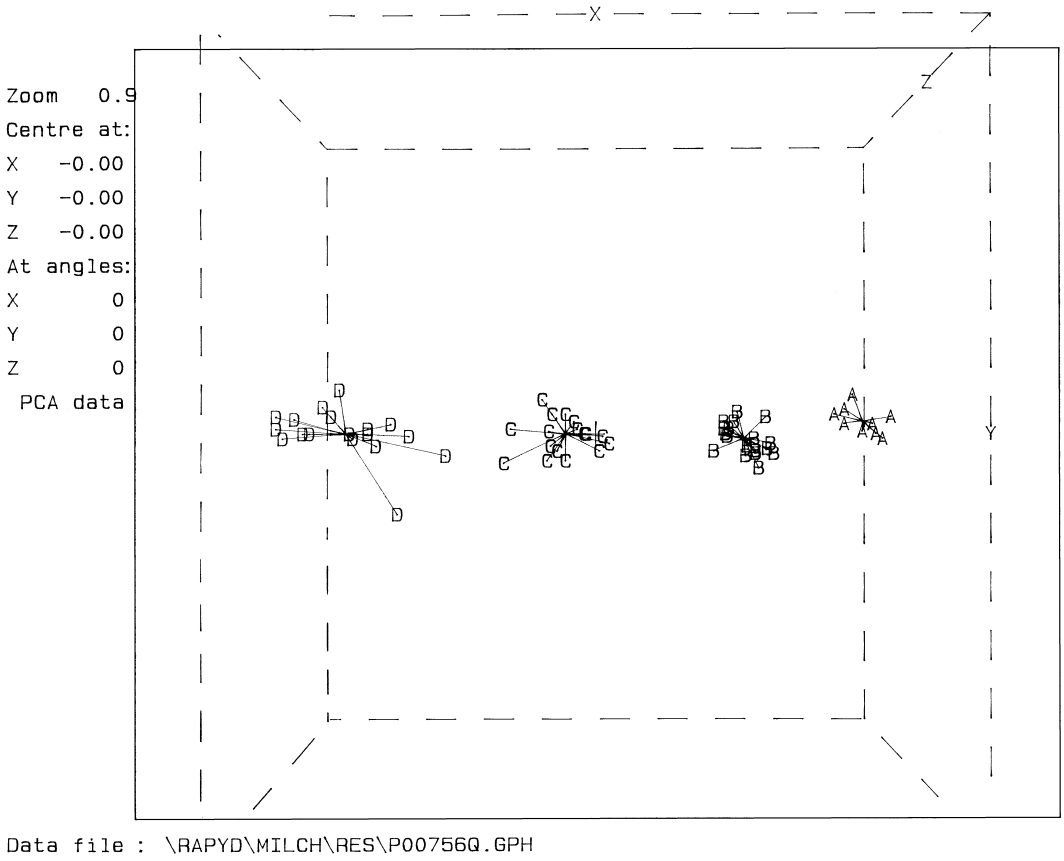

Fig. 4. Py–MS graph showing four groups of analysed milk samples; A: pure milk, B: milk with 2% added whey protein, C: milk with 5% added whey protein and D: milk with 10% added whey protein. Each group consists of diluted (1:10 v/v) and undiluted samples. Three replicates of each sample were analysed. 3D graph of the score vectors was obtained by the PCA based on the signals of 12 masses.

It is obvious that the results are based on a limited number of samples only. However, given the fact that care was taken to make samples using different kinds of milk samples and whey protein concentrates, it is expected that this method will give sufficient discrimination in all mixtures made from similar components.

4. Conclusions

These results suggest that pyrolysis–mass spectrometry provides a fast (analysis time less than 2 min) and versatile method for discrimination of pure milk samples from milk samples with added whey protein. Furthermore, Py–MS seems to be capable of discriminating between different quantities of added whey protein in milk. At the same time, results indicate that neither the fat content, total protein content, nor freezing of the samples are having a major influence on this discrimination.

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