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Effect of hydrogen peroxide on the amino acid composition of the proteins from cheese whey and evaporated milk

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Hydrogen peroxide has been used for the preservation of cheese whey for a long time (1). Preservation of whey is of great value to factories which use it in the manufacture of various products, such as lactose, whey powder and whey protein. Particularly during the past few years the use of whey powder has gained increasing interest for the substitution of skim milk powder in the production of artificial feeding stuffs for calves.

In this connection it is very important that the protein value of the whey powder is not affected by the treatment with hydrogen peroxide, because this would offset the effect of the improved preservation.

As described in the literature (2), hydrogen peroxide can also be used to improve the heat stability of evaporated milk. It might be expected that addition of peroxide combined with sterilization would have a maximum detrimental effect on those amino acids which are susceptible to oxidation, such as methionine, cystine and tyrosine.

In the present work are compared the amino acid compositions of the proteins from cheese whey and evaporated milk, with and without treatment with hydrogen peroxide. The whey powder used in this investigation was produced from Gouda cheese whey, derived from pasteurized milk. A 40% hydrogen peroxide solution was added in the usual amount of 200 ml per 1000 l cheese whey (3). Before evaporation the whey was pasteurized at 72°C for 15 s and concentrated in a falling-film evaporator to approx. 45% total solids at a temperature of 68°C. The concentrated whey was spray-dried at an inlet temperature of 180°C.

Evaporated milk (9% fat, 22% solids-not-fat) was produced as follows. The standardized milk was preheated at 115°C for 6 min. After concentration and standardization the evaporated milk was homogenized at 15 to 3.5 MN/m² at 60°C and sterilized (without addition of phosphate) at 118°C for 13 min.

Peroxide was added before sterilization in a concentration of 0.05% (w/v).

To prevent humin formation during acid hydrolysis, the lactose in the samples of whey powder and evaporated milk had to be removed. The proteins of whey were isolated by precipitation with TCA, as described by de Koning and van Rooijen (4). Evaporated milk samples were centrifuged in a Spinco L2 preparative ultracentrifuge (100,000 *g*). The sediment and the supernatant were treated by TCA as described before. The peroxide content of the stored whey powder was determined by the method of Gilliland (5). Hydrolysis of the protein samples was carried out in vacuo at 110°C for 24 h under the standard conditions described by Moore and Stein (6). Amino acid analyses were performed with a Jeol JLC 5 AH Amino Acid Analyser, with norleucine as an internal standard. The cysteine content of the reduced hydrolysates was determined with a Technicon Auto Analyser, as described by de Koning and van Rooijen (4, 7), and the nitrogen content of the hydrolysates by Kjeldahl analysis.

Table 1. Effect of hydrogen peroxide on the amino acid composition of the proteins from cheese whey (g amino acid/100 g protein)

Amino acid	After treatment with H_2O_2	Without treatment with H_2O_2
Aspartic acid	11.72	11.58
Threonine[2]	7.41	7.50
Serine[3]	6.15	6.17
Glutamic acid	19.74	19.71
Proline	5.91	6.15
Glycine	2.18	2.17
Alanine	5.25	5.22
Half-cystine[4]	2.62	2.54
Valine	6.57	6.49
Methionine	2.08	2.09
Isoleucine	6.43	6.42
Leucine	12.02	11.84
Tyrosine	3.72	3.70
Phenylalanine	3.77	3.66
Lysine	10.38	10.36
Histidine	2.07	2.10
Ammonia	1.74	1.79
Arginine	3.09	3.09

[1] These samples of whey powder were stored for two months at room temperature and contained 24 ppm of hydrogen peroxide. Immediately after production of the whey powder the concentration was 400 ppm.
[2] A correction of 3% was made to compensate for losses during hydrolysis.
[3] A correction of 10% was made to compensate for losses during hydrolysis.
[4] Half-cystine was determined by the method of de Koning and van Rooijen (4, 7).

The amino acid composition of the proteins derived from whey powder is given in Table 1. As appears from this table, close agreement is obtained between the amino acid composition of the samples with and without peroxide treatment. This is particularly true for the amino acids that are susceptible to oxidation, viz. methionine, cystine and tyrosine. Comparison of these amino acids did not show any decrease in concentration such as might have been caused by the peroxide treatment. The same conclusion can be drawn regarding the amino acid composition of the protein fractions derived from evaporated milk (see Table 2). As described before, the samples of evaporated milk were separated into two fractions, namely a sediment-containing (besides casein) part of the coprecipitated whey proteins, and a supernatant containing — in addition to the rest of the whey proteins — peptides split off from casein during sterilization of the evaporated milk (8). Separation of the various milk protein fractions was carried out to investigate their susceptibility to oxidation. Apparently, as can be observed in Tables 1 and 2, the concentration of hydrogen peroxide applied

Table 2. Effect of hydrogen peroxide on the amino acid composition of the casein and whey protein fractions isolated from evaporated milk (g amino acid/100 g protein)

Amino acid	Casein fraction		Whey protein fraction	
	from treated milk sample	from untreated milk sample	from treated milk sample	from untreated milk sample
Aspartic acid	8.20	8.17	9.05	8.83
Threonine[1]	4.64	4.57	5.50	5.46
Serine[2]	6.96	6.89	7.06	6.94
Glutamic acid	25.06	25.24	24.65	24.27
Proline	11.42	11.03	11.12	11.03
Glycine	2.17	2.12	2.17	2.09
Alanine	3.37	3.37	4.15	4.02
Half-cystine[3]	0.34	0.35	0.91	0.88
Valine	6.98	6.74	7.07	6.95
Methionine	2.81	2.86	2.79	2.64
Isoleucine	5.35	5.12	5.70	5.68
Leucine	11.14	10.90	11.40	11.29
Tyrosine	5.90	5.82	5.74	5.75
Phenylalanine	5.48	5.52	5.44	5.17
Lysine	7.62	7.64	7.81	7.56
Histidine	2.86	2.93	2.89	2.91
Ammonia	2.13	1.99	1.94	1.93
Arginine	3.72	3.60	3.78	3.48

[1] A correction of 3% was made to compensate for losses during hydrolysis.
[2] A correction of 10% was made to compensate for losses during hydrolysis.
[3] Half-cystine was determined by the method of de Koning and van Rooijen (4, 7).

in this investigation was too low to cause any difference in amino acid composition. This is in good agreement with the results of Barone and Krett (9), who found that milk treated with 0.05% hydrogen peroxide showed oxidation of methionine to the stage of methioninesulphoxide only. During acid hydrolysis methioninesulphoxide is retransformed to methionine. It is therefore clear that identical results will be obtained in acid hydrolysates if oxidation of methionine has not proceeded further than to methioninesulphoxide. Neither methioninesulphon nor cysteic acid was observed on our chromatograms during amino acid analysis. Consequently, our conclusion was that the effect of peroxide treatment, if it exists, is restricted to the oxidation of methionine to methioninesulphoxide. It is generally accepted, however, that this stage of oxidation has no effect on the protein value (10, 11, 12).

The foregoing leads to the conclusion that treatment of cheese whey and evaporated milk with hydrogen peroxide in a concentration of 0.02 and 0.05% (w/v) respectively did not appear to have any effect on the amino acid composition of the proteins. In addition, a concentration of 0.05% hydrogen peroxide is too low to demonstrate any possible difference in the susceptibility to oxidation between the sedimented casein and the whey protein fraction as isolated from evaporated milk.

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