Optimisation and validation of the protein content in liquid lactoserum by the kjeldahl method

Marie MEJEAN

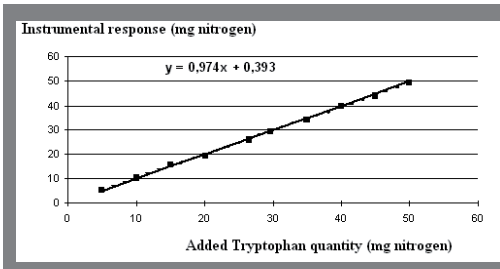
Introduction

The lactoserum is commonly used in the food industry. It is mainly constituted with the liquid fraction by-product of milk coagulation. The lactoserum analyses were performed in the food analysis unit of the Laboratory common service for custom classification purpose.
The nitrogen was measured following the Kjeldahl method. Samples were oxidized with sulfuric acid once a boiling point was reached. A catalyst was also added, in order to decompose the organic proteins into nitrogen and ammonium sulfate. Sodium hydroxyde was then added, and released vapors containing ammoniac were further distilled into a boric acid solution, which was finally titrated with a sulfuric acid solution.
The catalyst and the mineralization duration were chosen so as to give the best results. The distillation apparatus had to be validated following the ISO V03-110 standard: "Analysis of agri-foodstuffs". Trials were performed to optimize and validate the method.

Experimental methods

For this study, a mineralization apparatus (Foss Digestor 2006) was used followed by a distillation apparatus (Foss Kjeltec 2003). The mineralization apparatus contained 6 tubes of 250mLin which the samples were placed, a blank tube with sugar was used and another one with tryptophan (amino acid) to ensure that the mineralization process was properly carried out. Into the tubes the following chemicals were added: sulfuric acid (98%) and 1 of the 3 catalysts used in the laboratory. The first catalyst contained potassium sulphate (94.3%), copper sulphate (2.8%) and titanium oxide (2.8%), the second one selenium (2%), copper sulphate (1.5%) and sodium sulphate (96.5%), and the third one potassium (99.6%) and copper sulphate (0.4%). During the distillation process, boric acid (1%), sodium hydroxyde (32%) and sulfuric acid (0.2N) were used.
The parameters of the mineralization heating, which were recommended by the standard, were to increase heat with by steps, first reaching 200°C and then going up to 420°C.
One major problem encountered with digestion, was the presence of a considerable amount of foam, that disturbed mineralization and the recovery of nitrogen from the sample, as well as allowed for discharge of copper and selenium, which are toxic for aquatic environnment. When the selenium catalyst was utilized, little foam was created; it allowed for the recovery of 53 milligrams of nitrogen per 10 grams. In addition, the selenium quantity that would be disposed of within the wastewater, is between 16.8 and 33.6 gram per year; the environmental enforces regulation only when the level of disposal is above 5 grams a day.

The validation of the method was required to obtain reliable results. The first part demonstrated the linearity, the sensitivity, the detection and the quantification limits, with a range going from 0 to 100 milligrams of nitrogen. 6 trials with 5 repetitions were performed, to verify that the response increased gradually with the introduced quantity, using a pure tryptophan standard.
The second part determined the matrix effect, which could disturb the results (method specificity)
It was performed for 10 trials, by measuring out the addition of introduced tryptophan, within a range from 0 to 100 milligrams of nitrogen, into the lactoserum.



Legend : — Added content : tryptophan $C_{11}H_{12}N_2O_2$
■ Obtained content
(measured :Lactoserum+Tryptophan – initial: Lactoserum)

Graph about the specificity of the method (validation)

Results and conclusion

As seen on the above graph, the curve with the solid line corresponds to the added tryptophan; the curve of dots is the obtained content. Interestingly, both are linear and superimposing. These results show there were not any matrix effects.
The method has been optimized, and the selenium catalyst with the 150 minute mineralization duration was chosen. For practical reasons the mineralization step was rejected. The validation of the method has been accomplished.

Service commun des Laboratoires
10, Avenue des Saules, BP 74
69922 Oullins