At the meeting of the Management Committee on 22 May 1978, the representative of Spain handed to the secretariat the following communication. This communication, which comprises a note explaining the method used for analyzing milk powder denatured with blood flour and non-deodorized fish flour or fish solubles, is circulated for the information of delegations participating in the work of the Management Committee.

ANALYSIS OF MILK POWDER DENATURED WITH BLOOD FLOUR AND NON-DEODORIZED FISH FLOUR OR FISH SOLUBLES, IN ACCORDANCE WITH CIRCULAR NO. 789 OF THE GENERAL DIRECTORATE OF CUSTOMS

1. Determination of blood flour content in milk powder

1.1 Basis of the method

The determination is made by ultra-violet spectrometry, by measuring the specific absorbency of haemoglobin at 396 n.m. in solutions prepared for this purpose. In the first place, the absorption coefficient is determined for blood flour in solution at certain concentrations, although Beer's law applies to a broad range of concentrations (0.040-0.200 gr./litre) and once the coefficient is known, the concentrations are calculated by applying to the usual methods of absorption spectrometry.

1.2 Determination of the absorption coefficient of blood flour in solution

Three blood flour samples of approximately 100 mg. each are weighed and then each of them is brought up to a volume of 250 ml. by addition of distilled water.
20 ml. are taken from this solution and brought up to 100 ml. by the addition of acetic acid/water in the proportion of 1:1. In this way, the solutions prepared have an approximate concentration of 0.030 gr./litre.

The absorbency is read on the U.V. spectrometer at 396 n.m. and the absorption coefficient E is calculated according to the formula $E = \frac{A}{l \times c}$ in which $A$ is the absorbency measured, $l$ the thickness of the absorbing medium and $c$ the concentration in gr./litre.

The value of $E$ is the mean of the three results obtained.

The value of $E$ obtained in the laboratory is:

$E = 6.6 \text{ cm.}^{-1} \times \text{gr.}^{-1} \times \text{litre.}$

1.3 Assay of milk denatured with blood flour

Approximately 10 grs. of denatured milk (P) are weighed, and diluted with 150 ml. of water at 40°-50°C until a homogeneous dispersion is formed. This is brought up to 250 ml. and 20 ml. of this solution is taken, placed in a flask and brought up to 100 ml. with a solution of glacial acetic acid/water in a proportion of 1:1.

The absorbency of each solution is read, and the weight is calculated in gr./litre by means of the equation:

$$C = \frac{A}{l \times E}$$

Using the value of $C$, the percentage proportion ($x$) of blood flour contained in the milk powder is calculated by means of the equation:

$$x = \frac{125 \times c}{P}$$

2. Determination of presence of deodorized fish flour and fish solubles

In order to determine whether milk powder has indeed been denatured with these products, it is not necessary to make a quantitative determination by chemical means. It is merely necessary for the denatured milk powder to have a strong odour characteristic of fish derivatives, an odour that must be compared with that of samples prepared by mixing milk powder with genuine non-deodorized fish flour or fish solubles.
3. **Bacteriological tests**

Apart from the chemical tests, biological tests must be carried out to ensure that the denatured powder is free of pathogenic germs.

4. **Determination of the insoluble fraction of blood flour intended for denaturing milk**

A 250 ml. precipitation vessel is weighed, with a precision of not less than one-hundredth of a gram.

Thereafter 5 grs. of blood flour are weighed in the said vessel, 20 ml. of distilled water are added, and stirred carefully with a glass rod until there are no lumps or solid fragments left. The glass rod is rinsed under the jet of a washing bottle until completely clean of blood flour, and distilled water is then added to bring the mixture up to 50 grs. (5 grs. of blood flour plus 45 grs. of distilled water).

The magnetic core of an electromagnetic agitator is introduced into a vessel and the mixture is agitated for fifteen minutes without interruption, so that the soluble part of the blood is completely dissolved.

Immediately after this operation, 10 grs. of the solution are poured into a centrifugation tube which has previously been weighed with a precision of not less than one-tenth of a milligram (p1).

It is centrifuged for ten minutes at 200 revolutions per minute and the liquid part is carefully poured off. It is then rinsed with 9 ml. of an 0.9 per cent sodium chloride solution, the residue adhering to the sides of the tube being carefully stirred with a glass rod, and then centrifuged for a further ten minutes. Rinsing with 0.9 per cent sodium chloride solution is repeated three more times in the same conditions, and finally a fifth rinsing is carried out with distilled water.

The centrifugation tube containing the residue is dried at 105°C until weight constancy is obtained (p2).

Calculation: per cent of insoluble residue = (p2 - p1).100.

where: p1 is the weight of the centrifugation tube.
p2 is the weight of the centrifugation tube plus residue.