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## A SEMI-AUTOMATED METHOD FOR THE DETERMINATION OF TOTAL IODINE IN MILK

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### Résumé

MÉTHODE SEMI-AUTOMATIQUE DE DOSAGE DE L'IODE TOTAL DANS LE LAIT. — La matière organique est détruite par incinération alcaline à 500 °C. L'iode libéré est déterminé automatiquement à l'aide de la réaction de Sandell et Kolthoff. La récupération d'iodeure ajouté avant calcination varie de  $90,05 \pm 7,36$  % à  $97,14 \pm 4,56$  % (moyenne  $\pm$  écart-type) selon la quantité ajoutée et la teneur en iode du lait. Le coefficient de variation se situe entre 2,15 et 7,21 % selon la concentration en iode du lait. La limite de détection peut être estimée à 2  $\mu\text{g l/kg}$ .

The iodine content of milk is closely related to iodine intake (Miller *et al.*, 1975). The determination of total iodine in milk could therefore be useful in the detection of sub-clinical iodine deficiency in dairy animals. By contrast, the addition of iodine to feedstuffs (Miller and Swanson, 1973; Bruhn and Franke, 1978a; Hemken, 1979) or the use of iodine teat dips (Conrad and Hemken, 1978; Iwarson and Ekman, 1973; Joerin and Bowering, 1972) can lead to very high levels of iodine in milk (1 000  $\mu\text{g/kg}$ ). A method suitable for monitoring iodine is desirable since such concentrations can be dangerous for humans (Connally, 1971; Vidor *et al.*, 1973; Hemken, 1980) and can certainly exceed the upper limit set by Food and Nutrition Board (1970) and the National Health and Medical Research Council (1976): 500  $\mu\text{g/kg}$ .

However, while the determination of total iodine in milk is of great importance for both human and animal nutrition, the complexity of

the matrix, the low iodine content of milk, and the very large range in content complicate iodine determination. The numerous methods proposed have been reviewed by Wheeler (1979). Those most frequently employed depend on the catalytic reduction of the ceric ion by iodide in the reaction of Sandell and Kolthoff (1937) after destruction of organic matter.

The method of Bellanger *et al.* (1979) is suitable for the determination of iodine in plants but cannot be used, as such, for milk since some iodine is lost during the destruction of milk organic matter. The method reported therefore involves some modifications in the ashing of the milk, but retains the final determination by the Sandell and Kolthoff (1937) reaction as automated by Bellanger *et al.* (1979) for the Technicon Autoanalyser I.

Recoveries after iodide addition to different levels were studied in milks containing a range of iodine contents. Since the method described is

time-consuming, to improve flexibility, the conservation of the material from different steps of the ashing process was also tested.

## Materials and Methods

### Materials

A Technicon Autoanalyser model I with Gilson sampler, a muffle furnace, a drying oven and porcelain crucibles (30–40 ml) were used. The temperature of the drying oven was controlled by a thermocouple of constantan copper, the probes of which were placed near the crucibles. The manifold of Technicon AAI was described by Bellanger *et al.* (1979).

### Reagents

Deionized water, zinc sulfate solution (10% w/v) and potassium hydroxide solution (1 N) were used. Arsenious solution (AsIII), ceric sulphate solution (2 g/l), stock iodine standard (1 000 µg/ml) and working iodine solution were prepared according to the method described by Bellanger *et al.* (1979).

### Procedure

Ten grams of whole milk was weighed in a porcelain crucible. Zinc sulfate solution (1 ml) and potassium hydroxide solution (2 ml) were added and mixed well with a plastic rod. After drying at 80 °C for 8 h the matrix was unstuck and the milk layer broken up with a plastic rod. After a further dessication at 80 °C for 10 to 12 h, the crucibles were covered and heated at 170 °C for 2 h in a drying oven. The covered crucibles were then placed in a cold furnace with a closed chimney and ventilation hole. The temperature was increased to 500 °C for 30 min and the sample was kept at 500 °C for a further 90 min.

After cooling, zinc sulfate solution (1 ml) was added and the suspension was carefully mixed with a few millilitres of water. This was dried at 80 °C for 12 or 14 h, then, heated at 500 °C for 90 min as described previously (without ventilation and with a slow increase in temperature over 30 min in the covered crucible). The ash was then dissolved in 20 ml of water by stirring for 20 min. After centrifugation (20 min, 2 500 g) about 2 ml of supernatant was transferred to a Technicon capsule for the iodine determination. The subsequent automated determination was as described by Bellanger *et al.* (1979). Dilution of supernatant in potassium hydroxide (0.1 N) is necessary at concentrations greater than 100 µg/kg.

## Results

The different assays were carried out on two types of milk collected just after milking. They were homogenized and then kept frozen (at – 18 °C). Before weighing, samples were thawed out at 37 °C and homogenized with a vortex (and/or homogenizer).

The iodine content of the two batches used was 9.7 and 52.6 µg/kg for milk 1 and milk 2 respectively.

### Accuracy

Since no milk standard of iodine is available, the accuracy was estimated by recoveries after the addition of iodide.

Potassium iodide solution (1 ml) prepared in 0.1 N potassium hydroxide was added to milk before drying at 80 °C. The solutions used contained 50, 100, 500, 5 000 ng/ml.

The recoveries of the 0.1 and 0.5 µg added iodide were 95.6 % and 97.1 % respectively, while those from amounts of 0.05 and 5 µg were lower (table 1). The variability of recoveries decreases as the iodine content determined on the Technicon AAI increases.

### Precision

Ten runs of five to ten samples were carried out for each batch of milk. Repeatability was estimated as the mean of ten coefficients of variation determined from each run (within-run calculation), samples of each run being treated in the same way: the same reagent, the same iodine standard and the same operator. Reproducibility was estimated as the coefficient of variation determined from means of each run (between-run calculation) of samples that have been treated with different reagents, iodine standards and Technicon AAI parts. Precision was estimated as the coefficient of variation from all data. The coefficients of variation for the iodine standard at 10 and 30 ng/ml were 0.84 and 0.37 % respectively.

The results of the different precision assays are shown in table 2. The coefficients of variation for within-run, between-run and total assessments are small (< 3.4 %) except for between-run and total computation on milk 1: 6.92 and 7.21 % respectively.

### Sensitivity

Sensitivity is defined as the smallest difference that is statistically significant ( $P < 0.05$ ) between

the iodine content of two milks. The greatest discrepancies induced by reading off the peak height are 0.2, 0.5, 2.5  $\mu\text{g}/\text{kg}$  for concentrations ranging between 0-20, 20-100, 400-500  $\mu\text{g}/\text{kg}$  respectively. Sensitivities are 1.4  $\mu\text{g}/\text{kg}$  and 3.5  $\mu\text{g}/\text{kg}$  for iodine contents of 10 and 50  $\mu\text{g}/\text{kg}$  respectively.

#### Limit of detection

Since the sensitivity is 1.4  $\mu\text{g}/\text{kg}$  at very low concentration, the limit of detection can be estimated at 2  $\mu\text{g}/\text{kg}$ .

#### Versatility of the procedure

The effect of conservation of material from the different steps of the ashing process is shown in table 3. In the conditions indicated in this table, analytical results did not significantly differ between conserved and freshly treated samples.

#### Discussion

Iodine enters milk primarily as iodide in cows (Lengemans, 1963) and goats (Wright *et al.*, 1955)

Table 1. — Recoveries for different amounts of potassium iodide added to milk

Amount of added iodide ( $\mu\text{g}$ )	Nb of samples	Recovery of iodide (%)	
		mean $\pm$ sd <sup>a</sup>	range
<i>Milk 1 : 9.7 <math>\mu\text{g}/\text{kg}</math></i>			
0.05	18	90.05 $\pm$ 7.36	84.2-109.0
0.10	18	95.62 $\pm$ 4.86	89.1-106.3
<i>Milk 2 : 52.6 <math>\mu\text{g}/\text{kg}</math></i>			
0.50	15	97.14 $\pm$ 4.56	91.2-106.2
5.00	16	90.50 $\pm$ 2.28	86.5- 95.0

a : total calculation (within and between run).

Table 2. — Precision assays

	Within run calculation	Between run calculation	Total calculation
<i>Milk 1</i>			
Number of samples	a	10	80
Means ( $\mu\text{g}/\text{kg}$ )	9.7	9.7	9.7
Standard deviation ( $\mu\text{g}/\text{kg}$ )	0.3	0.7	0.7
Coefficient of variation (%)	3.34	6.92	7.21
Range ( $\mu\text{g}/\text{kg}$ )	9.3-10.2	8.7-11.0	8.3-11.6
<i>Milk 2</i>			
Number of samples	a	10	85
Means ( $\mu\text{g}/\text{kg}$ )	52.6	52.6	52.6
Standard deviation ( $\mu\text{g}/\text{kg}$ )	1.3	1.1	1.2
Coefficient of variation (%)	2.41	2.15	3.41
Range ( $\mu\text{g}/\text{kg}$ )	50.2-54.1	51.6-54.6	47.8-57.0

a : 5 to 10 samples in each run.

milk, but 10 % of the iodine naturally secreted in milk may be protein bound (Lengemans and Swanson, 1957 ; Miller and Swanson, 1973). The milk xanthine oxidase system can increase this fraction (Murphy and Campbell, 1967). Nearly 16 % of the iodine secreted in milk may be removed with the cream although this iodine is in the non-fat fraction (Miller *et al.*, 1975). Interestingly the main cause of increased iodine of milk, following the use of an iodophore teat dip, seems to be its entry into the organic iodine pool followed by its secretion into milk, rather than a direct contamination from the teat surface (Conrad and Hemken, 1978). However, direct contamination with iodine sanitizers can occur (Wheeler *et al.*, 1982).

Thus, methods for determination of iodine in milk must include all the forms of iodine present in the total milk. The recoveries of added potassium iodide are only an indicator of efficiency since the organic matrix affects the recovery of iodine after ashing (Binnerts and Das, 1974). Nevertheless, recovery of added iodide is the best and the most widely used method to estimate accuracy.

For routine analysis, the method described has very good recoveries, precision and sensitivity. Furthermore, modification of the ashing procedure may improve flexibility. But for iodine metabolic studies, two of three repetitions are necessary to avoid between-run variability at very low iodine contents (10 µg/kg).

The recoveries described are as high or higher than those obtained by different authors with kinetic methods, when the added iodide exceeds

0.1 µg for a 10 g sample. The recoveries of the method of Fisher and L'Abbé (1981) are somewhat higher (97-101 %) but these authors used a wet ashing procedure which is more complex than our dry ashing one. At very low levels of added iodide ( $0.015 \pm 0.03$  µg), Stolc and Nemeth (1961) found better recoveries ( $102 \pm 3$  %).

Generally, the recoveries using the neutron activation (Malvano *et al.*, 1972 ; Allegrini *et al.*, 1981) or the gas-liquid chromatographic method (Barker, 1977), or the X ray fluorescence method (Purdham *et al.*, 1975) are high (95-100 %) with low variability, but the results are rarely obtained with the very low iodine levels found in milk. Furthermore, these precise and highly sensitive methods are expensive and difficult in practice. The iodine selective electrode is a simple method with high recoveries from added iodine or iodophor (Wheeler *et al.*, 1980) but the limit of detection is also high (40 to 50 µg/kg ; Crecelius, 1975) and as the chloride (Convey *et al.*, 1977) or the sulphhydryl group (Bruhn and Franke, 1978b) interferes with the iodine determination, the sensitivity is lowered (Bruhn and Franke, 1978b) ; the electrode is also quickly coated and inoperative (Fisher and L'Abbé, 1981).

Convey *et al.* (1977) describe a method based upon polarographic iodine determination after dry ashing but recovery was only 79.2 % and precision only 10 %. Curtis and Hamming (1982) developed a method upon the same principle, but with a dry ashing procedure ; recoveries were high ( $98.6 \pm 2.9$  %) at concentrations higher than 200 µg/kg, but at lower concentrations they decrease (85-92 %). We have also tried to

Table 3. — Conditions of conservation of issues at the different stages of ashing

	Nature of container	Temperature (°C)	Conservation <sup>a</sup> period	Note
First drying	crucible	80	14 h to 8 d	
Residues from the first ashing	crucible	20	12 h	with lids
Second drying	crucible	80	10 to 24 h	
Residues from the second ashing	crucible	20	24 h	with lids
Ashes in 20 ml of water	polypropylene tube	20	24 h	slight indifferent
Supernatant	transparent polypropylene tube	20	48 h	slight indifferent

a : Results of each assay on two milks have been compared to results obtained from precision experiences. Comparison was made by Student unpaired t test : not any difference has been noted.

change sample size (5 g *versus* 10 g) at very high concentrations but recoveries were lower (71.2-91.5 %).

The estimated precision differs between authors : the repeatability is calculated only on milk with a middle or high iodine content, reproducibility is never estimated. The method proposed presents as good as or better precision than the other kinetics methods (see review of Wheeler, 1979) except for that of Lauber (1975). But this author only worked with blood, plasma, urine and glucose solutions.

The sensitivity obtained from the procedure

described is very high for a kinetic method, even for a high sample iodine content. Shvejkina (1975) indicates a sensitivity of 1 µg/kg but little further detail. In conclusion, the method proposed for determination of total iodine content of milk is simple, cheap and suitable for routine determinations or for iodine studies. It allows a sufficiently sensitive, lower limit of detection to estimate an iodine content as low as 10 µg/kg which can be considered as a level indicative of sub-clinical deficiency (Miller *et al.*, 1975).

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### Summary

A method for determination of iodine in milk is described. This method involves the destruction of organic matter by alkaline incineration and automated spectrophotometric determination of iodide based on the Sandell and Kolthoff's reaction.

The recoveries of added iodide before calcination were between  $90.05 \pm 7.36$  % and  $97.14 \pm 4.56$  % (mean  $\pm$  S.D.). The coefficient of variation ranged from 2.15 to 7.21 % according to the iodine content of the milk. The limit of detection was estimated to be around 2 µg/kg.

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