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11 Speciation of inorganic iodine in raw milk using ion-chromatography with Inductively Coupled Plasma-Mass Spectrometry detection

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A study was conducted within the experiment of iodine and selenium fortification of cow milk described in chapter 8. The aim of the study was to quantify by ion-chromatography the percentage of inorganic species of iodine (iodide and iodate) respect to different levels of total iodine content in raw milk. The milk samples were collected from Experiment 1 in Manuscript 1 (chapter 8), from here it will be named as M1. The ion-chromatography method described in this study was performed after preliminary study on speciation of iodine in milk and water according to Fernandez-Sanchez & Szpunar (1999) and Li Bing *et al.* (2006).

11.1 Material and methods

Twelve Holstein Friesian lactating dairy cows were randomly allotted to three diets in a completely randomized design ($n = 4$), and received experimental diets for 5 weeks as described in M1: control group fed basal diet containing 1.71 mg/kg DM of I (CTR); Group 1 (T1) and 2 (T2) fed basal diet supplemented daily with 23.2 and 45.3 mg/head of I, respectively for T1 and T2. Individual milk samples were collected at day 25 (proportionally mixed between a.m. and p.m. milking) and refrigerated at 8°C prior to be analysed (within 4 days) for iodide (I^-) and iodate (IO_3^-) content by ion chromatography. The samples for total iodine content were collected, frozen and analysed as described in M1. For major detail in animal management, please see M1.

The experimental work was performed on an Agilent 1100 HPLC equipped with the Agilent ICS A23 ionic column. The HPLC was inline coupled with the Agilent 7500ce ICP-MS. The total iodine content was analyzed as described in M1 using ICP-MS. The iodine speciation was performed as follows. The samples were defatted by centrifugation (4330 *g* for 10 min) at 10°C in a refrigerated centrifuge (4237R, ALC, Milan, Italy). Iodine was extracted as described for the total iodine determination in M1. After extraction and dilution to 50 ml, rhodium was spiked at 5 µg/L. An aliquot of diluted sample was withdrawal by HPLC syringe and then 0.45 µm filtered into the HPLC vials. The standard addition calibration method was used to determine the concentration of I^- and IO_3^- : 50 ml of a 0.15% ammonia solution were spiked with four different amounts of iodide (as KI) and iodate (as KIO_3): 5, 10, 20, 40 and 0.25, 0.5, 1 and 2 µg/L, respectively. Therefore, each point of calibration standard had an iodate:iodide ratio of 1:20. According to Li Bing *et al.* (2006), the mobile phase was a solution 0.03 mol/L of NH_4CO_3 , whose pH was

corrected at 9.4 using ammonium hydroxide. Internal standard addition was used to validate the trueness of methods: fresh milk samples were spiked with 100 and 10 µg/L of I⁻ and IO₃, respectively. The reference standard material skim milk powder BCR 151 was analysed for total iodine content and iodine speciation. Data were subjected to ANOVA (one-factor) using cow as experimental unit.

11.2 Results and discussion

In the preliminary setting of speciation method, we diluted five-fold defatted raw milk samples (previously added with 100 and 10 µg/L of iodide and iodate, respectively, as internal additions) with 0.01% potassium hydroxide (KOH), then samples were filtered by 0.45 µm filter and injected in the HPLC vials. The recovery of I⁻ and IO₃ were (mean ± SE) 78.3±5.7 and 72.7±10.3 (n = 11) percent, respectively. The alkaline extraction by 0.15% ammonia solution used by Fernandez-Sanchez & Szpunar (1999) should not produce any change in the chemical form I⁻ and IO₃, therefore, we decided to perform the total iodine extraction by ammonia before of the ionic chromatography. Applying the ammonia extraction, the recovery of I⁻ and IO₃ were 91.0±11.7 and 100.0±7.2 percent, respectively.

The calibration standard lines of I⁻ and IO₃ had the r² of 0.9997 and 0.9985, respectively. The pump flow was 0.6 ml/min and the peak of IO₃ and I⁻ were efficiently separated (figure 1) at 1.32 and 8.64 min, respectively. Also Li Bing *et al.* (2006) obtained similar separation at 2 and 9 min.

The concentration of iodide was influenced by treatment and increased linearly (P < 0.001) such as the total iodine content (M1). The percentage of iodide in milk samples were 77.9±4.5, 74.3±3.6, and 76.2±2.6 for CTR, T1, and T2 groups, respectively. No difference was found between groups (P = 0.7888) and iodate was never detected. Results were in agreements with Fernandez-Sanchez & Szpunar (1999), who found in commercial cow milk an iodide concentration ranging between 54 and 86%. Leiterer *et al.* (2001) found an iodide content of 89% respect to the total iodine.

Analysis on BCR 151 (n = 4) reported no detectable iodate, whereas the iodide content was 3.14±0.05 µg/g, corresponding to the 58.72±0.87 percent of total certified value of iodine (5.35±0.14 µg/g). Leiterer *et al.* (2001) found 0.7% of iodate compared to total iodine. However, BCR 151 is certified for the total iodine content, and no data are available in literature about its speciation.

11.3 Conclusions

Literature data clarify that the ionic chromatography coupled in line with ICP-MS is a sensitive technique for the speciation of iodine in milk. The sample preparation can be performed by simple dilution or SDS incubation of milk. In this study we demonstrated that the extraction of iodine by 0.15% ammonia solution from skimmed milk is feasible for the sample preparation prior to performing iodine speciation. Our procedure offered sensitive and rapid separation of the inorganic species iodate and iodide in defatted raw milk. Finally, the level of total iodine content did not influenced the percentage of iodide and iodate into milk.

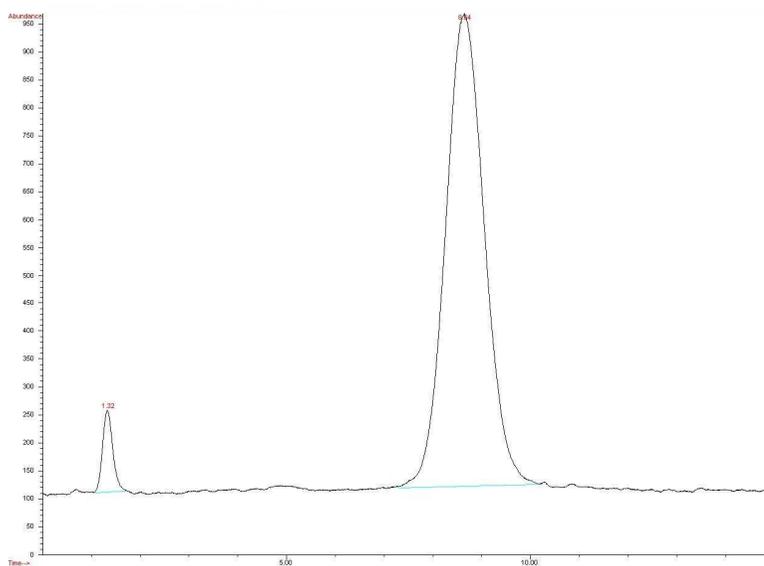


Figure 1. IC-ICP-MS chromatogram of milk spiked with 10 and 100 $\mu\text{g/L}$ of iodate (left) and iodide (right). Mobile phase: 0.03 mol/L ammonium carbonate.