Iodine content of semi-skimmed milk available in the Netherlands depending on farming (organic versus conventional) and heat treatment (pasteurized versus UHT) and implications for the consumer

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ABSTRACT

Purpose: To study the iodine concentration in semi-skimmed cow's milk available on the Dutch market.
Methods: The iodine content of 16 milk brands was determined at four times (June, September, December 2016 and March 2017) using inductively coupled plasma mass spectrometry. Brands included 12*pasteurized (n = 48 samples) and 4*UHT (n = 16 samples) milk, the brands subdivided into 8* organic and 8*conventional milk (each 32 samples).
Results: The median iodine concentration was 15.9 μg/100 g. The iodine concentration increased from June 2016 to March 2017 from 12.4 μg/100 g to 19.9 μg/100 g. The time trend with difference between summer and winter milk iodine content was stronger in organic than conventional producing farms indicating more uncertainties to supply iodized minerals by pasture feeding. Despite reflection of the time trend in the investigated kinds of milk, the distributions of iodine concentrations were similar between organic and conventional produced milk and between pasteurized and UHT milk.
Conclusions: Milk was shown as a valuable iodine source for the Dutch consumer. The significant time trend in the iodine concentration in favor of the milk from a stable feeding period (Dec and March) compared with a pasture feeding period (June and September) is a challenge to keep all year round the cow's iodine supply level constant, mainly in organic producing farms.

1. Introduction

Iodine is a trace mineral, essential for the synthesis of thyroid hormones and therefore crucial for growth and development [1]. Insufficient intake of iodine results in decreased thyroid hormone production and can cause enlargement of the thyroid gland (goiter) and impaired mental function [2]. Mild-to-moderate iodine deficiency during pregnancy may, and severe iodine deficiency will, result in impaired cognitive function of the offspring [3,4].

Worldwide, around 30 percent of the population suffers from iodine deficiency due to insufficient iodine intake, ranging from mild to severe deficiency [5]. Amongst them are also populations in several European countries [1]. In the Netherlands, people are overall considered to have an adequate iodine intake [6,7]. The Health Council of the Netherlands adopted the European Food Safety Authorities (EFSA) recommendations for iodine intake. The recommended intake (i.e. Adequate Intake (AI)) in these guidelines is 150 μg per day for adults [8].

Iodine is naturally present in foods [9]. However, as consumption of natural iodine sources generally does not meet iodine requirements, fortification is applied in many countries [10]. In the Netherlands, there is a long history of iodization of salt, with bread containing iodized salt as a main source [11]. In contrast, iodine intake through salt could be affected by the large worldwide effort to decrease salt consumption, taking place because high sodium intakes contribute to high blood pressure and thereby increase the risk for cardiovascular diseases [12]. In the Netherlands, dairy products are the most important contributor to iodine intake from non-fortified foods (where fortified foods include foods produced with iodized salt) [11]. Recently the value for iodine concentration in cow's milk in the Dutch Food Composition Database was increased from 7 to 14.9 μg/100 g [13,14], based on measurements in raw milk. Although factors that may influence the iodine content of cow's milk (such as type of feed and the use of iodine containing teat dips) have been previously identified [15], it remains unclear what caused this increase in iodine concentration of raw milk as measured in the Netherlands. However, it shows that dairy may be an even more important source of iodine than previously thought.

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Several studies show significant higher iodine concentrations in winter milk compared to summer milk [16–20]. This may be caused by differences in feeding practices across seasons [15]: in summer, cows can be pasture-fed while in winter they might receive more feed concentrates with a relatively high iodine content. In addition, organic milk has often been found to contain less iodine than conventional milk [16,18,20–24]. Possible explanations suggested in previous studies are the higher grazing/conserved forage intakes on organic farms [24] and the less frequent use of mineral mixtures and iodine containing teat dips [15]. Some studies also found an effect (direction remains unclear) of heat treatment on iodine concentration [20], whereas others did not find such effect [25].

In the Dutch Food Composition Database, no distinction is made between organic and conventional milk, or season of production [14]. In addition, measurements of the iodine concentration in milk have so far been performed in raw milk instead of retail milk, and thus also no distinction was made between pasteurized and ultra-heat temperature (UHT) treated milk. For an accurate estimation of the iodine intake of the Dutch population, it is important to gain insight in the variation in iodine concentration of retail milk. The aim of the present study was therefore to measure iodine concentrations in retail milk available on the Dutch market, at four time points spread over the year, and to study potential differences in iodine concentration between organic and conventional milk and pasteurized and UHT milk.

2. Materials and methods

2.1. Collection of milk samples

At four time points (June, September, December 2016, and March 2017), sixteen different semi-skimmed cow's milk containers were purchased on the same day in five conventional (including the two largest supermarket chains in the Netherlands) and one organic supermarkets in Amersfoort and Leusden, the Netherlands. Different supermarkets were included in the sampling, as specific supermarkets uniquely sell particular brands. In total 64 samples were collected for analysis. Only milk originating from the same dairy, milk was sampled using the European Community (EC) identification mark of dairies. In addition, both private label as well as supermarket owns brands were included. The brands selected for sampling in June 2016 were also sampled in September, December 2016, and March 2017, irrespective their EC identification mark. For two brands the EC identification mark was not equal in all months of purchase. One sample had a different origin in June, the other in March (Online Supplemental Material Table S.1). The brands varied in farming type, including organic (n = 8) and conventional milk (n = 8), and in heat treatment, including pasteurized (n = 12) and UHT milk (n = 4). In the supermarkets, UHT milk was stored unrefrigerated whereas pasteurized milk was stored refrigerated.

2.2. Sample preparation and analysis

After purchase, all milk containers were transported in isolated styrofoam boxes with ice packs to Qlip laboratory Leusden, the Netherlands, where they were coded and photographed. Storage of the milk and preparation of milk samples for laboratory analyses were performed according to NEN-EN-ISO-707:2008 [26]. The milk containers were stored at 4 °C until transported (also at 4 °C) on the next day to Qlip laboratory Zutphen, the Netherlands. The milk from each container was homogenized in a water bath of 40 °C by gently mixing the containers (all cartons or plastic bottles) every five minutes until they reached a temperature of at least 38 °C. Sample aliquots of 100 mL were obtained, cooled down in a water bath of 20 °C and stored at −18 °C until chemical analysis.

The chemical analysis of iodine [27] was performed at Qlip laboratory Zutphen, the Netherlands, within the month of purchase. Frozen milk samples were defrosted in a water bath of 20 °C and were warmed to at least 38 °C in a water bath of 40 °C, gently mixing them every five minutes for homogenization. The subsequent steps of sample preparation and analysis were performed in duplicate. First, 2.5 g of milk was thoroughly mixed with 5 mL water and 1 mL 25% tetramethylammonium hydroxide in H_2O (TMAH; density 1.016 g/ml at 25 degrees Celcius, 11.1 M) (Sigma-Aldrich, Darmstadt, Germany) and placed in a drying oven at 90 °C for three hours. After being cooled down to room temperature, samples were further diluted with water to a total volume of 50 mL and passed through a membrane filter of 0.45 μm to remove any coarse particles (i.e. potential contamination, iodine is in the solution) that may be present. The filtrate contained 0.5% TMAH (i.e. 1 ml 25% TMAH solution to 50 ml water). Potassium iodide solutions in 0.5% TMAH were prepared in iodine concentrations ranging from zero to 50 μg/L for calibration. Tellurium 1000 mg/l (Merck, Darmstadt, Germany) was used as internal standard and a blank sample without milk was analyzed. The iodine concentration in the milk samples was analyzed using inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher Scientific model i-CAP Q). Final iodine concentrations of milk samples were adjusted for the contribution of the blank. When duplicate measurements deviated more than 5%, measurement results were discarded and the analysis for that sample was repeated on the next day, using a second frozen aliquot of 100 mL. Iodine concentrations were expressed in μg/100 g.

Qlip laboratory Zutphen is accredited according to ISO-17025 (2005) [28]. No certified reference material is available for liquid milk, only for milk powder certified reference material is available, however this cannot be applied in this method. Therefore, a raw milk sample with known iodine content was used as internal reference by this laboratory. Validation characteristics of the method included an uncertainty of 8%, reproducibility of 8% and repeatability of 5%.

2.3. Statistical analysis

The iodine concentrations of each duplicate measurement were averaged, and medians were calculated per month of purchase and per milk type (organic/conventional or pasteurized/UHT) using Microsoft Office 2010 and R 3.4.3. Due to the small sample size, it was impossible to verify the assumptions of parametric statistical methods. Therefore, non-parametric analyses were performed, using the R-package nparLD (version 2.1) [29]. This statistics was designed for longitudinal data in factorial experiments. Longitudinal data are collected from the same subjects (in our study milk brands) over time (in our study different months of purchase). Factorial refers to treatments, in our study farm type and heat treatment. For each time-treatment group the rankmeans were estimated as well as the relative treatment effects. ANOVA-type statistics was applied to test the hypothesis for no time-treatment effect. The LD-F1 design (i.e. longitudinal data collected repeatedly at *t* different time points) was used to determine if the iodine concentration in semi skimmed milk was affected by month of purchase. The F1-LD-F1 design (i.e. longitudinal data collected repeatedly at *t* different time points, and 1 treatment) was used to study if the effect over the months was accompanied by a treatment effect and an interaction between month and treatment. In separate analyses pasteurized versus UHT and organic versus conventional milk samples were taken as treatment groups. If a statistically significant (P-value < 0.05) effect was observed, multiple comparisons were performed to study between which months of purchase these treatment effects were different. For these multiple comparisons, a Holm-Bonferroni adjustment was applied to get adjusted alpha. A P-value < adjusted alpha was considered statistically significant.

3. Results

When the observations from all types of milk were pooled, we found a median iodine concentration of 15.9 μg/100 g (min 3.8, max 35.8) over the year. The median iodine concentrations increased from

Table 1
Median (min, max)) of iodine concentrations in semi-skimmed cow's milk available on the Dutch market[1] overall and per month of sampling, for the total samples as well as separate for organic, conventional, pasteurized, and UHT[2] milk. For statistical comparisons, see Table 2 and Figs. 1–3.

	Sample size (per month)[3]	Iodine concentration: range (µg/100 g)				
		Overall (June 2016-March 2017)	June 2016	September 2016	December 2016	March 2017
Total	64 (16)	15.9 (3.8, 35.8)	12.4 (3.8, 21,0)	13.7 (6.0, 23.7)	17.3 (12.8, 24.1)	19.9 (13.1, 35.8)
Organic	32 (8)	15.9 (3.8, 35.8)	8.2 (3.8, 21.0)	11.8 (6.0, 23.7)	15.9 (14.0, 20.1)	20.1 (17.9, 35.8)
Conventional	32 (8)	16.2 (8.4, 26.3)	14.1 (8.7, 19.9)	14.1 (8.4, 23.2)	17.8 (12.8, 24.1)	17.4 (13.1, 26.3)
Pasteurized	48 (12)	15.9 (3.8, 35.8)	10.1 (3.8, 21.0)	12. (6.0, 23.7)	17.3 (13.1, 23.4)	19.9 (13.1, 35.8)
UHT[2]	16 (4)	16.0 (9.1, 26.3)	16.4 (9.7, 19.9)	14.0 (9.1, 18.8)	15.7 (12.8, 24.1)	19.5 (13.6, 26.3)

[1] six semi-skimmed milks originated from dairies in Germany (n = 5) or Belgium (n = 1), all UHT milk was from Germany or Belgium, as well as two pasteurized conventional milks (see Supplemental Material Table 1).
[2] UHT = ultra-heat temperature treated.
[3] Total sample size over all months of purchase, between brackets sample size for each month of purchase.
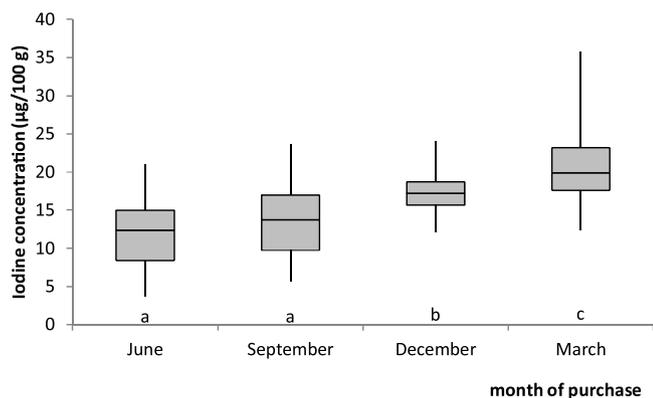

Fig. 1. Box plot of iodine concentration in semi-skimmed milk purchased in different months in the Netherlands, by month of purchase (N = 64, n = 16 each month). Box plot shows minimum, lower quartile, medium, upper quartile, maximum. Months with different index have statistically significant difference in iodine concentration.

12.4 µg/100 g in June 2016 to 19.9 µg/100 g in March 2017 (Table 1). The month effect on the milk iodine concentration was significant. Multiple comparisons did not show significance between the results in

June and September (Fig. 1 and Table 2).

Based on all samples, the median iodine concentration was similar for organic milk (15.9 µg/100 g) and conventionally produced milk (16.2 µg/100 g). There was no significant effect of farm type on iodine concentration (Table 2). For both organic and conventional semi-skimmed milk, there was a statistically significant rise in iodine concentration between June 2016 and March 2017 (Table1 and Fig. 2), except for the months June versus September. For organic milk, the iodine concentration increased from 8.2 µg/100 g in June to 20.1 µg/100 g in September. For conventional milk, the iodine concentrations in June and September were about 14 µg/100 g and raised to about 17.5 µg/100 g in December and March. The profiles over the months of purchase were not parallel between organic and conventional milk, as a statistically significant interaction was observed (Table 2). Significance was shown for the comparison of December versus March, and September versus March (Table 2). For these two periods, the profile of the increase in iodine concentration was different.

The median iodine concentration of pasteurized semi-skimmed milk was higher compared to UHT semi-skimmed milk in December and March reflecting the time trend of total samples (Table 1 and 2). Overall, the iodine concentrations did not differ for pasteurized and UHT milk (Fig. 3).

Table 2
P-values for differences in iodine concentration distribution of semi-skimmed cow's milk available on the Dutch market[1], presented for month effect, treatment effect and interactions of month and treatment effects; overall and per comparison of two months, for the total sample as well as for the comparison of organic versus conventional milk and pasteurized versus UHT[2] milk; analyzed with ANOVA-type statistics in nparLD package [29]. P-value < 0.05 (alpha) was considered statistically significant for total (in bold). Holm Bonferroni criterion was used to define adjusted alpha[3] to correct for multiple comparison between months P-value < adjusted alpha was considered statistically significant (in bold).

		Comparison						
		Overall	June vs. September	June vs. December	June vs. March	September vs. December	September vs. March	December vs. March
Total	Month effect	P < 0.001	P = 0.15	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.01
Organic vs. Conventional milk	Month effect	P < 0.001	P = 0.14	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
	Farm type effect	P = 0.89	P = 0.30	P = 0.53	P = 0.66	P = 0.44	P = 0.71	P = 0.63
	Interaction	P < 0.03	P = 0.29	P = 0.89	P = 0.02	P = 0.80	P < 0.01	P < 0.001
Pasteurized vs. UHT[2] milk[4]	Month effect	P < 0.001						
	Heat treatment effect	P = 0.76						
	Interaction	P < 0.03						

[1] six semi-skimmed milks originated from dairies in Germany (n = 5) or Belgium (n = 1), all UHT milk was from Germany or Belgium, as well as two pasteurized conventional milks (see Supplemental Material Table 1).
[2] UHT = ultra-heat temperature treated.
[3] P-values were ordered from smallest to greatest. For each P-value a new (adjusted) alpha was calculated based on the rank: 0.05/(n-rank + 1). Therefore, each P-value has a specific adjusted alpha, based on the ranking.
[4] The majority of samples was represented by pasteurized milk and the significances for the months are in line with that of the total samples in the first row. Due to the low number of UHT milk samples, comparisons between months are not considered.
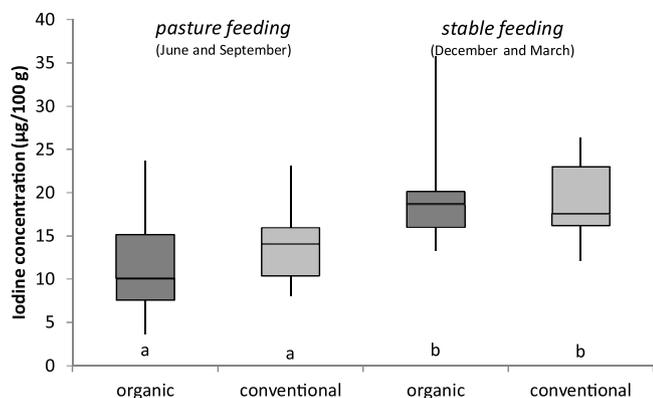
Fig. 2. Box plot of iodine concentration in organic and conventional semi-skimmed milk in the Netherlands (N = 64), separate for the pasture feeding (June and September) and stable feeding (December and March) period (each period N = 16 for both organic and conventional milk). Box plot shows minimum, lower quartile, medium, upper quartile, maximum. Box-plots with different index have statistically significant difference in iodine concentration.
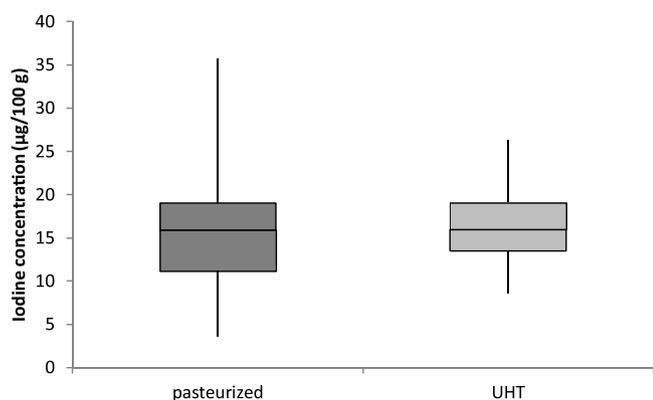

Fig. 3. Box plot of iodine concentration in semi-skimmed milk in the Netherlands separate for pasteurized (N = 48) and UHT (N = 16) treated milk. Box plot shows minimum, lower quartile, medium, upper quartile, maximum. The iodine concentrations are not statistically different (P > 0.05).

4. Discussion

As far as we know, this is the first study comparing the iodine concentrations in different types of retail semi-skimmed milk available on the Dutch market. The key finding is that iodine concentrations showed a seasonal variation, with lower values in summer and highest values at end of winter. There were no differences in iodine concentration between organic and conventional milk, or between pasteurized and UHT milk. However, the seasonal variation in iodine concentration was not parallel. The iodine concentration of organic milk showed a steeper increase compared to conventional milk. Only semi-skimmed milk was included, as this is the most consumed type of milk in the Netherlands [30]. As iodine is present in the aqueous phase of the milk, an effect of the fat or protein phase cannot be expected. Several studies did not find differences in iodine concentration between whole milk and skimmed milk [22,31]

4.1. Milk iodine concentration in the country comparison with consideration of seasonality

Some of our milk samples originated from Germany and Belgium (all UHT, few pasteurized). It is possible that the results of our study were affected by these different origins, as large differences in milk iodine concentration between countries have been observed. The median iodine concentration in semi-skimmed milk in our study was 15.9 μg/100 g. This is higher than the iodine concentration measured in Swiss (i.e. 8.7 μg/100 ml) [32] and Germany (i.e. 120 μg/L) [22], but lower than the iodine concentrations measured in UK (i.e. conventional 42.7 μg/100 ml, organic 24.1 μg/100 ml, UHT 31.4 μg/100 ml) [20]. We are not aware of studies on iodine content of consumer milk from Belgium. However, in our study, the iodine concentration in UHT milk was in each month higher (up to double) than the ones from German UHT milk.

Similar to the results of our study, seasonal effects on the iodine concentration in retail milk were also found in other studies [16–20,25,31–36]. Although most studies show a lowest iodine concentrations during summer and highest during winter, there is some variation in the month with the lowest/highest levels. A generally accepted main factor in the iodine content of milk is the iodine intake of the cow [15]. Most dairy cows in the Netherlands are pasture fed in spring and summer [37], and 15 of the 16 milk samples included in this study were so-called 'meadow milk' coming from cows that are pasture fed at least 6 h per day for 120 days a year [38]. During pasture feeding there is more uncertainty in the regular mineral supply compared to stable feeding, this may have resulted in seasonal difference in iodine concentration in the milk.

4.2. Milk iodine concentration and the farm type – conventional vs. organic

Several studies show a difference in iodine concentration between organic and conventionally produced milk [18,20–23,25]. However, others cannot detect a statistically significant difference [32], similar to our results. Again, differences in feeding practices between conventional and organic farming have been proposed as a possible explanation, and indeed EU regulations for organic farming state that a minimum of 60% of feed must be roughage, fresh or dried fodder, or silage [39,40]. In addition, organic farms are, similarly to conventional farms, allowed to use iodine-containing premixes throughout the year to provide for nutritional requirements [39,40]. Possibly, feeding practice on organic and conventional farms (grazing, the amount of iodine added to feed) are more similar in the Netherlands compared to other countries, like UK [20], where differences in iodine content of organic and conventional milk were larger. The stronger season effect in organic milk mentioned above will not be further discussed.

4.3. Milk iodine concentration depending on heat treatment – pasteurization vs UHT milk

We did not find a difference in iodine concentration between pasteurized and UHT milk. In addition, the significant interaction found between heat treatment and month of purchase cannot be explained, presumably this is a statistical artefact due to the limited sample size of UHT milk (4 per month). As presented in the introduction, the iodine concentration from raw milk in the Dutch food composition database (14.9 μg/100 ml) is comparable with the median iodine concentration found in our study (15.9 μg/100 ml), and equal if only pasteurized milk samples are considered (most originating from the Netherlands: 15.9 μg/100 ml). Comparison between un-treated and heat treated milk showed no difference in iodine concentration [25,31,41]. In contrast, other studies did find a difference in iodine concentration between different heat treatments [20,23,42]. It remains inconclusive to what extend heat treatment affects the iodine concentration.

4.4. Contribution of the milk beverages to the iodine supply of the Dutch consumer

According to the Dutch National Food Consumption Survey 2012–2014 (2 times 24-hr recall), adults aged 19–50 years have a mean intake of milk, milk beverages and fermented milk beverages of 198 g

per day [43]. Based on the median iodine concentration in milk of 15.9 μg/100 g as found in this study, this would imply an average intake of 31.5 μg of iodine per day coming from milk beverages. This is 21% of the AI set by the Dutch Health Council [8]. However, considering a similar dairy intake during the year, due to differences in iodine level throughout the year, the iodine intake can vary between 24.5 μg/d in June to 39.3 μg/d in March (based on median iodine concentration), representing 16% and 26% of the AI. Regarding the individual milk sample measured the iodine intake via milk beverages would contribute from 5% to 47% of the AI. In the Netherlands, the iodine intake of adults seems to be adequate, although the median intake decreased, mainly due to a change in iodine policy in 2008, with about 30% from 257 μg/d in 2006 to 179 μg/d in 2010 [6,7]. As mentioned in the introduction, in the Netherlands bread is a main source of iodine, as most bread contains iodized salt (average contribution almost 40%). Dairy is the second main source of iodine with an average contribution of circa 15% (i.e. about 30 μg/d for adults). With a decrease in bread consumption, as well as a trend in increasing consumption of organic foods (not all organic bread contain iodized salt), other foods like dairy may become more important in the iodine supply in the Netherlands.

Future research should focus on whether the seasonal variation in milk iodine concentration has a significant effect on total iodine intakes and the proportion of the population with intakes below requirements throughout the year. If milk consumption is constant throughout the year then higher iodine intakes from milk in winter could compensate for lower intakes in summer, since the body is able to store iodine and thyroidal iodine stores may be sufficient to least three months if iodine intake would be zero [1]. For Dutch adults milk consumption seems to be constant over the year, whereas children have somewhat lower intakes in spring and summer compared to winter [11]. Special attention should be paid to risk groups. The fetus is particularly vulnerable to damage from iodine deficiency early in pregnancy [44–46]. Therefore, it is worthwhile to investigate the iodine status of pregnant women focusing on the summer period with highest risk of inadequate intake. Future studies assessing adequacy of iodine intake should take into account not only the variability of the individual milk-products' consumption, but also that in the milk iodine concentrations during the year.

5. Conclusion

The median iodine concentration in semi-skimmed retail milk available on the Dutch market as found in this study was 15.9 μg/100 g. Significant differences in iodine concentrations over the year were found, with an increase from June to March. No differences in iodine concentration were found between organic and conventional milk samples. The time trend in the iodine content was strongest in organic milk, with the highest difference between June and March. It is a challenge to keep all year round the cow's iodine supply level constant, mainly in the organic producing farms. Future research could focus on the implications of the seasonal variation in milk iodine content for adequacy of iodine intakes on a population level and on vulnerable groups like pregnant women.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2019.08.008.

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