

RESEARCH PAPERS

Effect of Processing upon Concentration and Distribution of Natural and Iodophor-Derived Iodine in Milk

SALLY M. WHEELER, G. H. FLEET,¹ and R. J. ASHLEY

Dairy Research Centre
P. O. Box 217
Richmond, New South Wales 2753
Australia

ABSTRACT

Cream removal, pasteurization, and spray-drying of milk did not affect concentration of either natural or iodophor-derived iodine, as measured by both chemical and electrode methods, although electrode results were significantly higher. The use of iodine-131 labeled iodophor showed that only .02% of iodine was lost from milk on boiling and that 3.4% of iodophor-iodine became associated with milk casein.

INTRODUCTION

Iodine concentration of bovine milk has been of interest for many years because of the significance of iodine in the etiology of thyroid disease and also because radioactive fallout may lead to isotope ¹³¹I in milk. The normal physiological concentration of iodine in milk as secreted by the cow is generally below 300 μ g I/liter, although this concentration may be elevated to as high as 1000 μ g I/liter when iodine supplements are added to dairy cow rations (5, 8, 27). However, up to 2500 μ g I/liter has been reported in market milk, and elevated concentrations have been related to the use of iodine-based sanitizers (iodophors) in the dairy industry (5, 8). For example, in Australia milk from dairy farms where iodophors were not used had a mean concentration of 37 μ g I/liter, but milk from dairy farms using iodophors had a mean iodine concentration of 760 μ g I/liter (4).

Iodophor sanitizers depend upon available iodine (I₂) for their bactericidal properties and are made by dissolving iodine in a surface-active agent (often a polyethoxylated nonyl phenol)

(28). In the original method of manufacture, iodine was heated in a surfactant ("hot process"), but in a newer method ("cold process"), iodine first is dissolved in an iodide or hydriodic acid solution before surfactant is added (22). In hot process iodophors, the iodine is available iodine, iodide, and iodine chemically bound to the surfactant (22) whereas in cold process iodophors only available iodine and iodide are present (31).

Iodophor sanitizers may be used for a variety of tasks on the dairy farm, for example, teat-dipping (for mastitis prophylaxis) and equipment sanitization, and, thus, may contaminate milk as a consequence of inadequate drainage from equipment, or contamination may originate from milk-contact surfaces where iodine becomes absorbed during sanitization and later is released into milk by desorption from these surfaces (32).

Although results of different workers vary considerably, it appears that the major proportion (80 to 90%) of physiologically-derived iodine in milk exists in the inorganic or iodide form and is located in the water-soluble fraction of milk (2, 10, 12, 16, 17, 23, 25, 26). The remainder is associated with the protein fraction through either covalent bonds or loose physical associations (17, 19). The chemical nature of iodophor-derived iodine in milk and its association with milk components are largely unstudied, but one report indicated that such iodine is in the iodide form (6).

Most fresh milk today is processed, and yet little attention has been given to effects of processing upon either concentration of physiological iodine in milk or its association with milk components. Some studies showed that heating and boiling of milk reduced iodine concentration by 20% (1, 7, 14), whereas spray- and roller-drying reduced it by 40% (20). It is not clear how this reduction occurred if milk iodine is in the iodide form and, thus, unlikely

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¹ University of New South Wales, Sydney.

to volatilize. The effect of processing upon iodophor-derived iodine has not been studied.

The possibility that milk iodine concentration can be reduced by processing treatment such as pasteurization has important implications for public health and for regulatory authorities whose functions may include monitoring iodine in milk. This study aims to examine the effect of processing upon concentration and distribution of physiological and iodophor-derived iodine in milk. Because of the analytical problems associated with measurement of small concentrations of iodine in milk (29), two procedures were used for iodine analysis. A laboratory-prepared iodophor of known and constant composition (31) was used for controlled addition of iodophor to milk, and in some cases this iodophor was labeled with ^{131}I to provide further information on changes in concentration and distribution of iodine in milk.

EXPERIMENTAL PROCEDURES

Milk

Iodophor-free milk was obtained from two cows kept solely for this purpose, and no iodophor was used either for teat dipping or equipment sanitization. Where necessary, milk was centrifuged (BTL Bench Centrifuge, $4000 \times g/15$ min) and cream removed. Milk was refrigerated at 4°C until needed.

Acid Precipitation of Casein

With continuous stirring, 1M-hydrochloric acid was added to 100-ml aliquots of skim milk at 25°C by a micro-burette with its tip just below the milk surface; 1 ml acid was added each 7 min until 4.0 ml had been added, then .5 ml each 7 min until a pH of 4.5 to 4.6 was reached. The volume of acid was noted. After 30-min stirring, the mixture was centrifuged as before; the whey was decanted and its volume measured. Precipitated casein was macerated, mixed well with 80 ml distilled water for washing, and centrifuged (BTL Bench Centrifuge, $4000 \times g/15$ min). Wash water was removed and the washing procedure repeated. The casein was then solubilized: 50 ml water was added to the macerated casein and 1M-sodium hydroxide added during stirring. When the pH reached 7.0 to 7.2, the mixture was left

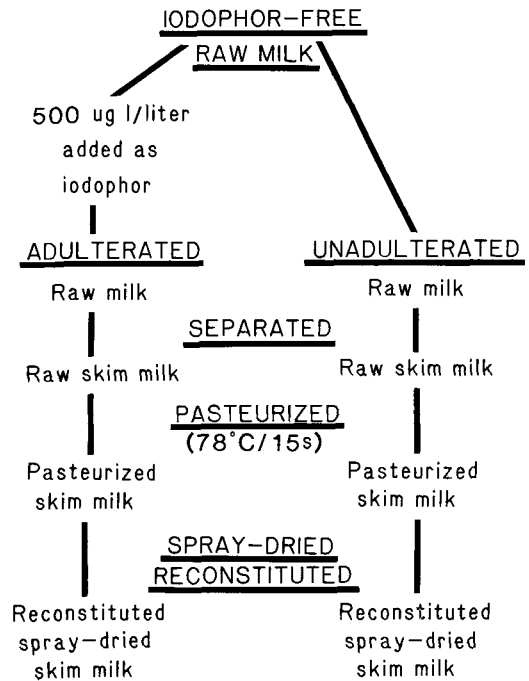


Figure 1. Milk processing procedure.

to stir overnight, and, if necessary, the pH was readjusted to 7.0 to 7.2. Volume of hydroxide was noted.

Milk Processing

Iodophor-free milk was treated as in Figure 1; the whole treatment was repeated three times on different days with an initial milk volume of 60 liters each time. Cream was removed with a centrifugal separator (Model 108, Alfa Laval, Sweden) and pasteurization was at $78 \pm 1^\circ\text{C}/15$ s in a pilot-scale plate heat exchanger (Model P20-HB, Alfa Laval, Sweden). A pilot-scale spray-drier (Type Lab S1, Anhydro, Denmark) was used for the final step, with atomizer speed 1600 rpm, inlet temperature $200 \pm 10^\circ\text{C}$, and outlet temperature $90 \pm 5^\circ\text{C}$. Samples of milk and cream were taken at each processing stage for later analysis. Total solids of both pasteurized skim milk and reconstituted spray-dried milk were measured.

Fractionation of Cream into Fat and Serum

Each cream sample was agitated in a commercial blender until separation of fat and

serum occurred. Serum was removed and sampled for later analysis; fat was washed seven times with distilled water in the blender and a fat sample then removed for analysis.

Analysis of Iodine in Milk, Casein, and Whey

Chemical Method. Samples for chemical iodine analysis were frozen in polypropylene vials and dispatched by air to Laboratory Services Pty. Ltd., Melbourne, Vic., Australia. Duplicate analyses were performed on each sample by a semi-automatic version of the Sandell-Kolthoff reaction (13).

Electrode Method. Samples were refrigerated until analyzed. The method (30) was an adaptation of one designed for plant material (9). An Orion iodide electrode (Model 94-53A, Orion Research Inc., USA) and a single junction reference electrode (Orion 90-01, filled with Orion solution 90-00-01) were used with a millivolt meter (Digital Ionalyzer 701A, Orion Inc.).

Iodophor

A laboratory-formulated iodophor of known iodine concentration was used (31). Dilute iodophor was labeled with Na-¹³¹I (Australian Atomic Energy Commission, Lucas Heights, NSW, Australia) (32).

Apparatus for Counting ¹³¹I Label

Gamma counts were by sodium iodide crystal mounted in a lead tower attached by a photomultiplier relay to an IDL Scaler Mk II.

Statistical Procedures

Analysis of variance was according to Snedecor and Cochran (24).

RESULTS

Effect of Distillation upon Concentration of Iodophor-Derived Iodine in Milk

Iodophor labeled by ¹³¹I was added to skim milk at 25°C to give an addition of 300 µg I/liter. One sample of milk was removed for counting. Another 100-ml sample was distilled in a glass distillation apparatus until its volume was reduced by about half. Samples of both residue and distillate were counted, and results of these counts are in Table 1 together with calculated recovery. The bulk (94%) of the iodine remained in the residue after distillation and was not volatilized and lost during heating and distillation of milk.

Effect of Acid Precipitation of Casein upon Distribution of Iodophor-Derived Iodine in Milk

Radioactively-labeled iodophor was added to iodophor-free skim milk at 25°C. The milk was refrigerated overnight at 4°C, and after 18-h contact with iodophor, casein was precipitated from 3 × 100-ml samples by addition of dilute hydrochloric acid. Aliquots of whey and solubilized casein and both wash-waters were counted, and counts from each fraction for each milk sample were subjected to one-way analysis of variance. There was no significant difference ($P < .01$) between the iodine distribution in each fraction of each of the three

TABLE 1. Distribution of ¹³¹I after distillation of skim milk containing labeled iodophor.

Fraction	Volume (ml)	¹³¹ I count on 2 ml		Mean total count	Proportion of milk count (%)
		\bar{X}^a	SD		
Skim milk	100	1.015×10^5	4.92×10^2	5.077×10^6	
Distillate	41	57	11.9	1.169×10^3	.02
Residue	56	1.709×10^5	1.119×10^3	4.786×10^6	94.28
Recovery	97 ^b				94.30

^aMean of 5 counts.

^bThree milliliters of sample remained as fine droplets in the apparatus and could not be recovered.

replicate samples, indicating that the method of milk fractionation and label counting was highly reproducible. Mean counts for each fraction (Table 2) show that only a small percentage (3.4%) of iodine was associated with casein under these conditions, and of the remaining ^{131}I , 77.9% was recovered from the whey. The 17.7% measured in the wash-waters also would enter the whey fraction.

Effect of Cream Removal, Pasteurization, and Spray-drying upon Concentration and Distribution of Iodine in Milk

Iodophor-free raw milk was adulterated by addition of laboratory-prepared iodophor to give 500 $\mu\text{g/liter}$ of added iodine, and refrigerated overnight at 4°C. The milk then was processed as described. A parallel processing procedure was performed on a sample of the same raw milk that was not adulterated by iodophor.

Effect of Processing upon Iodine Concentration of Milk. Iodine concentration of each sample of milk was determined by both a chemical and an electrode method, and results of both analyses are in Table 3. To discover whether processing caused a significant change of iodine concentration in either adulterated or unadulterated milk, and in addition to determine whether the two analytical methods gave the same results, both measures for milk iodine concentration were subjected to analysis of variance. There was no preferential association of iodine with the cream component of either adulterated or unadulterated milk ($P < .05$) as measured by either analytical method because

the iodine concentration of skim milk remained the same as that of whole milk. Similarly, pasteurization of skim milk (either adulterated or unadulterated) followed by spray drying and reconstitution did not change significantly iodine concentration of milk. Any differences in Table 3 are not statistically significant at 5%. However, the two methods used for analysis of iodine did give significantly different iodine concentrations of milks ($P < .01$) with the mean electrode result being higher than that by the chemical method. The large standard deviations ($>10\%$ in some cases) for both analytical methods may be attributed partly to processing runs being on different days with different batches of milk, but statistical analyses showed no significant difference ($P < .05$) between iodine concentrations of milks in the three trials. The large variance also may reflect difficulties with analysis of iodine in milk.

Recovery of the added iodophor-iodine is also in Table 3, and recovery of this iodine was 7% higher by the electrode method than by the chemical method.

Effect of Processing upon Iodine Concentration of Whey and Casein. At each stage of the processing experiment, samples of both adulterated and unadulterated milk were subjected to acid precipitation of casein. The whey and solubilized casein fractions were analyzed for iodine concentration by both chemical and electrode methods. Results of these analyses were converted to give the iodine concentration of each fraction in terms of the original milk sample.

TABLE 2. Distribution of iodine after acid precipitation of casein from milk containing labeled iodophor.

Fraction	^{131}I count on 2 ml sample		Mean total count of ^{131}I	Proportion of count in skim milk (%)
	\bar{X}^a	SD		
Skim milk	9.274×10^4	1.79×10^3	4.173×10^6	
Whey	8.262×10^4	1.06×10^3	3.250×10^6	77.9
Wash 1	1.490×10^4	6.44×10^2	5.984×10^5	14.3
Wash 2	3.495×10^3	2.85×10^2	1.398×10^5	3.4
Casein	4.185×10^3	1.26×10^2	1.413×10^5	3.4
Recovery of ^{131}I				99.0

^aMean of five replicates from each of three samples.

Milk for the processing runs was obtained at weekly intervals from the same two cows. On the two occasions where the processed milk was fractionated into whey and casein components, the casein content of the milks varied by some 14% (by volume). The results of iodine determinations on whey and casein samples from two processing runs are in Tables 4 and 5.

Iodine concentrations in whey samples (Table 4) showed large and inconsistent variations, and the only conclusion was that whey from iodophor-adulterated milk showed (as might be expected) higher iodine concentration than whey from unadulterated milk. There was no significant ($P < .05$) association of either physiological or iodophor-derived iodine with the whey fraction as a result of any processing treatment, but this statistic was influenced by the large variation between samples.

Iodine concentrations in various samples by the two methods of analysis also were compared. Although, on some occasions, methods gave similar results for a particular sample, on other occasions variations were apparent. There was no difference ($P < .05$) between the two methods

for measuring iodine in whey samples.

Table 5 shows iodine concentrations from casein samples. As in the experiment using the ^{131}I label, the amount of iodine associated with casein fractions is much less than that in whey fractions. For a given sample, higher iodine concentrations were generally when measurements were with electrode than by chemical method. This difference was significant at 95%. For unadulterated milk, iodine associated with each casein fraction was not significantly different ($P < .05$), and, within the experimental error, processing does not cause any preferential association of physiological iodine with casein. For iodophor-adulterated milk, iodine concentration of casein was higher ($P < .05$) than that of casein from unadulterated milk. Overall, there was no significant difference ($P < .05$) between iodine concentration of casein fractions from each processing stage. It seemed that a small proportion (about 2%) of added iodophor-iodine became associated with casein on initial adulteration, but there was no tendency for this association to change as a result of processing.

TABLE 3. Iodine concentration of milk subjected to processing.

Milk sample	Iodine concentration of milk ($\mu\text{g I/liter}$)							
	Unadulterated milk				Adulterated ^b milk			
	Electrode method		Chemical method		Electrode method		Chemical method	
	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD
Whole milk	268.3	46.46	156.0	12.00	753.3	55.08	524.0	52.00
Raw skim milk	246.7	28.43	177.3	30.55	626.7	68.07	628.0	84.85
Pasteurized skim milk	248.3	40.41	176.0	36.66	705.0	65.38	616.0	28.84
Reconstituted skim milk after spray drying	243.3	41.63	182.7	40.07	740.0	105.83	602.7	120.95
Mean iodine concentration of milk	251.65		173.00		706.25		592.7	
Mean recovery of iodine added as iodophor (%)					90.9		83.9	

^aMean of duplicate analyses from each of three separate processing trials; iodine concentration measured on the same sample by both a chemical and an electrode method.

^bAddition of 500 $\mu\text{g I/liter}$, as iodophor, to unadulterated milk.

TABLE 4. Iodine concentration of whey obtained from processed milks.

Source of whey		Iodine concentration of whey ($\mu\text{g I/liter milk}$)			
		Unadulterated		Adulterated ^a	
		Electrode method	Chemical method	Electrode method	Chemical method
Whole milk	(1) ^b	102.5	96.3	355.0	415.0
	(2)	100.0	122.5	288.0	371.6
Raw skim milk	(1)	70.0	96.3	331.3	457.5
	(2)	48.8	166.3	732.5	446.6
Pasteurized skim milk	(1)	81.3	92.5	402.5	447.5
	(2)	77.5	165.0	265.0	556.3
Skim milk reconstituted after spray drying	(1)	103.8	78.8	451.3	435.0
	(2)	157.5	154.7	566.3	463.8

^aAddition of 500 $\mu\text{g I/liter}$ as iodophor to unadulterated milk.

^b(1) and (2) indicate the sets of data from each of the two processing runs.

Distribution of Physiological and Iodophor-Derived Iodine in Cream. Cream from centrifugal separation of the original whole adulterated and unadulterated milk was fractionated further into milkfat and buttermilk (serum) by agitation. Iodine concentration of each fraction was measured by the chemical method only, and iodine concentration of cream was measured

by both chemical and electrode methods. Results in Table 6 show that iodine concentration of milkfat is unaffected by adulteration of milk with iodophor and that the increase of iodine concentration in cream after adulteration may be accounted for by the increased iodine concentration of the serum.

TABLE 5. Iodine concentration of casein from processed milks.

Source of casein		Iodine concentration of casein ($\mu\text{g I/liter milk}$)			
		Unadulterated		Adulterated ^a	
		Electrode method	Chemical method	Electrode method	Chemical method
Whole milk	(1)	52.5	37.5	63.8	103.8
	(2)	41.3	18.6	40.0	34.9
Raw skim milk	(1)	47.5	35.5	55.0	35.0
	(2)	35.0	24.4	36.3	50.8
Pasteurized skim milk	(1)	53.8	16.3	65.0	57.5
	(2)	41.3	37.1	51.3	21.6
Skim milk reconstituted after spray drying	(1)	60.0	45.0	67.5	48.8
	(2)	50.0	32.4	70.0	39.1

^aAddition of 500 $\mu\text{g I/liter}$, as iodophor, to unadulterated milk.

^b(1) and (2) indicate the sets of data from each of the two processing runs.

TABLE 6. Iodine concentration ($\mu\text{g l/liter}$) of cream and cream fractions from raw unadulterated milk and milk adulterated by addition of iodophor.

Fraction	Mean ^a iodine concentration of cream and cream fractions ($\mu\text{g l/liter}$)							
	Unadulterated milk				Adulterated milk			
	Electrode method		Chemical method		Electrode method		Chemical method	
	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD
Cream	236.7	33.29	186.0	64.09	591.7	120.03	418.3	179.44
Milkfat	b		105.0	17.32	b		92.7	27.50
Serum	b		170.0	44.06	b		549.3	85.45

^aMean of two replicates from three samples.

^bNot analyzed.

DISCUSSION

The loss of milk iodine during processing (1, 7, 14, 20) was reexamined, and the fate of iodophor-iodine during milk processing also was examined.

In initial experiments, ¹³¹I-labeled iodophor was added to milk, which was boiled subsequently. The label was recovered virtually quantitatively in the milk and not the distillate. Previous work (32) showed that the ¹³¹I label is incorporated into the volatile available iodine fraction of the iodophor, and, thus, it appears that this fraction is converted to the nonvolatile iodide form on addition of iodophor to milk. In another experiment, after addition of ¹³¹I-labeled iodophor to skim milk, the label was recovered mostly from the whey fraction after precipitation and washing of casein, and only a small percentage (3.4%) of the label was recovered from the precipitated protein. Thus, it seems that iodophor-iodine exhibited little tendency to associate with milk casein and remained in solution in the whey fraction.

In subsequent experiments, unadulterated milk and milk deliberately adulterated with iodophor were processed, and the iodine content of milk, whey, and casein were measured at each step by two analytical procedures. Although the milk processing experiment was repeated three times, and iodine determinations were duplicated on each sample by each analytical procedure, reproducibility of results was not as close as desired. However, statistical examination showed that certain conclusions could be

drawn. First, milk iodine concentration remained constant throughout processing, and this conclusion was noted for both adulterated and unadulterated milks and was obtained by two analytical methods for milk iodine (the significant difference in the absolute measures of the two methods is discussed later). This conclusion is at variance with results of previous workers who reported losses of milk iodine during processing (1, 7, 14, 20); this discrepancy may reflect the difficulty of measuring iodine concentration of milk accurately. Nevertheless, if milk iodine exists largely in the iodide form with the remainder covalently or physically bound to casein, it is difficult to explain losses from volatilization of iodine as reported.

Examination of iodine concentration of whey fractions showed that the bulk of milk iodine, whether physiological or iodophor-derived, was associated with whey, although the proportion associated with this fraction varied according to analytical method. This result was similar to that obtained with ¹³¹I-labeled iodophor (Table 2). Statistical analysis of whey iodine concentrations showed no preferential association of either physiological or iodophor-derived iodine with the whey during processing.

About 20% of the physiological milk iodine was recovered from the casein fractions of the processed milks, and, despite some variation in results, statistically there was no tendency for this association of iodine with casein to increase as a result of milk processing. The association of a proportion (5 to 10%) of physiological

milk iodine with milk protein has been established (18). The relatively small but statistically significant differences in the absolute iodine concentration of casein fractions by the two analytical methods pose an interesting problem, because the results from the electrode were higher than those from the chemical method. Iodine covalently bound to protein should not induce a response in the electrode, and it might be expected, therefore, that the chemical method would give results higher than the electrode (see later for further discussion of this point). Consistent with results with ^{131}I -labeled iodophor (Table 4), increase of iodine concentration was small in the casein from iodophor-adulterated milks, and, according to statistical analysis, this amount did not alter as a result of processing. The nature of this iodine-casein association is not known, but addition of ^{131}I to milk involves inclusion of ^{131}I into monoiodotyrosine (17, 18).

Iodine determinations on cream from both unadulterated and iodophor-adulterated milks showed variations greater than was desired; this again may be related to method of analysis (see later). However, there appeared to be no preferential association of iodine with cream, because iodine concentration of cream fraction was similar to that of the whole milk from which it was derived, and these two concentrations were similar to the concentration of the skim milk. When cream was subfractionated into milkfat and serum, the physiological iodine was approximately equally distributed between the two fractions. This equal distribution was not observed with cream from the iodophor-adulterated milk where the bulk of the iodine was recovered in the serum. The effect of added iodide on milk fat has been studied (19), and iodine content of milkfat was unaffected by addition of iodide to milk, supporting conclusions of our study. However, other workers found that of ^{131}I -labeled iodide added to milk 22% was recovered in the milk fat (11). The reasons for these conflicting observations are not clear but may reside in difficulties of measuring iodine in cream samples. Iodine concentrations are relatively low normally in dairy products of high milkfat content (5).

Results were inconsistent on some occasions by both methods of analysis for iodine. The two methods gave different absolute iodine concentrations of samples. Although both

methods have been used for measuring iodine in milk, neither has been used for cream, casein, or whey; it is not known, therefore, whether the variability of results reflects inadequacies of the methods in their application to these specific milk fractions. It was for this reason that two differing methods were chosen at the outset to examine iodine concentrations.

The electrode method gave significantly higher milk iodine concentrations than the chemical method. This was for both unadulterated and iodophor adulterated milks, before and after processing. A similar discrepancy was noted (3, 15) and attributed to either loss of iodine during ashing in the chemical method (15) or an elevated response of the electrode from a nonspecificity between iodide and sulphhydryl groups in milk (3).

A more comprehensive evaluation of methods for measurement of iodine in milk and its fractions is required. Nevertheless, heat-treatment and processing of milk cannot be relied upon to reduce the iodine concentration of manufactured dairy products, and initial iodine contamination, therefore, must be avoided.

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