Possibilities for Radiocesium Decontamination of Kefalograviera Cheese Through Modifications of the Standard Manufacture Method

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ABSTRACT

The transfer of radiocesium during all phases of kefalograviera cheese making was monitored, and the corresponding transfer coefficients were determined. To reduce the radiocesium concentration of the final product, modifications of the standard cheese making procedure, as single and double curd washing, were investigated.

The transfer coefficients from milk to cheese were determined as .33 and .22, .15 for the standard and the modified (single and double curd washing) procedures, respectively. Milk to curd transfer coefficients were also measured and total Cs recovery was verified.

Changes in the composition and taste of the product due to the modifications of the standard procedure were also investigated and remedies are proposed.

(Key words: radiocesium, decontamination, kefalograviera)

INTRODUCTION

The contamination of pastures by fallout from the nuclear accident at Chernobyl on April 26, 1986 caused serious problems in the use of milk from free-grazing animals (mainly sheep and goats) in Greece. Due to the high radioactivity content (1), severe restrictions were placed on the distribution and consumption of ovine milk during May and June 1986. All sheep milk produced during that period was processed into soft cheese (primarily feta) and hard cheese (mainly kefalograviera). This eliminated health hazards from the short-lived $^{131}I$ ($T_{1/2} = 8.04$ d), because this isotope's activity became negligible during the maturation period of the cheese. However, a problem still remained with regard to the long-lived isotopes $^{134}Cs$ ($T_{1/2} = 2.06$ yr) and $^{137}Cs$ ($T_{1/2} = 30.1$ yr). For these isotopes, the European community limit was set at 600 Bq/kg, but the measured contamination in some cheeses were higher. Assuming a daily consumption of 100 g of cheese and a contamination of 600 Bq/kg ($^{134}Cs$:200 Bq/kg; $^{137}Cs$:400 Bq/kg) a resultant effective dose of .3 mSv is calculated for 1 yr (10). This level suggests 15 new cases of cancer will occur for the population of Greece (6). It should be noted, however, that in nuclear accident situations, like the one following the Chernobyl accident, levels 10 times higher were measured in cheese.

The transfer of the radiocesium isotopes during the cheese making process has been recently investigated (2, 9, 17, 18), yet few attempts have been made to study those operations during cheese making, which could possibly reduce the radiocesium content of the final product. In a more recent paper, Pappas et al. (15) have reported on the effects of several processes contributing to the radiocesium decontamination of feta, a soft cheese. The object of the present work was to investigate processes that may reduce the radiocesium concentration of a hard cheese made from contami-
DECONTAMINATION OF KEFALOGRAVIERA

MATERIALS AND METHODS

Milk Contamination

Thirty ewes were selected from a free-grazing flock at the Agricultural Research Station of Ioannina and confined in an isolated pen. Because the aim of this procedure was to produce contaminated milk, feeding of the animals was on an individual basis. The animals were allowed to feed ad libitum on contaminated wheat and dried grass (Medicago sativa) harvested in Central Macedonia during the 1st wk of June 1986, which was thus strongly affected by the Chernobyl fallout. Samples of this grass and wheat were measured for Cs contamination at the Nuclear Physics Laboratory, University of Ioannina. Average contamination was 9000 Bq/kg for grass and 3000 Bq/kg for wheat. The average consumption of wheat and grass (total weight divided by the number of animals) was 500 and 150 g per animal per day. To supplement their daily allowance, the ewes were also allowed to graze on uncontaminated grass.

The sheep were milked in the morning and in the evening. The evening milk, kept at 4°C overnight, was mixed with next morning’s milk at the laboratories of the Dairy Research Institute, where it was processed into kefalograviera cheese.

Before cheese making, samples of milk were delivered to the Nuclear Physics Laboratory, where the contamination was determined. The measured activity concentration ranged from 130 to 230 Bq/L, following variations in the consumption of contaminated feed.

Cheese Making by the Standard Method

Thirty kilograms of radiocontaminated milk of 5.6% fat and 11.5% SNF were pasteurized at 63 to 65°C for 20 min. Yogurt starter culture of Streptococcus thermophilus and Lactobacillus bulgaricus (Chr. Hansen’s Laboratory, Denmark) at .5% (wt/wt) was added 15 min before renneting. Fifteen milliliters of 40% CaCl2 solution and liquid rennet were added to achieve the coagulation of milk at 37°C in approximately 25 to 30 min. After coagulation, the curd was cut to 2.5- to 3.0-cm cubes and left to rest for 1 to 2 min. Cutting lasted about 10 min, until the pieces of curd were sliced down to the size of a maize grain. Following this, the curd was promptly scalded with gentle stirring, until the final temperature of 48°C was reached in about 15 to 20 min (the rates of heating were: 1°C for 2 min, 1°C for 1.5 min, and 1°C for 1 min for temperatures 37 to 41°C, 41 to 45°C, and 45 to 48°C, respectively). Stirring was continued for an additional 5 min at this temperature. Then all the curd was wrapped in a cheesecloth, placed into a metal hoop, and a pressure of 1.38 N/cm2 was applied for 2.5 h. During the pressing of the curd, the cloth was changed twice. Following this treatment, the cloth was removed and the cheese was left overnight in molds at 14 to 15°C. The dimensions of cheese, which the next morning weighed 5.5 to 5.8 kg, were 30 cm in diameter and 13 cm in height. The quantity of the whey remaining in the cheese vat after hooping the curd was collected and mixed with the amount of whey drained off during the pressing procedure of the cheese. The next morning, the radiocesium concentration of cheese and whey were measured. The cheese was then salted by immersion in brine of 15% NaCl; the cheese stayed in brine for 5 d without turning. The ratio of cheese weight to brine volume was .5. When the cheese was removed from the brine, it was placed on a clean board, and a small quantity of salt was spread on the surface. It was allowed to remain in this state at 14 to 16°C for 2 to 3 d. Each day the curd was wiped dry with a cloth dipped in salt. During the next 2 mo the cheese was allowed to ripen stored at 14 to 16°C. Finally, the cheese was coated with paraffin and stored at 4°C.

Investigation of the Salting Phase

It has been demonstrated by Pappas et al. (15) that periodic replacements of brine effectively contribute to the decontamination of feta cheese during storage. Because salting in brine is one of the standard phases of kefalograviera cheese making, it was considered worthwhile to investigate the transport of radiocesium during this phase and to experiment with brine replacements. The radiocesium content of the curd was thus monitored every 24 h for 5 d during the salting phase of the cheese.

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According to the model developed by Pappas et al. (15), when a mass (M1, kg) of curd with initial contamination concentration (C0, Bq/kg) is immersed in brine of volume (V2, L), the reduction of radiocontamination concentration [C1(t)] of the curd with respect to time is described by the relation:

\[ C_1(t) = \frac{A C_0}{B} + C_0(1 - \frac{A}{B})\exp(-Bt) \]  

where:

\[ A = \frac{k_2}{V_2} \]  

\[ B = \frac{k_1}{M_1} + \frac{k_2}{V_2} \]

and \( k_1 \) (kg/h) and \( k_2 \) (L/h) are rate constants. Equation [1] is an exponentially decreasing function, which eventually reaches the equilibrium value of

\[ C_{\text{eq}} = \frac{k_2 M_1 C_0}{k_1 V_2 + k_2 M_1} \]  

with a characteristic half-life of

\[ T_5 = \frac{0.693 M_1 V_2}{k_1 V_2 + k_2 M_1} \]

Because the equilibrium contamination of the curd in Equation [4] is essentially reached within \( 3T_5 \), periodic replacements of the brine could effectively reduce the contamination of the product only if the time interval \( 3T_5 \) is substantially smaller than the salting period (5 d for kefalograviera). It was therefore undertaken, by a series of measurements, to determine the values of the rate coefficients \( k_1 \) and \( k_2 \) and, through them, the values of \( C_{\text{eq}} \) and \( T_5 \) in Equations [4] and [5].

**Modification of the Standard Method**

In order to reduce the radioesium concentration of kefalograviera cheese, whey was partially replaced by water during the scalding phase of the curd.

It is common practice in the manufacture of Edam cheese to replace half of the whey by hot water while scalding the curd (16). The application of this procedure to the manufacture of kefalograviera cheese could possibly reduce the radioesium concentration in the curd. In a series of experiments, the curd was scalded for 10 to 15 min and, subsequently, quantities of whey, either 40 or 50% (wt/wt) of the starting milk volume, were replaced by equal quantities of water that had been previously heated at the final scalding temperature (48°C), while stirring vigorously to avoid the consolidation of cheese grains (single curd washing). The stirring was continued for 5 to 7 min at scalding temperature.

Several variations of this method were explored. In one treatment, 50% of whey was twice replaced by water (double curd washing). In another double curd washing experiment, lactose monohydrate was added in a quantity sufficient to restore the concentration of lactose in the whey after the second addition of water (1.08%) to the level measured in the original whey (4.53%). Both levels were determined in preliminary experiments by the method described by the International Dairy Federation (7). Moreover, starter culture (the same yogurt culture used in the standard manufacture process) at .5% and 15 ml of 40% CaCl2 solution were simultaneously added with lactose. The addition of lactose and starter culture was considered necessary to ensure adequate lactose and microorganisms in the curd for the normal ripening of cheese and with acceptable final organoleptic and nutritional properties.

**Radioactivity Measurements**

All liquid and solid samples were measured in a standard geometry of 400 ml with a 1.9 keV resolution (for the 661.65 keV line of \(^{137}\text{Cs}\)), 15% efficiency, intrinsic Ge detector. The detector was shielded with 5 cm of lead against background radiation. Standard electronics were used, and the spectra, accumulated in 1024 channels, were stored for analysis in the Nuclear Physics Laboratory's computer. The detector was calibrated for efficiency with a standard \(^{152}\text{Eu}\) source. The accumulation of a spectrum with adequate (less than 10%) statistics required 1000 to 3000 s. In the spectra obtained through measurements of the dairy samples, the peaks due to \(^{134}\text{Cs}\) and \(^{137}\text{Cs}\) were identified and analyzed with a modified version of code ANNA (3). The average ratio of the activity concentrations of \(^{134}\text{Cs}\) to \(^{137}\text{Cs}\) in the samples was determined as .2 ± .1. Because the
TABLE 1. Transfer of $^{137}$Cs from sheep milk into curd and whey during the manufacture of kefalograviera cheese according to the standard method.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Milk (Bq/L)</th>
<th>Curd (Bq/kg)</th>
<th>Whey (Bq/L)</th>
<th>Milk to curd (L/kg)</th>
<th>Milk to whey (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>127 ± 12</td>
<td>62 ± 5</td>
<td>144 ± 13</td>
<td>.49 ± .06</td>
<td>1.13 ± .15</td>
</tr>
<tr>
<td>2</td>
<td>132 ± 4</td>
<td>60 ± 5</td>
<td>133 ± 9</td>
<td>.46 ± .04</td>
<td>1.01 ± .07</td>
</tr>
<tr>
<td>3</td>
<td>132 ± 13</td>
<td>69 ± 5</td>
<td>152 ± 11</td>
<td>.52 ± .06</td>
<td>1.15 ± .14</td>
</tr>
<tr>
<td>X</td>
<td>130 ± 3</td>
<td>64 ± 5</td>
<td>143 ± 10</td>
<td>.49 ± .04</td>
<td>1.10 ± .08</td>
</tr>
</tbody>
</table>

Chemical Analysis and Sensory Evaluation of the Cheese

Three months after cheese making, the cheese was subjected to chemical analysis and sensory evaluation. Moisture was determined by the method adopted by the International Dairy Federation (8). The fat content was measured by the Gerber method (5). Total nitrogen and soluble nitrogen of ripened cheese were determined by the Kjeldahl method as described by Kosikowski (11). Ash was determined according to the analytical methods of the Association of Official Analytical Chemists (4). Acidity, expressed by the lactic acid content of cheese, was measured according to the method described by Ling (14). The pH of cheese was also measured.

After ripening, cheese produced according to the standard method and cheese processed through variations of the standard method were subjected to sensory evaluation by a group of four panelists for taste and texture. The rating was based on a seven-point hedonic scale (12).

RESULTS

Investigation of the Standard Method

Cheese Making Phase. Results on the transfer of $^{137}$Cs from ovine milk to curd and whey during the manufacture of kefalograviera cheese according to the standard method described in the previous section are presented in Table 1. In Table 2, the recovery of $^{137}$Cs from milk to its products, curd and whey, is also shown. In all three cases, the recovery is 100% within experimental errors. The errors associated with mean values contained in Tables 1 and 2, as well as errors appearing elsewhere in the paper, denote 1 SD. The transfer coefficients $f$ (L/kg) are also contained in Table 1. Following the definition of Pappas et al. (15), the transfer coefficients provide the measure of the percentage radioesium transfer as the ratio:

$$f = \frac{C_P}{C_M}$$

where $C_P$ and $C_M$ are the radioesium concentrations in the final product and the original milk, respectively. For the standard cheese making method, the milk to curd ($f_{MC}$) and the milk to whey ($f_{MW}$) transfer coefficients were determined as:

$$f_{MC} = .49 \pm .04 \text{ L/kg}$$
$$f_{MW} = 1.10 \pm .08.$$

The value found for the milk to curd transfer coefficient is in agreement with the results of earlier studies. Thus, from an investigation by Assimakopoulos et al. (2), it is inferred that the transfer of radioesium into curd during the routine cheese making of Gruyère cheese may be described by a transfer coefficient equal to .42 ± .02 L/kg. Similarly, Kandarakis and Annyfantakis (9) have reported a transfer coefficient of .47 L/kg to describe the transfer of radioesium into kefalotyri, a cheese similar to kefalograviera. However, Pappas et al. (15)
TABLE 2. Recovery of $^{137}\text{Cs}$ from milk, to the products curd and whey of the three separate experiments presented in Table 1.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Milk (Bq)</th>
<th>Curd and whey (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\overline{X}$</td>
<td>SD</td>
</tr>
<tr>
<td>1</td>
<td>3678</td>
<td>347</td>
</tr>
<tr>
<td>2</td>
<td>3823</td>
<td>115</td>
</tr>
<tr>
<td>3</td>
<td>3823</td>
<td>377</td>
</tr>
</tbody>
</table>

have found that the milk to curd transfer coefficient of radiocesium during the processing of sheep milk to feta cheese is $0.79 \pm 0.04$ L/kg. Because the transfer of cesium into the products of cheese making follows closely the subdivision of moisture (15), this marked difference with the findings of the present and other studies on different types of cheeses is attributed to the different moisture content of the cheeses (e.g., the moisture of feta, determined the next day of manufacturing, is 60 to 61%; for kefalograviera, 43 to 44%).

The distribution of radiocesium among the products of cheese making for kefalograviera from sheep milk is also expressed through the percentage retention of radiocesium calculated from the data in Table 1 and presented in Table 3. In routine production, $30 \text{ L}$ of milk yield, on the average, $5.5 \text{ kg}$ of curd and $23.6 \text{ L}$ of whey; thus, the mean percentage retention of the radiocesium contamination in the curd and whey are $9.3 \pm 0.6$ and $82 \pm 7$, respectively. Once more it is verified that the retention of cesium follows closely the distribution of water from milk into the products of cheese making ($9.6 \pm 0.4$ and $90 \pm 5$, respectively).

The Salting Phase. The percentage reduction of $^{137}\text{Cs}$ concentration in curd during the salting phase is presented in Figure 1. The best fit of Equation [1] to the data determined the values of the parameters:

$$k_1 = 0.047 \pm 0.015 \text{ kg/h}$$
$$k_2 = 0.172 \pm 0.066 \text{ L/h}.$$

For an average curd weight of $M_1 = 5.54 \text{ kg}$ and brine volume of $V_2 = 11.08 \text{ L}$, these values may be used to calculate the final radiocesium concentration level in the curd (see Equations [4] and [5]):

$$C_{1eq} = (0.6 \pm 0.2)C_0 \text{ Bq/kg}$$

as well as the half-life time constant with which the equilibrium state is attained:

$$T_5 = 28 \pm 9 \text{ h}.$$

Because the equilibrium is effectively achieved within $3T_5$, to maximize the extraction of radiocesium from kefalograviera through
TABLE 4. The effects of curd washing and curd salting on the transfer of $^{137}$Cs from milk to curd. Measurements on curd were performed before and after curd salting.

<table>
<thead>
<tr>
<th>Whey replaced by water (%) of initial milk</th>
<th>$^{137}$Cs transfer coefficient (L/kg)</th>
<th>Reduction (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>$SD$</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>0</td>
<td>.49</td>
<td>.04</td>
<td>.29</td>
</tr>
<tr>
<td>40</td>
<td>.30</td>
<td>.03</td>
<td>.21</td>
</tr>
<tr>
<td>50</td>
<td>.26</td>
<td>.03</td>
<td>.15</td>
</tr>
<tr>
<td>50</td>
<td>.19</td>
<td>.03</td>
<td>.12</td>
</tr>
<tr>
<td>50</td>
<td>.18</td>
<td>.03</td>
<td>.11</td>
</tr>
</tbody>
</table>

$^1$Weighted mean of three independent measurements.

Modifications of the Standard Method

The effect of washing the curd by replacing varying volumes of whey, expressed as percentages (wt/wt) of the original milk, on the milk to curd radiocesium transfer coefficient is presented in Table 4. From these results, it is deduced that the partial replacement of whey with water, amounting to 40% of the original milk at scalding temperature (48°C), reduces the radiocesium concentration at the 67 ± 12% of the (control) cheese prepared according to the standard method. A further increase in the quantity of water to 50% of the original milk decreases radiocesium concentration down to 61 ± 12%. However, when a second curd washing step is introduced (double washing), the concentration of radiocesium in the curd dropped to 48 ± 11%. A similar reduction of 42 ± 8% is measured when the double washing treatment is repeated with the addition of culture, lactose, and calcium into whey after the second washing in order to compensate for the degradation of the quality of cheese due to the washings.

Effect of Different Treatments on the Properties of the Ripened Cheese

To examine if the modifications of the standard method have any effect on the composition and the organoleptic properties of cheese, we determined the fat, moisture, protein, and ash content as well as the acidity and the pH of the final product (Table 5).

Fat is lost during the treatment in which water is added to the original milk or the curd is washed with water. The loss is proportional to the amount of excess water involved in each treatment.

The moisture of the cheese is not significantly affected by the different processes considered herein. It should be noted, however, that the moisture content of all cheeses prepared in this study was lower than the content of kefalograviera produced in dairy factories (on the average, 37.5%) due to excessive evaporation of moisture during ripening in the storage room, where the relative humidity was low (uncontrolled atmosphere). As a result of the evaporation of moisture and the subsequent losses of the cheese weight, the radiocesium concentration of cheese increased during the ripening phase before coating with paraffin.

The total nitrogen content of cheese, expressed as a percentage of dry matter, was not
effectively influenced by the modifications imposed on the standard method. However, the soluble nitrogen content, expressed as a percentage of total nitrogen, was less when cheese was manufactured by a nonstandard treatment. The difference was more pronounced when a considerable amount of whey was replaced by water (e.g., in double curding washing). The soluble nitrogen content was almost the same as that measured in the cheese produced by the standard method when culture starter and lactose was added to the cheese vat (as described in Materials and Methods). Because soluble nitrogen is used as an index of cheese ripening (12), the modifications applied to the standard method likely affect the ripening of cheese. It is assumed that during the washings, some of the factors contributing to ripening (such as rennet remains, the milk proteolytic enzymes or lactose, and microorganisms) are partially removed.

The washing of the curd reduced the acidity of cheese. Again, the observed decrease in acidity is proportional to the amount of water employed in the washings. The addition of starter culture and lactose to the cheese vat during the last washing increased the acidity from .58 to .68%. However, this acidity level was considerably lower than the 1.34% acidity determined by the standard method cheese. There are corresponding variations of the pH of the ripened cheese. All modifications of the standard method resulted in cheese with increased pH. Addition of starter culture and lactose lowered pH, but the pH of the standard was not reached.

Moreover, the water washing of the curd reduces the ash content of cheese, apparently because of losses of water-soluble salts. Ash content was increased when calcium was added to the cheese vat during the last washing.

No appreciable differences were observed in cheese yield produced by the various treatments.

The results of the sensory evaluation of cheese are presented in Table 5. The lack of good statistics prevented definite conclusions. However, the mean values indicate a minor decrease of the organoleptic properties in cheese, which was produced by the variations of the standard method. This was described by the panelists as the loss of the pleasant taste of the standard cheese. The deterioration in taste
was more pronounced in the cheese which had been subjected to double curd washing with 50% of whey replaced by water. The addition of lactose and culture restored the organoleptic properties which approached those of the standard. Nevertheless, in all cases, the product was found to be acceptable.

CONCLUSIONS

Variations of the standard cheese making method were effective in reducing the radiocesium activity concentration of kefalograviera cheese produced from contaminated sheep milk. Thus, although the transfer coefficient of $^{137}$Cs to cheese produced by the standard treatment was determined as $0.33 \pm 0.04$ L/kg, the transfer coefficient produced by the modification of double curd washing was $0.15 \pm 0.02$. These values express the quality of the product with respect to radiocontamination. They suggest that, from milk with 100 Bq/L in radioactive cesium, the cheese produced through each procedure contains $33 \pm 4$ and $15 \pm 2$ Bq/kg correspondingly.

The technique proposed herein for the removal of radiocesium is easily applicable; it does not require additional treatment or equipment that could significantly raise the production cost. The effect of the proposed technique on the organoleptic properties and to some physicochemical characteristics of the final product may be compensated by adding culture, lactose, and calcium.

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REFERENCES