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Environmental and surface hygienic quality of small dairies in mountain areas: suggestion to improve food safety

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Abstract
This survey investigates the quality of the environment and any work surface (floors, walls, worktops) in small family-run dairies to identify frequently neglected critical factors, despite their potentially decisive role in improving the hygiene standards of dairy processing. The major problems of small dairies are related to the microbial contamination and the environmental conditions that are essential for quality cheese-making. Hygiene both in the environment and on the work surfaces is important to avoid microbial contamination, and if food-environment interactions are not monitored correctly, food safety can be at risk.
The choice of the sample fell upon family-run farms still characterized by craft work and traditional methods. In every dairy and in all processing premises, surveys were carried out to assess microbes and eumycetes role in air and work surface contamination. Air contamination was checked by means of a microbiological air sampler followed by colony count. The microbial surface contamination was determined by three different techniques (swab method, sponge method and bioluminescence method).
In general air quality appeared to be good. A small number of microbiological health indicators, found just outside the entrance to the dairy, were due to cross-contamination coming from the cattle- shed. In some cases work equipment surfaces revealed unsatisfactory hygienic conditions due to ineffective cleaning operations. In this respect we underline that it would be useful to use boiling whey especially in dairies where running water is not available.

Keywords: work equipment surface, microbial air contamination.

Introduction
To produce hygienically safe food, an environment clean and free from any source of contamination is essential. Even more care is needed in the case of handmade or craft products, exposed to air for a long time while being processed. An essential element to decrease microbial contamination of the product, is good quality of both the air and of the surfaces where this process occurs; this can be obtained by an adequate design of the premises followed by the adoption of correct hygienic procedures. It means that both the layout and the building must be apt to avoid or reduce filth settling and scattering and to facilitate cleaning; also periodical hygienic programmes will ensure constant cleaning. It is hard for dairies set in isolated areas, such as those in mountain areas, to keep a properly cleaned environment; this is due to the inadequacy of the premises to a thorough cleaning and to scarce availability of water. The aim of this survey was to examine the hygienic quality of production premises in small mountain dairies and to supply producers with practical suggestions for more hygienic procedures.

Material and methods

Several dairies located in different mountain areas, that is in the valley floor or on mountain pasture, were examined. Most of them were small sized, while some in the valley floor were medium sized.

On one hand, the smaller typology consists of family-run businesses where two people are involved, one with the breeding and the other with the cheese-making; or even where only one worker can conduct all activities, and - if necessary - other components of the family can help.

On the other hand, dairies in the valley floor may employ 4-5 workers. Equipment and plants there consist of several steel basins in case of natural milk skimming; of a small steel churn to make butter; of one or two gas or wood heated copper or steel vats; rarely of steam double layer vats; of steel or wooden tables to purge the whey; of food grade plastic or metal curd moulds; of plastic tanks for brine salting, and of wooden boards for cheese drying and ripening. The biggest dairies can also be provided with stainless steel tanks where to chill-storage milk; centrifuges for mechanical milk skimming; pasteurizing machines (raw milk is rarely used); machines for butter moulding and packaging, and plants for washing curd moulds.

Microbial air contamination

To check quality and quantity of air bacterial contamination it is necessary to sample microbial cells suspended in the air, to have them multiplied on proper culture media, counted on the plate, and following insulation to identify micro-organisms. It is a technique that highlights metabolically active micro-organisms, i.e. able to reproduce themselves and give shape to visible colonies; so data obtained might underestimate the real air contamination.

The equipment used was Microbial Air Monitoring System (Merck, mod. MAS-100 Eco®), a single-stage aspiration orthogonal impact sampler able to aspirate fixed air volumes. Within its head, to be sterilized before each sampling, there is a 90 mm Ø Petri dishes with a specific culture medium to collide with aspired air. The plates are stove-incubated according to time and temperatures depending on the microbial group searched. The air-dispersed microbial concentration is calculated as to the number of colonies developed, and to the volume of aspirated air.

Processing premises (skimming, cheese-making, ripening) were sampled in different points and at different heights. Each figure obtained derives at least from the mean of three samplings. They were performed while processing was in progress to consider its actual hygienic conditions. Obtained concentrations were compared with the European Community Board indications (*European Collaborative Action*) suggesting the following guiding levels of air contamination for indoor environment:

- Very low, for colony-forming units less than 50;
- Low, for colony-forming units between 50 and 100;
- Medium, for colony-forming units between 100 and 500;
- High, for colony-forming units over 500.

Work surface contamination

Two techniques were used to control work surface contamination: sponge and swap methods, both implying the development of vital micro-organisms on the plate. Bioluminescence was used when traditional microbiological analyses were not possible.

The sponge method required the use of small sponges inside sterile sealable bags containing some sterile diluent (100 ml triptone salt). Sampling consisted in passing a well-

wrung sponge across a 10x10 cm surface to analyze. The sample obtained was refrigerated up to its analysis, to be performed within 24 hours. In the laboratory, decimal progressive dilutions of the sample were pour plated using different culture media. Plates were incubated at variable time and temperature in relation to the microbial group searched. Surface contamination (CFU/cm^2) was calculated according to the number of colonies grown on the plate, to the plated decimal dilution, and divided by the sampled surface.

The swap method was used when it was necessary to measure contamination on surfaces of difficult reach, e.g. pipes. Sterilized sticks with a cotton wool end were fit for the purpose. The surface to check for contamination was rubbed down with a stick dampened with triptone salt; the stick was then reinserted into the test tube and refrigerated up to its analysis, to be performed within 24 hours. In the laboratory, the content from the test tubes was spread plated. Plates were incubated at variable time and temperature in relation to the microbial group searched. The surface contamination value was given by the number of colonies grown per unit of sampled surface.

Bioluminescence technique was used to measure on the field the overall level of microbial contamination of the work surface. This method is based on the oxidation reaction between luciferine and ATP, which, catalysed by luciferase enzyme, in aerobe environment produces light. Such a technique measures total ATP deriving from microbes and others (remnant food, human and animal by-products). Therefore, bioluminescence outlines how clean a work surface can be as to microbes and non-microbial remnants that facilitate the growth of any micro-organisms.

A bioluminometer (Merck, mod. HY-LiTE® 2) and a sample kit were used. The methodology was based on the following steps:

- Sample taking using suitable kit;
- ATP reaction by luciferase enzyme;
- Instrument reading (within 10 seconds from reaction).

The instrument measures the light intensity emitted by obtained ATP reaction and gives results in RLU (Relative Light Unit). The result can be converted into CFU using a calibration straight line previously stated for surfaces whose contamination is given. Literature suggests standards that on one hand give values of microbial charge (aerobic mesophils) above which the surface denotes incorrect sanification; on the other hand threshold values above which there is risk of impairing the quality of the food in contact with the surface. Correct sanification of the surfaces is indicated by few CFU/ cm^2 , generally not over 50 CFU/cm^2 for mesophil aerobic bacterial charge, and 1 CFU/cm^2 for other indicators (*Enterococcus faecalis*). Instead, the highest values of microbial charge above which food quality is at risk, enhancing its deterioration, equal 10^4-10^5 CFU/cm^2.

The surfaces of milk skimming basins, vats, curd, tables to purge whey, and maturing boards, were checked before and after their cleaning, with the aim to verify the efficacy of sanification procedures.

Results

Microbial air contamination

Microbial surveys were performed inside milk skimming room, if any, cheese-making and ripening premises, and were repeated at least three times each sampling session, as well as in different periods, e.g. in spring and in winter. Microbial air contamination data, as seen in figure 1, represent mean values of air contamination from sampled dairies.

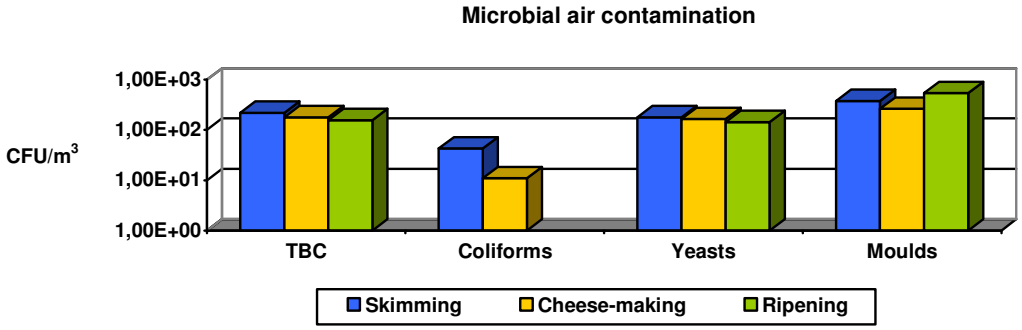

Figure 1. Mean values of microbial air contamination in the main work premises, expressed in colony-forming units per cubic metre of air

Measured values for TBC (Total Bacterial Count), yeasts, and moulds were slightly over 10^2 CFU/m^3, which indicated a mid-low contamination level, though not important.

Presence of Coliforms, about 10 CFU/m^3, called for more attention even if found in few dairies, as they indicated faecal contamination. They were localised in skimming and cheese-making premises. The cross-contamination of the processing premises came from the nearby cattle-shed or was due to the overlap between cheese-making and breeding operations performed without hygienic work procedures. On observing every single result obtained from each dairy, it is clear that contamination levels are different (Figure 2).

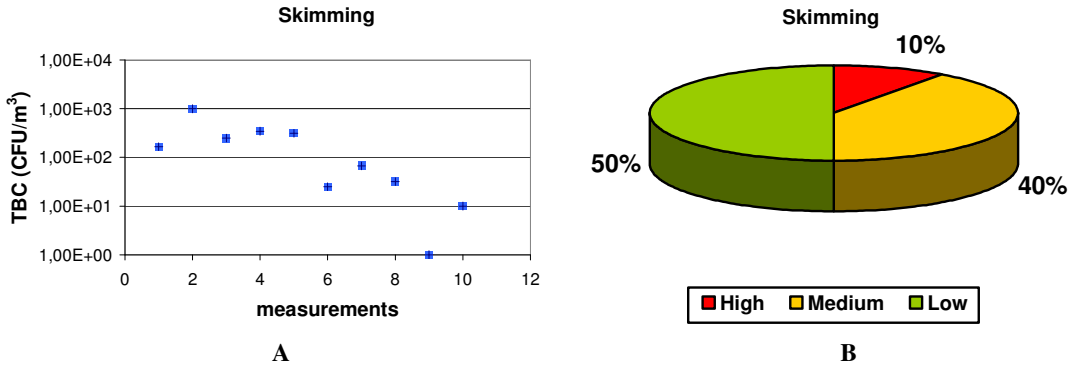

Figure 2. Air-dispersed microbial charge data obtained in skimming rooms (A) and percentage related to dairies considered according to their contamination levels (B)

10% of skimming rooms was clearly contaminated, with TBC being above 500 CFU/m^3, mainly due to structural and procedural reasons. Critical situations showed (i) windows without anti-insect nets or with nets so damaged to be unable to protect against flies and other insects transmitting faecal contamination; (ii) skimming room close to dirty rooms, or milk skimming basins set inside premises used for activities other than production; (iii) a filthy room because of frequent coming and going of workers carrying milk cans. Good air quality was anyway found in the other rooms, which is important to keep milk quality unaltered.

Similar situation was found in cheese-making premises (Figure 3).

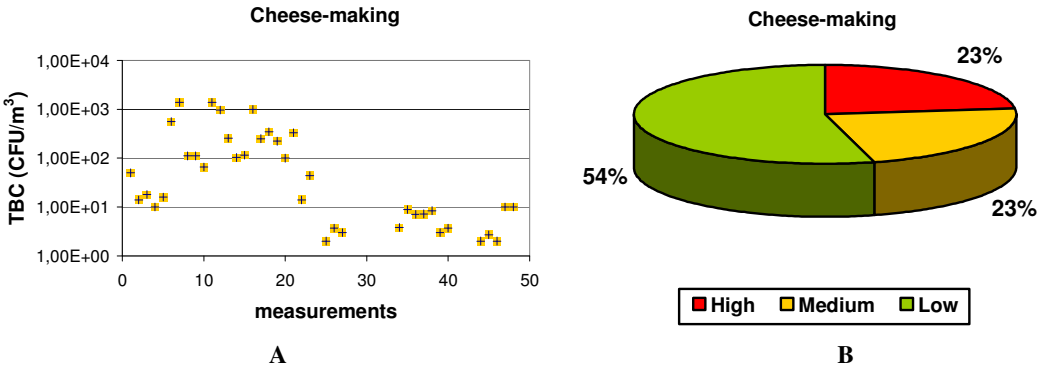

Figure 3. Air-dispersed microbial charge data obtained in cheese-making rooms (A) and percentage related to dairies considered according to their contamination levels (B)

Total microbial charge (TBC) measured showed contrasting situations. In the most contaminated dairies (23%), contamination derived from the cattle-shed, then it was due to their casual layout and the state of neglected work premises, which made it hard to sanitize them also because no drinking water was available. Steady cleaning through deterging and sanification of the premises can improve the hygienic quality of the environment. For example, in a dairy whose environment was highly colonised by *Enterobacteriaceae*, their concentration was significantly decreased by simply foam washing its walls. From the details of our data, dairies in the valley floor generally resulted to be the dirtiest, being bigger and employing more workers who could spread contamination. It was also evident that workers who know hygienic procedures can determine a better environmental quality.

In the ripening rooms, moulds are the predominant microbial forms (Figure 4) and difference in concentration levels can be relevant in case of refrigerated cells or cellars.

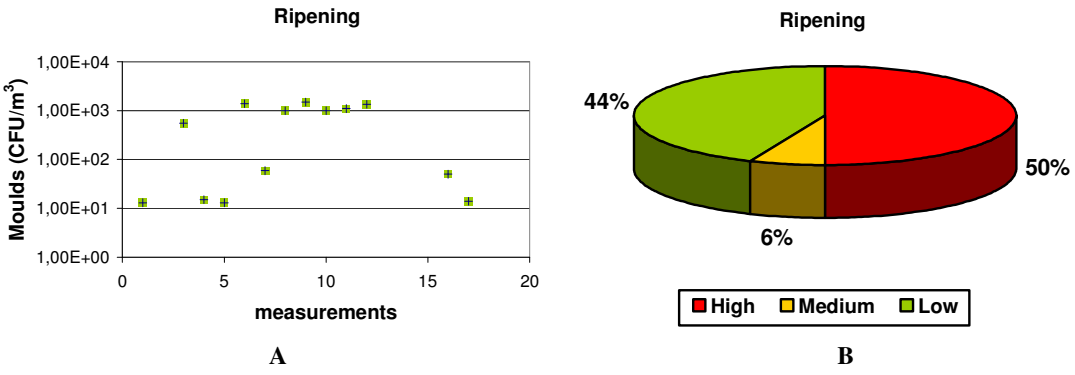

Figure 4. Air-dispersed microbial charge data obtained in ripening rooms (A) and percentage related to dairies considered according to their contamination levels (B)

Higher contamination was observed in cellars (50%), almost all equipped with wood maturing boards. It was mainly due to white or green moulds deriving from each product rind, moulds for which constant microclimate was an ideal habitat.

The peculiar flavour of the product is significantly determined by the presence of such moulds and their metabolic activity. A different situation was found in cells where the microbial charge resulted to be low (44%) and scarcely due to moulds and to trivial environmental saprophyte bacteria, mainly deriving from workers (cross-contamination) or from incorrect use of entrance doors.

Microbial surface contamination

Microbial charge on the surface of most tools used in cheese-making (milk skimming basins, vats, curd moulds, tables to purge whey and maturing boards) was measured as well as on the walls near the processing areas (Figure 5).

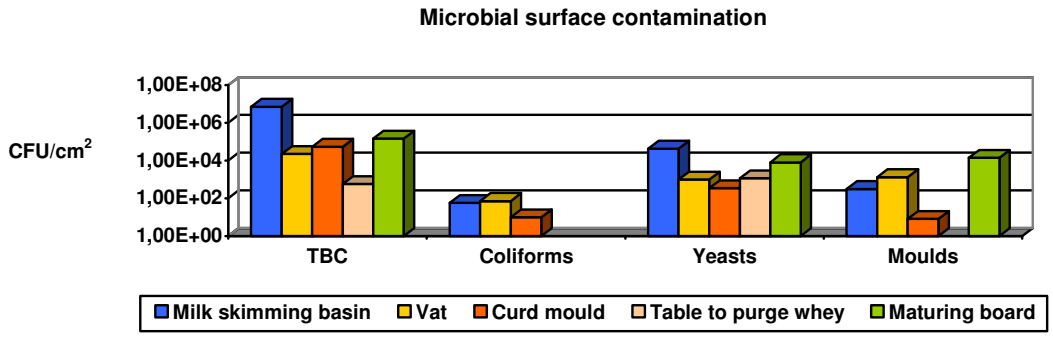

Figure 5. Mean values of microbial charge on main tools for cheese-making and ripening, expressed in colony-forming units per square centimetre

The milk skimming basins resulted to be the dirtiest, their measured values were always above 10^3 CFU/cm^2. This was probably due to their being washed with only cold water, sometimes high pressured. In fact, only few dairies used detergents. If water for washing was highly contaminated, basins resulted contaminated too as a consequence. Residual microbial charge could develop as basins were let to dry naturally.

As for vats, lower values were measured, and no particular difference was noted between copper and steel vats. Surface hygiene was highly dependent on washing procedures. The vats where hot whey was used were generally perfectly clean, which could not be obtained by only water-washing.

A similar situation was found for curd moulds. They were usually washed with water and detergents or sanitized in acid solution at 70°C followed by an alkaline treatment. If moulds were not kept in clean places or simply watered with tap water after their use, surface microbial charge reached values between 10^3-10^5 CFU/cm^2.

Tables to purge whey as well as boards for cheese ripening were clean thanks to the bactericide action of the acid whey. Wood is usually considered a scarcely hygienic material; actually, when brushed to be cleaned it is less contaminated than other surfaces. Replacing tiled walls improved hygiene level (TBC lower than 10 CFU/cm^2) when compared with those obtained by periodical cleaning of the walls (once a week or every 2 weeks). Highest values were shown at dairies where air contamination was moderate.

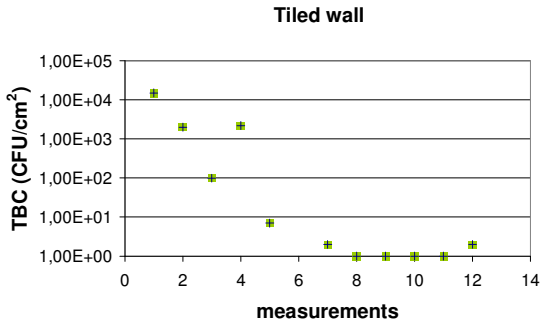

Figure 6. Surface microbial charge from walls, expressed in colony-forming units per square centimetre

Conclusions

From a microbiological point of view the quality of processing premises was generally satisfactory; this mainly in small alpine pasture environment, for which objective difficulties were to be considered. No pathogenic forms (*Listeria monocytogenes* and *Salmonella* sp.) were detected, which is extremely positive. To assure proper uncontaminated air, particular care is necessary as to the location of doors and to the closing and protection of windows; moreover, access to the premises is to be limited only to the staff.

One main critical factor consists in water availability for cheese-making and for washing. In many situations mains water is not available, so surface water must be treated. Such an operation supplies microbiologically acceptable water at first, but its characteristics cannot be kept for long, which implies increased contamination of the surfaces. In such a case, hygiene can be ensured by the use of boiling water to clean milk skimming basins and other various tools and by promptly drying them with clean cloths, while whey could be used for vats.

As to ripening premises, cellars can affect the typical characteristics of cheese positively, while cold stores can alter the environmental ecosystem because of tiles, PVC, and stainless steel surfaces, thus contrasting the development of all the sensorial peculiarities so important to make a craft product unique.

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