The influence of air hygiene on microbiological safety of butter

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Abstract. In this work, the microbial air quality in a butter factory was studied, and the microbiological safety of packaged butter during its shelf-life is presented. Control of air hygiene was performed in two halls in one butter factory. The production hall housed the butter churner, and here, 20 kg blocks of butter were packaged in cardboard boxes (8 different locations were examined). The packaging hall housed a line for splitting the 20 kg blocks of butter into 125 g amounts and a butter packaging machine producing smaller cardboard boxes containing the 125 g packets of butter (6 locations were examined). The hygienic quality of air in the two halls was assessed by determining the numbers of mesophilic aerobic bacteria, yeasts and molds. Microbiological examination of bulk 20 kg butter blocks and 125 g packets of butter was conducted every tenth day over 60 days. The air in the production plant contained mesophilic bacteria that settled on plates during 20 minutes’ exposure; numbers were from 6-20 at churning, and 12-36 colonies at packing. The mycological profile of packaged butter is largely a result of the presence of molds in the packaging hall air and their direct incorporation onto the finished product.

1. Introduction

The technological processing of food products in food processing plants leads to contamination of the air with phages, bacteria, yeasts and molds. Movement of air currents from ventilation systems can contribute to the spread of microbial aerosols in food plants. Therefore, great attention should be paid to air filtration in food plants. The passage of air through efficient filters retained up to 99.99% of microorganisms [1]. To this end, in food production plants, the use of filtration transmission devices creating laminar flow of sterile air in the vertical and horizontal planes is recommended [2].

During operations such as churning and packing, butter is exposed to the effects of contamination from the ambient air in the production plant and from the staff working in these areas, as well as to the impact of the ambient temperature. Therefore, in terms of maintaining good microbiological quality of the butter, control of the microbiological air quality in rooms where butter is churned and packaged is necessary [3].

Manipulations during butter production and packaging can lead to undesirable water and residual or dispersed product on the floor. Subsequent movement and activity of workers at the facility and/or the processes of washing and cleaning equipment can result in dispersion of microorganisms in the form of aerosols into the air. Activities that promote increases in aerosols often produce unacceptable levels of air contamination [4]. Hence, it is desirable to maintain dry conditions in the production area.

Considering the packaging process, special attention must be given to the choice of the packaging material. Cardboard, as a secondary material, can be a significant source of mold spores, especially if...
it is recycled. To prevent contamination, cardboard packaging material should not be handled in the production area but in a separated area. Condensation or humidity, if present, will initiate the growth of mold spores. Some authors [2] state there is a connection between microbiological contamination of food products and aerial spread of microorganisms. For example, the level of contamination of food increases by 120% after 48 h exposure to air at 32 °C, compared with 24 h exposure to the same conditions. Contamination of butter increases with increasing humidity, with 80-95% of isolated microorganisms being Gram-positive airborne bacteria (coci, bacilli). Air quality is especially important for butter manufactured in continuous-type mixers, which can incorporate ambient air into the product at levels up to 5% of product volume [5].

2. Materials and methods
Control of air hygiene was performed in two halls in one butter factory. The production hall housed the butter churning machine. Butter was accepted from the churning machine, and 20 kg blocks of butter were packaged by hand in polyethylene bags that were then placed in protective cardboard boxes (8 different locations were examined in this hall). After boxing, the butter blocks were stored at <6 °C. In the butter packaging hall, retail 125 g packs of butter were formed from the 20 kg blocks of butter and packaged in smaller cardboard boxes (6 locations).

Air sampling was carried out by exposure of open Petri dishes with the appropriate nutrient medium (nutrient agar for total viable count; TVC, and Sabouraud maltose agar with streptomycin (from 0.01 to 0.02%) for the total number of yeasts and molds). Petri dishes were exposed for 20 mins, after which the plates were incubated (TVC: 2-3 days, 30 °C; yeasts: 3-5 days, 25 °C; molds: 7 days, 25 °C), and colonies were counted.

Blocks of butter (20 kg) (60 samples) and 125g packs of butter (60 samples) were kept in refrigerated storage at 4 °C in order to monitor expiration dates. Microbiological control of these samples was conducted every ten days from the date of production, for a total of 60 days. The microbiological tests conducted were in accordance with: (a) TVC [6]; b) Lipolytic bacteria [7]; c) Proteolytic bacteria [7]; d) Salmonella spp. [8]; e) Escherichia coli [9]; f) Sulphate-reducing clostridia [10]; g) Enterobacteriaceae [11]; h) Coagulase positive staphylococci [12]; i) Listeria spp. and identification to species level using biochemical systems API List (BioMerieux, France) [13] and j) Yeasts and molds [14].

3. Results and discussion

TVC, yeasts, and molds expressed as colony-forming unit (cfu) determined in the air in the butter processing halls are shown in Table 1.

| Production hall location | TVC (cfu) | Yeast (cfu) | Molds (cfu) |
|-------------------------|-----------|-------------|-------------|
| 1                       | 10        | –           | 5           |
| 2                       | 6         | 1           | 3           |
| 3                       | 7         | –           | 5           |
| 4                       | 18        | –           | 4           |
| 5                       | 17        | –           | 6           |
| 6                       | 7         | –           | 9           |
| 7                       | 14        | 1           | 7           |
| 8                       | 20        | –           | 8           |
The results in Table 1 show the TVC ranged from 6-20 colonies (production hall) to 12-36 colonies (packaging hall), suggesting poorer microbiological quality of air in the packaging hall. This suggests a potentially negative impact of the packaging hall on the microbiological safety of packaged butter, since all the manipulative packaging operations are conducted here.

A similar trend was evident in the total number of airborne molds, since air in the packaging hall contained more molds than air in the production hall. However, any airborne contamination in the production hall could have negative effects on the microbiological quality of bulk and packaged butter, due to the fact that up to 5% of the volume of churned butter can be ambient air with its accompanying load of microorganisms, incorporated into the butter at churning [5]. Similar values for TVC were found [15] in an examination of air hygiene in dairy plants in Belgrade. These authors stated the TVC in air in the locations examined ranged from 12 to 36 cfu, while the number of yeasts and molds was 2 to 7cfu [15].

Examination of air contaminated with molds in cheese ripening plants [16] resulted in identification of numerous molds that were classified in 11 genera and 32 species. The greatest number of species (45.16%) belonged to the genera *Penicillium* (mostly *Penicillium verrucosum* var. *cyclopium*) and *Aspergillus*. Some (38.71%) species of molds were indoor air contaminants in the cheese ripening plants studied [16]. Since 61.29% of molds appeared simultaneously as cheese contaminants during ripening and as airborne contaminants in these premises, the author concluded the air was the main source of contamination for these molds.

Results of microbiological testing of the two different butter pack types (butter blocks of 20 kg and butter packs of 125 g) during storage at 4 °C are shown in Tables 2 and 3, respectively.

### Table 2. Results of microbiological examination (cfu/g or cfu/0.1 g or presence/absence) of 20 kg butter blocks during storage at 4 °C to determine shelf life

| Microorganisms                          | Storage time (days) |
|----------------------------------------|---------------------|
|                                        | 1  | 10 | 20 | 30 | 40 | 50 | 60 |
| **TVC per g**                          |    |    |    |    |    |    |    |
| **Proteolytic bacteria per g**         |    |    |    |    |    |    |    |
| **Lipolytic bacteria per g**           |    |    |    |    |    |    |    |
| **Salmonella spp. presence/absence in 25 g** |    |    |    |    |    |    |    |
| **Escherichia coli per 0.1 g**         |    |    |    |    |    |    |    |
| **Sulphate-reducing clostridia per 0.1 g** |    |    |    |    |    |    |    |
| **Coagulase positive staphylococci per 0.1 g** |    |    |    |    |    |    |    |
| **Listeria spp. presence/absence in 25 g** |    |    |    |    |    |    |    |
| **Enterobacteriaceae per g**           |    |    |    |    |    |    |    |
| **Yeasts per g**                       |    |    |    |    |    |    |    |
| **Molds per g**                        |    |    |    |    |    |    |    |
Table 3. Results of microbiological examination (cfu/g or cfu/0.1 g or presence/absence) of 125 g butter packs during storage at 4 °C to determine shelf life

| Microorganisms                                      | Storage time (days) |
|-----------------------------------------------------|---------------------|
|                                                     | 1  | 10 | 20 | 30 | 40 | 50 | 60 |
| TVC per g                                            | –  | –  | –  | 2  | 80 | 4000 | 12000 |
| Proteolytic bacteria per g                           | –  | –  | –  | –  | –  | –  | –  |
| Lipolytic bacteria per g                             | –  | –  | –  | –  | –  | –  | –  |
| *Salmonella* spp. presence/absence in 25 g           | –  | –  | –  | –  | –  | –  | –  |
| *Escherichia coli* per 0.1 g                         | –  | –  | –  | –  | –  | –  | –  |
| Sulphate-reducing clostridia per 0.1 g               | –  | –  | –  | –  | –  | –  | –  |
| Coagulase positive staphylococci per 0.1 g           | –  | –  | –  | –  | –  | –  | –  |
| *Listeria* spp. presence/absence in 25 g             | –  | –  | –  | –  | –  | –  | –  |
| *Enterobacteriaceae* per g                           | –  | –  | –  | –  | 1  | 8  | 12 |
| Yeasts per g                                         | –  | –  | –  | –  | –  | –  | 1  |
| Molds per g                                          | –  | –  | –  | 4  | 15 | 52 | 89 |

The microbiological results of testing the butter during storage at 4 °C (storage duration test) showed the butter produced was of high microbiological quality (Tables 2 and 3). Pathogenic bacteria were not detected in the analyzed samples of butter, which indicates good manufacturing and good hygienic practices were applied during the butter manufacturing process. The resultant butter product was microbiologically safe and fit for human consumption.

In the 125 g packs of butter (Table 3), very small numbers of lipolytic bacteria were detected between 30 and 40 days of storage (2-11 cfu/g), while in the 20 kg blocks during the same timeframe, no lipolytic bacteria were detected. These lipolytic bacteria in the 125 g packs are the result, above all, of handling and the various actions that are performed before and during the packaging process and that lead to increased numbers/presence of these microorganisms. The 20 kg blocks of butter are much less handled than the smaller 125 g packs. The current study also confirmed the 125 g packs of butter contained molds, which were likely largely derived from their being airborne in the packaging hall (Table 1). As explained above, airborne contamination in production hall air could be directly incorporated in the butter via churning. The maximum number of molds determined in 125 g packs of butter during 60 days storage was 89 cfu/g, while in 20 kg blocks of butter, that number was lower and amounted to 22 cfu/g.

The results obtained showed airborne mold contamination was greater in the packaging hall than in the production hall housing the butter churning process [17]. Butter packed in 125 g portions are riskier forms of commercial packaging than the 20 kg blocks of butter as a result of various handling operations carried out before and during the machine packing. Also, the molds in the 125 g packs of butter were largely derived from the air in the packaging room, and these molds were likely directly deposited on the butter surfaces.

4. Conclusion
Butter producers should conduct microbiological examination of final product as part of controls to determine product safety and appropriate expiration dates. Butter producers are required to have appropriate microclimate conditions (temperature and humidity), to document potential sources of microbiological contamination of air, and to safeguard against air contamination in order to obtain microbiologically acceptable products. Personnel hygiene and aspects of the premises’ construction should be addressed, while special projects utilizing performance aeromicrobiology could be
implemented to solve any specific problems arising. Finally, methods of purifying air (conditioning, filtration, laminar flow, etc.) must be considered.

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