Occurrence of Enterobacteria and Clostridium During Gelatin Manufacture

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The study was made for the occurrence and isolation of enterobacteria and Clostridium during different stages of gelatin manufacture in an industry. Samples were collected during each stage of manufacturing, i.e., from the raw material ossein to the finished product and analysed for the enterobacterial and clostridial species. The enterobacterial species identified were Escherichia coli, Klebsiella oxytoca, Proteus mirabilis, Salmonella typhi and Shigella sonnei. Among the genus Clostridium, the species identified were C.perfringens and C.bolitnulinat. The finished product barring technical grade gelatin did not show the presence of any bacterial species. Presence of these bacterial species may be responsible for severe health hazards, since gelatin is commonly used in the preparation of various food products.

Introduction

Gelatin is the product of denaturation or disintegration of collagen. It is extracted from degreased and demineralised crushed bones (termed as ossein) following their acid and alkali treatment in the form of weak liquor\(^1\). The weak liquor is concentrated and dried to yield gelatin in the form of granules. Because of its unique properties such as solubility, solution viscosity and thermally reversible gelation properties gelatin finds use in pharmaceutical, food, photographic and other industries such as, cosmetics, and safety matches.

Gelatin being an excellent nutrient for most bacteria, the manufacturing process has to carefully avoid contamination. Since most of the gelatin manufactured is used in food industry the present work was concentrated mainly on the occurrence and isolation of enterobacteria and Clostridium, as their members are the potent causative agents for food poisoning and food borne diseases.

Materials and Methods

The samples used for the present study were from water used in the manufacturing process, ossein (before and after pre-treatment), weak liquor, strong liquor (concentrated weak liquor), wet noodles, different zones of drying and the finished product.

Standard procedures were followed for the analysis of enterobacteria and Clostridium described elsewhere\(^2\).\(^4\).

Gelatin and liquid samples (i.e., water and liquor), were enriched using Nutrient broth, while ossein was washed with distilled water and phosphate buffer and then enriched in phosphate buffer. This enriched culture was then inoculated on Sulphite Polymixin Sulphadiazine (SPS) Agar for the isolation of Clostridium. The inoculated media was incubated at 37 ± 2 °C for 24-48 h. The isolated colonies were then picked from SPS agar and identified on the basis of biochemical characters with the help of Bergey's manual and also by the PIB kit.
Results and Discussion

The enterobacterial species identified were *Escherichia coli*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Salmonella typhi* and *Shigella sonnei*. *Escherichia coli*, *Klebsiella oxytoca* and *Proteus mirabilis* had the same occurrence frequency of 13.4 per cent each while that of *Salmonella typhi* and *Shigella sonnei* were somewhat different at 8.4 and 4.7 per cent, respectively. All the water samples which were to be used during the gelatin manufacturing process were free of coliforms or clostridial species, thus they were in accordance to the WHO guidelines for the potability of water. Therefore the possibility of occurrence of these bacteria through the water used during manufacturing was ruled out.

The occurrence frequency (per cent) of *E. coli*, *K. oxytoca* and *P. mirabilis* in ossein samples before treatment was 45.4 each, while that of *S. typhi* and *Sh. sonnei* were 36.4 each. However the ossein samples after treatment did not show the presence of any of these species. This may be due to drastic change in the conditions, *i.e.*, during the pre-treatment, a low pH was maintained which was increased considerably after the pre-treatment. In weak liquor, *E. coli*, *K. oxytoca*, *P. mirabilis* and *S. typhi* each had the occurrence frequency of 50 per cent while *S. sonnei* was absent. Addition of appropriate preservatives in weak liquor might have inhibited the growth of *S. sonnei*. In the next steps, *i.e.*, the strong liquor and drying zones, none of these species were recovered, due to sterilization prior to the concentration of weak liquor to strong liquor.

The microbial analysis of the plant using swabs revealed the occurrence frequency of 9.1 per cent each for *E. coli*, *K. oxytoca* and *P. mirabilis* and 6.1 per cent for *S. typhi* while *Sh. sonnei* was absent. Among the finished product Pure grade, Edible grade and Pharmaceutical grade gelatine, all were free of any bacteria. In the case of Technical grade gelatin the occurrence frequency of *E. coli*, *K. oxytoca* and *P. mirabilis* was 80 per cent while that of *S. typhi* was 60 per cent and *S. sonnei* 40 per cent, respectively (Table 1).

The clostridial species isolated and identified were *Clostridium perfringens* and *Clostridium botulinum*. While *C. perfringens* had the frequency occurrence of 68.2 per cent in ossein samples, and *C. botulinum* had 54.6 per cent. *C. perfringens* was found in all the samples of weak liquor examined (100 per cent), while *C. botulinum* was found in 83.3 per cent samples examined. None of the species were recovered in the strong liquor or the drying zones due to sterilization. Microbial analysis of the plant by swab technique revealed 9.1 per cent occurrence of each of the species. Among the finished products, Pure grade, Edible grade and Pharmaceutical grade Gelatin were free of any clostridial species. Also all the water samples examined during the process were free of *Clostridium*. Both the species identified were gelatinase positive (Figure 1).

In total, 149 samples were analysed, out of which 51 per cent showed viable growth. *Enterobacteria* were present in 13.4 per cent samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total</th>
<th>Entero-bacteria</th>
<th>Occurrence, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water used during the process</td>
<td>20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ossein before pretreatment</td>
<td>22</td>
<td>45.4</td>
<td>45.4</td>
</tr>
<tr>
<td>Ossein after pretreatment</td>
<td>12</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Weak liquor</td>
<td>6</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Strong liquor</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Drying zones</td>
<td>30</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Swabs of plant</td>
<td>33</td>
<td>9.1</td>
<td>9.1</td>
</tr>
<tr>
<td>Finished product (pure, edible and pharmaceutical grade gelatin)</td>
<td>15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Technical grade gelatin</td>
<td>5</td>
<td>80.0</td>
<td>80.0</td>
</tr>
</tbody>
</table>

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Table 1 — Occurrence frequency of Enterobacteria at different stages of gelatin manufacture
and *Clostridium* in 36.2% while both were collectively present in 12.8% per cent samples. Thus, enterobacateria contributed very little to the total viable count. The higher occurrence of *Clostridium* may be attributed to the fact that the enterobacateria could not survive the drying conditions and high temperature during the drying stage and the pH changes during different stages while the spores of *Clostridium* could tide over these conditions (Figure 2).

Out of the five species of enterobacateria identified, only two of the species viz. *P. mirabilis* and *K. oxytoca* were gelatinase positive which gives a clue regarding liquefaction/deterioration of gelatin, if present after treatment. Since *Klebsiella* and *Proteus* are non-spore formers, their possibility of occurrence in finished product, gelatin, is very low. Also these are facultative aerobes, hence their presence in air cannot be ruled out. However, maintenance of sterile working environment is suggested as a preventive measure.

*Clostridium* may play a more significant role in the contamination and spoilage of gelatin than enterobacateria since they are spore formers and both the species isolated and identified were gelatinase positive. Also the occurrence of *Clostridium* was much higher than that of enterobacateria, i.e., 36.2% as against 13.4% per cent for enterobacateria. Thus, special preventive measure is suggested against contamination of product by the spores of the former and sterile working conditions and instruments are suggested to prevent their occurrence. Also since the *Clostridium* is an anaerobe, its population in the initial stage can be controlled by proper aeration of ossein during the maturation period.

The finished products barring technical grade gelatin were free of any bacteria. Thus in accordance with the International Commission on Micro-
biological Specifications for Foods. The occurrence of enterobacteria and clostridial species in technical grade gelatin is insignificant as it is never used for consumption.

Conclusion

The enterobacterial species isolated and identified from different samples during present investigation were E. coli, K. oxytox, P. mirabilis, S. typhi and S. sonnei. Since the water used during the process were free of these bacteria, their occurrence due to water can be ruled out. The clostridium species identified were C. perfringens and C. botulinum.

The occurrence of enterobacteria in weak liquor was very significant, since this denoted some cross-contamination as they were absent in the previous step, i.e., ossein after treatment. But their growth was again checked in the next stage, i.e., in strong liquor where all sorts of bacteria were absent.

The drying conditions of the plant did not favour the growth of enterobacteria though Clostridium species were present.

Barring technical grade gelatin the finished product had excellent microbial quality, since they were free of both aerobic and anaerobic bacteria. Technical grade gelatin was manufactured during plant breakdown situations, power failure or when the plant malfunctioned due to some reason and hence the high frequency of Clostridium and enterobacteria in the same. Also the microbial quality of technical grade gelatin is insignificant from the public health point of view, since they are never used for human or animal consumption.

Acknowledgement

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