EFFECT OF CLIMATIC CONDITIONS ON THE SUCCESSION OF MICROFLORA ON VEGETABLE TANNED LEATHER(SHEEP) DURING STORAGE

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Received June 01, 2009
Accepted August 23, 2009

ABSTRACT

Biodeterioration of leather by its definition, is concerned with the interaction of leather with microorganisms. Thus materials, organisms and environment, all intimately concerned in biodeterioration. So, the climate of storage place is of great importance to the activity of the microflora of stored leather. Therefore, it is quite necessary to investigate various types of leather infesting fungi, their succession and ecological conditions which play an important role in the development of these organisms on such products. In the present study attempts have been made to study the succession of microflora on vegetable tanned (Sheep) leather under varying storage conditions. The conducive factors which are taken into consideration include varying levels of the relative humidity and storage conditions at suitable temperature. Thus during the present studies three basic attempts were taken under considerations (a) Qualitatively-what kind, (b) Quantitatively-how many living microbial forms inhabit the leather and (c) how do, relative humidity and duration of storage affect fungi.

Key Words: Biodeterioration, Fungi, Succession, Climatic factors.

INTRODUCTION

Leather is utilized in making a large number of commercial commodities and it has gained a status symbol as one of the topmost foreign exchange earner and belongs to the elite of society. India is fortunate in having a good raw hide base but biodeterioration of leather in industries by aeromycoflora is fairly common during leather manufacture, finishing, storage and in use. Leather, organisms and environment are intimately concerned in biodeterioration.

The number and kinds of microorganisms on leather vary according to the storage conditions and presence of spores in the air of storage places. The results clearly indicated that a high relative humidity of storage not only helped in the multiplication of fungal species but it also favoured the establishment and development of the fungi during storage. The storage duration also affected the succession of microflora. Many fungal species which were isolated initially showed decreased frequency and some fungal forms appeared in due course of time.

Thus, certain fungal forms showed preferential requirements of relative humidity and storage time to colonize the finished leather, at suitable temperature.

MATERIAL AND METHODS

Vegetable tanned (sheep) leather was collected from leather factories and tanneries, inoculated with isolated fungi prior to storage. The exposed leather samples were stored at varying levels of relative humidity.
at suitable temperature to determine the succession of fungi.

**Maintenance of relative humidity:** The relative humidity was maintained in desiccators (8" diameter) using salt saturated solutions (Anonymous, 1968). The different i.e. Chroium trioxide (Cr₂O₃), Magnesium acetate [Mg(C₅H₃O₂)₂·4H₂O], Sodium chlorate (NaClO₃) and Zinc sulphate (ZnSO₄·7H₂O) were used to control 30, 60, 80 and 95% relative humidity, respectively. The temperature is maintained in incubator.

**Determination of succession of microflora:** The finished leather was stored at different relative humidity levels i.e., 60, 80 and 95% and 28±1°C temperature. A control set was also maintained at 30% relative humidity and 28°C temperature for both sets of experiments.

Samples were examined visually for fungal growth on grain and flesh side of leather. The species composition of each sample was determined regularly after 30, 60, 90, 120, 150 and 180 days intervals. The increase or decrease in number of fungal species was noted and compared with that of control. The effect of different relative humidity was observed on the succession of fungi on leather samples throughout the storage period.

**RESULTS AND DISCUSSIONS**

The close examinations of table shows the succession of microflora on vegetable tanned (sheep) leather under varying relative humidity and duration of storage at suitable temperature. Total 46 fungal species were recorded from sample. Profuse fungal growth was observed on grain and flesh sides of leather. (Table 1)

**Table 1:** Effect of relative humidity on succession of microflora on vegetable tanned (sheep) leather during storage visual observation of the fungal growth at Temp. 28°C

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fungi</th>
<th>Percent relative humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-180</td>
</tr>
<tr>
<td>1</td>
<td>Aspergillus niger</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>A. chevalieri</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>A. nidulans</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>A. fumigatus</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>A. conicus</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>A. humicola</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>A. flavus</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>A. terreus</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>A. repens</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>A. sulphureus</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>A. tamarii</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>A. luchuensis</td>
<td>0</td>
</tr>
</tbody>
</table>

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| No. | Species | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | No. 7 | No. 8 | No. 9 | No. 10 | No. 11 | No. 12 | No. 13 | No. 14 | No. 15 | No. 16 | No. 17 | No. 18 | No. 19 | No. 20 | No. 21 | No. 22 | No. 23 | No. 24 | No. 25 | No. 26 | No. 27 | No. 28 | No. 29 | No. 30 | No. 31 | No. 32 | No. 33 | No. 34 | No. 35 | No. 36 | No. 37 | No. 38 | No. 39 | No. 40 | No. 41 | No. 42 | No. 43 | No. 44 | No. 45 | No. 46 | Total species |
|------|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 13.  | A.amstelodami | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 1     | 1     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 2     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | 3     | Total species 33 40 41 44 45 46 46 46 46 46 46 46 46 45 45 44 39 32 |

Fungi like *Aspergillus chevalieri*, *Alternariahumicola*, *A.alternata*, *Drechslera papendorfii*, *Fusarium* sp., and *F.solani* were appeared after 60 days.

*Helminthosporium* sp., first appear after 90 days of storage. *Aspergillusluchuensis*, *Penicillium asperum* and *Rhizopus nigricans* were appeared after 120 days of storage.

*Cunninghamella* sp., was appear after 150 days of storage. Total 45 species were found to grow after 180 days of storage at 60% relative humidity. Some of them shows moderate growth e.g. *Aspergillus niger*, *A.fumigatus*, *A.flavus*, *A.repens*, *Botryoderma* sp., *Mucor ambiguus*, *M.mucedo*, *Penicillium camemberti*, *P.funiculosum* and Paecilomyces varioti.

At 80% relative humidity all 46 fungi were appeared and *Aspergillus amstelodemi* appear at 80% relative humidity after 30 days. Fungus showed luxuriant growth like *Aspergillus niger*.

Total number of fungi 46 retained up to the 95% relative humidity after the storage of 30s days but *Botryoderma* sp. and *Aspergillus candidus* disappear after 60 and 120 days respectively and some fungi showed luxuriant growth.

After 150 days many other fungi were disappeared like *Aspergillus repens*, *A.sydowii*, *Fusarium solani*, *Mucor mucedo*, *Torula lucifuga*.

After 180 days many other fungi were disappeared like *Aspergillus sulphureus*, Curvularia pallescens, *Cladosporium herbarum*, *Fusarium* sp., *Penicillium asperum*, *P.funiculosum*, Rhizopus oryzae.

<table>
<thead>
<tr>
<th>Temp.</th>
<th>60% R.H.</th>
<th>80% R.H.</th>
<th>95% R.H.</th>
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<tbody>
<tr>
<td>28°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Graph showing the number of fungal species over storage period in days](chart.png)
On the other hand many fungi showed luxurient growth as indicated in the table.

Most of the fungal species recorded at 95% relative humidity and 28°C temperature and growth pattern is very active at this relative humidity in comparison to those at 60 and 80%. Some fungus grew slowly at 80% relative humidity when showed active growth at 95% relative humidity.

The results of this study shows the succession of microflora of vegetable tanned (sheep) leather, stored at different level of relative humidity and duration of storage. These conditions are of physiological interest for their effect on the fundamental process of growth and development. The development of spores and germ tube, is, for instance, influenced by temperature, relative humidity and duration of storage. Various fungi have different time to appear and multiply during storage and rendered leather unfit for commercial purpose. Succession of leather microflora is also influenced by type of leather tanning and also the animal from which the leather was obtained.

Succession of various fungal species were studied on vegetable tanned (sheep) leather under different environmental conditions. Total 46 fungal species were studied during present study. Fungal succession is the sequential occupation of the same site by either of different fungi or of different associations of fungi (Fryar et.al. 2004). According to Kallinberger (1967) during warm wheather these natural substances would begin to deteriorate within an hour. He stated that after using most commercial hides contain nearly 50% water. This water is important to the microbes that potentially may attack the hide and destroy it.

It is clear from these results that Aspergillus niger, A.fumigatus, A.flavus, A.terreus, A.sulphureus, A.tamarii, A nidulans, A.sydowii, Penicillium citrinum, P.purpurogenum, P.funiculosum and Paecilomyces varioti were the most persistent fungi and occurred on leather sample at various level of relative humidity during different duration of storage. These fungi appeared with luxuriant growth on both the surface of leather throughout the storage period at various level of relative humidity is found, influenced spore germination\textsuperscript{11}. Experiments on 15 days in high moisture and 29°C temperature stated that the species of Aspergillus, Penicillium, Paecilomyces and Trichoderma can be considered as frequent and damaging one for leather industry products\textsuperscript{10}. Orlita (1968) gave much stress to find out ecological factors and succession of mycoflora at different stages of the processing of leather in industry till its storage and usage of finished products. It was found during the present studies that higher number of fungi (46 species) was recorded at 95% relative humidity and 30°C temperature even after 30 days of storage. The number of fungi were comparatively low in the sample stored at 80% relative humidity, while those of 60% relative humidity did not support such large number as evident from the table. The increase in number of fungi was due to increase in the leather moisture at higher relative humidity which helps the multiplication and further development of fungi on leather surface. According to Galloway (1935) atmospheric moisture is more useful than moisture in the substrate for germination of mould spores\textsuperscript{5}. By the growth of Aspergillus flavus, A.flavips, A.repens, A.versicolor, Penicillium diverssum, P.chrysogenum, Botryts trichemipituteforum, Basipetospora rubra, Paecilomyces varioti, Mortierella polycephala, Scopulariopsis brevicaulis and Cladosporium herbarum on leather and parchment it was observed that A.flavus and A.repens were the most resistant to decreased humidity\textsuperscript{10}. A.repens grew on parchment in dry conditions at 62.5% relative humidity\textsuperscript{1,9,13,17}. 

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Fungal growth, development and their severity during succession were also influenced by duration of storage. Different fungi appeared at different stages of storage at the same relative humidity and temperature. Increasing the duration of storage increased the number of microfungi. This number increased up to 60 days. It was reported that some fungal species appeared only after 60 days duration. These fungi were *Penicillium citrinum*, *Alternaria alternata*, *Aspergillus chevalieri*, *Fusarium solani* and *Curvularia* sp. known as secondary colonizers. According to Ainsworth and Sussam (1968) the fungi grow at initial stage cause hydrolysis of the leather surface by hydrolysing enzymes. This hydrolysed products is utilized by these fungi to some extent. It is well established fact that all the hydrolysis products are not totally utilized by the developing fungi. Sometimes the higher concentration of such substances show inhibitory effect on these fungi. During this time some other fungi (Secondary colonizers) utilize this accumulated food materials for their growth. Because these fungi are not able to utilize substances as food, which are incorporated into the leather due to their finishing. Disappearance of some fungi may be due to the selective and gradual elimination process adopted by dominant species previously colonizing the leather and relative availability of nutritional substances on the finished leather. This is fact that the growth of fungus is faster during initial stages but become slow in due course due to utilization of nutrients from materials. Strezekzyk et al. (1987) observed that various fungi develop abundantly on calf leather kept in humid conditions. The dominant fungal species changed with time, after 19 months *Chaetomium* sp. and *Trichoderma* sp. were dominant. The predominance of *Penicillium* and Phycomycetes indicated that the material was attacked by microflora characteristic of the first stage of leather biodeterioration when the water soluble compounds and tannins are the main source of nutrition for microorganisms. The interactions among microorganisms (i.e., dealing with nutrient competitors, antagonism) also effect the process of succession.

Similarly, Jelinski et al. (1992) stated that some of the organisms use sunlight, water and minerals to grow while others consume the first, alive or dead, along with minerals and gases and produce waste of their own. These wastes are in turn food for other organisms, some of which may converts the wastes into the mineral used by the primary procedures, and some of which consume each other in a complex network of process in which everything produced is used by some organism for its own metabolism.

The climate of the storage place is of great importance for activities of microflora of stored leather. Because the development of spore and germ tube is, influenced by temperature, relative humidity and duration of storage. Besides these factors, the success of species or an individual is determined by its ability to find the resources at needs, food water air, sunlight space and shelter in its surroundings and to process them in a way that causes the individual or species to function effectively, to grow to remain healthy, to live a long life. During the study of biodeterioration of PHBV, it was observed that biodeterioration rates depend on a variety of factors, including surface area microbial activity of the disposal environment, pH, temperature, moisture level and the presence of other nutrient materials.

**CONCLUSION**

It has been concluded by this studies that various fungi required different relative humidities, temperature and time period to grow upon the finished leather. So, the studies on succession of leather
microflora showed a clear spectrum about the behaviour of fungi in relation to their need about relative humidity, and duration at suitable temperature.

ACKNOWLEDGEMENT

We are grateful to the Principal, Govt. K.R.G.P.G. (Autonomous) College, Gwalior for providing facilities. The director, CMI, Kew, England for identification of fungi.

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