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The fungistatic action of oleic acid

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Oleic acid (C 18:1) has been found to be fungistatic against a wide spectrum of saprophytic moulds and yeasts. The fatty acid causes a delay of 6–8 h in the germination of fungal spores and is very effective at a low concentration of 0.7% (v/v). The application of this property of oleic acid finds great use in preserving foodstuffs including bakery products like cakes and pastries with sweet soft cream as a topping which are prone to quick spoilage under conditions of non-refrigeration.

FATTY acids of varying chain lengths are known for their antimicrobial action primarily against Gram-positive bacteria and yeasts at low pH1⁴. The observed inhibition is explained as a consequence of the uptake of undissociated fatty acids which dissipate the transmembrane proton gradient and thereby affect ATPase activity1⁵. The undissociated form of fatty acids is highly soluble in membrane phospholipids and has been shown to enter the cell by passive diffusion⁶⁷.

Toxicity studies using fatty acids have been well documented during bacterial and yeast alcoholic fermentations of grape must⁸⁹. In the present communication, we report the wider ability of oleic acid to inhibit a variety of moulds and yeasts. The fungistasis is effected at the germination stage of the spores. This observation can find use in the preservation of foodstuffs, as oleic acid can be metabolized easily in vivo in the same manner as fatty acids normally found in food.

A number of saprophytic yeasts and moulds (as listed in Table 1) were grown and maintained on slants of potato dextrose agar (PDA) medium1⁰ at 30 ± 1°C or 37 ± 1°C. Following incubation for five days, the cultures were then stored at 4 ± 1°C till use. The organisms were subcultured once in every fifteen days and the purity of the cultures was checked regularly under microscope.

The inhibition tests were carried out in two stages. (i) The plate method; where oleic acid (0.2 ml) was loaded in wells (8 mm diameter) on PDA plates containing a luxuriant lawn of the different yeasts and moulds; (ii) *Candida albicans* and *Aspergillus* sp. (showing largest zones of inhibition) were grown in potato dextrose broth containing 0.1–2% (v/v) oleic acid at 30 ± 1°C and 37 ± 1°C, respectively in a controlled environment, using new Brunswick shaker (G25-KC) at 250 rpm. Biomass in each case was measured using predried (80°C for 48 h) and preweighed discs of Whatman filter paper no.1 or aluminium foil cups.

For the study of inhibition of aerial microflora, PDA plates containing 1%, 2%, and 3% (w/v) of dextrose were exposed to air for 1 h. A high concentration of 3% dextrose was tried, as bakery products often contain a
high concentration of sugar and this supports the growth of osmophilic yeasts and moulds. The air-exposed plates were incubated at 37 ± 1°C and observed for microbial growth after 24, 48 and 72 h.

For determination of the exact stage of growth at which oleic acid inhibited the fungi, spores of *Aspergillus* sp. were observed in cavity slides containing 3% dextrose solution with oleic acid. Control slides were devoid of the fatty acid. The slides were observed under a Nikon microscope (Type 102) at regular intervals of 30 min.

Oleic acid and all other chemicals used in the present investigation were of analytical grade and purchased locally from E. Merck, India.

The addition of oleic acid to the cultures of *C. albicans* (Figure 1) and *Aspergillus* sp. resulted in a deviation from the growth curves, normally obtained in a defined medium. The effects observed can be generally described as a decrease in the specific growth rate and an increase in the length of the lag phase, which were particularly well marked in the presence of higher concentrations of oleic acid. The specific growth rate dropped to a low of 0.03–0.04 h⁻¹, and the observed lag period was of 7–8 h in case of *C. albicans*.

It has earlier been reported that the effects of sublethal concentrations of organic acids are the expressions of a complex interaction between the acid molecule and the microorganism. The toxicity level of the compound is associated with the concentration of the unionized form of the acid and the size of the carbon chain. The resistance of the microorganism to acid toxicity depends on the effectiveness of its tolerance mechanism, and varies from one species to another. Acid stress results in changes at all levels of cell physiology leading to a modification of the microbial growth profile¹¹.

When a variety of yeasts and moulds were grown on plates containing a central well loaded with 0.2 ml of oleic acid, zones of inhibition ranging from 1–9 mm were observed after 48 h (Table 1; Figure 2). This finding clearly showed that oleic acid has antimicrobial effect over a wide spectrum of yeasts, a good number of aspergilli, penicilli, and many other moulds.

![Figure 1](image1.png) **Figure 1.** Effect of oleic acid (0.7% v/v) on growth of *Candida albicans* under shake culture conditions.

![Figure 2](image2.png) **Figure 2.** Zone of inhibition caused by oleic acid against yeasts and moulds after 48 h in (a) *Cryptococcus neoformans*; (b) *Candida albicans*; (c) *Aspergillus* sp. and (d) *Curvularia lunata*.

![Figure 3](image3.png) **Figure 3.** Potato dextrose agar plates exposed to air for 1 h and incubated at 37 ± 1°C for 48 h. (a) control (without oleic acid); and (b) test (with oleic acid).

Extending this experiment further, PDA plates containing 1, 2, and 3% (w/v) dextrose and 0.7% (v/v) oleic acid incorporated in the medium were prepared. In addition, PDA plates with the same concentrations of dextrose after solidification were overlaid with a film of oleic acid. These sets of plates were exposed to air for 1 h with a view to trap the aerial microflora.

Figure 3 clearly shows that oleic acid was extremely effective in preventing/reducing the growth of aerial microflora. In particular, the plates with oleic acid incorporated in them showed better results. This might be due to the inability of oleic acid to form a uniform film
Table 1. Effect of oleic acid (0.7% v/v) on growth of yeasts and moulds

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus parasiticus</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>A. flavus</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>8.0 ± 0.2</td>
</tr>
<tr>
<td>Penicillium nigricans</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>7.2 ± 0.3</td>
</tr>
<tr>
<td>Curvularia lunata</td>
<td>6.1 ± 0.4</td>
</tr>
<tr>
<td>Glomerella sp.</td>
<td>7.3 ± 0.3</td>
</tr>
<tr>
<td>Microsporum gypsum</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>9.0 ± 0.2</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>4.5 ± 0.1</td>
</tr>
</tbody>
</table>

Figure 4. Fungistatic effect of different concentrations of oleic acid on Candida albicans after 8 h.

on the surface of the PDA plates owing to its hydrophobic nature. Our study showed that while lower concentrations of oleic acid were effective when incorporated in the medium, higher concentrations were required for the spread plate method. Similar results were also obtained using milk cream.

Oleic acid acts in a concentration-dependent manner with 0.7% (v/v) being the least concentration which is most effective (Figure 4). At high concentrations, growth is largely inhibited.

It was observed that the time of addition of oleic acid was an important factor. Oleic acid added at the time of incubation (0 h) was very effective in causing inhibition of growth. Addition of the fatty acid later during incubation of the organisms was not determined to be a useful measure. Thus, the fungistatic nature of oleic acid was identified. In order to confirm this property of oleic acid, germination of spores of Aspergillus sp. was observed in cavity slides. Figure 5 clearly shows the delay in the germination of the fungal spores by 8 h in presence of oleic acid. This clearly established the fungistatic nature of oleic acid.

It can hence be concluded that oleic acid can be used in combination with other compounds routinely in the preservation of cakes and pastries which are easily prone to spoilage, particularly during power breakdowns in developing countries. Oleic acid is not highly reactive, and is also easily miscible with any lipophilic preparation. In addition, it causes no interference with the preservative effect of other chemicals. Oleic acid can be metabolized easily in vivo in the same manner as fatty acids normally found in food and thus the use of oleic acid as a preservative tool for processed foods is advocated.

Figure 5. Inhibition of germination of spores of Aspergillus sp. by oleic acid (0.7% v/v). (a) control (without oleic acid); and (b) test (with oleic acid).

Enhanced wheat germ agglutinin accumulation in the germinating embryos of wheat (*Triticum aestivum* L.) appears to be a general stress response

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The effect of osmotic-, heat- and salinity-stress on accumulation of wheat germ agglutinin (WGA) in the germinating embryos of wheat (*Triticum aestivum* L.) was studied. Imposition of different stresses correspondingly resulted in differential enhancement in WGA levels; the effect being maximum during the initial 4 h period. Exposure of germinating embryos to salt-stress resulted in highest accumulation of WGA. Our studies suggest that WGA enhancement in the germinating embryos appears to be a general stress response, and is differentially regulated by different stress conditions.

Wheat germ agglutinin (WGA) is a *N*-acetylglucosamine specific lectin and is synthesized in developing and germinating embryos and roots and meristematic part of leaves of wheat. WGA is the best characterized of all the gramineous lectins and its accumulation in different tissues of wheat is enhanced by different stress conditions. Although based on *in vitro* studies, WGA has been implicated in dormancy and plant defence, but these roles fail to account for its characteristic biochemical properties, its predominance in growing and meristematic tissues, and its osmotic- and drought-stress inducibility. Therefore, based on earlier and our own studies, a novel in vivo role of this lectin in scavenging of free radicals, especially under stressful conditions, was proposed. Since other stress conditions, viz. heat and salinity have also been reported to elevate the production of free radicals, and if WGA is involved in their scavenging, enhanced accumulation of WGA is also expected under these conditions as well. To test this possibility and to gain insight into the proposed role of WGA as a free-radical scavenger, WGA accumulation in the embryos germinated under osmotic-, heat-, and salinity-stress conditions was studied at regular intervals.

Wheat (*Triticum aestivum* L. cv PBW-154) seeds obtained from Department of Plant Breeding, Punjab Agricultural University, Ludhiana (Punjab), were washed and surface-sterilized with 1% (w/v) mercuric chloride followed by 70% ethanol for two min each. Seeds were thoroughly rinsed and imbibed in sterile deionized water for 6 h. After imbibition, 100 seeds in ten replicates each, were placed on sterile Whatman no. 1 filter papers in petri plates and incubated in the seed germinator at 25°C after irrigating with different solutions. Control seeds were irrigated with deionized water whereas osmotic- and salt-stresses were imposed by irrigating the seeds with 0.75 M mannitol and 0.41 M NaCI solutions, respectively. Osmotic potential of both the solutions, as calculated by von’ Hoff equation, was −1.86 MPa. Effect of temperature stress was studied by incubating the plates at 35°C. Embryos were collected at regular intervals of 4 h for up to 24 h of germination. Samples were immediately frozen and stored in liquid nitrogen till further analysis.

Total acid soluble proteins (TASP) were extracted from 50–60 embryos by grinding in 50 mM HCl (3 ml g⁻¹ fresh wt) according to Singh et al. and estimated by Lowry’s method. WGA was quantified by enzyme-linked immunosorbent assay (ELISA) with polyclonal anti-WGA antibodies (Sigma Chemical Co., USA) in eight replicates in the presence of *N*-acetylglucosamine as described earlier. Western blotting was performed according to Bhagla et al. Ten µg of TASP were resolved on 15.5% discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (ref. 12) followed by transfer onto nitrocellulose membrane (Hybond) using semi-dry western blot system (Pharmacia-LKB Multiphor II). WGA protein bands were detected by using WGA-specific rabbit polyclonal antibodies (Sigma Chemical Co., USA) and sheep anti-rabbit IgG-alkaline phosphate conjugate, with nitroblue tetrazolium and X-phosphate as the substrates.

Relative to control embryos, where the TASP (µg per embryo) increased up to 8 h, imposition of different stress treatments resulted in highest TASP accumulation during the initial 4 h of germination (Figure 1).