NOTES ON SOME ORGANISMS OF TOMATO PULP.

By G. L. Windred.

(Communicated by Gilbert Wright.)

(Read before the Royal Society of New South Wales, Dec. 5, 1928.)

Introduction.

The manufacture of tomato products is now quite an important business in this State. Whereas, formerly such products were mostly imported from the United States and England now the local production is gradually supplanting such imported goods and with more inquiry into canning and preserving methods there is no reason why we should not be wholly self-supporting in this direction. Indeed, there may be opportunities to export surpluses.

In the manufacture of tomato-pulp, the tomatoes are first thoroughly washed as it has been found that this process gives a better product due to a decrease in mould growth compared with those that are untreated (1). Rotted portions are also cut out and the tomatoes drained of surplus water. The treated tomatoes are then put through a machine which removes the skin, pulps the tomatoes and passes the resulting pulp to a large wooden vat where it is heated by steam pipes. This vat is exposed to the air and is shallow, so that a large proportion of the pulp comes into direct contact with the air. Now, when large quantities of tomatoes are being put through the pulping machine, the pulp only remains in the vat for a short time before it is drawn out with buckets and poured into kerosene cans and sealed. Consequently, the temperature of the pulp in the
vat varies considerably in these rush periods and may not rise high enough to kill off vegetative forms of microorganisms which may be present.

After the pulp is sealed in the bulk cans these are stored for further use. Usually, no provision is made for cooling these stacks of cans and often the temperature of the storage rooms is considerably above normal, so that an opportunity is provided for the growth of microorganisms, either from spores or from vegetative cells, which have escaped being killed in the heating vat. It is during this period of storage that losses due to spoilage occur and these losses are by no means slight. A rough estimate shows from 5 to 15 per cent. spoilage, or even more in warm weather.

For the most part spoilage is manifest by a "sliminess" of the pulp and by the bursting of the cans, the latter being the more important. The object of this investigation has been to find the origin of such spoilage.

Tomato-pulp has a very rich flora of microorganisms including moulds, yeasts and bacteria, all of which, especially the last group, bring about profound changes in the pulp, rendering it, in many cases, unfit for human consumption. Although a good deal of work in this direction has been done in the United States, as yet little has been done here. It has been the aim of the investigator to make counts of the microorganisms occurring in the pulp and also to identify them if possible, especially that organism causing "sliminess."

The material was collected in a sterile aluminium ladle and placed in sterile flasks. Samples were taken of the unheated pulp immediately after it left the pulping machine. Other samples were taken from the heating vat at various levels, from burst cans and from slimy cans. All the samples were subsequently stored at room temperature.
It will be noticed that there is a striking decrease in numbers in the heated pulp and the 4,500,000 per c.c. probably results, for the most part, from the subsequent germination of the spores. The rise to 6,000,000 in sample (3) may be similarly accounted for or may be due to contact with unsterile surfaces as would be presented by the buckets and the containers. With such numbers of bacteria as occurred in samples (4) and (5) it is only to be expected that profound changes would occur in the pulp, spoiling it for further use.

Quantitative Determinations.

Counts were made both by the dilution method and by a direct method formulated by B. J. Howard (1). In making counts by the dilution method, standard agar medium was used and the plates inoculated at 32°C. for 48 hours. Dilution of 1:10,000, 1:100,000 and 1:1,000,000 were plated, there being six plates to each dilution. Only the plates which showed not more than 200 colonies and not fewer than 50 colonies were counted. The average was taken for each set of dilutions. The following table shows the results of the counts:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
<th>Dilution Method</th>
<th>Direct Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bacteria</td>
<td>Moulds</td>
</tr>
<tr>
<td></td>
<td></td>
<td>per cc.</td>
<td>per cc.</td>
</tr>
<tr>
<td>1*</td>
<td>Unheated Pulp</td>
<td>40</td>
<td>16</td>
</tr>
<tr>
<td>2**</td>
<td>Heated Pulp</td>
<td>4.5</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Pulp from good can</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>“Slimy” Pulp</td>
<td>400</td>
<td>130</td>
</tr>
<tr>
<td>5</td>
<td>Pulp from burst can</td>
<td>274</td>
<td>23</td>
</tr>
</tbody>
</table>

*From pulping machine.
**From vat.

These counts agree fairly well with those made in the United States although the direct method shows slightly greater numbers. Samples (4) and (5) would be quite unfit for human consumption.

The higher counts by the direct method are probably to be accounted for by the fact that in this method all cells in the field are counted, regardless of whether they are dead or alive, whereas in the dilution method, only the live cells produce colonies.
It will be noticed that there is a striking decrease in numbers in the heated pulp and the 4,500,000 per c.c. probably results, for the most part, from the subsequent germination of the spores. The rise to 6,000,000 in sample (3) may be similarly accounted for or may be due to contact with unsterile surfaces as would be presented by the buckets and the containers. With such numbers of bacteria as occurred in samples (4) and (5) it is only to be expected that profound changes would occur in the pulp, spoiling it for further use.

Qualitative Determinations.

From the plates used in the counting by the dilution method, nine different colonies were selected for identification. Standard agar slopes were made from the colonies and after incubating at 32°C, for 24 hours were replated in order to test purity of the cultures.

A pure culture of each organism having been obtained, the cultural, morphological and biochemical characters of each were studied according to the procedure advised by the Society of American Bacteriologists. The organisms were named according to the scheme set out in Bergey's Manual (2) further corroboration being obtained from the more detailed descriptions in the Journal of Bacteriology (3).

The following organisms were identified:—

1. *Bacillus vulgatus* Flügge.
2. *B. megatherium* De Bary.
5. *B. ellenbachiensis* Stutzer.
9. *Aerobacter cloacae.*
It will be noted that none of these are known to be pathogenic, and also, that all except *Aerobacter cloacae* are spore formers and therefore quite capable of withstanding the temperature of the heating vat and so of being able to germinate when the temperature of the pulp falls, which is after the cans have been sealed. It is also significant that all of them produce acid from carbohydrates and as will be seen later this has a bearing on the bursting of the cans. Now since *Aerobacter cloacae* does not produce spores its presence in a sample of heated pulp has to be accounted for. Members of this group have been found in pasteurised milk so that it is capable of withstanding fairly high temperatures. Otherwise it may gain access to the cans by leaks in faulty cans, or may enter before the can is sealed and after it has cooled considerably.

**Slime Production.**

Pulp which has become slimy has a very characteristic appearance somewhat resembling a thick starch paste but more coherent. When the slimy condition is at its maximum and most viscous, it is not possible to lift it up with a fork or glass rod as it slips off or breaks away. The cohesion is sufficient, however, to allow slime-threads of about 10 c.m. to be drawn out.

An organism was isolated from a sample of slimy pulp and numbered 10. Small portions of the slimy pulp were plated by the usual methods and with the exception of a few colonies of a *mucor* species, which always seems to be associated with the slimy condition, the bacterial colonies were all of the same appearance. Thus the slimy pulp was practically a pure culture of No. 10.

After the isolation of this organism and its transfer to standard agar slopes a test tube full of sterile pulp was inoculated with a heavy dose of organism. The slimy condi-
tion appeared in 72 hours at room temperature (18°C.) After a period of about 17 days the condition began to disappear (of course the duration of the sliminess would depend on many factors such as temperature, mass of pulp, amount of inoculum, etc.). With the gradual disappearance of the slime, a layer of clear amber coloured liquid appeared on the surface of the pulp. At the end of 32 days the sliminess had quite disappeared and the layer of clear liquid occupied about one quarter of the test-tube. The sedimented pulp became much lighter in colour and had a flocculent appearance. There was a very noticeable sour odour following the disappearance of the slime, otherwise the material remained differentiated into clear supernatant fluid and flocculent "precipitate" till the end of the experiment, i.e., for six weeks, without marked change. On plating out some of this material the same colony formation was noticed as at first and on re-inoculating some sterile pulp with this inoculum the slimy condition was again produced. Thus it is highly probable that this organism, No. 10, is the cause of the sliminess. However, since no capsule or envelope could be demonstrated round the organism it is assumed that it is not the organism itself which brings about the slimy condition, but rather some product of its metabolism.

The following is a brief description of No. 10:—

*Morphology.*—Long rods with rounded ends, measuring 4μ by .75μ on an average. Shadow forms common. Arranged singly or in long chains.

*Spore-formation.* Forms spores early, central in position and sometimes excentric. Cause slight bulge in organism. Average measurement of 1.5μ by .5μ.

*Mobility.*—Very active in young cultures. Flagella peritrichous and numerous.
**Agar Slope.**—Moderate growth with a well defined ridge. Tends to spread giving in older cultures a rhizoid appearance. Opaque, raised, smooth, membranous, moist and pure white.

**Agar Colonies.**—Rapid growth. Different forms, some round and regular, others amoeboid. Surface smooth, moist, glistening, raised, opaque and pure white. A ridge appears near the periphery giving a shallow crater-like appearance.

**Gelatine Stab.**—Growth best at the top. Line of puncture filiform. Liquefaction infundibuliform. Medium liquefied fairly rapidly.

**Broth.**—A fragile pellicle formed with slight turbidity near surface. Clears by sedimentation. Long chains.

**Potato.**—Creamy-white profuse growth, spreading, raised, glistening, very rugose, slimy, membranous consistency. Decided odour.

**Glucose Agar.**—Rapid growth, filiform but spreading. Flat, dull, rugose, opaque, cream, butyrous.

**Gram Stain.**—Positive.

**Glucose Broth.**—Acid, no gas.

**Lactose Broth.**—Alkaline, no gas.

**Sucrose Broth.**—Acid, no gas.

**Milk.**—Rapid casein digestion with clear, amber-coloured fluid in upper part of tube.

**Litmus Milk.**—Slightly acid in 48 hours with slight coagulation followed by digestion.

**Pigment.**—None.

This description resembles closely that of *Bacillus ruminatus* Gottheil except that it forms long chains in broth and milk. Also, in agar colonies, no shell-like periphery
was observed as has been attributed to *B. ruminatus*. In all other characters, however, it resembles fairly closely this species and may be a variety of it.

Many cans, both the large kerosene cans and the smaller sizes show a swelling due to increase of internal pressure. At times this pressure increases to such an extent that the can bursts, and in the case of the large bulk cans, with such a force that the whole stack may be thrown down. A large proportion of kerosene-cans of pulp burst, owing, probably, to the fact that they are not so well made as the smaller two-pound tins.

The gas may be produced in two ways: (1) by the action of the *Coli* group of organisms on the carbohydrates of the pulp thus liberating CO₂ and H₂, and (2) by the action of the acid juices on the metal of the container (4).

In the first case when tins of sterile pulp were inoculated with a vigorous culture of *Aerobacter cloacae* and incubated at 37°C. the cans became swollen and burst in 17 days. Since this organism produces both CO₂ and acid in the pulp, the pressure caused by the CO₂ is augmented by the liberation of hydrogen by the action of the acid produced on the metal of the container. This pressure is sufficient to burst open the seams of a two-pound can.

In the second case all the organisms isolated produced acid so that if any great number of organisms remain in the can after processing there is the possibility of them producing enough acid to attack untinned portions of the can and thus liberate hydrogen. The pulp itself shows an acidity of 0.45%, calculated as citric acid, so that together with the products of the bacteria present a considerable acidity may develop, which, if not sufficient to produce enough gas to burst the can, may bulge the ends of the can considerably.
NOTES ON SOME AUSTRALIAN TIMBERS
OF THE MONIMIACEAE.

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(With Plates XXVI.-XXIX.)

(Read before the Royal Society of New South Wales, Dec. 5, 1928.)

Several Australian genera belonging to the Monimiaceae, & family principally occurring in tropical and subtropical regions, yield useful timbers. Of the eight Australian genera recorded by Bentham, two are woody climbers, whilst several are too rare to be of commercial importance. The Australian representatives are chiefly confined to the eastern rain forest areas of the mainland, with one genus, Atlierosperma, occurring in Tasmania.

The following anatomical descriptions apply to specimens of the various woods in the Technological Museum collection.

Doryphora sassafras, Endlicher.

Sassafras, Grey or Black Sassafras.

A medium-sized tree found in the brush forests and on alluvial pockets in gullies, throughout eastern New South Wales and extending into southern Queensland. The wood is very close textured, almost "pine-like," and pale yellowish in colour, becoming darker on exposure. Occasionally dark, irregular streaks are present, especially near the heart, which is occasionally almost jet black. The freshly sawn wood, or even a fresh surface on seasoned wood, usually possesses a pleasant safrol-like


Gas production causing bursting of the cans is due to two causes, (1) the action of acid on the metal of the container, and (2) the production of CO₂ by bacteria.

(Communicated by Gilbert Wright.)

This investigation was carried out in the Faculty of Agriculture, University of Sydney, under the direction of Mr. G. Wright. Acknowledgments are due to Professor R. D. Watt for reviewing the manuscript.

LITERATURE CITATIONS.


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DOI: https://doi.org/10.5962/p.359979
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