SOME OF THE CHARACTERISTICS OF YEASTS FOUND IN FERMENTING HONEY

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INTRODUCTION

Honey is produced for the market in two forms, so-called comb or section honey and extracted honey. In Wisconsin alone from 6 to 10 million pounds of extracted honey are produced each year. The common method of marketing honey is to extract it from the combs, strain, and put it in 5 and 10 pound pails for retail trade and 60-pound cans for wholesale trade. A part of the honey put in 5 and 10 pound pails is pasteurized by heating to 160° F., but by far the greater portion of the honey that goes into the wholesale market is not pasteurized. In from four to eight weeks after removal from the comb, the honey begins to crystallize and by the time it is delivered to the broker or wholesale dealer it is completely granulated. Under conditions as they existed a few years ago, honey found its way to the consumer within six to eight months after it was produced. Under present conditions of greatly increased production, it is often stored for many months, perhaps years, and, when stored under unfavorable conditions, may undergo decided changes in flavor and color which reduce it to an inferior market grade.

But still another problem of greater importance in the storage of honey is that of fermentation. The majority of beekeepers do not make any attempt to pasteurize honey and consequently fermented honey is of frequent occurrence. It was formerly supposed that when fermentation took place, the honey had not been properly prepared by the beekeeper or perhaps had been extracted from the comb before it was thoroughly ripened. However, many recent cases have been noted which show that fermentation is not a result of contamination due to unsanitary methods of handling. The organisms which cause the fermentation are found normally in association with bees and the bees are responsible for their spread in new honeys (14). These organisms are not killed during the ripening of the honey, and therefore develop at certain temperatures when the physical conditions of the honey are changed through granulation.

REVIEW OF LITERATURE

Since honey fermentation and spoilage have not been of much economic importance until recent years, the literature on the subject is meager. A few papers have been published in scientific journals and a few references have been made to honey fermentation in popular journals on beekeeping.

1 Received for publication Feb. 2, 1931; issued August, 1931.
2 Reference is made by number (italic) to Literature Cited, p. 131.
At the request of the Beekeepers’ Association of Ontario, Shutt (12) undertook to ascertain the difference in composition between honey taken from uncapped and capped combs. Honey from uncapped combs is known as immature or unripe. Honey from fully capped combs or ripe honey contains from 4 to 5 per cent less moisture than that from the partially or entirely uncapped combs. Shutt found that honey from the uncapped combs has decidedly poorer keeping qualities than that from fully capped combs.

Klöcker (3, 4) isolated an organism which he named *Zygosaccharomyces priorianus* from the bodies and honey sacs of honeybees. The cells of this yeast were oval or elongated and were firmly bound together so that when sedimented the yeast formed a coherent mass. The spores were round or oval and generally numbered from two to four in a cell. This species fermented glucose, fructose, maltose, and sucrose.

Cook (1) states that unripe honey is an excellent medium for microorganisms, and that during fermentation there is an evolution of carbon dioxide. In a Chicago warehouse he found barrels of honey bursting as a result of the formation of this gas.

One of the early investigations of fermented honey was made by Nussbaumer (9), who worked with a sample of Canadian honey. From this he isolated two strains of *Zygosaccharomyces*, which he called strains A and B. The two strains fermented glucose, fructose, mannose, sucrose, and dextrin. Strain A fermented maltose while B did not, and B fermented galactose while A did not. In some of his later experiments Nussbaumer diluted 61 samples of honey and incubated these for several months. Strains of *Zygosaccharomyces* were obtained from all the samples that fermented—37 in number. He advised heating honey to 70° C. for 30 minutes to prevent fermentation.

Richter (10) observed the souring and fermentation of honey in the combs before it was removed from the hive and in cells sealed by the bees. The development of gas in the cells was so great that the cappings ruptured and the contents were forced out, flowing over the combs in foaming streams. From this fermenting comb honey he isolated *Zygosaccharomyces mellis acidi*. The fermentation products of this organism were alcohol and acid. This yeast fermented glucose, fructose, and sucrose strongly and galactose feebly.

According to Root (11), not one beekeeper in a hundred can tell by the taste or appearance of extracted honey whether or not it is proof against fermentation when placed in storage. It is impossible for all honey to reach the consumer within six to nine months from the time it is produced, and therefore the supreme test comes when honey is kept past the spring months. Root found that granulated honey kept in a warm room underwent fermentation, and as a result of gas production burst the cans. While the honey was not very sour to the taste, there had been enough change practically to ruin it for the market. The mere fact that honey is granulated is not by any means proof that it is entirely ripe or that it will keep.

Marvin (7, 8) isolated five types of yeastlike organisms from samples of fermenting Wisconsin honey. All of these organisms were found capable of fermenting honey after it had granulated. When the honey was heated to 160° F. fermentation was prevented. Fabian and Quinet (2) isolated four species of *Zygosaccharomyces* and one strain of *Torula* from samples of fermenting honey. Three were iden-
Characteristics of Yeast Found in Fermenting Honey

Identification of Yeast Species

Lockhead and Heron (5) isolated four types of yeasts from fermented honey and found that all belonged to the genus Zygosaccharomyces. Two types were identified with previously described yeasts, namely, Z. barkeri and Z. mellis. The other two were considered new and designated as Z. nussbaumeri and Z. richgeri.

EXPERIMENTAL WORK

FIELD OBSERVATIONS ON FERMENTED HONEY

Late in 1925 a study of the causes of fermentation in honey and methods of preventing this loss while honey is in storage was started. Since the beginning of this investigation 49 samples of fermented honey from the following regions have been received and studied: California 2, Colorado 1, Connecticut 1, Florida 1, Illinois 4, Kentucky 1, Maine 2, Ohio 1, Utah 3, Wisconsin 28, Hawaii 1, Norway 1, Porto Rico 1, and Canada 2. From these samples 50 cultures of yeast have been isolated and 5 of these have been studied in detail.

Tall glass containers were filled with the fermenting honey. All of the samples had a soft granulation. Soon they became plainly divided into a lower granulated layer and an upper fluid layer with the surface covered with foam. The odor resembled that of sweet wine or fermenting canned fruit, and on microscopic examination the honey showed many yeast cells. As fermentation progressed, the fluid layer increased in depth and the granulated layer decreased.

ISOLATION OF YEASTS FROM HONEY

At first some difficulty was encountered in getting a suitable medium for the growth of the honey yeasts, but after several preliminary experiments a medium was made up of the following composition:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast water</td>
<td>1,000 c. c.</td>
</tr>
<tr>
<td>Honey</td>
<td>200 c. c.</td>
</tr>
<tr>
<td>Agar</td>
<td>36 gm.</td>
</tr>
</tbody>
</table>

The agar was dissolved in the yeast water and the honey was added just before the medium was tubed or placed in 125 c. c. flasks. These flask cultures were for giant colonies and for pouring plates. The medium was then sterilized for 20 minutes at 15 pounds pressure. If sterilized for a longer time, the medium will become dark because of caramelization, the agar will be partly hydrolyzed by the acid of the honey, and the medium will not harden properly.

The reaction of this medium was about pH 4.5. As honey yeasts grow better on a slightly acid medium than on a neutralized one, the medium was never brought to neutrality.

Three different methods were used in isolating yeasts from a sample of honey. (1) A loopful of fermenting honey was thoroughly mixed with the melted honey agar and the mixture was poured into a sterile Petri dish and allowed to harden. (2) The medium was liquefied, poured into a sterile Petri dish, and allowed to harden.

3 Starch-free yeast 100 gm. and 1,000 c.c. of water, steamed for two hours and sterilized. After standing for two weeks the clear supernatant liquid was used as a nutrient.
The hardened surface was then streaked with a needle which had been inserted previously in fermenting honey. (3) Ten or twelve loopfuls of fermented honey were put into a tube of sterile water, which was agitated to dissolve the honey, and centrifuged. The clear liquid on top was drawn off. A loopful of the heavier material on the bottom was used as an inoculum and the first procedure was followed.

The Petri dishes inoculated as described were incubated (1) at room temperature and at 28° C. under aerobic conditions, (2) in anaerobic jars at room temperature and at 28° C. and (3) under vacuum at room temperature and at 28°.

Within two or three days the plates incubated under aerobic conditions at 28° C. showed growth, while those incubated at room temperature required from five to six days for the colonies to appear. Microscopic examination revealed that the colonies were yeasts. No growth at all was noticed on the plates incubated in anaerobic or vacuum jars.

Plating operations were repeated many times and different samples of fermenting honey from various sources were used. Each time yeast colonies and molds were the only microorganisms that appeared on the plates. When some honeys were plated, so many molds appeared on the plate that the entire surface was covered before a yeast colony could be selected. Under these circumstances, a few loopfuls of fermenting honey were put in a sterile mixture of 50-50 honey and yeast water. When the mixture began to ferment, a loopful was plated out and usually no molds appeared.

Isolated colonies of yeasts were picked from the plates and transferred to slopes of the same medium. From these slopes, material was used for stock cultures and for further inoculation.

Giant colonies of each yeast were grown. Erlenmeyer flasks of 125 c. c. capacity were used. Fifty cubic centimeters of the honey-yeast-water-agar medium were put into each flask, after which the flasks were plugged and sterilized. Some of the yeast culture was transferred by means of a needle to the center of the hardened surface. Care was taken not to break the surface, for if this is done the medium cracks at this point on evaporation and ruins the giant colony.

Giant colonies of five strains of honey yeasts were grown very successfully in this way, and illustrations of these are found in Plates 1 and 2. The problem of maintaining uncontaminated colonies is reduced to a minimum by means of the flask cultures.

Three of the yeasts, strains 6, 7, and 8, were isolated from pollen which the bees had stored in the comb for brood food. Strain 9 was isolated from a sample of fermenting honey received from Hawaii. It belongs to the genus Zygosaccharomyces. Cultures of Saccharomyces cerevisiae S. logos, and Z. priorianus were also carried to see if there were any differences between certain known species and the honey yeasts, and were designated as strains 11, 12, and 13, respectively.

EXPLANATORY LEGEND FOR PLATE 1

A.—Morphology in young (a) and old (b) cultures of strain 1 which apparently corresponds with Zygosaccharomyces mellis, Fabian et Quinet.
B.—Two-month old giant colony of strain 1, enlarged 2 diameters.
C.—Morphology in young (a) and old (b) cultures of strain 2.
D.—Giant colony of strain 2, 2 months old, enlarged 2 diameters.
E.—Morphology in young (a) and old (b) cultures of strain 3.
F.—Giant colony of strain 3, 2 months old, enlarged 2 diameters.
For explanatory legend see opposite page
For explanatory legend see opposite page
RELATION BETWEEN CONCENTRATION OF HONEY AND INITIATION OF FERMENTATION

An experiment was set up to determine whether natural unheated honeys when diluted would undergo fermentation. Honey and sterilized water were mixed in small sterilized bottles in the following proportions:

<table>
<thead>
<tr>
<th>Honey (parts by weight)</th>
<th>20</th>
<th>18</th>
<th>16</th>
<th>14</th>
<th>12</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (parts by weight)</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

Seven Wisconsin honeys, all unheated and marketable, were used in this dilution experiment. One set of bottles from each of the seven honeys so diluted was inoculated by adding to each bottle a large loopful of a young vigorous growing culture of each of the five yeasts isolated from fermenting Wisconsin honey. A second set of bottles from each of the seven honeys so diluted was left uninoculated and kept as controls. Lead foil was wrapped around the top of each bottle to prevent evaporation. A strong fermentation was under way after 48 to 72 hours in the inoculated diluted samples. In the uninoculated controls fermentation was started in the more dilute samples after a week and in the more concentrated ones after two to three weeks. These results show that fermentation is promoted by a high percentage of water in the honey.

All of the diluted honeys which fermented were discarded and the undiluted ones were kept. As long as the honey remained liquid no noticeable fermentation took place, but after it had been stored a few months at room temperature, granulation began, and with this physical change in the honey signs of fermentation appeared in the inoculated samples.

A second series of samples of pasteurized honey was inoculated with five pure cultures of yeasts isolated from fermenting honey. As long as the honey remained liquid there were no signs of fermentation, but after a year and a half in storage the samples granulated and fermentation became apparent. Another series of samples of the same pasteurized honey was inoculated with small amounts of fermenting honey already granulated. These granules hastened crystallization in the samples and all fermented within a year. From this experiment it appears that crystallization of honey and fermentation are related to each other.

To study further this apparent relation of crystallization of honey to fermentation, moisture determinations were made on the syrup and crystal layers of honey. In the samples studied the moisture of the syrup layer was 9.11 per cent higher than that in the crystal layer, and the moisture was greater in the liquid layer than in the original honey.

When honey granulates, the water formerly serving as a solvent for the glucose crystals is retained in the liquid part of the honey and thus increases the moisture content of the syrup. Crystallization is

EXPLANATORY LEGEND FOR PLATE 2

A.—Morphology in young (a) and old (b) Zygossaccharomyces cultures of strain 4 which apparently corresponds with Z. mellis, Fabian et Quinet.
B.—Giant colony of strain 4, 2 months old; enlarged 2 diameters.
C.—Giant colony of strain 5, which is apparently the same as Z. nussbaumeri, Lochhead et Heron, 2 months old and enlarged slightly over 2 diameters.
D.—Morphology in young (a) and old (b) cultures of Z. nussbaumeri, Lochhead et Heron.
E.—Giant colony of Z. nussbaumeri, 1 month old, enlarged 2 diameters; the colony is smooth at first, later a rough secondary growth breaks out at the center and gradually covers the entire surface, as appears in the older colony at C.
F.—A streak culture of strain 3 (a) and Z. barkeri (b).
thus equivalent to diluting the uncrystallized honey with water. The syrup phase becomes dilute enough to permit the growth of yeasts that could not grow in the uncrystallized honey.

FERMENTATION CHARACTERISTICS OF HONEY YEAST

The fermentation reaction of the yeasts isolated from honey was studied in a medium containing about 2 gm. of sugar dissolved in 100 c. c. of 10 per cent yeast water. Ten cubic centimeter portions of this medium were placed in test tubes and sterilized, and each was inoculated with a loopful of a vigorous 4-day-old culture. These tubes were incubated at 28° C. and after seven days were analyzed for unfermented sugars by the method of Stiles, Peterson and Fred (13). By this time the medium in the tubes had ceased to produce gas and had begun to clear up. The results are given in Table 1.

From the table it is evident that there was some variation in the fermentability of the sugars used. Glucose, fructose, and mannose were easily fermented by the five strains of honey yeasts, while approximately one-third of the galactose was fermented, and lactose was very slightly utilized. A variation was noted in the fermentability of sucrose. Strains 1 and 4 fermented approximately only one-third while strain 2 utilized one-half. Strains 3 and 5 fermented sucrose more energetically than did the other honey yeasts. With maltose, strains 1 and 4 utilized approximately the same amounts. Strain 5 fermented it energetically. Strain 2 fermented about one-third and strain 3 utilized over one-half.

TABLE 1.—Fermentation of various sugars by yeast strains isolated from honey and pollen

<table>
<thead>
<tr>
<th>Sugar</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>96</td>
<td>97</td>
<td>97</td>
<td>99</td>
<td>96</td>
<td>96</td>
<td>97</td>
<td>97</td>
<td>95</td>
<td>97</td>
<td>99</td>
<td>99</td>
<td>98</td>
</tr>
<tr>
<td>Fructose</td>
<td>96</td>
<td>96</td>
<td>95</td>
<td>95</td>
<td>94</td>
<td>95</td>
<td>94</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>Mannose</td>
<td>96</td>
<td>96</td>
<td>95</td>
<td>96</td>
<td>93</td>
<td>93</td>
<td>94</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>90</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>Galactose</td>
<td>35</td>
<td>32</td>
<td>28</td>
<td>30</td>
<td>33</td>
<td>34</td>
<td>30</td>
<td>63</td>
<td>26</td>
<td>30</td>
<td>67</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>Sucrose</td>
<td>37</td>
<td>30</td>
<td>75</td>
<td>36</td>
<td>90</td>
<td>95</td>
<td>92</td>
<td>98</td>
<td>44</td>
<td>90</td>
<td>98</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>Maltose</td>
<td>58</td>
<td>30</td>
<td>55</td>
<td>50</td>
<td>81</td>
<td>98</td>
<td>96</td>
<td>48</td>
<td>65</td>
<td>58</td>
<td>71</td>
<td>60</td>
<td>55</td>
</tr>
<tr>
<td>Lactose</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

* Concentration of sugar before fermentation, about 2 per cent.

As the amount of raffinose, melezitose, soluble starch, and dextrin fermented could not be determined directly, the production of gas as indicated by the vaseline-seal method of Loewe and Strauss (6) was taken as a measure of fermentation. The yeasts isolated from fermenting honey did not form gas with these carbohydrates and apparently do not ferment them.

FERMENTATION PRODUCTS

METHODS OF ANALYSIS

In order to study the products of honey fermentation, 450 c. c. of 1 part honey and 1 part of 10 per cent yeast water were placed in liter flasks and sterilized. Small flasks containing 50 c. c. of the same medium were heavily inoculated with the honey yeasts from
slopes, and incubated. The fermenting material in the small flasks was used for inoculating the media in the large flasks. Sterile fermentation traps were inserted through corks and the whole waxed tightly into the mouth of the flask. Enough 20 per cent sulphuric acid solution was poured into the trap so that the opening of the tube leading from the interior of the flask was below the surface of the acid. The flasks were incubated at 28°C. After 40 days, when the production of gas had ceased and the material in the flasks had begun to clear, the liquid was analyzed for alcohol and acids.

**Alcohol.**—One hundred cubic centimeters of the honey medium, after fermentation was completed, were put into a distilling flask, a small amount of dry phenolphthalein was added, and the medium brought to neutrality. Next, 50 c. c. of distilled water, 5 gm. of NaCl, and a little tannic acid were added. The latter substance prevented frothing during distillation. One hundred cubic centimeters of distillate were collected, cooled to 15½°C., and the specific gravity determined by means of a pycnometer.

**Titratable Acid.**—Fifty cubic centimeters of distilled water were added to 10 c. c. of the fermented medium and the mixture was brought to boiling. Phenolphthalein was used as an indicator and titration continued until the first permanent pink color appeared.

**Volatile Acid.**—Two hundred cubic centimeters of the fermented medium were acidified with 5 c. c. of N/5 sulphuric acid, distilled with steam, and 1,500 c. c. distillate collected. The distillate was brought to boiling and titrated with N/10 NaOH to the phenolphthalein end point.

**Nonvolatile Acids.**—One hundred cubic centimeters of honey medium after fermentation was extracted for four days with ether. After the ether had been removed water was added and the extract titrated with N/10 barium hydroxide.

**Carbon Dioxide.**—The amount of carbon dioxide given off by each yeast during fermentation was determined by weighing the flask of fermenting honey at intervals. The flasks were sealed with fermentation traps and the carbon dioxide escaped through the sulphuric acid.

### Results of Analyses

Carbon dioxide was measured quantitatively on 10 of the honey and pollen yeasts with the results that appear in Table 2. Strains 11, 12, and 13 failed to grow in a 50 per cent concentration of honey. From observations, fermentation in a 50 per cent honey medium is relatively rapid compared with what it would be in an ordinary sample of honey. In the case of the latter, it requires a long time for the fermentation to begin and it extends over a period of six months to a year. Evolution of carbon dioxide is very slow. In the 50 per cent honey medium the fermentation appeared to be over after 40 days and clearing was noticed. The evolution of carbon dioxide in 40 days varied from 3.0 to 6.3 gm. per 100 c. c. of 50 per cent honey for the 10 strains, as shown in Table 2.

In experimental fermentations of 50 per cent honey about equal quantities (4 to 5 gm. per 100 c. c.) of carbon dioxide and alcohol were produced in 40 days, as shown in Table 3. Small quantities of acid, mainly nonvolatile, were also produced. It was thought at first that on account of the large amounts of sugar present in the 50 per
cent honey medium, a depressing action was caused on the yeasts, which accounted for the small amounts of alcohol and acids formed. The concentration of honey was reduced to 20 per cent, and here again approximately the same amounts of alcohol and acid were formed. The acids and alcohol probably contribute the products which apparently cause the off-flavor of fermented or slightly fermented honey.

Table 2.—Evolution of carbon dioxide from 50 per cent honey solution when fermented with 10 strains of yeast isolated from honey and pollen

<table>
<thead>
<tr>
<th>Duration of fermentation (days)</th>
<th>Grams of CO₂ evolved per 106 c. c. of honey when fermented with strain No.—</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>0.9</td>
</tr>
<tr>
<td>10</td>
<td>1.6</td>
</tr>
<tr>
<td>20</td>
<td>1.4</td>
</tr>
<tr>
<td>27</td>
<td>1.1</td>
</tr>
<tr>
<td>40</td>
<td>0.7</td>
</tr>
<tr>
<td>Total</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Table 3.—Fermentation products of five typical honey yeasts produced in a 50 per cent honey fermentation lasting 40 days

<table>
<thead>
<tr>
<th>Yeast strain No.</th>
<th>Production per 100 c. c. of 50 per cent honey of—</th>
<th>0.1 N acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbon dioxide</td>
<td>Alcohol</td>
</tr>
<tr>
<td>Gram</td>
<td>Gram</td>
<td>C. c.</td>
</tr>
<tr>
<td>1</td>
<td>4.8</td>
<td>4.4</td>
</tr>
<tr>
<td>2</td>
<td>5.6</td>
<td>4.7</td>
</tr>
<tr>
<td>3</td>
<td>4.5</td>
<td>3.8</td>
</tr>
<tr>
<td>4</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>5.2</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Morphological Characteristics of Honey Yeasts

The published descriptions of species are frequently incomplete, but the writers have had the opportunity to compare their strains with cultures isolated by Fabian, formerly of Michigan, and Lochhead of Ottawa, Canada, who have been kind enough to send transfers of their honey yeasts. Cultures of Zygosaccharomyces barkeri and Z. priorianus have been obtained from Tanner of Illinois, Kluyver of Delft, Holland, and the American type culture collection in Chicago.

The morphological characters of the five yeasts isolated from fermented honey were studied from 96-hour-old streaks on 20 per cent honey yeast-water agar incubated at room temperature. As suggested by Lochhead, a concentrated honey gelatin was used for spore formation. This consisted of 100 c. c. of honey diluted with the same amount of water, to which was added 50 gm. of gelatin. Cultures were examined in liquid mounts of 0.1 N iodine. Vaseline was smeared around the cover slip and put over the drop of liquid to pre-
Characteristics of Yeast Found in Fermenting Honey

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The strains were classified according to differences in (1) morphological characters of their giant colonies, (2) ring formation in flasks of fermenting wort, (3) type of growth on slopes, (4) appearance of cells under the microscope, and (5) their ability to ferment various sugars. The size of the cells of the different strains when grown on separate media is shown in Table 4:

**Table 4.** Relative size of cells of five yeast strains isolated from honey when grown on different media

<table>
<thead>
<tr>
<th>Measurement</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average length μ</td>
<td>5.59</td>
<td>9.46</td>
<td>4.16</td>
<td>5.35</td>
<td>4.66</td>
</tr>
<tr>
<td>Average width μ</td>
<td>3.94</td>
<td>2.73-3.44</td>
<td>3.71</td>
<td>3.8</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Grown in 50 per cent honey 6 days at room temperature

<table>
<thead>
<tr>
<th>Measurement</th>
<th>4.8</th>
<th>8.12-4.25</th>
<th>4.13</th>
<th>4.78</th>
<th>4.14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average length μ</td>
<td>3.65</td>
<td>2.62-3.9</td>
<td>3.32</td>
<td>3.75</td>
<td>4</td>
</tr>
<tr>
<td>Average width μ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Strain 2 persisted in having two types of cells, elongated and round.

**Description of Yeasts Isolated from Fermented Honey**

**Type 1.** (Pls. 1 and 2, A, B.)—Strains 1 and 4 form medium heavy rings in flasks of fermenting wort. Glucose, fructose, and mannose are fermented energetically, while galactose, sucrose, and maltose are fermented more weakly. Four-day-old cultures incubated at room temperature on 20 per cent honey agar show mostly elliptical cells, single or in groups of two and three. When a 50 per cent honey broth is used the cells are smaller. Conjugation and spore formation were observed on concentrated honey gelatin. The giant colonies of the two strains have not the same appearance, yet cultures have the same fermentative properties as type 3 of Fabian and Quinet and culture D1 of Lochhead and Heron, and so may be classed as *Zygosaccharomyces mellis* Fabian and Quinet.

**Type 2.** (Pl. 2, C, D, E.)—Strain 5 forms an extremely heavy ring in a flask of fermenting wort. Glucose, fructose, mannose, sucrose, and maltose are fermented strongly, while galactose is fermented more weakly. The cells are round, generally in pairs. The giant colony appears smooth at first with a folded and wrinkled secondary growth, as shown in Plate 2, E. This yeast corresponds with culture J7 of Lochhead and Heron, named by them *Zygosaccharomyces nussbaumeri*.

**Type 3.** (Pl. 1, C, D.)—Strain 2 forms medium heavy rings in flasks of fermenting wort. Glucose, fructose, and mannose are fermented energetically, while galactose and maltose are utilized to a less degree. This strain possesses cells of varying shapes, round or elliptical, and very much elongated. Elongated cells are always found attached to round ones, which gives the culture a characteristic
appearance. Conjugation preceding sporulation has been observed, but cells form spores without having undergone conjugation, only the beginning of a canal being noticed. This fact, which was not observed in the rest, and its different morphological and cultural characteristics on 20 per cent honey-agar medium entitle it to a separate classification. It appears, therefore, that the writers' strain 2 is not identical with culture D1 of Lochhead and Heron nor with type 3 of Fabian and Quinet. It does not appear to have been described before.

Type 4. (Pl. 1, E, F, Pl. 2, F.)—Strain 3 forms a very light ring in flasks of fermenting wort. Glucose, fructose, mannose, and sucrose are fermented strongly, while galactose and maltose are fermented more weakly. The cells of this yeast are mostly round, though some are elliptical. Usually two cells are joined, although they may appear singly. Conjugation between cells has been observed before spore formation on concentrated honey gelatin. This yeast has the same fermentative properties as type 2 of Fabian and Quinet and culture E6 of Lochhead and Heron and has been placed by them with the previously described *Zygosaccharomyces barkeri*. Pure cultures of *Z. barkeri* have been obtained from Professor Tanner of Illinois and from the American type culture collection. The type of growth of *Z. barkeri* from these sources on slants and in giant colonies is not in the least like that of the cultures of Fabian and Lochhead, which are the same as the writers' strain 3. In Plate 2, F, strain 3 is shown on the left and *Z. barkeri* on the right. It appears that strain 3 is a new species.

**Prevention of Fermentation in Honey**

The writers have found it possible to prevent fermentation of honey by holding it at 100° F. for several months, 122° for 24 hours, or heating it to 160°. Nussbaumer (9) recommended heating honey to 70° C. for 30 minutes, and Fabian and Quinet (2) found that honey heated to 145° F. and held there for 30 minutes would not ferment. These last temperatures are rather difficult to maintain with the equipment found in the average commercial beekeeper's honey house. Honey kept at points above room temperature for a period of time has a tendency to darken in color and to lose some of its flavor. If the honey is heated very quickly to 160° F., poured in containers, sealed while hot and cooled soon afterwards the color and the flavor will not be changed to any great extent. Fermentation, provided the honey has been heated all the way through to this temperature, is immediately and completely stopped. Granulation is delayed for some time, but when this honey does granulate the crystals are usually large. Honey which has not been heated should be held in storage at temperatures below 52°, for honey yeasts will not grow below this temperature.

**Summary**

This paper reports the results of a study of yeasts isolated from fermenting honey.

It is shown that when honey granulates the water serving as a solvent for the glucose is retained in the liquid part of the honey, and increases the moisture content of the syrup. Crystallization is
thus equivalent to diluting the uncrystallized honey with water. The syrup becomes dilute enough to permit the growth of yeasts that could not grow in the unchanged honey.

The fermentation process in honey is slow, extending over a period of six months to several years. About equal quantities of carbon dioxide and alcohol (rarely over 5 per cent), together with small amounts of nonvolatile acids, form the chief fermentation products.

Some of the yeasts isolated from fermenting honey correspond with previously described species. What appear to be two new species are described in this paper.

Fermentation of honey can be prevented by heating the honey to 160° F., pailing it while hot, and cooling it immediately. Honey in storage will not ferment if kept at temperatures below 52° F.

LITERATURE CITED

(10) Richter, A. A. V. 1912. ÜBER EINEN OSMOPHILEN ORGANISMUS, DEN HEFEPILZ ZYGOSACCHAROMYCES MELLIS ACIDI SP. N. Mycol. Centbl. 1: [67]-76, illus.