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# The Utilization of Pentoses by Yeasts and the Composition of Plant Gums

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# The Utilization of Pentoses by Yeasts, and the Composition of Plant Gums*

OUIDA DAVIS ABBOTT

**Abstract**—About one-half of the yeasts and related organisms tested were able to destroy the five-carbon sugar, arabinose, in a mineral nutrient solution under pure culture conditions. Xylose and arabinose disappeared in solutions to which Fleischmann's yeast cake was added. Reactions more acid than pH 5.0 interfered with the destruction of the pentoses by the yeasts used. Traces of $CO_2$ and alcohol and some non-volatile acid were formed by the yeast in the utilization of pentoses. A substance of high reducing power, which may be glyceric or glycollic aldehyde, was also produced in some cases. The fermentation method as commonly used for the determination of pentoses may be inaccurate because of the presence of foreign organisms, because the common varieties of yeast such as are found in Fleischmann's yeast cake can utilize pentoses and because galactose is as resistant to utilization by the yeasts in Fleischmann's yeast cake as are the pentoses. Orange gum could be completely hydrolyzed by taka-diastase. A combustion on orange gum gave the empirical formula $C_{32}H_{41}O_{35}$.

## INTRODUCTION

While pentose sugars seldom appear free in nature, they have been well known in the form of pentosans, as components of the cell walls and vessels of plants, and as constituents of various gums and mucilages. In 1891 Tollens[57]† pointed out the wide distribution of pentosans in the plant kingdom, and McDougall, Spoehr, Hooker, Levene and Rosa have indicated their physiological importance in plant economy.

McDougall[30] states that the "plant protoplasm consists of a comparatively inert base of pentosans in colloidal combination with proteins, amino acids, lipins, and salts."

Spoehr[56] found that the pentosan content of *Opuntia* increased under xerophytic conditions and suggested that the large water-retaining power of the pentosans is largely responsible for the well known ability of the cacti to survive under arid conditions.

Hooker[24] has shown that the hardier parts of apple shoots, the bases, have a greater water-retaining power than the tips, which are less cold-resistant. He attributed the greater water-retaining power of the

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†Superscript numerals refer to Bibliography on page 27.

hardy tissues to a larger percentage content of total pentosans or of some specific hydrophilous colloid.

Rosa[49] found the total pentosan content to be greater in hardened than in tender plants. He suggested that hot-water-soluble pentosans might represent more nearly the amount of pentosan in the protoplasm and that these might function more specifically as water-retaining material. The hot-water-soluble pentosans were considered by Rosa to function in the cold resistance of plants.

Another great physiological importance of the pentoses becomes evident in the light of recent investigations on the chemistry of the cell nucleus. Levene and Jacobs[28] have shown that in the plant nucleic acids so far studied a pentose group is always present.

Pentosans have usually been estimated by one of two methods, the furfural method or the fermentation method.

Spoehr[56] and Rosa[49] used the fermentation method in determining pentosans. When Bartholomew and Robbins[1] tried to use this method in determining the pentosan content of normal and declined lemons, they found that in 17 to 44 hours their cultures became contaminated with bacteria and mycoderma. They overcame this difficulty by adding 8% of 95% alcohol to the plant extracts which were to be fermented. Even under such conditions a known quantity of xylose added to the plant extracts inoculated with Fleischmann's yeast could not always be recovered.

In view of the wide distribution of pentosans in the plant kingdom and of their physiological importance to the plant, information regarding the accuracy of the methods for pentosan determination is evidently needed.

The purpose of this work was primarily to determine the accuracy of the fermentation method as it is generally used, by determining the utilization of pentoses by different yeasts under varied environmental conditions.

## METHODS OF DETECTING AND DETERMINING PENTOSES

Pentosans may be detected in plant extracts by warming the extracts with concentrated hydrochloric acid (sp. gr. 1.2) and a little orcinol. They produce a green color that varies from greenish yellow to a clear green. By substituting phloroglucinol for orcinol in the above test, a red color is produced which changes to a brown precipitate. Both of these tests depend on the fact that pentose sugars yield furfural with hydrochloric acid, and the action of the furfural on the orcinol and phloroglucinol produces the characteristic color.

Pentosans are usually estimated by one of two methods: (1) distillation with hydrochloric or sulphuric acid; (2) fermentation by yeast. The first method is based upon the conversion of the pentose into furfural and the second is based upon the belief that pentose sugars are not fermentable by yeast.

**The Furfural Method.**—The official method[37] for estimating pentosans is to heat the sample with 12% HCl at such a rate that 30 c.c. pass over in ten minutes. Thirty cubic centimeters more of 12% HCl are then added and the process is repeated until 360 c.c. of the distillate have been collected. Pervier and Gortner[41] in their investigations found that in the official method for determining pentosans the reaction takes place in 18 to 20% HCl instead of 12%, and that hydrochloric acid of this concentration has considerable destructive effects on furfural. They concluded that this is perhaps responsible for the low yields of furfural that are obtained when the official method is used. They found that a slow current of steam passed through the distilling mixture carried the furfural off as rapidly as it was formed. The destruction of furfural by long contact with strong acid was thereby avoided.

Numerous methods have been suggested for the determination of the furfural. Jolles's[25] method of titration with sodium bisulphite has been recommended. Titration with phenyl-hydrazine and with Fehling's solution have been tried but all of these methods, according to Spoehr[56], are open to serious error and are not reliable in a quantitative sense. Pervier and Gortner[41] tried several new methods for the determination of furfural in dilute aqueous solutions. They made unsuccessful trials with iodine in alkaline solutions and with acid permanganate. They report that with potassium bromate in acidified furfural solutions containing potassium bromide they were successful, and that 95 to 99% of the estimated furfural was obtained.

It should be mentioned that pentoses are by no means the only substances that yield furfural on treatment with mineral acids. Hexoses, cane sugar, and other substances found in plants also form furfural under these conditions. Pervier and Gortner[41] believe, however, that furfural obtained from such sources as glucose is not sufficiently great to interfere seriously with the method.

According to Spoehr[56] the fermentation method is the only reliable one for estimating pentosans. This method is based upon a separation of the pentose sugars from other substances by fermentation with yeast. Spoehr fermented away the hexoses by using a good culture of baker's yeast. He incubated his cultures for thirty to forty hours at 35°C. Rosa[49] used Fleischmann's yeast cake and incubated his cultures over night at 35 to 40° Centigrade.

## OCCURRENCE OF PENTOSES AND PENTOSANS

The pentoses are sugars containing five carbon atoms and having the general formula $C_5H_{10}O_5$. In plants they occur chiefly as condensation products formed with the elimination of water. These products are termed pentosans and are widely distributed in nature. On hydrolysis they yield pentoses. Various gums found in plants consist largely of pentosans and on treatment with acid the pectins also yield pentose groups. Cherry gum yields almost entirely arabinose, and gum tragacanth yields a mixture of complex acids and various sugars such as arabinose, galactose and xylose. (Onslow[38])

The pentoses were first found in the animal kingdom by Salkowski[50] in the urine of a person addicted to the morphine habit. Hammarsten[21] found them among the cleavage products of a nucleo-protein obtained from the pancreas. Blumenthal[4] observed pentoses to be a constituent of nucleo-proteins of various organs, such as thymus, thyroid, brain, spleen and liver. Pentoses have been found as the carbohydrate group in guanylic acid, inosinic acid, and the plant nucleic acid of yeast and wheat. The pentose in inosinic acid is, according to Neuberg and Brahn[35], l-xylose, but Levene and Jacobs[28] have shown that the pentose is not l-xylose, but that it is d-ribose. Yeast nucleic acid, according to Levene[27], also contains d-ribose as the pentose.

## UTILIZATION OF PENTOSES

In regard to the value of pentoses as a source of carbon, the researches of Salkowski[51], Cremer[9], Neuberg and Wohlgemuth[36] upon rabbits and hens have shown that these animals can use pentoses. The pentoses seem to be absorbed by human beings and in part used, but some of them pass into the urine even when small quantities are taken (Salkowski). Frankland and MacGregor[16] found that a solution of arabinose, inoculated with *B. aethaceticus* forms alcohol, acetic acid, formic acid, succinic acid, carbon dioxide, and water. Salkowski[52] has described the effect of a fermenting mass of meat on arabinose and xylose. From arabinose arose ethyl alcohol, volatile acids, particularly acetic, and a non-volatile acid, succinic acid. From xylose the same products are produced. Bendix[3] reported that arabinose, xylose, and rhamnose with a bacterial culture from pressed yeast or from feces produced alcohol, volatile fatty acids, and lactic acid. Behrens[2] has shown that *Botrytis vulgaris* and *Penicillium sp.* can use both arabinose and xylose as a source of carbon. Went[59] obtained good results growing *Monila sitophila* on xylose. Peterson, Fred and Schmidt[44] were able to ferment pentoses by the use of the molds *Aspergillus sp.* and *Penicillium*

*glaucum*. They were also able to ferment pentoses by the use of *B. granulobacter pectinovorum*[45]. Kruger[26] found that *Gloeosporium sp.*, parasitic on the apple, can use arabinose. Hawkins[23] observed that *Glomerella cingulata*, parasitic on the apple, was able to utilize pentose. He concluded from his work that the pentoses, especially xylose, were a slightly better source of carbon for this fungus than glucose, and that pentoses were readily used by the fungi.

Czapek[12] gives the following values for *Aspergillus niger* in weight of yield:

| | mg. | | mg. |
|---|---|---|---|
| d-fructose | 523.7 | d-glucose | 477.1 |
| l-xylose | 512.7 | l-arabinose | 350.0 |
| d-galactose | 489.3 | d-mannose | 286.8 |

Spoehr[56] mentions that many bacteria and molds are capable of utilizing pentoses as the only source of carbon. Cross and Tollens[11] were able to produce lactic acid from pentoses by inoculating with an organism from sour milk.

The authors of many organic and plant chemistries state that pentoses are not capable of being fermented by yeast. Haas and Hill[19] in discussing the general properties of pentoses say that they are not fermented* by yeasts. Plimmer[46] states that pentoses are not fermented by yeasts. Spoehr considers that the yeasts "are quite incapable of utilizing any of the pentose sugars."

Cross and Tollens[11] conclude from the results of experiments of von Schriebler, Lippman, Stone and Tollens, Schone and Tollens, Fischer and Thierfelder, and Cross, Bevan and Smith[10] that the pentoses are not capable of alcoholic fermentation except under unusual conditions. They are, however, of the opinion that the pentoses can be used by the yeasts as material for building new cells. They think that assimilation of pentoses by yeasts takes place when fermentation occurs in the absence of sufficient nutrient material for the yeasts. They found that the disappearance of pentoses during fermentation of pentoses and hexoses takes place when there is a relative starvation of the yeasts, but not when the fermentation is vigorous and rapid. Cross and Tollens[11] observed that when they used an artificial nutrient solution the amount of pentose was reduced during fermentation. When washed yeast was

*The terms *fermentation, assimilation,* and *utilization* are used in this paper to indicate the following:

*Fermentation*—chemical changes in the compound fermented, brought about by microorganisms with the formation of compounds intermediate to $CO_2$ and $H_2O$ such as ethyl alcohol, lactic acid, or acetic acid, usually accompanied by a release of energy which is used by the microorganism.

*Assimilation*—the use of a compound for the construction of body parts.

*Utilization*—the use of a compound in any way whether by assimilation or by fermentation, or in some other way not fully understood.

used they considered that the pentose was used by the yeast to build new cells. They concluded that in nutrient solutions poor in organic matter, the pentose decreased in the presence of yeast without the formation of alcohol. Schone and Tollens[53] found that pentose disappeared from cultures fermented with lager beer yeast. They concluded, however, that because foreign organisms were present they might have been responsible for the disappearance of the pentose. They found that when a solution containing arabinose was inoculated with a pure culture of yeast and the culture grown under favorable vegetative conditions, the sugar did not disappear in 10 days. Cross, Bevan and Smith[10] observed that the pentoses in contact with yeast disappeared without being fermented; a decrease of 95% was noticed when starved yeast was used. They concluded that under certain conditions pentoses can be used as food by the yeasts and that this assimilation is attended by a change in constitution of the pentose.

Pentoses were found by Bokorny[6] to serve as a source of carbon for yeasts, but not to be fermented by them. Englis and Hale[14] found that when *S. cerevisiae* was placed in a solution containing pentose the reduction method gave only a fraction of the quantity of the apparent pentose found by the Krober-Tollens method. Englis and Hale did not consider this to indicate that the yeast had used the pentose. Conrad[8] reported that when plant extracts containing pentoses were inoculated with suspensions of Fleischmann's yeast and incubated at 30°, the pentoses as well as the hexoses were destroyed. The small amount of material remaining after such a fermentation was much less than that corresponding to the original pentoses as determined by the phloroglucinol method. Guillermond[18] mentions that *Saccharomyces thermentitonium* ferments the pentoses (arabinose and xylose) and that according to Lindner the same seems to be true for *Saccharomyces octosporus*. Davis and Sawyer[13] concluded from a larger number of experiments that baker's yeast does not ferment nor assimilate the pentoses but they report that the maltase free yeasts *S. Marxianus*, *S. exiguus* and *S. anomalus* gradually but slowly destroy these sugars.

Bartholomew and Robbins[1] working on normal and declined lemons found that when a known amount of xylose was added to the solutions fermented by yeast it could not always be recovered. When 0.025 g. of xylose was added .0148 g. was recovered after fermentation with washed Fleischmann's yeast for 20 hours at 30°. When 0.025 g. of xylose was added to a solution none of it was recovered after fermentation with yeast for 5 days, under sterile conditions.

Pellet[40] observed that appreciable amounts of xylose or arabinose were destroyed in a mixture containing 200 g. of molasses (i. e. 90-100 g. of sucrose and 1-2 g. of pentose in a liter of water) when 5-10 g. of yeast

were added and incubated at 28-32° for 48-60 hours provided 5 grams of yeast were added daily to such a mixture.

At the end of 24 hours_____arabinose unchanged.
At the end of 48 hours_____48% arabinose destroyed.

At the end of 24 hours with 50 grams of yeast all the arabinose was destroyed. Arabinose was more rapidly fermented in the presence of molasses. It disappeared at the end of 6 hours. Arabinose and xylose were found by Pellet to be completely destroyed in solutions containing as other constituents only the nutrients necessary for the life of yeasts.

From the above review of literature it is evident that while the utilization of pentoses by bacteria and fungi is well established considerable differences of opinion exists concerning the relation of the yeast to these sugars. The various views may be briefly summarized as follows:

1. Yeasts can not utilize pentoses. (Spoehr)
2. Pentoses are good nutrients for yeasts. (Bokorny)
3. Pentoses are utilized by yeasts under certain conditions. (Cross and Tollens, Conrad, Pellet, Cross, Bevan and Tollens)
4. Only some yeasts can utilize pentoses. (Davis and Sawyer and Lindner).
5. Yeasts can not ferment pentoses. (Haas and Hill, Plimmer)
6. Yeasts ferment pentoses under certain conditions. (Cross and Tollens).

## MATERIALS

The orange gum used in this investigation was collected by Bartholomew near Riverside, California. The gum was dried and ground in a mortar. Chemically pure sugars prepared by the Digestive Ferments Co. were used in all determinations.

The strains and varieties of yeasts and related organisms used were as follows: No. 1, 2 and 4, strains isolated from Fleischmann's yeast cake; No. 5, Mycoderma, 6, pink yeast, 7, distiller's yeast, 8, Burgundy yeast, No. 11, *Sach. Porupe*, No. 12, *Sach. ellipsoidae*, No. 16 bottom yeast, No. 17 *Sach. cerevisiae*, No. 19 *Sach. apiculatus*, No. 20 top yeast.*

The nutrient solutions used for the study of the utilization of the pentose sugars were:

**No. 1**

$NH_4NO_3$ 1g.; $KH_2PO_4$ 1g.
$MgSO_4$ 0.5g.; $CaCl_2$ 0.5g.
Distilled water 1000 c.c.; 2 c.c. of 1% autolyzed yeast
Reaction adjusted by addition of $\frac{N}{5}$ NaOH

*Organisms No. 5 to 8 were furnished by Dr. E. B. Fred, University of Wisconsin and No. 11, 12, 16, 17, 19 and 20 by Dr. Edwin LeFevre, Micro-biological Laboratory, Bureau of Chemistry, United States Department of Agriculture. The writer's thanks are due them for furnishing these organisms and also to Dr. E. T. Bartholomew for supplying the orange gum.

**No. 2**

(a) $NH_4NO_3$ 1g.; $MgSO_4$ 0.5g.
$CaCl_2$ 0.5g.; 2 c.c. 1% autolyzed yeast
Distilled water 500 c.c.

(b) $KH_2PO_4$ 0.5g.; Distilled water 500 c.c.
Potassium acid phthalate $\frac{M}{10}$
$\frac{N}{5}$ NaOH to adjust to required reaction.

Solution No. 2 was prepared by sterilizing 50 c.c. of (a)+ the sugar and 50 c.c. of (b) in separate flasks. These two solutions were then mixed under aseptic conditions after sterilization. This procedure was followed to prevent precipitation of salts in solutions alkaline to $p_H$ 5.0.

The pure cultures of yeasts were maintained on a water extract of dried peaches plus 2% of agar.

## METHODS

The normality of the copper sulphate solution used for making Fehling's solution was found by the electrolytic method. The reducing value of the sugar solutions was determined by heating with Fehling's solution, centrifuging, and determining the copper sulphate left in an aliquot of the supernatant liquid by the use of potassium iodide and $\frac{N}{20}$ sodium thiosulphate. This method is described by Peters.

As shown by the very careful work of Peters[42], the factor for transforming the mgms. of copper reduced to mgms. of dextrose is 0.522 if 25 mgms. or more of dextrose are added to the Fehling's solution. When the amount of dextrose is less than 25 mgms. the dextrose-copper factor is larger. Thus for 10 mgms. of dextrose it is 0.575 and for 1 mgm. it is 0.833. The possibility of introducing a considerable error by failing to consider the variation in the dextrose-copper factor is evident. It is a possibility which has been frequently neglected by investigators. To facilitate securing the proper dextrose-copper factor, the writer has constructed a curve from the data of Peters by plotting the mgms. of copper against the dextrose-copper factor (Fig. 1). By referring to this curve the proper factor for transforming the copper reduced to dextrose can easily be secured. In the present investigation such procedure has been followed in every case. According to Spoehr[56] if the reducing value of

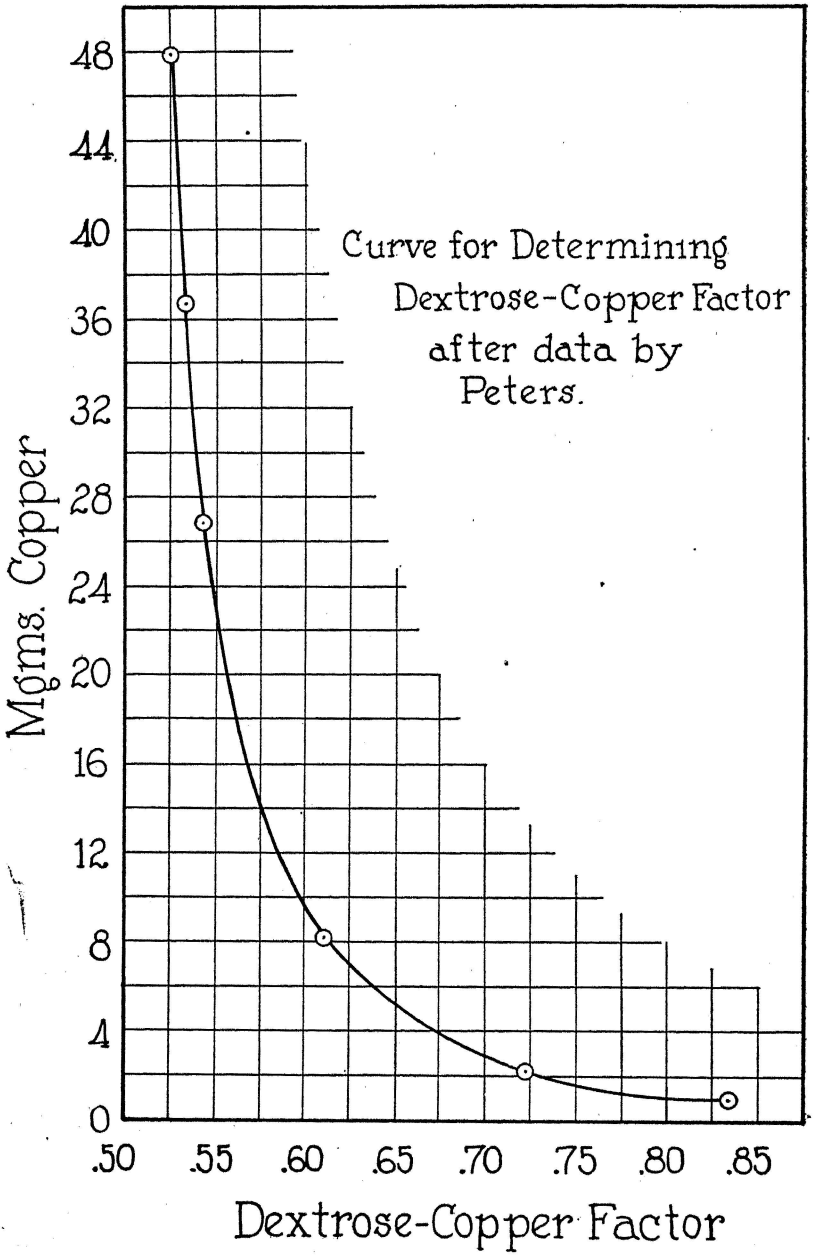


Fig. 1.—Curve for determining the dextrose-copper factor.

dextrose is 1, that of the pentoses (xylose) is 0.9. Therefore, mgms. of dextrose = mgms. of copper reduced x suitable factor; and

mgms. of pentose = mgms. of copper reduced x suitable factor x 0.9.

The methods used in determining the pentoses of orange gum were essentially those used by Spoehr[56] in his study of the carbohydrate economy of the cacti. The powdered gum was heated for three hours under a reflux condenser with 1% HCl. Forty cubic centimeters of the 1% HCl were used for each gram of gum. The hydrolyzed extract was divided into aliquots and adjusted to the desired hydrogen-ion concentration with NaOH. All experiments were carried out in triplicate or in fives.

In the experiments with pure cultures of yeast the solutions were sterilized in an Arnold steam sterilizer for twenty minutes on three consecutive days.

Gillespie's[17] colorimetric method was used in determining the hydrogen-ion concentrations. The hydrogen-ion concentration was determined before sterilization, after sterilization and after fermentation.

In inoculating with pure cultures 2 loopfuls of a pure culture of yeast were added to each 100 c.c. of solution. The cultures were incubated with the controls at 35°C. When Fleischmann's yeast cake was used, the solutions were not sterilized, but 8% of 95% alcohol was added before fermentation. The alcohol prevented the growth of surface films unless fermentation continued longer than 5 days.

All inoculations were made under sterile conditions in a special inoculating room. This room was usually filled with steam for 30 minutes and the vapor was allowed to settle before the room was used.

## RELATION OF DIFFERENT SPECIES OR RACES OF YEASTS TO PENTOSES

It is well known that the ability to assimilate or to ferment a given sugar varies with the species or race of yeast. The investigations of Lindner[29] indicated that maltose is best adapted to yeast metabolism. This sugar is assimilated by practically all yeasts. However, there are certain yeasts, as *Saccharomyces Marxianus*, *S. exiguus*, *S. Jorgensenii*, and *S. guttulatus*, that are unable to ferment maltose. Guillermond[18] states that sucrose, which is so easily fermented by most yeasts, does not play any role in assimilation. *Saccharomyces Ludwigii*, *S. exiguus*, *S. cartilaginosus*, and *Schizosaccharomyces Pombe*, and *Sch. mellacei* can

produce an active fermentation of glucose, levulose, and sucrose, but are unable to assimilate any of them. *Schizosaccharomyces octosporus*, *Saccharomyces apiculatus*, and *Pichia membranoefaciens* do not possess sucrase and can not ferment cane sugar.

Galactose is not readily fermented by most yeasts. Sohngen and Coolhaas[54] found however, that if *Saccharomyces cerevisiae* were placed in a liquid medium containing galactose the newly formed yeast cells had the property of fermenting this sugar.

Lactose is neither assimilated nor fermented except by a few species of yeast. Yeasts possessing the enzyme lactase are not common. Only a small number are known. Lactase has been found in *Saccharomyces Kepir.*, *Saccharomyces fragilis* and various species of Torula and mycodermae. (Guillermond[18].)

That there is a difference in the ability of various yeasts to act on pentoses is also indicated by the literature summarized above.

In view, therefore, of the differences in the relation of various yeasts to other sugars and the reported differences in the relation of various yeasts to pentoses, it was thought advisable to determine the effect of different yeasts in pure culture on the pentoses. It was found that considerable variation in the relation of various yeasts to the use of the pentoses exists.

One hundred cubic centimeters of nutrient solution No. 1 containing 0.1% arabinose were placed in 150-c.c. Erlenmeyer flasks of Pyrex and sterilized as previously described. They were inoculated with two loopfuls of a pure culture of yeast and incubated 20 days at 35°C. Twelve different yeasts (Table 1) were used.

TABLE 1.—THE EFFECT OF PURE CULTURES OF VARIOUS YEASTS AND RELATED ORGANISMS ON ARABINOSE UNDER STERILE CONDITIONS.

(100 c.c. quantities of nutrient solution No. 1 adjusted to $p_H$ 6.0 and containing 100 mgms. of arabinose, incubated 20 days at 35°C.)

| Yeasts | Cu. reduced | Sugar Recovered | Final $p_H$ |
|---|---|---|---|
| | mgms. | mgms. | |
| No. 1 | 7.8 | 4.36 | 5.9 |
| 4 | 7.9 | 4.41 | 5.8 |
| 5 | 8.0 | 4.47 | 5.9 |
| 6 | 7.8 | 4.39 | 5.9 |
| 7 | 7.9 | 4.41 | 5.8 |
| 8 | 8.0 | 4.47 | 5.9 |
| 11 | none | none | 5.7 |
| 12 | none | none | 5.6 |
| 16 | none | none | 5.7 |
| 17 | none | none | 5.5 |
| 19 | none | none | 5.5 |
| 20 | none | none | 5.6 |
| arabinose without yeast | 8.14 | 4.46 | 6.0 |

Table 1 shows that yeasts Nos. 1, 4, 5, 6, 7, and 8 were not able to utilize arabinose at $p_H$ 6.0 in 20 days. This reaction had previously been found favorable for pentose utilization. Yeasts Nos. 11, 12, 16, 17, 19, and 20 completely destroyed 100 mgms. of arabinose in 100 c.c. of nutrient solution at $p_H$ 6.0 within 20 days at 35°C. The sugar recovered from the uninoculated flasks shows that sterilization and incubation had not affected the sugar.

Solutions similar to the ones used with pure cultures were prepared. The reaction was adjusted to $p_H$ 5.2 and 6.6. The development of foreign organisms was largely prevented by adding 8% of 95% alcohol. To each 100 c.c. of solution 1/9 of a cake of Fleischmann's yeast was added and the mixture incubated at 35°C. for 4 days. All the arabinose was utilized in four days.

The effect of time on the utilization of glucose, galactose and xylose at $p_H$ 6.7 when inoculated with 1/6 cake of Fleischmann's yeast is shown in Table 2. In this case 100 c.c. quantities of nutrient solution No. 1 containing 200 mgms. of sugar were used. The glucose completely disappeared in 9 hours, while xylose and galactose had apparently not been used at all. At the end of 12 hours about 12% of the xylose and galactose had been used; at the end of 36 hours about 1/3 of the sugar remained, and at the end of 48 hours both the galactose and xylose had entirely disappeared.

TABLE 2.—THE EFFECT OF 1/6 OF A FLEISCHMANN'S YEAST CAKE ADDED TO 100 C.C. OF NUTRIENT SOLUTION NO. 1 ADJUSTED TO $p_H$ 6.7 AND CONTAINING 200 MGMS. OF SUGAR AND 8% ALCOHOL AND INCUBATED AT 35°C.

| Sugar | Cu. reduced 9 hrs. | Sugar recovered 9 hrs. | Cu. reduced 12 hrs. | Sugar recovered 12 hrs. | Cu. reduced 36 hrs. | Sugar recovered 36 hrs. | Cu. reduced 48 hrs. | Sugar recovered |
|---|---|---|---|---|---|---|---|---|
| | *mgms.* | *mgms.* | *mgms.* | *mgms.* | *mgms.* | *mgms.* | *mgms.* | *mgms.* |
| Glucose inoculated | --- | --- | --- | --- | --- | --- | --- | --- |
| Uninoculated | 13.6 | 7.89 | --- | --- | --- | --- | --- | --- |
| Galactose inoculated | 13.7 | 7.89 | 12 | 6.96 | 4.26 | 2.46 | --- | --- |
| Uninoculated | 13.7 | 7.94 | --- | --- | --- | --- | 13.6 | 7.93 |
| Xylose inoculated | 15.31 | 7.984 | 12.6 | 6.97 | 5.21 | 2.70 | --- | --- |
| Uninoculated | 15.3 | 7.983 | --- | --- | --- | --- | 15.2 | 7.93 |

The fact that some strains of yeast are able to utilize pentoses and others are not, offers one explanation for the conflicting statements on the relation of yeasts to the pentoses. It is evident that one of the necessary qualifications in asserting the ability or inability of yeast to utilize the pentoses is to indicate the variety or race which is investigated. The fact

that half of the yeasts used in this investigation were found capable of using arabinose also indicates that the property is probably not limited to a few yeasts but under suitable conditions is common to a comparatively large number.

## THE EFFECT OF ENVIRONMENTAL CONDITIONS ON THE UTILIZATION OF PENTOSES BY YEASTS

That the ability of a given yeast to utilize pentose is influenced by the conditions under which the pentose is presented is indicated in the literature cited earlier. Starvation was the condition suggested as favorably affecting the destruction of pentose. Other factors such as oxygen supply, temperature, reaction, presence of other carbohydrates, quantity of inoculum and mineral composition of the nutrient medium might be suggested as possibly concerned.

**Effects of Reaction.**—Preliminary experiments indicated that reaction is a factor of considerable importance in determining the utilization of pentoses by yeast. An experiment may be cited to show the effect of this factor.

TABLE 3.—THE EFFECT OF REACTION ON THE UTILIZATION OF ARABINOSE AND XYLOSE BY *Saccharomyces cerevisiae* IN PURE CULTURE AND UNDER STERILE CONDITIONS.

(In 100 c.c. quantities of nutrient solution No. 1 containing 100 mgms. of sugar and incubated 15 days at 35°C.)

| Initial $p_H$ | Final $p_H$ | Sugar | Cu. reduced | Sugar rec. |
|---|---|---|---|---|
| | | | mgms. | mgms. |
| 4.5 | 4.6 | arabinose with yeast | 8.02 | 4.47 |
| 4.5 | 4.6 | arabinose without yeast | 7.8 | 4.36 |
| 5.5 | 5.3 | arabinose with yeast | none | none |
| 5.5 | 5.5 | arabinose without yeast | 8.01 | 4.47 |
| 6.6 | 5.6 | arabinose with yeast | none | none |
| 6.6 | 5.6 | arabinose without yeast | 8.0 | 4.47 |
| 7.0 | 5.5 | arabinose with yeast | none | none |
| 7.0 | 7.0 | arabinose without yeast | 8.0 | 4.47 |
| 4.5 | 4.5 | xylose with yeast | 7.9 | 4.41 |
| 4.5 | 4.5 | xylose without yeast | 7.9 | 4.41 |
| 5.5 | 5.3 | xylose with yeast | none | none |
| 5.5 | 5.5 | xylose without yeast | 7.8 | 4.36 |
| 6.6 | 5.6 | xylose with yeast | none | none |
| 6.6 | 6.6 | xylose without yeast | 8.0 | 4.47 |
| 7.0 | 6.5 | xylose with yeast | none | none |
| 7.0 | 7.0 | xylose without yeast | 7.8 | 4.36 |

*Saccharomyces cerevisiae* was selected as a typical yeast to use in the determination of the effect of reaction on pentose utilization. The experiments were carried out in nutrient solution No. 1 adjusted to reactions of $p_H$ 4.5, 5.5, 6.6 and 7.0 with NaOH and containing 1 gram of arabinose or 1 gram of xylose per liter. One hundred cubic centimeters

of the solution adjusted to the reactions given above were placed in 150-c.c. Erlenmeyer flasks and sterilized intermittently in an Arnold sterilizer. The experiment was performed in triplicate. The inoculated solutions were incubated 15 days at 35°C. The results (Table 3) show that arabinose and xylose were completely destroyed by *Saccharomyces cerevisiae* in solutions of initial reactions of $p_H$ 5.5, 6.6, and 7.0, but remained unaffected at an initial $p_H$ of 4.5. The reactions of those solutions at which destruction of the pentoses occurred became more acid. This change in reaction was apparently not due to the production of carbon dioxide but to a non-volatile acid or to the absorption of cations by the yeast cells, as no change in reaction was observed after boiling. This experiment was repeated 5 times with identical results.

Experiments were also performed on the effect of reaction on the utilization of pentoses by Fleischmann's yeast. When 1/9 of a cake of Fleischmann's yeast was added to 100 c.c. of solution No. 1 at $p_H$ 4.5 it was found that the reaction was changed to about p 5.2. At this reaction the pentoses were utilized by the Fleischmann's yeast. This change in reaction when Fleischmann's yeast was used was due to the fact that in solution No. 1 the buffer action is produced by the potassium or sodium phosphates and at $p_H$ 4.5 the buffer action of potassium or sodium phosphate is slight. To increase the buffer action a nutrient solution was prepared containing $\frac{M}{20}$ potassium acid phthalate (solution No. 2). Comparatively little change in the reaction was produced when Fleischmann's yeast was added to this solution in the proportions given above. A solution of initial $p_H$ 4.0 was changed to $p_H$ 4.3 and one of $p_H$ 6.5 to $p_H$ 6.6. In 72 hours at 35°C. Fleischmann's yeast produced no effect on arabinose at an initial reaction of $p_H$ 4.3 but at $p_H$ 6.6 it completely destroyed it. Attempts to use solution No. 2 with pure cultures of yeast were unsuccessful because the small quantity of yeast added in such cases failed to grow at any reactions probably because of the toxicity of the phthalate radical. A more dilute solution of the phthalate than that used might permit the growth of yeasts when a small amount of inoculum is used.

From the data given it is evident that the utilization of pentose sugars by certain yeasts depends on the reaction of the medium. At a reaction of about $p_H$ 4.5 neither *Saccharomyces cerevisiae* nor the yeasts of Fleischmann's yeast cake were able to utilize pentoses but at $p_H$ 5.5 to 7.0 they were able to do so. The critical point appears to be near $p_H$ 5.0. In more acid solutions *Saccharomyces cerevisiae* and the yeasts of Fleischmann's yeast cake do not appear capable of utilizing pentoses though

they can ferment glucose. At more alkaline reactions they are able to utilize both pentoses and glucose.

To what is the effect of the reaction of the medium on the utilization of pentoses by yeast due? Is it an effect on the sugar, changing it from a more to a less fermentable form, or is it an effect on the metabolism of the yeast?

At a reaction of $p_H$ 4.5 the yeast plant is not killed nor injured, as Hagglund and Augustson[20] report that the optimum reaction for the fermentation of hexoses is $p_H$ 4.5.

A possible explanation is that the reaction changes the pentose molecule from a more easily utilizable form existing at $p_H$ 5.5 to 7.0 to a less easily utilizable form at $p_H$ 4.5. There is considerable evidence to indicate that alkalinity favors the utilization of glucose by living organisms because of a change in the glucose molecule under alkaline conditions.

Rona and Wilkenko[48] found that an increase in the hydrogen-ion concentration of a perfusion fluid greatly reduced the utilization of glucose by an excised beating heart.

Murlin and Kramer[34] obtained results which indicated that if depancreatized dogs be given alkali they are able to utilize glucose.

Shaffer[55] has advanced the theory that before glucose can be oxidized the molecule must first undergo progressive conversions into other more reactive substances and it is these products resulting from molecular rearrangement which are finally oxidized with the liberation of energy.

Glucose behaves like a weak acid, and in the presence of alkali mutarotation with the production of isomeric forms occurs. The presence of traces of acids prevents these transformations as well as any oxidative changes in glucose. To the invulnerability of the glucose molecule under these circumstances has been ascribed its non-destructibility in diabetes.

It is doubtful whether such an explanation is permissible in this case. The reactions at which the pentose becomes available are still slightly acid, $p_H$ 5.5 to 7.0 and it is only under alkaline conditions that the phenomena of mutarotation referred to above occur. If the pentose molecule is changed to a less reactive form at $p_H$ 4.5 the change is not permanent. The writer adjusted the reaction of a solution of $p_H$ 4.5 in which yeast had failed to utilize the pentose to a reaction of $p_H$ 6.7 by the addition of sterile NaOH and found that the yeast would then utilize the pentose.

Another explanation which might be suggested for the results is that the reaction affects the rate of oxidation of the sugar. This is suggested by the literature.

McGuigan[32] found that when progressive amounts of acetic acid were added to cupric acetate, the oxidizing power was gradually lost.

Levulose, galactose, glucose, maltose, and lactose were easily oxidized in neutral solutions, but very small amounts of acid prevented the oxidation of lactose, while levulose required much more acid to prevent its oxidation.

When equal quantities of various body tissues dried and powdered were used, he found[31] that they required different quantities of acid to slow the catalysis of hydrogen peroxide. The kidney required more acid than the liver, spleen, pancreas, and muscle.

According to McGuigan[31] an organism may not have sufficient energy to bring about the oxidation of the sugar after dissociation of the latter. In other words the oxidation potential of the organisms is not high enough to affect one sugar while it may be high enough for some other. In case the potential is high enough the catalytic agent for oxidation or dissociation may be lacking. In this case the organism is able to decompose the sugar as far as energy is concerned, but lacks agents necessary to cause the process to take place with sufficient velocity to make the sugar available.

Bunzel[7] has suggested that hydrogen-ions retard the oxidation of sugar because they decrease the total number of free oxygen-ions in solution and diminish the dissociation of the sugar molecule.

Any explanation involving the effect of reaction on the oxidation of the sugars must also include the assumption that the oxidation of a pentose is more easily affected by acids than glucose, since the latter is fermented at a reaction at which the pentose remains unchanged. Particularly interesting in this regard are the results of McGuigan, quoted above, which show a difference between sugars in the amount of acetic acid required to inhibit their oxidation by cupric acetate.

It may also be suggested that acidity may decrease the permeability of yeast cells to the pentoses more readily than it does that for glucose.

Boaz and Leberle[5] have suggested that different sugars have different effects on the plasma membrane in such a way that the utilization of sugar by yeast stands in the following series: maltose > dextrose > levulose. The different sugars according to Hammarsten[22] are absorbed by the intestines with varying degrees of rapidity. It is generally admitted that the simpler sugars are more quickly absorbed than the disaccharides, and of the disaccharides, lactose is more slowly absorbed than the others. Pentoses are said to be[47] more slowly absorbed by the intestines than the hexoses. It is conceivable that in the less acid solutions both the pentose and glucose penetrate the plasma membrane of the yeast cell but that in slightly more acid solution the absorption of the pentose is more seriously interfered with than that of the glucose.

It may also be suggested that the enzymes concerned in the utilization of the pentoses may be more sensitive to the effects of acidity than those which are concerned in the utilization of dextrose.

Another explanation which might be suggested could be based on the laws of mass action. It is well known that glucose is fermented to alcohol, acetic acid, glycerol, and $CO_2$ in alkaline media. In acid media neither acetic acid nor glycerol are formed —only alcohol and $CO_2$. If pentoses always give rise to acids on fermentation by yeasts as is suggested by the increase in acidity observed below when pentoses are utilized then fermentation would be decreased in acid media since hydrogen-ions are one of the end products of the reaction.

What the specific effects of the reaction of the medium on pentose utilization by yeasts are cannot be fully explained at this time. The observation, however, is extremely interesting. The fact that a given strain of yeast can utilize pentoses at one reaction and cannot affect them at another may account for some of the differences in opinion regarding the relation of yeasts to the pentoses which were summarized in the literature reviewed earlier. It may also be a factor in the accumulation of pentoses in plant tissues. Pentoses appear to be less readily utilized at slightly acid reactions. There may be a correlation between the accumulation of pentosans in plant tissue and the acidity of the cell sap. Acidity may favor the accumulation of pentosans and slightly acid, neutral, or alkaline reactions may favor their utilization.

**Effect of Quantity of Inoculum.**—Two very marked effects were noticed when 1/9 to 1/6 cake of Fleischmann's yeast was used instead of 2 loopfuls of pure culture. When the larger amount of yeast was added to nutrient solution No. 1 containing pentoses whose initial reaction was $p_H$ 4.5, the reaction was changed to near $p_H$ 5.0.

The time factor was also affected by the quantity of inoculation. When 2 loopfuls of a pure culture were used, 15 to 20 days were required for the complete disappearance of the pentoses, but with 1/6 to 1/9 of a cake of Fleischmann's yeast the pentoses disappeared in 48 to 72 hours. That the sugar was removed from the solution by being absorbed by the yeast without utilization was considered as a possible explanation for the quick disappearance of the sugar when Fleischmann's yeast cake was used. A freshly inoculated solution was placed with an uninoculated one in a shaking machine and shaken for one hour to hasten the absorption. At the end of the hour the solutions were analyzed. No sugar had been absorbed; the reducing values of the two solutions were identical. It was concluded therefore that the pentose is utilized by the yeast and not merely removed from solution.

**Effects of Variations in Oxygen Supply.**—It was suggested earlier in this paper that the specific effects of the reaction of the medium might concern oxidation of the pentose. To determine the effect of increased and decreased oxygen supply on pentose utilization 50 c.c. of nutrient solution No. 1 containing 100 mgms. of pentose per liter were placed in 750 c.c. flasks and a similar amount was placed in a 75 c.c. test tube. Both cultures were inoculated with Fleischmann's yeast and incubated 4 days. The sugar disappeared much sooner in the flask where the oxygen supply was better because of the thinner layer of liquid. It was thought, however, that because the yeast formed a layer in the bottom of the vessel slowness of diffusion in the test tube might account for the results.

## THE PRODUCTS OF PENTOSE FERMENTATION

How does the yeast cell utilize pentoses? Is their utilization accompanied by an alcoholic fermentation, a lactic acid fermentation, or is the pentose used in metabolism without the production of decomposition products?

The utilization of pentoses by organisms other than yeast is often accompanied by the production of alcohol, acetic acid, formic acid, succinic acid, carbon dioxide, and water. Bendix[3] obtained alcohol, lactic acid, and volatile fatty acids from arabinose and xylose inoculated with a bacterial culture from pressed yeast. Peterson and Fred[43] found that the fermentation of arabinose and xylose by certain lactic acid bacteria resulted in the production of acetic and lactic acids, with 90% of the sugar destroyed. The relation of the acetic acid to the lactic acid was one molecule to one molecule.

Nutrient solution No. 1 adjusted to $p_H$ 6.7 and containing 2% xylose was placed in fermentation tubes and inoculated with pure cultures of yeast, or with Fleischmann's yeast cake. Only a trace of $CO_2$ was produced. The iodoform test on sterile solutions inoculated with pure cultures of yeasts that utilize pentoses was always positive, though there was never more than a trace. From the fact that only very small amounts of $CO_2$ and alcohol were produced, pentose utilization by these yeasts was considered not to be of the alcoholic type.

Pentose utilization was always accompanied by a slight increase in acidity (Table 1). Boiling did not change the hydrogen-ion concentration This indicated that the acidity was due to non-volatile acids and not to carbon dioxide nor volatile acids. As lactic acid is a non-volatile acid, the Hopkins[33] test for lactic acid was made. This gave positive results but as lactic acid is not the only compound that produces a red color with thiophene it was not conclusive. The amount of acid produced from the quantity of sugar present was so small that it could not be isolated.

While the examination made for alcohol, lactic acid and carbon-dioxide showed little production of these substances or gave questionable results, some observations made in the course of the investigation indicated the formation of material of very high reducing power in the utilization of pentose by yeast.

In the fermentation of pentoses alone, in the fermentation of pentoses in the presence of glucose, and in the fermentation of orange gum hydrolyzed with hydrochloric acid, a substance with very strong reducing power sometimes appeared after 25 to 30 days fermentation. It was produced in cultures inoculated with yeasts No. 1 or No. 17. The appearance of this substance was variable. Sometimes it appeared and again under apparently the same conditions it did not.

This substance gave the following reactions:

1. It reduced Fehling's solution in the cold. Reduction began immediately.
2. The solution in which this substance appeared had a maximum of about 70 times the reducing value of the solution of pentose from which it was formed.
3. It was not removed nor destroyed by boiling.
4. It was not precipitated by basic lead acetate nor by neutral lead acetate.
5. With $AgNO_3$ it produced the mirror characteristic of aldehydes.
6. It was not removed by shaking the solution with ether.

This material was developed after the disappearance of the pentose and its formation was associated with a decrease in the acidity of the solution. This is illustrated in Table 4 where 100 c.c. quantities of nutrient solution No. 1 containing 100 mgms. of rhamnose were inoculated with pure cultures of *Saccharomyces cerevisiae* and incubated at 35° C. At the end of 15 days the solutions showed a $p_H$ of 6.0 and no reducing power but after 25 days the $p_H$ had risen to 6.4 and the reducing power was almost 6 times that of the original solution.

TABLE 4.—THE REDUCING VALUES OF ALIQUOTS OF 100 C.C. QUANTITIES OF NUTRIENT SOLUTION NO. 1 CONTAINING 100 MGMS. OF RHAMNOSE, INOCULATED WITH PURE CULTURES OF *Saccharomyces cerevisiae* AND INCUBATED AT 35°C.

| Time | Initial $p_H$ | Cu. reduced | Sugar recovered |
|---|---|---|---|
| | $p_H$ | *mgms.* | *mgms.* |
| Yeast 15 days | 6.0 | none | none |
| no yeast 15 days | 6.6 | 7.8 | 4.40 |
| Yeast 25 days | 6.4 | 40.95 | 21.96 |
| no yeast 25 days | 6.6 | 7.9 | 4.41 |

This reduction in acidity which apparently accompanied the development of the highly reducing material suggested that it was not

acidic but might have been formed from an acid decomposition product of the pentose.

It was thought that this highly-reducing substance might be an aldehyde—formaldehyde, acetaldehyde, glyceric aldehyde or glycollic aldehyde. There are several acids from which formic acid could be produced and the reduction of formic acid would give formaldehyde. It was found, however, that formaldehyde reduced Fehling's solution in the cold only after long standing and then the reduction was slight. In addition formaldehyde is a volatile aldehyde and would be removed by boiling. Acetaldehyde is also volatile, and does not reduce Fehling's solution in the cold.

Glyceric aldehyde or glycollic aldehyde could very readily be formed from certain acids. They can reduce Fehling's solution in the cold; are not volatile; are not acidic; and are not precipitated by basic lead acetate. These compounds were not available so the comparative reducing values could not be determined.

Warkany[58] found that yeast formed a carbohydrate which is not destroyed in 60% KOH, is soluble in hot water, precipitated by alcohol and not precipitated by Fehling's solution. On hydrolysis with 2.7% hydrochloric acid this carbohydrate yields a non-volatile substance which reduces Fehling's solution in the cold. He suggests that it may be glyceric aldehyde or dihydroxyacetone.

## ORANGE GUM

The natural gums were formerly thought to be carbohydrates of the general formula $(C_6H_{10}O_5)n$ but the researches of O'Sullivan[39] on gum arabic have shown that they are not simple carbohydrates. He considered gum arabic to be a mixture of calcium, magnesium, and potassium salts of a diaraban—tetragalactan—arabic acid. He assigns arabic acid the formula $C_{23}H_{38}O_{22}$ and considers one molecule of this acid to serve as a nucleus for the union of 4 galactose and 2 arabinose groups. The formula for the compound exclusive of the mineral elements with which it is loosely united is given by O'Sullivan as $C_{91}H_{150}O_{75}$.

Orange gum is a translucent amorphous substance, which dissolves in water, giving a slightly sticky solution.

A combustion on orange gum, that had previously been dissolved in water, filtered, and dried to constant weight in an oven at 50°C. indicated the following percentage composition:

C = 39 H = 4.4 O = 56.6 Empiric formula = $C_{32}H_{44}O_{35}$

The orange gum could not be purified as O'Sullivan purified gum arabic. The following compounds were used in an unsuccessful attempt to

precipitate it: 95% alcohol, ether, aluminum sulphate, barium sulphate, basic lead acetate, ferric chloride and alkali.

When the gum was hydrolyzed for 3 hours with 1% HCl, carbon dioxide was given off. One gram of orange gum produced 58.14 mgms. $CO_2$, representing 6.5% of the dry weight of the gum. This indicates that acid treatment of orange gum may involve decomposition as well as hydrolysis.

To determine whether enzymes could hydrolyze orange gum and whether $CO_2$ was a product of this hydrolysis, one gram of gum was dissolved in 100 c.c. of distilled water and 3 grams of taka-diastase added. The flask was connected with flasks containing KOH and $Ba(OH)_2$. Check solutions containing gum and water, and water and enzyme were also set up. These solutions were kept free from contamination by an inch layer of toluene. The solutions were aspirated before incubation, to remove the $CO_2$ that might be contained in the flasks; and after 20 days incubation, to force the $CO_2$ that might have formed into the $Ba(OH)_2$ solution. The solutions were then boiled to drive off the toluene, made up to volume, and prepared for analysis as previously described. The reducing value of one gram of gum hydrolyzed for 2 hours with 1% HCl and expressed as glucose in percent of dry weight was 85.72%. The net reducing value (less the reducing power of taka-diastase) for orange gum hydrolyzed with taka-diastase was 87.55%. The gum in water and toluene showed a reducing value, expressed as glucose, of 2.59% of the dry weight. This shows that orange gum can be completely hydrolyzed by the enzymes of taka-diastase and suggests since, no carbon dioxide was evolved as in acid hydrolysis, that acid treatment involves decomposition.

On warming a solution of hydrolyzed gum with HCl and phloroglucinol a cherry red color appeared indicating the presence of furfural.

Oxidation of the hydrolyzed gum with nitric acid yielded mucic acid ($C_6H_{10}O_8$), indicating the presence of galactose, galactonic or galacturonic acid.

When the hydrolyzed gum was fermented 24 hours with yeast No. 1, there was only a slight loss in reducing value. This may indicate that there is formed through the hydrolysis of orange gum by HCl no glucose or easily fermentable sugar, as glucose is readily fermented by yeast No. 1. Fermented 5 days at 35°C. with 1/9 cake of Fleischmann's yeast in presence of 8% of alcohol 1 gram of the hydrolyzed gum with an original reducing value, expressed as glucose, of 84.01 to 84.4% showed a reducing value of 4.08 to 5.60%. This indicates that most of the reducing materials formed in the acid hydrolysis of this plant gum can be utilized by the yeasts and associated organisms in Fleischmann's yeast cake. Most of

the reducing material remaining after fermentation was volatile. A solution fermented for 5 days showed before boiling a reducing value expressed as glucose of 4.08% of the dry weight of the gum and but 0.63% after boiling.

## DISCUSSION OF THE FERMENTATION METHOD OF DETERMINING PENTOSES

It would appear from the investigations reported in this paper as well as from the results found by Bartholomew and Robbins that the fermentation method of determining pentoses as it is commonly used may be unreliable for several reasons. The introduction with baker's yeast or Fleischmann's yeast cake of foreign organisms capable of utilizing pentoses, the utilization of pentoses by the yeast itself, the fact that galactose appears to be as resistant to fermentation as the pentoses and the possible presence of unfermentable or difficultly fermentable reducing material other than galactose and the pentoses all militate against the accuracy and reliability of the method. The difficultly fermentable residues found by various investigators in the hydrolyzed extracts of plant tissues and interpreted by them as representing pentoses probably include, in many cases, galactose as well as pentoses. They also probably represent only a larger or smaller fraction of the total quantity of one or both of these sugars or groups of sugar actually present in the extracts. It is possible, however, that such results represent more or less accurately the relative quantities of original mucilages or gums many of which are reported to yield galactose as well as pentoses on hydrolysis. The determination of the reducing value of fermented hydrolyzed plant extracts may be valuable for that reason even though the results do not equal the pentoses present.

Is it possible to determine pentoses in a mixture of sugars by yeast fermentation?

From the data given in this paper, two possible methods for pentose determination by the fermentation method may be suggested; first, by controlling the reaction of the medium and second, by using pure cultures of yeast which ferment hexoses but do not ferment pentoses. In either case the presence of galactose would be a complicating factor difficult to eliminate as galactose is as resistant to the action of the yeasts used as are the pentoses.

As a method which might prove practical but which requires further investigation the following is proposed:

(1) Buffer the solution to $p_H$ 4.5 and add 8% of 95% alcohol. Ferment 9 to 12 hours at 35°C. with ⅓ of a cake of Fleischmann's yeast

per 100 c.c. of solution. This should remove all easily fermentable sugars.

(2) Determine the presence or absence of galactose by the mucic acid test. If positive estimate the amount of galactose present.

(3) Adjust the solution to $p_H$ 6.7 and ferment as under (1) for 4 to 5 days to determine whether any unfermentable residue reducing Fehling's solution is present.

The difference between the results of 1 and 3 less the galactose present will represent the pentose and methyl pentose.

In place of the Fleischmann's yeast and alcohol pure cultures of a yeast known to ferment pentoses and solutions sterilized by the intermittent method might be used.

## SUMMARY

1. Two yeasts isolated from Fleischmann's yeast cake (No. 1 and 4) a mycoderma, a pink yeast, distillers' yeast, and Burgundy yeast were not able to utilize arabinose in a nutrient solution (solution No. 1) in 20 days at $p_H$ 6.0, but *Sach. porupe*, *Sach. ellipsoidae*, *Sach. cerevisiae*, *Sach. apiculatus*, and top yeast were able to destroy arabinose completely in 20 days at $p_H$ 6.0 in nutrient solution No. 1.

2. *Sach. cerevisiae* was not able to utilize arabinose or xylose in 15 days at $p_H$ 4.5 but was able to utilize 0.1 gram of xylose or arabinose in 100 c.c. of solution at $p_H$ 5.5 to 7.0 in 15 days.

3. *Sach. cerevisiae* was able to utilize 0.1 gram of xylose or arabinose in 100 c.c. of solution in the presence of glucose in 20 days at $p_H$ 6.6.

4. Fleischmann's yeast was able to utilize xylose and arabinose at $p_H$ 5.0 to 6.6 in 48 hours to 5 days depending on the amount of yeast used and the amount of sugar present.

5. The utilization of pentose by yeast is accompanied by the production of traces of $CO_2$ and alcohol, and mainly of non-volatile acid or acids.

6. A high reducing substance that was variable in its appearance was produced during the utilization of pentoses by yeasts in cultures containing nutrient solution plus xylose, arabinose, or rhamnose alone or in the presence of glucose, inoculated with yeast No. 1, for 20 days at $p_H$ 4.8. It also appeared in nutrient solution No. 1, containing rhamnose inoculated with *Sach. cerevisiae*, 25 days at p 6.6, and in hydrolyzed orange gum inoculated with yeast No. 1 for 20 days.

7. This reducing substance reduced in the cold, and had a maximum reducing value of 70 times the original reducing value of the sugar solutions in which it was formed. It is suggested that the substance, being

a non-volatile, and non-acidic substance was perhaps glyceric or glycollic aldehyde.

8. The fermentation method as commonly used was found inaccurate for the determination of pentoses because of the following reasons: (a) The presence of foreign organisms. (b) The common varieties of yeast such as are found in Fleischmann's yeast cake can utilize pentoses. There was some utilization in 12 hours and the pentoses had completely disappeared in 48 hours to 5 days depending on the amount of yeast used. (c) Galactose is about as resistant to fermentation by the yeasts in Fleischmann's yeast cake as the pentoses.

9. Orange gum was found to give the phloroglucin test and the mucic acid test.

10. Orange gum hydrolyzed in 3 hours with 1% HCl. During hydrolysis $CO_2$ equivalent to 6.5% of the dry weight of the gum was given off. It was also hydrolyzed in 20 days by taka-diastase and no $CO_2$ was produced.

11. A combustion on orange gum, that had previously been dissolved in water, filtered and dried to constant weight at 50°C. showed the following percentage composition:

C=39 H=4.4 O=56.6 corresponding to the empiric formula $C_{32}H_{41}O_{35}$.

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