that the air-cured, heat-cured, and sun-cured tobaccos fall into natural groups with respect to these physical properties. The range of isosteric net heats of desorption is from about 700 to about 16,000 B.t.u. per pound mole, depending on the type of tobacco and its moisture content.

The isosteric net heats of desorption of bright stems are higher than the leaf, whereas those of burley stems are somewhat lower than the leaf.

The effect of temperature on equilibrium moisture content for a given relative humidity is pronounced, and may amount to as much as 0.06 weight fraction of water for a 60° F. increase in temperature in the region of 70% relative humidity.

Nomenclature

A = a constant
P = total pressure, cm. of mercury
p = vapor pressure, cm. of mercury
P_0 = saturation vapor pressure of water, cm. mercury
°R = degrees Rankine
R = gas constant, B.t.u./lb. mole/°R
T = absolute temperature, °R
T^* = absolute reference temperature, °R
x = relative humidity, %
x^* = relative humidity at temperature T^*, %
λ_T = total heat of desorption, B.t.u./lb. mole
λ_N = isosteric net heat of desorption, B.t.u./lb. mole

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BEER ANALYSIS

Chromatographic Determination of Trace Amounts of Sucrose in Beer

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In a study of the useful limits of the paper chromatographic method as applied to materials of biological origin, beer was examined for sucrose. Sucrose could not be found in a sample of bottled beer, but it occurred in draft beer to the extent of about 20 p.p.m. Suggestions for the occurrence of sucrose in draft beer are made. Recovery experiments show that 5 p.p.m. of sucrose in beer is detectable. In attempts to lower this limit, difficulties were encountered as a result of interference of materials present in the filter paper.

A METHOD OF ISOLATING AND DETERMINING TRACES of sucrose would be of general application to the determination of traces of other sugars. Sucrose offers certain analytical difficulties, as it is harder to detect than most sugars. It does not react with the very sensitive reagents that are available for detecting the reducing sugars. Furthermore, sucrose decomposes comparatively easily, and this can impose certain limitations on the methods used to isolate it.

The brewing industry determines "residual fermentable extract" by a method in which a large amount of yeast is added to the beer and the extent of further fermentation measured by changes in the "real extract" or in the copper-reducing substances. This value has been taken to indicate the residual sugar content of the beer, although the possibility of large inaccuracies in these methods has been recently pointed out (*20*, *23*). It was of interest, therefore, to see whether sucrose contributed to this value. McFarlane and Held (*11*) found sucrose in some American and Canadian bottled ales and beers. British beers are known to contain considerable amounts of added sucrose.

In recent years, paper chromatography has had successful application in studies of the fate of sugars in the brewing of beer (*1*, *2*, *4*, *5*, *8*, *11*, *13-18*), in dough and bread making (*7*, *10*), and in investigations of the sugars present in various plant materials (*13-18*, *25*). These studies, for the most part, have not attempted to measure sugars in extreme dilutions, and the lower level of most determinations has not been less than 0.01% and is usually much higher.

The paper chromatographic studies cited have shown that, with a few exceptions, the simple sugars present in wort are assimilated rapidly on fermentation. In one study (*1*, *8*) of top fermentation, glucose and fructose could not be found after 24 hours nor sucrose after only 9 hours. The corresponding times for bottom fermentation were roughly doubled. Obviously, these sugars might have been found if the sensitivity of the method had been greater. The present study was undertaken in an effort to increase the sensitivity from the usual 100 to 1000-p.p.m. range to 1 p.p.m. so that there could be a corresponding increase in the certitude of negative findings.

Sucrose Content of Bottled Beer

A sample of bottled beer was examined for sucrose in the following manner. A quart (947 cc.) was degassed by holding it under vacuum at room temperature. It was then deproteinized according to Somogyi (*22*) and subjected to an alcohol precipitation scheme in which the beer was brought successively to ethyl alcohol concentrations of 67, 80, and 85%. The sirups thrown down by these treatments and by concentration of the alcohol solutions to small volume were saved for examination.

The 85% ethyl alcohol–soluble material was taken to dryness and extracted with pyridine, according to the procedure of Malpress (*19*), to separate the salts. The pyridine was taken off under vacuum, and the resulting residue was dissolved in 80% ethyl alcohol. It was then made up to 86% ethyl alcohol and filtered from gummy material. The final volume was made exactly 10 cc.

Various amounts of the final concentrate up to 100 μl. were spotted on Whatman No. 1 paper by repeated application and chromatographed alongside known amounts of sucrose. The ethyl acetate–pyridine-water solvent system of McFarren (*12*) was used with a naphthoresorcinol–trichloroacetic acid spray (*3*) for detecting sucrose.

It was shown that 40 γ of sucrose was required for a definite color reaction under these conditions and that this amount of sucrose was not in as much as 100 μl. of the concentrate. Correcting for various aliquots removed during the fractionation scheme, this means that the beer could not have contained more than 4.7 mg. of sucrose in the original quart—that is, more than about 5 p.p.m. This figure is based on the assumption that all the sucrose originally present survived the analysis scheme and was contained in the final 10-cc. concentrate.

In an effort to ensure this, each fractionation step throughout the analysis scheme was accompanied by paper chromatography of the rejected material. Obviously, the problem in finding traces of sucrose in rejected material approaches that of finding traces of sucrose in the beer. It can be assumed, however, that an 80% ethyl alcoholic extract of the rejected material—prepared by dissolving the sirup in water and making up to 80% with respect to ethyl alcohol—will reveal, on chromatography, any gross loss of sucrose.

Chromatograms were run in duplicate for all the various fractions, using naphthoresorcinol as the visualizing agent for one, and ammoniacal silver nitrate for the other. McFarren's system (*12*) of dissolving the silver nitrate in the irrigating solvent was employed.

The naphthoresorcinol reagent, which was found to be the most sensitive of the various chromogenic agents for detecting sucrose (*3*), was invariably negative. The ammoniacal silver nitrate, however, revealed gradually increasing amounts of reducing materials in the sirups thrown out in the course of the fractionation scheme. The first sirup obtained had only traces of reducing material, but the later sirups contained considerable amounts.

The scheme was stopped when small amounts of maltose began to appear. At this stage maltose was the chief constituent of the alcoholic concentrate. The maltose was useful as a guide, as sucrose was unlikely to be thrown out by alcohol while large amounts of maltose remained in solution.

In addition to the maltose, the alcohol concentrate contained appreciable amounts of reducing material with the same R_f values as ribose. Two other reducing substances with R_f values similar to xylose and arabinose were present in trace amounts. These three substances may be the pentoses whose presence in wort and beer has been disputed (*1*, *2*, *4-6*, *21*, *24*).

In the alcohol-precipitated sirups, there were, as would be expected (*11*), several reducing substances with R_f values below—less mobile than—maltose. A twice-irrigated chromatogram of one fraction showed at least five of these substances, such as maltobiose and maltotetraose. The "maltose" spot was seen, after the second irrigation, to be composed of two incompletely resolved substances. The slower one, which was in rather greater concentration, was probably isomaltose. Other fractions showed an unidentified reducing substance with the same R_f value as galactose and another with an R_f value slightly greater than that of glucose.

Recovery Experiments

To test the validity of the alcohol fractionation and chromatographic method, a series of bottled beer samples was made up containing added sucrose to make solutions of 1, 2, 5, 7.5, 10, 20, and 40 p.p.m. Aliquots of each were taken such that each sample contained 100 γ of sucrose. Each sample was worked up individually, and half of the final fraction was analyzed by paper chromatography. At concentrations above 10 p.p.m., the sucrose was easily observable; at 7.5 p.p.m., it was just observable. At lower concentrations, the final concentrate contained too much material to be applied as one spot on the paper.

Sucrose Content of Draft Beer

A sample of draft beer was obtained and analyzed by this method. It was found to contain sucrose in amounts greater than 20 p.p.m.

It was known that the only difference between this draft beer and the bottled beer was a pasteurization step undergone by the latter. It was later learned that sucrose, to the extent of 32 p.p.m., had been added to both beers in a final step called chill-proofing. This consists of adding proteolytic enzymes to the beer to prevent "protein haze" formation on refrigeration. The enzymes are supplied commercially as a mixture with powdered sucrose to facilitate measurement. It seemed probable that the sucrose found in draft beer came from this source, and that it was not found in the bottled beer because of inversion due to the temperature of the pasteurization step (140° F.) and the pH (4.3) of the beer. Pasteurization of a 20-p.p.m. solution of sucrose in water at pH 4.3 failed to substantiate this theory, since 95% of the sucrose survived. After 9 days at room temperature, 92% still remained. Another objection to the inversion theory is that fructose, which is more easily picked up by the naphthoresorcinol reagent than sucrose, should have been produced. It would seem, then, that pasteurization in beer is more destructive to sucrose than pasteurization in water of the same acidity.

The occurrence of sucrose in draft beers may arise from the addition of "priming" or "finishing" sirups. Sometimes these sirups are added to draft beer, as it is believed that they serve to stabilize the foam and to counteract any rawness in the flavor. Pasteurization is said to have the same effect as priming, and bottled beers of the lager type are not usually considered to need the addition of these sirups. The sirup is usually partly inverted sucrose. About 70 to 100 p.p.m. of sucrose may be added to the beer in this fashion.

Possibility of Decreasing Detectable Amount of Sucrose

The largest limitation of the method as described is in the amount of final concentrate that can be applied to the filter paper without overloading. Only 1% of the 10-cc. final concentrate was applied in the chromatography of the bottled beer. In subsequent experiments the paper chromatograms were used in a fashion which permitted a larger sample of the concentrate to be examined for sucrose. A quart of bottled beer was fractionated by the scheme described earlier. The final concentrate was adjusted to 20 cc., and exactly 2 cc. of this was applied with the Gilmont ultramicroburet in the form of horizontal streaks at the starting line of eight large paper chromatograms. Guide strips, on which was chromatographed a mixture of 40 γ of sucrose with 50 μl. or more

of the concentrate, were used to indicate the zones where material with the R_f of sucrose was to be found on the irrigated chromatogram. These zones were wide, owing to overloading. Horizontal strips were cut out of the large chromatograms, pointed, and eluted with water as described by MacLeod (*13-18*).

The eluates from eight strips were combined, taken to dryness, dissolved in 80% ethyl alcohol, and reapplied as streaks to new chromatograms. This cycle was repeated four times, reducing the size of the cut-out zones each time. The material obtained was sufficiently reduced in amount to be applied as one spot on the paper. Standards containing 10 to 50 γ of sucrose were run simultaneously. The developed chromatogram was treated with an improved (*9*) naphthoresorcinol reagent, but only the standards were revealed. Recovery experiments were run to measure the efficiency of the chromatographic cycle, and it was found that 80 to 95% recovery could be obtained on eluting sucrose from the chromatogram. Using 80% as the recovery figure for one cycle, the recovery after four cycles will be 41%. The sample of concentrate analyzed contained 10% of any sucrose originally present in the quart of beer. Using a new value of 20 γ for the least detectable amount of sucrose, the sucrose content in this beer sample was calculated to be not more than 0.5 p.p.m.

However, in attempts to refine this method by rigid standardization of the variables involved—e.g., by running chromatograms in the constant temperature room, by using purified filter papers, and freshly prepared solvents—certain difficulties were encountered. The recovery of sucrose on elution from a chromatogram had dropped to 48 to 50%. Obviously, four cycles at this figure return only $^1/_{16}$th of the original sucrose, which is a high price to pay for the ability to handle 20 times the amount of concentrate on the chromatogram.

A study of the factors influencing the recovery of sucrose from chromatograms has since shown that materials eluted from filter paper can cause severe losses of sucrose, especially during the subsequent evaporation to dryness of the eluate. Prewashing the paper is not an entirely satisfactory solution, since the paper then gives poorer separation of sugars. The elution of sugars with water, after spotting on the washed paper, also elutes substantially more of these interfering substances. Some papers seem to have a strong affinity for sucrose and other sugars. One of the best ways to combat these difficulties is to pretreat the paper with a sucrose or glucose wash—which will not produce a color reaction with naphthoresorcinol to any great extent—followed by a water wash to satisfy the paper's capacity for binding the sugars. However, this in turn can produce additional difficulties.

The bulk of the unknown interfering materials is efficiently removed from the paper by irrigating the paper before use with a sucrose (or glucose) solution. The paper is then irrigated with water to remove the sucrose and dried carefully to avoid wrinkling. While the recovery of sucrose, spotted on such purified paper and eluted with water by MacLeod's technique (*13-18*), was materially increased, some interference was observable.

The interference is not apparently merely a question of preventing or retarding the development of color with the naphthoresorcinol reagent. The chromatograms of sucrose, eluted from paper (such as Whatman No. 1), run on a variety of papers and visualized with naphthoresorcinol reagent, show considerable streaking, often with the appearance of two or three centers of density, as if definitely different molecular species were involved. Whether these arise from complexes formed by sucrose with the impurities or whether the color reactions are caused by sucrose that has had its normal partition equilibria disturbed by the presence of impurities has yet to be resolved. An aqueous eluate of fresh paper strip will show traces of impurities upon chromatography. The amount appears to be increased by eluting paper which has previously been irrigated with the normal solvent used for the chromatography of the sugars.

Many variables in the chromatographic system remain to be evaluated. It is clear, however, that this elution technique should not be employed, especially where rechromatography is involved, without a critical evaluation of all factors. In the case described, the color given by the eluate with a naphthoresorcinol reagent indicates a complete recovery of sucrose from the paper on elution with water, but upon rechromatography it becomes apparent that only part of this naphthoresorcinol-reacting material has the chromatographic behavior expected of sucrose.

One serious difficulty may remain, even when the problem of sucrose loss has been overcome. Irrigation with sucrose is perhaps the theoretically ideal way to remove impurities from the paper which is to be used for the quantitative estimation of sucrose. However, chromatograms run on such purified paper are distinctly inferior to those run on untreated paper, both in clarity of color reactions and in definition, the background color of the treated paper is uneven, and R_f values are not as reproducible. The situation then seems to be that the impurities in the paper are needed for clean chromatographic separations, while they are undesirable for high recoveries of sucrose from the paper for quantitative work.

These studies are still in progress, and the authors hope to publish the results shortly.

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