

of the total nitrogen contained in the crop from the inoculated unfertilized plot (1) was secured from the atmosphere by the alfalfa bacteria. It should be borne in mind that nitrogen is required for root growth as well as for growth above ground and also that these amounts were obtained from a single crop of alfalfa, and that three or four crops will be cut during the season. From the data already given, it will be seen that on the unfertilized soil four such crops as that cut on May 28th from the inoculated plot would mean at least 160 pounds of atmospheric nitrogen fixed by an acre of alfalfa during a single year, and this would require a total yield of only about five tons of alfalfa hay for the season, which is by no means a maximum yield for alfalfa on Illinois soil under the most favorable conditions, as has been shown in the preceding pages.

The percentage of nitrogen is much higher in the crops from the inoculated plots, the average being 1.97 per cent. in the dry matter for the uninoculated plots and 2.69 for the inoculated plots. This means, of course, that the hay produced on the inoculated plots is not only more in quantity, but it is also much better in quality, the percentage of protein averaging only 12.29 in the dry matter of the uninoculated crops, while 16.84 is the average percentage for the inoculated plots.

UNIVERSITY OF ILLINOIS AGRICULTURAL
EXPERIMENT STATION.

THE IDENTIFICATION AND COMPOSITION OF MALT LIQUORS.¹

BY CHARLES LATHROP PARSONS.

Received July 3, 1902.

THREE of our northeastern states have laws which, if enforced, strictly prohibit the sale of all intoxicating liquors and of some which are, perhaps, not intoxicating. In two of these States the statutes use the phrase "Malt Liquors" of any kind, and prosecutions have been brought in large numbers under this section. To obtain convictions, therefore, it has been necessary to convince the judge or jury that the beer in question was brewed

¹ Read at the Pittsburg Meeting of the American Chemical Society.

in part, at least, from malt, for it has quite properly been held in the lower courts that a malt liquor is one which has had germinated grain or some preparation of malt used in its brewing. The question, therefore, submitted to chemists has not been the simple one of alcohol content.

In looking over the literature of the subject almost nothing will be found bearing on this phrase of the question. Much has been written on the essential constitution of pure malt liquors and many analyses have been made to prove that certain beverages were up to standard and that foreign substances had not been used in their preparation. Here, however, it is not to find adulteration but to prove positively that the main source of a first quality beer was used. This would perhaps seem easy from appearance alone but there are beers on the market in these States, with the main physical properties of lager or ale, which analyze quite differently from the pure malt liquors and the representative of at least one of the manufacturers has frequently taken oath that absolutely no malt or preparation of malt was used in their manufacture. The point at issue becomes then very much the same as if the chemist was asked to prove, by analysis, that oleomargarine contained no butter and, if the amounts of malt or butter are small, the solution in either case is, at the present time, equally impossible. The claim has been made that maltose is a characteristic which, if found, would satisfy the meaning of a malt liquor but the contention has naturally met with about as much success as if the presence of butter in oleomargarine was claimed because stearine was present.

If we try to find any one constituent of malt which is absolutely characteristic of that substance and which will be unchanged in the finished beer we immediately encounter difficulties. Diastase, formed in the germinating grain, is destroyed when the beer wort is boiled previous to its fermentation. Maltose and dextrine are large constituents of the glucose which, as the analyses to follow will indicate, and as most brewers admit, is now quite generally used in brewing. Alcohol can arise from sugar of any kind. Sykes,¹ Osborne,² Osborne and Campbell,³ E. Erich,⁴ and H.

¹ Transactions Institute of Brewing, 4, 173 (1891).

² *Am. Chem. J.*, 17, 429, 539 (1895).

³ *Ibid.*, 18, 542.

⁴ *J. Soc. Chem. Ind.* (1896), p. 366.

Schjerning¹ have given much careful study to the various albuminoses, proteoses, etc., of grain, malt and beer wort but none of these substances appears to persist in an unchanged form in the beer, so that its individual presence could be used as a proof of its source. The bitter principle present has no bearing on the case. The extended investigations of O'Sullivan published in the Journal of the Chemical Society and elsewhere throw no light on this problem. The study of the spectra of the coloring-matter of malt by H. C. Sorby² shows it to be characteristic but again it is much modified in beer and, while this field seems worthy of exploration, has not been sufficiently investigated.

Grain and sugar of some form, generally glucose, are the only forms of malt substitutes from which beers are brewed. Of the various sugars used, all have one characteristic in common in that they contain little or no nitrogenous matter or phosphates. Also, while glucose has a considerable and varying amount of ash it is of a distinctly different character from that derived from malt. On the contrary, we have in grain of any kind the essential constituents of malt with the exception of diastase. Fortunately grain can only be used in brewing when mixed with malt in order that the necessary diastase may be furnished. Therefore it may be considered as malt in determining whether or not the beer under examination was a malt liquor. The ash of grain, malted or unmalted, is rich in phosphorus and potassium and poor in sulphates. The ash of glucose is poor in the first-mentioned constituents but is usually rich in sulphates. The nitrogenous matter, the phosphates, which are also characteristic of seed products, and many of the constituents of the ash are conveyed in quite considerable quantities to the beer and give it its chief nutritive value. The beers which are claimed to be brewed mainly from glucose show, on analysis, little nutritive value. This is to be expected if the claims are true. The hops carry some of the above constituents in both cases to the beer. Also I am told by the manufacturer of the only really successful imitation malt liquor on the market, which is claimed to use glucose as its sole source of alcohol, that they are obliged to add some soluble "French albumin" in order to get their yeast to work and that then, although they

¹ *Ztschr. anal. Chem.*, **35**, 285 (1896).

² *Deut. Viertel. Jahreschrift. öffentl. Gesundheitspfög.*, II, Heft 1.

IDENTIFICATION AND COMPOSITION OF MALT LIQUORS. 1173

boil off part of the alcohol and at the same time Pasteurize the beer, they have great difficulty in preserving it for any great length of time.

The following summary of 172 samples of American lager and 199 samples of American ale, published by Dr. F. E. Englehardt in the Report for the New York Board of Health for 1886 and taken in part from Bulletin 13, part 3, U. S. Department of Agriculture, Division of Chemistry, give a good idea of the average composition of the commercial malt liquors of this country as they were made eighteen years ago.

COMPOSITION OF 172 SAMPLES OF AMERICAN LAGER BEER.

	Sp. gr.	Alcohol by volume.	Extract.	Ash.	Phosphoric acid (P ₂ O ₅).
Average	1.016	4.70	5.86	0.259	0.096
Maximum		8.78	9.64	0.412	0.120
Minimum		0.85	3.65	0.172	0.050

COMPOSITION OF 199 SAMPLES OF AMERICAN ALE.

Average	1.013	5.77	5.42	0.307	0.083
Maximum		11.16	9.50	0.552	0.166
Minimum		3.02	2.70	0.197	0.0405

Further an average of 28 samples of American malt liquors analyzed by Dr. C. A. Crampton¹ shows the following composition:

COMPOSITION OF 28 SAMPLES OF AMERICAN MALT LIQUORS.

	Sp. gr.	Alcohol by volume.	Ex-tract.	Ash.	Albumi- noid (n × 6.25).	Phos- phoric acid (P ₂ O ₅).	Reducing sugars as maltose.	Dex- trine.
Average	1.0161	5.79	5.53	0.279	0.563	0.077	1.65	2.33
Maximum	1.0241	8.63	8.19	0.472	0.763	0.104	2.67	3.12
Minimum	1.0077	4.85	3.46	0.183	0.400	0.056	0.59	0.90

These analyses are all characterized by notable quantities of albuminoids and phosphates, the lowest amounts being 0.400 and 0.0405 per cent. respectively while the averages are about 0.563 and 0.085 per cent. The larger the quantity of glucose used, the lower, relatively, will be the amounts of nitrogenous and phosphatic material. My own experience leads me to believe that no true lager or ale on the market as such will be found to contain less than 0.04 per cent. of phosphoric acid and 0.25 per cent. of albuminoids, even when these facts are taken into consideration. An amount of glucose equivalent to from 15 per cent. to 20 per

¹ Division of Chemistry, Bulletin 13, Part 3.

cent. of the mashing material appears to be about the maximum quantity used even with the poorest beers derived from malt. König, in his *Menschliche Nahrungs u. Genussmittel*, gives 0.05 per cent. as the minimum amount of phosphoric acid allowable in a beer containing 5 per cent. of extract or over, while the Association of Bavarian chemists¹ gives the minimum amount of albuminoids as 4 per cent. of the extract. It will be seen from the above-quoted analyses and those to follow that their figures are lower than will probably be found in an American malt liquor, even with a considerable proportion of glucose used.

As a further contribution to the literature of this subject I think it will be well to offer the following summary of 76 analyses of beers recently made by me, each of which I considered to give sufficient evidence to warrant a chemist in pronouncing them to have been brewed from malt. This judgment would rest largely upon their percentage of albuminoids and phosphoric acid but is supplemented by the composition in general.

COMPOSITION OF 76 SAMPLES OF AMERICAN MALT LIQUORS.

	Sp. gr.	Alcohol by volume.	Ex- tract.	Albumi- noid ($n \times 6.25$).	Phos- phoric acid.	Ash.	Sulphates in ash.	Free acid.
Average ..	1.0100	5.61	4.61	0.470	0.061	0.209	6.34	0.26
Maximum	1.0210	7.85	7.64	0.614	0.095	0.296	12.67	0.87
Minimum.	1.0047	0.35	3.15	0.290	0.045	0.147	2.44	0.10

Approximately 50 per cent. of these malt liquors contained salicylic acid.

A glance over this last summary will show at once that the beers analyzed are not equal in quality to those whose analyses have been before quoted. The high percentages of sulphates, the comparatively low specific gravity and extract, point to a much more general use of glucose than formerly existed. This question of quality was also frequently apparent in the physical properties of the beer and it would be a curious thing to know whether or not their sale in so-called prohibition States had anything to do with this quality. In one or two instances only have I any knowledge of the brands.

The average high percentage of free acid shows that some of these beers reached me in anything but the best of condition and the percentages of alcohol were probably modified from the

original in many instances. The alcohol is reported in per cent. by volume for the reason that it is the volume per cent. which is usually the legal one. The per cent. of albuminoids and phosphoric acid, is, however, unmodified from that of the perfectly fresh beer for when any yeast was present the container was thoroughly shaken before the sample was taken and the contents of the yeast, which had been derived from the beer, included in the results. It is particularly worthy of note that in some samples not included above, where the alcohol had been wholly changed into acetic acid, the special characters derived from the malt still stood calculated. While the above summary of analyses shows some interesting facts the mistake should not be made of using it as a basis of judging the composition as a whole of fresh American beer and ale. They indicate more closely the kind of material that is apt to be sent to a chemist as the result of a police seizure—material which is sometimes kept several days or even weeks before reaching his hands. If, however, the sample can be treated, when first taken, with eight to ten drops of formaldehyde to the liter of beer, it will keep it in excellent condition, for analytical purposes, for weeks.

If a beer shows the composition given in the analyses already quoted it can safely be pronounced a malt liquor. The converse is not necessarily true. A beer might have had a small amount of malt used in its production and the fact be absolutely undeterminable. In such case, and that is probably just the case with many of the "temperance beers" on the market, chemists can say only "no evidence of malt." The analyst can only be sure of his statements that malt was used when a considerable proportion of that material was used in the brewing. Fortunately the "no malt" beers which have come under my own observation do not even approach the composition of the ordinary part glucose beer.

The following are typical analyses of beer which in my opinion do not furnish sufficient evidence in warranting a chemist in stating that they were brewed from malt.

No.	Sp. gr.	Alcohol by volume.	Ex-tract.	Albumi- noid ($\mu \times 6.25$).	Phos- phoric acid.	Ash.	Sul- phates.	Free acid.
1	1.0074	1.68	2.52	0.114	0.010	0.19	21.22	normal
2	1.0098	2.63	3.40	0.215	0.023	0.189	normal
3	1.0062	2.27	2.25	0.150	0.015	0.124	11.30	normal
4	1.0112	2.11	3.53	0.133	0.015	0.140	10.81	normal
5	1.0041	1.85	1.73	0.031	0.010	0.088	12.50	normal

The percentages of sulphates are high but a glance at the previous table will show at once that too much dependence must not be placed upon this fact. It is simply circumstantial evidence.

The composition of the ash of beers is of great importance in doubtful cases but unfortunately a complete analysis of ash is tedious and would make undesirable expense. Its actual amount tells but little, its composition being the important consideration. Unfortunately, also, more beer is required to obtain enough ash for analysis than is generally sent to the analyst. Blyth¹ gives the following average composition of beer ash:

I have made but one fairly complete ash analysis of any one of the above samples, that of No. 5 of the "no malt" beers, which I place beside that of Blyth's for comparison.

	Malt beer (Blyth).	"No malt" beer (Parsons).
K ₂ O	37.22	12.93
Na ₂ O	8.04	19.61
CaO	1.93	undetermined
MgO.....	5.51	"
Fe ₂ O ₃	trace	"
SO ₃	1.44	10.81
P ₂ O ₅	32.09	10.71
Cl	2.91	21.76
SiO ₂	10.82	7.50

The differences are very prominent and would be a great help in pronouncing judgment. Here again would arise the same difficulty as before, if only small amounts of malt had been used and the verdict would probably always be in accord with that rendered by the albuminoids and phosphoric acid.

METHODS OF ANALYSIS.

Specific Gravity.—Official method.¹ Pycnometer with thermometer stopper used.

¹ "Foods," Fourth edition, p. 518.

² Bulletin No. 65, Department of Agriculture, Bureau of Chemistry.

Alcohol.—Pycnometer with thermometer stopper. Pycnometer carefully calibrated with distilled water at 15.6°, 17°, 20°, and 22° C., according to temperature of atmosphere. Alcohol solution weighed at same temperature. The departure from 15.6° may be criticized but a study of the coefficients of expansion of pure water and mixtures of alcohol and water¹ will show that with such dilute solutions of alcohol as are under consideration the error would be less than 0.01 per cent. This is less than would result from evaporation due to capillarity around the ground-glass stopper if the pycnometer is filled at 15.6° and brought to room temperature to avoid condensation on the glass, is much less than would arise from the use of a hydrometer, even the most delicate made, and is fully as accurate as by calculation from the tables of Quibb. When distinctly acid, the beer was, of course, always neutralized before distillation.

Extract.—Provisional official method,² using the formula $1 - \text{sp. gr. (of alc. dist.)} + \text{sp. gr. (of beer)}$ and using the tables of Oestermann and Schultze. Many comparative results were made by evaporation and by direct determination of the specific gravity of the dealcoholized beer. The results from the use of the tables never varied greatly from the others and are probably much nearer correct owing to the hydrolysis of sugar in the first case and the precipitation of albuminoids, etc., in the second.

Albuminoids (total $n \times 6.25$).—Official Gunning method, with the additional precaution of always first boiling down the beer taken to a small bulk, but not to dryness, after acidification with a few drops of sulphuric acid, before the main quantity of acid or potassium sulphate was added. This did away with the foaming which was otherwise uncontrollable.

Phosphoric Acid.—Uranium acetate method. Solution of uranium acetate in which 1 cc. was equivalent to 0.01 per cent. P_2O_5 when 50 cc. of beer were used. When the beer was light-colored the method was applied both to the beer itself and to the ash; otherwise to the ash alone. The results on the ash were generally 0.1 or 0.2 cc. higher than on the beer itself and sometimes even more when any considerable quantity of yeast had

¹ See Makins: *J. Chem. Soc.*, 90, 224; Silbermann: *Compt. rend.*, October, 1848.

² Bulletin No. 65, Bureau of Chemistry.

developed. Results were compared with the molybdate method and agreed closely. There was no difficulty in determining the point of precipitation of the phosphate within 0.1 cc. when the ash was used. The ash was moistened with concentrated hydrochloric acid and the acid evaporated on the water-bath. It was then boiled with 50 cc. of distilled water and titrated with uranium solution. Under these conditions the end-point was always sharp. If the ash was not treated with hydrochloric acid, if acetic acid was substituted for hydrochloric acid or if, after treatment with hydrochloric acid, the ash was heated much above the temperature of boiling water, the phosphates were insoluble or, at least, remained in a form where they were not attacked by the uranium solution. With these precautions, the uranium method gives on a beer ash results which are quite accurate to 0.1 cc., and which are quickly obtained.

Sulphates.—The photometric method of Hinds¹ was used. In my hands his tables give percentages in beer ash more closely agreeing with the gravimetric than when the tables of Jackson² were used, as the latter yielded too high results. The solution of ash in hot, dilute hydrochloric acid, was either taken direct after filtering, or the filtered solution from the phosphoric acid determination could be equally well used.

Ash, Salicylic Acid, and Free Acid were determined by the usual official methods.

NEW HAMPSHIRE COLLEGE,
July 2, 1902.

ON AZOXYBENZENE.

Received September 5, 1900.

BY ARTHUR LACHMAN.

A FEW years ago, I endeavored to deduce the structure of the nitro-group, and came to certain conclusions.³ Subsequent developments, however, showed the line of reasoning then adopted to be erroneous; and these conclusions have since been withdrawn.⁴ A new method of investigation became necessary, and a

¹ This Journal, **22**, 269.

² *Ibid.*, **23**, 799.

³ *Am. Chem. J.*, **21**, 433 (1899).

⁴ This Journal, **23**, 897 (1901).