results are obtained when the soda reagent is added in such quantities that not more than 20 per cent of it enters into the reaction, and that the results are within 5 per cent of the true amounts when not more than 40 per cent of the alkali is used up.

MAGNESIUM HARDNESS BY DIFFERENCE

It has been shown that the soda reagent method for total hardness is quite satisfactory for ordinary work if care be taken to have sufficient excess of the alkali present, and that the permanganate titration for calcium is very accurate. When the magnesium is computed by difference, it suffers from the errors of the 2 direct methods and low results are to be expected because the total hardness figures are themselves low, due to the appreciable solubility of normal calcium carbonate and magnesium hydroxide. With certain waters, however, very close results can be obtained by this method. Seven of the samples, showing a range in hardness encountered in the Ohio River at this locality (48 to 130 p. p. m. total hardness; 15 to 37 p. p. m. magnesium hardness), gave very satisfactory results for magnesium by difference, the average percentage of the true amount being 98.6 per cent. With waters ranging in total hardness from 136 to 655.5 p. p. m. and in magnesium hardness from 63.8 to 295.6 p. p. m., however, the average accuracy was but 90.3 per cent. In such hard waters a comparatively small percentage error in the total hardness affects the magnesium to a greater extent, especially if the sample contains a relatively small quantity of this base in comparison to the lime content.

It appears then, that with comparatively soft waters a procedure, whereby the total hardness and calcium hardness are determined volumetrically as outlined above and the magnesium by difference, is satisfactory for ordinary purposes. This method can also be applied to hard waters the general character of which is familiar to the analyst, provided he applies a correction factor determined by occasional comparisons of the volumetric with gravimetric results. Hard waters very low in magnesium as compared to calcium content will give the least satisfactory results, and the gravimetric method for magnesium must be resorted to in this case if accurate figures are desired.

SUMMARY

I. No volumetric methods for calcium or magnesium are given in the 1912 edition of Standard Methods of Water Analysis.

II. For ordinary purposes, rapidity is preferred to extreme accuracy, and volumetric methods are desired.

III. The estimation of calcium by titration with permanganate is easily and quickly performed and the results are very accurate.

IV. The estimation of magnesium with lime water is entirely unreliable.

V. The best results for total hardness are obtained when less than 20 per cent of the soda reagent is used up in the reaction, and the results will be within 5 per cent of the true amount when not more than 40 per cent of the soda reagent is consumed. The original

strength of the soda reagent must be determined by a blank determination.

VI. An ordinarily satisfactory procedure for examination of waters of low or medium hardness consists in determining the total hardness with soda reagent, the lime hardness with permanganate and ascertaining the magnesium content by difference. This method can also be used for hard waters by applying a correction factor. For very hard waters containing only small amounts of magnesium, this base must be determined gravimetrically if accurate results are desired.

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THE QUANTITATIVE ESTIMATION OF THE SALT-SOLUBLE PROTEINS IN WHEAT FLOUR

By GEO. A. OLSON

Received October 2, 1913

It has been repeatedly pointed out¹ that the strengths of alcohol suitable for the extraction of gliadin from flour, extracts other proteins besides gliadin. That this is unquestionably true was brought out by the writer in a previous article² on the estimation of gliadin in flour and gluten where it was found that the direct method for the extraction of the alcohol-soluble proteins gave considerably higher yield of nitrogen (38.3 per cent more) than could be obtained by either the indirect or coagulation methods.

Likewise it has been found³ that 1 per cent sodium chloride extracts, besides edestin, leucosin and amino bodies, and some gliadin. Osborne⁴ states that gliadin is practically insoluble in 10 per cent sodium chloride. From a quantitative point of view, it appears reasonable that a 10 per cent salt solution is the proper strength to use. But we find that a 10 per cent salt solution is impractical to work with owing to the large amount of salt present, and for this reason Teller,⁵ and subsequently others, adopted strengths less objectionable. It was admitted by Teller and confirmed later by others by indirect methods that gliadin is partly soluble in 1 per cent salt solution, but just how soluble gliadin is no one has stated, nor even corrected for, when using this strength solution. A correction has been made for amide bodies by assuming that amide bodies are not precipitated by phosphotungstic acid. In the article referred to, the writer has pointed out the method for the estimation of the gliadin extracted by a 1 per cent salt solution and in connection with this the following experiments were conducted with the view of establishing a correct method for the estimation of edestin and leucosin proteins in flour.

Three different methods of procedure were adopted for the estimation of the salt-soluble proteins in flour. The first series of results was obtained by the method which is annually recommended to be followed by the

¹ U. S. Dept. of Agr., Bur. of Chem., *Bull.* No. 81 and 90.

² *This Journal*, 5, 917.

³ *Ark. Bull.* No. 53; U. S. Dept. Agr., Bur. of Chem., No. 81 and No. 90.

⁴ "The Proteins of the Wheat Kernel."

⁵ *Ark. Bull.* No. 53.

referee on vegetable proteins for the A. O. A. C.¹ with a view to its final adoption. The second series of results was obtained from a modified method of the first where the coagulable nitrogen was considered. The third series of results was obtained from a still further differentiation than that given in the second method in that the alcohol-soluble in the salt-soluble which is coagulable was considered. Besides this a series of results are given according to Method III where for comparison a 10 per cent sodium chloride solution was used instead of the 1 per cent solution.

Method I—Instead of using 5 g. of flour and 250 cc. of one per cent salt solution twice the amount was used. The mixture was shaken at intervals of 5 minutes for the first two hours and then let stand for 24 hours when filtered clear. Nitrogen determinations in the total and phosphotungstic acid precipitates were made and the difference between these two determinations was regarded as amide nitrogen. The results were as follows:

TABLE I—1 PER CENT SALT-SOLUBLE NITROGEN IN FLOUR (PERCENTAGES)

Number	Total N	Precipitated N	Amide N
O. C.	0.43	0.39	0.04
R. W. B.	0.38	0.29	0.09
B. L.	0.33	0.30	0.03
P.	0.50	0.41	0.09
L. C.	0.34	0.31	0.03
M. 15.	0.40	0.36	0.04

The larger part of the nitrogen carrying bodies soluble in 1 per cent salt solution are precipitated by phosphotungstic acid. In the proposed method there is no differentiation made between the gliadin and edestin and leucosin nitrogens soluble in 1 per cent salt solution.

Method II—Instead of precipitating the salt-soluble proteins with phosphotungstic acid directly, aliquots of the salt-soluble were boiled down to within 20 cc. of liquid. The precipitate formed was filtered off and washed with water. The resulting filtrate was then treated with sufficient phosphotungstic acid, thoroughly mixed and allowed to stand one hour, when the resultant precipitate was removed and washed with water containing phosphotungstic acid. Nitrogen determinations were made in the total and the two precipitates. The difference of the sum of the nitrogens from these precipitates and the total nitrogen was reckoned as amide nitrogen. According to this method of procedure, the following results were obtained:

TABLE II—DIFFERENTIATION OF THE COAGULABLE NITROGEN IN 1 PER CENT SALT-SOLUBLE (PERCENTAGES)

Number	Total N	Coagulable N	Pptd. N	Amide N
O. C.	0.43	0.130	0.250	0.050
R. W. B.	0.38	0.045	0.230	0.105
B. L.	0.33	0.055	0.240	0.035
P.	0.50	0.125	0.275	0.100
L. C.	0.34	0.120	0.230	0.000
M. 15.	0.40	0.065	0.270	0.065

Since gliadin is appreciably soluble in 1 per cent salt solution, one would expect to find this material included with what has been generally estimated as albumin and globulin. The resulting data given in Table II do not show what portion of the nitrogenous

bodies are gliadin bodies. Method II is better for the separation of the protein bodies soluble in one per cent salt solution than Method I, because the nitrogenous bodies coagulable by heat are separated from those not coagulable. The amounts of amide nitrogen obtained by difference in this method are practically identical with the amounts obtained in Method I.

The writer found in earlier investigations that it was possible to separate gliadin from the other salt-soluble proteins, and as a result of these investigations the following method for the estimation of the edestin and leucosin fractions in flour was adopted.

Method III—200 cc. aliquots of salt-soluble (10 grams of flour digested with 500 cc. of 1 per cent sodium chloride) were boiled down to within 20 cc. of liquid, then slowly evaporated to dryness on an iron hot plate. The solid was digested with 100 cc. of 55 per cent alcohol¹ by volume, filtered, then washed with same strength alcohol. The precipitate obtained was regarded as albumin. The alcohol filtrate was evaporated to within 10 cc. and 50 cc. of water added, boiled down to 35 cc., 15 cc. more water added, allowed to cool to room temperature and then filtered. The coagulum was washed with cold distilled water and reckoned as gliadin nitrogen. The coagulum filtrate was precipitated with phosphotungstic acid and reckoned as globulin nitrogen. The sum of the nitrogens obtained in the precipitates deducted from the total nitrogen leaves a small amount of unaccounted-for nitrogen which may be regarded as amide nitrogen. In Table III are given the results found according to this method.

TABLE III—INCLUDES THE ESTIMATION OF THE GLIADIN NITROGEN IN 1 PER CENT SALT-SOLUBLE (PERCENTAGES)

Number	Total N	Coag. N Insol. in alcohol	Gliadin N	Pptd. N	Amide N
O. C.	0.43	0.070	0.100	0.190	0.070
R. W. B.	0.38	0.080	0.095	0.140	0.065
B. L.	0.33	0.060	0.115	0.125	0.030
P.	0.50	0.125	0.160	0.165	0.050
L. C.	0.34	0.075	0.135	0.130	0.000
M. 15.	0.40	0.080	0.085	0.175	0.060

According to the results found and recorded in Table III it will be noted that the largest amount of nitrogen material soluble in 1 per cent salt solution was precipitated by phosphotungstic acid, which is nearly double that found in the coagulable insoluble in alcohol. The per cent of the alcohol-soluble which is coagulable is as much or more than the amount of nitrogen material insoluble in alcohol. In other words, there is very little nitrogen material extracted with 1 per cent salt solution that is not soluble in 55 per cent alcohol by volume.

In another series of experiments the same flours were digested with 10 per cent sodium chloride instead of 1 per cent solution. The method of procedure for the separation of the different nitrogen bodies was the same as that given in Method III. Owing to the large amount of salt present the alcohol separation method was tedious and slow. The various fractions for the nitrogen bodies soluble in 10 per cent salt solution are recorded in Table IV.

¹ See proceedings of Association, issued as bulletins, Bureau of Chemistry, Department of Agriculture.

¹ Fifty per cent alcohol is just as efficient as alcohol of higher strengths.

TABLE IV—INCLUDES THE ESTIMATION OF THE GLIADIN NITROGEN IN 10 PER CENT SALT-SOLUBLE (PERCENTAGES)

No.	Total N	Coag. N insol. in alcohol	Gliadin N	Pptd. N	Amide N
O. C.....	0.290	0.215	0.015	0.043	0.017
R. W. B.....	0.210	0.175	0.010	0.005	0.020
B. L.....	0.160	0.125	0.005	0.025	0.005
P.....	0.310	0.268	0.010	0.020	0.012
L. C.....	0.220	0.165	0.023	0.008	0.024
M. 15.....	0.260	0.203	0.007	0.040	0.010

As would naturally be expected, the per cent of nitrogen extracted with a 10 per cent salt solution is considerably less than that extracted with a 1 per cent salt solution. The reason for a much lower yield of nitrogen bodies is due to the increased concentration of the solute. It will be noted from the data given in Table IV that there is very little nitrogen material extracted which is soluble in 55 per cent alcohol by volume, and about 80 per cent of the total proteins soluble in 10 per cent salt solution are coagulated upon boiling down or increasing the salt concentration.

When the data for the total nitrogen and the various fractions obtained in the 1 per cent salt extraction are compared with those obtained in the 10 per cent salt extraction (see data in Tables III and IV), it will be noted that the amount of nitrogen material separated varies in any one group, depending upon the concentration of the solvent used for extracting these bodies. In the one case it is found that there is considerable gliadin and phosphotungstic acid precipitated nitrogen extracted, while in the other only a limited quantity is found which is not directly coagulable upon boiling the solution. The large amount of nitrogen bodies extracted by 10 per cent salt solution insoluble in 55 per cent alcohol by volume are insoluble in this strength of alcohol, owing to the concentration of the salt present. In Table V the sum of the figures given in Tables III and IV for alcohol insoluble and phosphotungstic acid precipitated nitrogen are compared with each other. In addition the total nitrogen in the 10 per cent salt solution with the gliadin correction has been included.

TABLE V—RESULTS FOR ALCOHOL INSOLUBLE AND PRECIPITATED NITROGEN IN 1 AND 10 PER CENT SALT-SOLUBLE (PERCENTAGES)

No.	1 Per cent NaCl	10 Per cent NaCl	
	Alcohol insol. and pptd. N	Alcohol insol. and pptd. N.	Salt-sol. N — gliadin N
O. C.....	0.260	0.258	0.275
R. W. B.....	0.220	0.180	0.200
B. L.....	0.185	0.150	0.155
P.....	0.290	0.288	0.300
L. C.....	0.205	0.173	0.197
M. 15.....	0.255	0.243	0.253

Having a method for estimating the gliadin nitrogen that may be extracted from wheat or its product by salt solution, it will be noted from the data presented in Table V that the amount of edestin and leucosin nitrogen is the same whether the concentration of sodium chloride be 1 or 10 per cent, providing the gliadin nitrogen is accounted for.

Referring again to the data given in Tables III and IV, it will be noted that the amount of nitrogen insoluble in 55 per cent alcohol in the 1 per cent salt extract is quite small and that of the nitrogen bodies precipitated by phosphotungstic acid is very large, while in

case of the 10 per cent salt solution the per cent alcohol insoluble nitrogen is very large while only a noticeable amount of nitrogen is found in the phosphotungstic acid precipitate. These facts naturally raise the question, Are we dealing with true globulins and albumins? The quantitative data obtained and the method of procedure followed indicate that the substances spoken of as globulin and albumin are only such in 10 per cent sodium chloride solution, while in lower per cents of sodium chloride there is a smaller quantity of such material.

Again, if the substances extracted from flour with 10 per cent salt solution are globulin and albumin, then either one or both of these two materials in less concentrated salt solution, or water, are wholly soluble or partly soluble in 55 per cent alcohol or alcohol of higher strengths. This thought has weight in the preceding article on the estimation of the gliadin nitrogen in flour where it was found that approximately 68 per cent of the alcohol-soluble was coagulable by the direct method and corroborated in the indirect method of procedure and subsequent analyses. The data given for the alcohol-soluble uncoagulable nitrogen, where different strengths of alcohol were used, should also be considered in connection with this view.

There is sufficient reason to believe that the globulin and albumin of wheat are appreciably soluble in diluted alcohols even in the presence of 1 per cent sodium chloride solution. And since gliadin is appreciably soluble in 1 per cent salt solution, the methods that have been followed in the past where 1 per cent salt solution is used, includes gliadin as a part of globulin and albumin nitrogen and in the direct alcohol extraction of flour albumin and globulin are included as a part of the gliadin nitrogen. With 10 per cent salt solution only a very small part of the gliadin nitrogen is considered.

The amount of gliadin nitrogen extracted in salt solution depends upon the amount of salt used, being approximately 90 per cent more in a 1 per cent sodium chloride solution than was found to be the case in 10 per cent sodium chloride. The fact that the gliadin nitrogen can be removed from the salt solution regardless of the strength of salt used up to and including 10 per cent sodium chloride, makes the 1 per cent salt extraction method, owing to the more rapid filtering, more rapid digestion, etc., the most desirable one to follow. Omitting the determination for gliadin nitrogen and making the one for amide nitrogen in its place, renders the method of procedure for the estimation of edestin and leucosin nitrogen less difficult and is as follows:

Digest 10 grams of flour with 500 cc. of 1 per cent sodium chloride solution, shaking at intervals of 5 minutes for the first two hours, then allow to stand over night in a cool place. Filter clear and use aliquots of 200 cc. each. Boil to within 20 cc. of liquid; then slowly evaporate to dryness on iron hot plate. Digest the solid mass in each beaker with 100 cc. portions of 55 per cent alcohol by volume, filter, wash precipitate on filter paper with same strength alcohol. Determine nitrogen in this precipitate by the Kjeldahl method,

and after correcting for filter paper, etc., by blank determinations, the difference found should be regarded as albumin nitrogen.¹

Evaporate the alcohol filtrate to within 10 cc., add 50 cc. of distilled water, boil down to 35 cc., then add 15 cc. more of distilled water, allow to cool to room temperature and filter. Wash precipitate with cold distilled water. Owing to the sticky nature of this material and the difficulty in transferring the same to the filter paper, it is not advisable to make this determination.

The resulting filtrate obtained in the gliadin separation is treated with sufficient phosphotungstic acid to cause precipitation of the albumin filtered and washed with distilled water containing phosphotungstic acid. Nitrogen determinations in this precipitate should be corrected for by running blanks containing filter paper washed in phosphotungstic acid. The nitrogen obtained by difference is regarded as globulin nitrogen.

The nitrogen found in the phosphotungstic acid precipitate filtrate when properly corrected for is considered amide nitrogen. The total nitrogen found as amide, globulin and albumin when deducted from the total salt-soluble nitrogen is gliadin nitrogen extracted in the 1 per cent salt solution.

CONCLUSIONS

1. One per cent sodium chloride extracts from flour edestin, leucosin and gliadin.
2. The amount of gliadin extracted by 1 per cent sodium chloride solution approximately amounts to about 29 per cent of the total proteins.
3. The amount of gliadin extracted by 10 per cent sodium chloride solution approximately amounts to about 5 per cent of the total proteins.
4. The gliadin nitrogen extracted by solutions containing sodium chloride can be determined and corrected for in making edestin and leucosin nitrogen estimations of flour and its products.
5. The amount of nitrogen bodies extracted with salt solutions and directly coagulated by heat varies with the concentration of the solvent. The 10 per cent concentration gives higher results than was found possible with a 1 per cent salt solution.
6. The nitrogen bodies soluble in salt solution are partly or wholly soluble in diluted alcohols varying with the concentration of the sodium chloride used.

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AN INVESTIGATION OF THE PRESENCE OF FURFURAL IN CIDER VINEGAR

By AGNES A. ANDERSON

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That pure cider vinegar contains volatile reducing substances has been known for some time and several methods have been given² for eliminating these substances in order to get the true amount of reducing sugars. The presence of furfural in these volatile reducing substances was discovered while doing routine vinegar analyses in the Kansas State Food Laboratory.

¹ Globulin does not coagulate at temperatures below 100° C. (see "Proteins of Wheat Kernel," Osborne, Carnegie issue, p. 116).

² THIS JOURNAL, 6, 845, 928.

Since nothing on the subject could be found in the literature an investigation was begun to determine its presence in normal cider vinegars. The results seem to warrant publication at this time.

Twenty-eight samples of cider vinegar of known purity were tested for furfural by the aniline acetate method as given by Leach.¹ Nineteen gave a positive test, nine being negative. These vinegars were made at the laboratory and their history known. In addition to these samples some farmers' vinegars were tested with similar results. From these results it is seen that furfural may be present normally in cider vinegars and its presence does not necessarily indicate added wood acetic acid.

In the *Abstract Journal* (7, 666) appeared a method by Ronnet for detecting added caramel in vinegar. This method is based upon the formation of furfural when sugar is heated in the process of making caramel, and makes use of the Fiehe resorcinol reagent.²

Since it appears that furfural is normally present in some cider vinegars, its presence can again be said not to indicate adulteration from caramel. This test for caramel was applied to the vinegars of known purity and in every case an affirmative result was obtained. Four of the samples tested for caramel did not give a test for furfural upon distillation, but gave a very positive caramel test. This may be due to decomposition of the sugar during the drying on the water bath. The water bath was carefully maintained at a temperature below 70° C. to avoid this possible decomposition, so this fact adds to the unreliability of the method. Caramel was added to some of the vinegars, but the resulting tests were not different from those to which none had been added.

TABLE I

No.	Variety	Furfural	No.	Variety	Furfural
P1	Winesap Pomace...	Neg.	18	Gano.....	Aff.
P2	Pomace.....	Neg.	P18	Gano Pomace.....	Aff.
3	Jeneton.....	Neg.	19	Jeneton.....	Aff.
P3	Jeneton Pomace...	Neg.	20	Winesap.....	Neg.
6	Jeneton.....	Aff.	21	Jeneton.....	Neg.
7	Clayton.....	Aff.	22	Roman Beauty.....	Neg.
8	Black Twig.....	Aff.	23	Winesap.....	Aff.
9	York Imperial.....	Aff.	24	Black Twig.....	Aff.
10	Ben Davis.....	Aff.	25	Missouri Pippin.....	Aff.
P10	Ben Davis Pomace..	Aff.	A	Unknown.....	Aff.
12	Limbirtwig.....	Aff.		Pomace of above ...	Neg.
P12	Limbirtwig Pomace.	Aff.	70284	Farmers' Vinegar....	Aff.
14	Winter Greening....	Aff.		Rotten apples.....	Neg.
15	York Imperial.....	Aff.	70284A	Farmers' Vinegar....	Neg.
17	Winesap.....	Aff.	9373	Farmers' Vinegar....	Aff.
P17	Winesap Pomace....	Neg.	5159	Farmers' Vinegar....	Neg.

TABLE II

No.	Variety	Caramel	Furfural test
A	Unknown.....	Good test	Aff.
9	York Imperial.....	Good test	Aff.
	York Imperial + Caramel....	Good test	
70284	Farmers' Vinegar	Good test	Aff.
5159	Farmers' Vinegar.....	Good test	Neg.
9373	Farmers' Vinegar.....	Good test	Aff.
	Rotten Apples.....	Good test	Neg.
70284A	Farmers' Vinegar.....	Good test	Neg.
21	Jeneton.....	Good test	Neg.

The above tables give the results; duplicate determinations are not included since in every case the results checked.

¹ Leach, "Food Inspection and Analysis," p. 777.

² Bur. of Chem., Bull. 154, 15.