

ON THE CHEMICAL CHANGES PRODUCED IN  
FLOUR BY BLEACHING.

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WHEN flour is shaken with air containing a small quantity of nitrogen peroxide gas, the gas is almost immediately absorbed, and the golden-yellow tint of the flour is destroyed. If considerable quantities of the gas are used the flour acquires at the same time a musty odour, which increases with the amount of gas absorbed.

In starting a series of laboratory experiments to determine the changes which may take place in the nature of the flour owing to this treatment, it was necessary to employ a substantial quantity of the gas, considerably more than, as appears from the examination of recent trade samples, is at present employed in British flour mills. Judged by a series of results obtained in the examination of commercial flours for nitrites, it would seem that the bleaching at present adopted in the trade may be effected by gas which on the average is applied only in the proportion of 1 to 2 c.c. of nitrogen peroxide per kilogramme of flour. Inquiries were made at several mills, and the composition of the gas at the inlet and outlet of the agitator where the bleaching takes place was ascertained by analysis in several instances. No data were, however, available as to the quantity of flour treated per unit of gas,

except in one instance. Here the data, while too uncertain to warrant any definite conclusion as to the amount of gas absorbed by the flour, tended generally to show that this was of the same order as that indicated by the results obtained in the examination of commercial flour for nitrites. Considerably larger quantities, such as 20 to 30 c.c. of nitrogen peroxide per kilogramme of flour, appear to have been used in the past, and the possibility of local overbleaching has to be borne in mind.

For the purpose of the inquiry, it seemed desirable to ascertain by laboratory experiment how far the degree of bleaching was affected by a progressive increase in the quantity of nitrogen peroxide used and at what point the maximum bleaching effect could be produced, so that the experimental work, though carried out with larger quantities than are at present used by the millers, might nevertheless be restricted so far as possible to quantities which can be considered to be of possible utility in bleaching processes.

The method adopted in the laboratory for treating flour with accurately measured quantities of nitrogen peroxide gas was as follows:—

200 grms. of a freshly-milled high grade patent flour were introduced into a  $2\frac{1}{2}$  litre vessel fitted with a rubber stopper carrying a capillary tube with glass cock, and a measured quantity of pure nitric oxide gas introduced from a gas-holder. Red fumes of nitrogen peroxide were formed in the vessel, and on shaking for a few minutes were absorbed by the flour. To arrive at the actual quantity of gas taken up by the flour, the vessel was connected with another of equal capacity which had previously been exhausted as completely as possible by a Fleuss pump. Approximately half of the nitrogen peroxide still remaining in the bleaching vessel was in this way collected in the second vessel, in which it was absorbed by shaking with dilute caustic soda solution, and the nitrogen estimated as ammonia by means of Nessler's solution after reduction with aluminium foil and subsequent distillation.

The nitric oxide used was prepared by heating a solution of ferrous sulphate which had previously been saturated with the gas evolved from nitric acid and copper turnings, and contained about 99% of NO. It was stored in a gas-holder over water, and measured in a mercury burette. In the following table, Column I gives the number of c.c. of nitric oxide used per kilogramme of flour; Column II the corresponding weight of nitrogen, after correcting for atmospheric

pressure (753 mm.), temperature (25° C.), moisture, and 1% impurity in the gas; Column III the amount of gas remaining unabsorbed under the conditions of the experiment; and Column IV the nitrogen actually absorbed by the flour as nitrogen peroxide. 200 grammes of flour were used in every case.

NO introduced: c.c. per kgm. flour	NO introduced: grammes Nitrogen per kgm. flour (corrected)	Residual NO <sub>2</sub> : grammes Nitrogen per kgm. flour	NO <sub>2</sub> absorbed: grammes Nitrogen per kgm. flour
5	·00272	·0016	·0011
10	·00544	·0014	·0040
15	·00817	·0018	·0064
20	·01089	·0026	·0083
30	·01633	·0030	·0133
40	·02178	·0026	·0192
60	·03266	·0050	·0277
100	·05445	·0042	·0502
160	·08711	·0030	·0841
230	·12523	·0070	·1182
300	·16335	·0054	·1579

The completeness of the absorption appears to depend upon the length of time the shaking is continued, upon the relative volume of the bleaching vessel compared with the amount of flour present, and upon the amount of bleaching gas used. The absorption is practically complete on prolonged shaking in a small vessel.

The bleached samples were kept in the dark in loosely stoppered glass jars, or in glazed paper bags, and the following series of experiments carried out:—

- (i) Examination of colour by Lovibond's tintometer.
- (ii) Determination of nitrous acid and nitrites by the Griess-Ilosvay method.
- (iii) Determination of nitrogen recoverable as ammonia by reduction of the aqueous extract of the flour with a copper-zinc couple, and subsequent distillation.
- (iv) Determination of the total nitrogen rendered soluble by bleaching, and of the cold water extract of the flour.
- (v) Characteristics of the oil from bleached and unbleached flour.
- (vi) Measurement of the decrease in volume of the air in the bleaching vessel, due to absorption of nitrogen peroxide, with a view to ascertaining whether free nitrogen is produced during bleaching, and tests for the presence of diazo-compounds.
- (vii) Action of dilute nitric acid of various strengths upon pure glutenin and gliadin.

(viii) Salivary digestion of bleached and unbleached flour, and of starch treated with varying amounts of sodium nitrite and nitrogen peroxide.

## I.—COLOUR.

The samples of bleached flour were examined as to colour in a Lovibond tintometer, being pressed into small wooden trays so as to give a flat surface, and then "Pekardised," *i.e.*, dipped carefully into water and slowly withdrawn. All the bleached samples were practically the same colour, the following being the colour equivalents in Lovibond's units of the unbleached flour, the same flour as bleached at the mill, and as bleached in the laboratory.

	Yellow	Red
Unbleached ... ..	1·20	·60
Bleached at mill ... ..	1·05	·57
Bleached in laboratory ... ..	·90	·50

The laboratory bleached samples were kept in the dark in loosely stoppered glass jars, and examined again by the tintometer after the lapse of 17 days, when the colour equivalents in Lovibond units were as follows:—

	Yellow	Red
Unbleached flour ... ..	1·20	·60
5 c.c. NO per kgm. flour ... ..	·90	·50
10 " " " " " " " " " " " "	·85	·47
15 " " " " " " " " " " " "	·82	·46
20 " " " " " " " " " " " "	·80	·46
30 " " " " " " " " " " " "	·76	·45
40 " " " " " " " " " " " "	·75	·45
60 " " " " " " " " " " " "	·75	·45
100 " " " " " " " " " " " "	·75	·45
160 " " " " " " " " " " " "	·80	·45
230 " " " " " " " " " " " "	·85	·45
300 " " " " " " " " " " " "	·90	·45

From the above table it will be seen that a considerable change takes place in the colour on keeping. The majority of the bleached samples tend to become still whiter, the maximum of bleaching effect being attained with 30 to 100 c.c. of gas per kilogramme, while with quantities greater than this there is a gradual increase of yellow, but not of red.

## II.—NITROUS ACID AND NITRITES.

A large number of analyses have been published giving the amount of nitrites in the cold water extract from bleached flours (cf. Dr Hamill's report). Extremely small quantities of nitrites can be detected by the Griess-Ilosvay method in which the colour produced with sulphanilic acid and  $\alpha$ -naphthylamine hydrochloride in acetic acid solution is compared with standards containing known amounts of sodium nitrite. It has been stated that up to a certain point the amount of nitrogen present as nitrite corresponds to the amount of nitrogen in the bleaching gas employed, but that above this point the ratio of nitrite nitrogen to total nitrogen absorbed decreases, becoming still smaller on keeping the flour for several days. With a view to ascertaining the ratio of nitrogen present as nitrous acid or nitrites to the total nitrogen absorbed, and also the rate of disappearance of the nitrites, weighed quantities of the samples were mixed with ten times their weight of well boiled distilled water free from nitrites<sup>1</sup> at the ordinary temperature, allowed to stand for 12 hours with frequent shaking, and centrifuged. Nitrite estimations were then carried out on the clear liquor by the Griess-Ilosvay method. Three series of determinations were made, the first on the samples exactly one hour after bleaching, the second on the same samples after keeping for 20 days, and the third on the same samples after the lapse of 62 days. In the first series the quantity of nitrogen present as nitrites was found to be proportional to the quantity of bleaching gas employed. In the lower members of the series the amount corresponded to about 30% of the total quantity of nitrogen absorbed by the flour, rising to about 40% in the more highly bleached samples. On keeping for 20 days the amount of nitrite present remained approximately the same in the samples bleached with 5 to 60 c.c. of gas per kilogramme, but decreased considerably in the higher members of the series. After keeping for 62 days the nitrites had decreased in amount very greatly, except in the lowest members of the series, while the samples bleached with 230 and 300 c.c. of gas per kilogramme contained slightly less nitrite than those bleached with 100 and 160 c.c. of gas.

<sup>1</sup> It is not easy to obtain distilled water quite free from nitrites. In examining commercial samples of flour it is preferable to use ordinary tap water as this can generally be obtained free from any traces of nitrites.

The following table gives the amount of nitrogen as nitrous acid or nitrites per kilogramme of flour compared with the total quantity of nitrogen absorbed by the flour:—

c.c. Nitric Oxide per kgm. of flour	NO <sub>2</sub> absorbed, grammes N per kgm. flour	Nitrogen present as Nitrites or Nitrous Acid					
		One hour after bleaching		20 days after bleaching		62 days after bleaching	
		Grammes N per kgm. flour	Pts. NaNO <sub>2</sub> per million flour	Grammes N per kgm. flour	Pts. NaNO <sub>2</sub> per million flour	Grammes N per kgm. flour	Pts. NaNO <sub>2</sub> per million flour
0	—	nil	nil	nil	nil	nil	nil
5	·0011	·0008	4	·0006	3	·0006	3
10	·0040	·0012	6	·0010	5	·0010	5
15	·0064	·0020	10	·0014	7	·0012	6
20	·0083	·0025	12·5	·0024	12	·0016	8
30	·0133	·0040	20	·0040	20	·0022	11
40	·0192	·0060	30	·0060	30	·0030	15
60	·0277	·0090	45	·0084	42	·0035	17·5
100	·0502	·0180	90	·0126	63	·0050	25
160	·0841	·0300	150	·0184	92	·0050	25
230	·1182	·0450	225	·0200	100	·0040	20
300	·1579	·0660	330	·0206	103	·0035	17·5

### III.—NITROGEN RECOVERABLE AS AMMONIA BY REDUCTION OF THE AQUEOUS EXTRACT.

When nitrogen peroxide is absorbed by a powder, such as flour, containing a small quantity of moisture, the first result should be combination with the water present to form nitric and nitrous acids. If no further action takes place, the whole of the nitrogen present as nitric and nitrous acids should be recoverable from the aqueous extract of the flour by reduction to ammonia and subsequent distillation, as in the ordinary estimation of nitrates in water analysis. With this object in view weighed quantities of the samples were mixed with ten times their weight of ammonia-free distilled water and thoroughly shaken for several hours in a rotary shaking apparatus. They were then centrifuged as clear as possible, and 200 c.c., corresponding to 20 grammes of flour, treated with a Gladstone-Tribe copper-zinc couple in a stoppered bottle at 37° C., 1 c.c. of toluol being added to prevent decomposition<sup>1</sup>. The couples were prepared by immersing a piece of

<sup>1</sup> In this connection it is interesting to note that bleaching may exercise a considerable preservative action on flour. In a series of extracts containing no toluol the unbleached or slightly bleached samples became sour very much sooner than those which had been highly bleached. (Cf. E. Fleurent, *Bull. Soc. Chim.* [iii.], xxxv. pp. 381–396. 1906.)

sheet zinc measuring  $12 \times 5$  cm. in a 3% copper sulphate solution for 15 minutes, and afterwards washing in distilled water. The bottles were allowed to remain in the incubator until the liquid ceased to give a reaction for nitrites, generally about one or two days. The contents were then diluted to 500 c.c. with ammonia-free distilled water, one gramme of calcium carbonate added, and the ammonia distilled off and estimated colorimetrically by Nessler's solution. Unbleached flour naturally yields a small quantity of ammonia on reduction in this way<sup>1</sup>, and accordingly a blank experiment was always carried out with the unbleached flour under exactly the same conditions, and the amount of ammonia obtained subtracted from that obtained from the bleached samples. It was found that calcium carbonate will liberate the whole of the ammonia from very dilute solutions of ammonium salts on boiling, without acting on the proteins in solution, whereas if a stronger alkali is used a considerable quantity of ammonia is formed by protein decomposition. The following table gives the amount of nitrogen recovered as ammonia compared with the nitrogen actually absorbed by the flour as  $\text{NO}_2$  :—

c.c. Nitric Oxide per kgm. flour	$\text{NO}_2$ absorbed, grammes N per kgm. flour	Nitrogen recovered as Ammonia : Grammes Nitrogen per kgm. flour			
		One hour after bleaching		22 days after bleaching	
		Total quantity	Subtracting blank	Total quantity	Subtracting blank
0	—	·027	nil	·028	nil
5	·0011	·028	·001	·028	nil
10	·0040	·029	·002	·028	nil
15	·0064	·030	·003	·028	nil
20	·0083	·032	·005	·028	nil
30	·0133	·036	·009	·030	·002
40	·0192	·038	·011	·034	·006
60	·0277	·044	·017	·038	·010
100	·0502	·058	·031	·044	·016
160	·0841	·076	·049	·056	·028
230	·1182	·096	·069	·068	·040
300	·1579	·124	·097	·080	·052

It will be seen from the above results, that the nitrogen recoverable as ammonia is approximately proportional to the total nitrogen absorbed as  $\text{NO}_2$ , and amounts, in the case of the samples examined soon after bleaching, to about 60% of the total nitrogen absorbed, falling to about 33% after the lapse of 22 days.

The error of experiment is of course relatively great, especially in the lower members of the series, but the results indicate that the

<sup>1</sup> Cf. J. E. Purvis and R. M. Courtauld, *Proc. Camb. Phil. Soc.* xiv. pp. 441–446. 1908.

greater part of the nitrogen peroxide absorbed by the flour can be recovered as ammonia by reduction of the aqueous extract, and may therefore be assumed to be present as nitric and nitrous acids or as nitrates and nitrites. The nitric acid seems to disappear more quickly than the nitrous acid, since after keeping the flour 22 days the nitrogen recovered as ammonia is approximately equal to that present as nitrous acid or nitrites, except in the case of the three most highly bleached samples. As will be shown later, the oil of the flour absorbs about 6 to 7% of the nitrogen peroxide added, so that somewhat over 30% of the gas remains to be accounted for. This may possibly be present as nitric or nitrous acid in combination with, or mechanically "adsorbed" by, the gluten or starch of the flour, and so escape extraction with cold water. The quantity of water used for extraction did not appear to affect the results. Flour bleached with 300 c.c. of gas per kilogramme was extracted with 100, 200, 400 and 800 c.c. of water per 10 grammes of flour, and the extracts treated with copper-zinc couples, blank experiments being carried out in the same way on the unbleached flour. The amount of ammonia obtained increased with the dilution, but the difference, representing the excess of ammonia due to bleaching, was approximately the same in all cases as the following table shows:—

Flour bleached with 300 c.c. NO per kilogramme, corresponding to an actual absorption of 0.1579 grms. N per kilogramme:—

Number of grammes flour corresponding to one litre extract	Ammonia from bleached flour, grammes N per kgm.	Ammonia from unbleached flour, grammes N per kgm.	Difference, grammes N per kgm.
100	.130	.056	.074
50	.160	.075	.085
25	.176	.088	.088
12.5	.208	.128	.080

The same flour was also extracted in 10% solution immediately after bleaching and compared with the sample extracted one hour after bleaching, the same amount of ammonia being obtained in both cases.

#### IV.—TOTAL NITROGEN RENDERED SOLUBLE BY BLEACHING, AND COLD WATER EXTRACT OF THE FLOUR.

During the reduction of the aqueous extracts by the copper-zinc couple it was noticed that a white precipitate was formed in the liquid, the solution becoming quite opaque, and that this precipitate increased considerably with the amount of gas employed for bleaching. This appeared to be due to the gradual precipitation of proteins by the

ammonia formed in the solution, and it seemed desirable to ascertain the effect of bleaching on the solubility of the proteins of flour. Estimations of total soluble nitrogen and soluble extract were therefore carried out on all the samples. Weighed quantities of bleached flours were shaken with ten times their weight of water, with addition of toluol, in a rotary shaking machine on two or three successive days at the ordinary temperature, and the extracts obtained as clear as possible by means of a centrifuge. In these extracts, total nitrogen was estimated on 40 c.c. by Kjeldahl's method and total solids by evaporating 40 c.c. in nickel basins on the water bath and drying in a water-oven at 96° C.

Two series of estimations were done, the first on a patent flour bleached 21 days after milling, the extracts being made one hour after bleaching, and the second series on the same flour bleached the day after milling, and extracted 16 days after bleaching. Blank estimations were in both cases carried out on the unbleached flour. The results are given in the following table:—

c.c. Nitric Oxide per kgm. flour	Flour bleached three weeks after milling and extracted one hour after bleaching			Flour bleached one day after milling and extracted 16 days after bleaching		
	Total soluble Nitrogen, grammes per kgm.	Soluble Proteins (N×57), grammes per kgm.	Extract, grammes per kgm.	Total soluble Nitrogen, grammes per kgm.	Soluble Proteins (N×57), grammes per kgm.	Extract, grammes per kgm.
0	4.16	23.7	81.3	3.01	17.1	96.0
5	4.16	23.7	81.5	3.13	17.8	96.3
10	4.27	24.3	81.3	3.15	18.0	96.6
15	4.10	23.4	80.6	2.99	17.0	96.9
20	4.27	24.3	83.1	3.19	18.2	97.8
30	4.31	24.6	83.9	3.32	18.9	99.0
40	4.37	24.9	85.2	3.26	18.6	101.4
60	4.55	25.9	88.0	3.29	18.8	105.1
100	5.25	29.9	94.0	3.50	20.0	110.6
160	6.37	36.3	103.9	4.88	27.8	127.0
230	7.84	44.7	118.9	5.98	34.1	138.9
300	8.82	50.3	127.6	7.31	41.7	145.6

From the above it will be seen that although in the lowest members of the series an increase in soluble nitrogen and total extract can hardly be detected, in the more highly bleached samples a very large amount of nitrogen is rendered soluble<sup>1</sup>, far more than can be accounted for by

<sup>1</sup> The action of extremely dilute acids and alkalis on the gluten of flour has been investigated by Wood and Hardy (*Proc. Roy. Soc.* 1909, Series B, lxxxii. pp. 38-43), and will be referred to again when dealing with the action of dilute nitric acid on pure glutenin and gliadin.

the nitrogen introduced into the flour during bleaching. Moreover the increase in total soluble matter is greater than the increase in soluble proteins, assuming the nitrogen to be present in the form of protein.

Subsequent experiments on the solubility of the glutenin and gliadin of wheat flour in very dilute nitric acid confirmed the above results as regards the increase of soluble proteins. The aqueous extracts were therefore further investigated in order to account, if possible, for the still greater increase of total soluble matter.

Two of the extracts, one from the unbleached flour and one from the same flour bleached with 300 c.c. of gas per kilogramme and extracted 16 days after bleaching, were treated with mercuric chloride and alumina cream to remove proteins, the mercury removed from the filtered liquids by sulphuretted hydrogen, and the resulting solutions, measuring 75 c.c., boiled for 15 minutes with  $12\frac{1}{2}$  c.c. of concentrated hydrochloric acid. They were then cooled, neutralised, made up to 100 c.c., and 10 c.c. titrated for dextrose by Ivar Bang's method (*Biochem. Zeitsch.*, II. p. 271, 1906). The amount of dextrose found corresponded in the unbleached sample to 54.0 and in the bleached sample to 81.5 grammes per kilogramme of flour. Calculated to dextrin this gives 48.6 and 73.3 grammes dextrin per kilogramme respectively.

It appears therefore that a considerable amount of carbohydrate as well as protein is rendered soluble by bleaching. The following table shows the relation of carbohydrate to protein in the extract:—

	Total soluble matter, grammes per kgm. flour	Soluble proteins, grammes per kgm. flour	Soluble carbohydrate as Dextrin, grammes per kgm. flour	Dextrin + proteins grammes per kgm.
Unbleached flour	96.0	17.1	48.6	65.7
Bleached flour	145.6	41.7	73.3	115.0
Difference	49.6	24.6	24.7	49.3

It has been pointed out (Allen, *Commercial Organic Analysis*, 1898, IV. p. 79) that a constant ratio exists between the proportion of soluble proteins and soluble carbohydrates in flour. From the above results it appears that the actual increase in soluble carbohydrate due to bleaching is approximately equal to the increase in soluble protein, while the original ratio of soluble carbohydrate to soluble protein decreases. The amount of soluble matter other than carbohydrate and protein does not appear to be altered by bleaching, being in the above case 30.3 grammes per kilogramme for the unbleached, and 30.6 grammes per kilogramme for the bleached flour.

## V.—CHARACTERISTICS OF THE OIL FROM BLEACHED AND UNBLEACHED FLOUR.

One kilogramme of a patent flour was extracted with petroleum ether and the solvent evaporated off on a water bath in a current of carbonic acid gas in order to avoid oxidation of the oil. The oil was also extracted in the same way from samples of the same flour which had been bleached with 150 and 300 c.c. of gas respectively per kilogramme, the extraction being made one hour after bleaching. The oil from the unbleached sample was opaque and golden-yellow in colour, and gave an intense golden-yellow solution in petroleum ether and chloroform, while the oils from the bleached samples were reddish-yellow and gave an almost colourless solution in the same solvents. The following constants were obtained for the oils:—

c.c. NO <sub>2</sub> per kgm. flour	Iodine value (Hübl)	Saponification value (Köttstorfer)	Saponification Index (C. I.) <sup>1</sup>	Total Nitrogen
0	{ 102·2 101·8	170·0	303·6	1·12 per cent.
150	{ 98·7 100·3	170·1	303·8	1·20 „
300	{ 100·6 101·2	170·6	304·7	1·23 „

The total nitrogen was estimated by Jodlbauer's modification of Kjeldahl's method, using zinc dust and salicylic acid.

From the above it appears that if the oil is examined immediately after bleaching, no alteration except as regards colour, and a slight increase in the nitrogen content, can be detected. It being possible, however, that if any action on the oil takes place, it would be more evident after the lapse of several days from the time of bleaching, another sample of flour was bleached and allowed to stand in closed glass jars in the dark for seven days. The flour yielded 0·8% of oil (petroleum ether extract). 2000 grammes were bleached with 100 and 300 c.c. of gas per kilogramme respectively, and the oil extracted with petroleum ether. The solvent was evaporated off on the water bath under diminished pressure in a current of carbonic acid gas.

The oils showed the same characteristics in regard to colour as in the previous case, that from the unbleached flour being golden-yellow,

<sup>1</sup> The letters C.I. indicate the methods of expression recommended by the International Conference for the Unification of Methods of Analysis (Paris, 1910).

that from the flour bleached with 100 c.c. per kilogramme reddish-yellow, and that from the more highly bleached sample dark reddish-yellow. The viscosity had been increased by bleaching, especially with the more highly bleached sample which was exceedingly viscous.

As many of the constants as possible were determined with the limited quantity of material at disposal, and very great differences were observed, especially with the oil from the highly bleached sample. The following numbers were obtained on analysis of the oils:—

	Unbleached flour	100 c.c. NO <sub>2</sub> per kgm. flour	300 c.c. NO <sub>2</sub> per kgm. flour
Specific gravity $\frac{20^\circ}{15^\circ}$ C. ...	0.946	0.952	0.985
Iodine value (Hübl) ...	{ 96.7 97.1	{ 92.6 94.5	{ 60.7 61.9
Saponification value (Köttstorfer)	158.1	159.4	166.3
Saponification index (C.I.) <sup>1</sup> ...	282.4	284.7	297.0
Solidifying point of fatty acids ...	26.5° C.	28.0° C.	32.4° C.
Reichert-Wollny number ...	0.8	0.9	3.5
Acid value ...	12.5	11.2	12.4
Acidity in c.c. $\frac{N}{I}$ alkali per 100 grammes			
oil (C.I.) <sup>1</sup> ...	22.3	20.0	22.1
Total nitrogen ...	0.83 %	0.89 %	0.96 %

It is evident that a very considerable oxidation of the oil takes place when flour is highly bleached, although it is possible that the effects produced may be to a certain extent due to the "elaidin" reaction having taken place under the influence of the nitrous acid present. The length of time that the flour is kept after bleaching appears to be an important factor in the effect produced on the oil, and it is possible that even with slightly bleached flours the characteristics of the oil would be gradually altered by long storage. The increase in nitrogen corresponds roughly to about 6% of the total nitrogen absorbed by the flour in the form of nitrogen peroxide, and the same increase is also apparent in the oil extracted immediately after the flour has been bleached, although oxidation has not yet taken place<sup>2</sup>.

<sup>1</sup> The letters C.I. indicate the methods of expression recommended by the International Conference for the Unification of Methods of Analysis (Paris, 1910).

<sup>2</sup> Egoroff (*Journ. Russ. Phys. Chem. Soc.* 1903, xxxv. pp. 973-997), has shown that small quantities of nitrogen peroxide act on oleic acid giving an additive product, and it is only where this latter is present in a certain proportion, for the formation of which a certain minimum quantity of nitrogen peroxide is required, that the isomeric change of oleic into elaidic acid can take place.

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VI.—CHANGE OF VOLUME IN AIR CONTAINING NITROGEN PEROXIDE  
DUE TO ABSORPTION OF THE GAS BY FLOUR, AND TESTS FOR  
THE PRESENCE OF DIAZO-COMPOUNDS.

In view of the assertion that free nitrogen may be produced by the action of nitrogen peroxide on flour, the following experiment was devised with a view to ascertaining whether this is in fact the case.

200 grammes of flour were introduced into a glass globe of 1500 c.c. capacity, fitted with a capillary tube and glass cock at one side, and a wide tube with glass cock at the other, and the globe was placed in a water thermostat which could be kept constant to within  $0.2^{\circ}$  C. The capillary tube was connected with a water manometer, and the vessel allowed to remain in the thermostat until equilibrium was attained at atmospheric pressure. It was then removed from the thermostat, 60 c.c. of pure nitric oxide introduced, and the vessel well shaken. It was then replaced in the thermostat and allowed to attain equilibrium. On connecting again with the manometer a decrease in pressure was observed owing to the absorption of the nitrogen peroxide. Air was then introduced from a gas burette until the whole was again at atmospheric pressure.

Since 60 c.c. of nitric oxide combine with 30 c.c. of oxygen to give 60 c.c. of nitrogen peroxide, there will be a diminution in the volume of the whole system of 30 c.c. if the whole of the nitrogen peroxide is absorbed by the flour. If nitrogen or any other gas is evolved during bleaching, the diminution in volume will be less than 30 c.c. and less than 30 c.c. of air will have to be added to bring the whole to atmospheric pressure. The error due to changes in temperature may be considerable, but by keeping the thermostat constant to within  $0.2^{\circ}$  C., this error will not amount under the conditions of the experiment to more than 1 c.c. If the production of nitrogen were an essential part of the reaction the quantity produced would probably be considerably more than this when 60 c.c. of bleaching gas are employed. In no case could an evolution of nitrogen be detected. It being conceivable that an actual absorption of free oxygen by the oil of the flour might take place under the influence of the bleaching gas, and thereby mask any evolution of nitrogen, the experiment was repeated with fat-free flour, but the same result was obtained as with ordinary flour.

The nitrogen peroxide remaining unabsorbed in a globe of 1500 c.c. containing 200 grammes of flour is so small that it may be neglected.

The following were the quantities of air which had to be added to bring the vessel to atmospheric pressure:—

Ordinary flour	Fat-free flour
(i) 29·2 c.c.	(i) 29·6 c.c.
(ii) 30·2 c.c.	(ii) 29·9 c.c.
(iii) 30·2 c.c.	

The above experiment shows, therefore, that no appreciable quantity of nitrogen is evolved during bleaching, provided that there is no simultaneous absorption of free oxygen by any constituent of the flour other than the fat, which would compensate for the change in volume due to the production of nitrogen.

In view of the possibility of diazo-compounds being formed by the action of the nitrogen peroxide on the proteins of the flour, samples of bleached and unbleached flour were subjected to the tests given by Treves and Pellizza (*Atti R. Accad. Sci. Torino*, xxxix. pp. 429–434, 1904). These observers found that certain proteins react with nitrous acid to give diazo-compounds, which give brownish-red azo-compounds with alkaline  $\beta$ -naphthol and salicylic acid. No difference in colour, however, could be observed between highly bleached and unbleached flour when treated with these reagents.

#### VII.—ACTION OF DILUTE NITRIC ACID UPON PURE GLUTENIN AND GLIADIN.

In order to ascertain, if possible, the nature of the protein rendered soluble by bleaching, and to study the effect of dilute nitric acid solutions upon the proteins of flour, it appeared essential to work upon the pure substances. For this purpose a considerable quantity of glutenin and gliadin was prepared for me by Dr S. B. Schryver, to whom I am much indebted for advice in connection with this part of the inquiry. The proteins were prepared by Osborne's method from a large quantity of flour, and were obtained as dry white powders, which could be kept without change in stoppered vessels over calcium chloride.

Weighed quantities of each protein were allowed to remain in contact with excess of  $\frac{N}{10}$ ,  $\frac{N}{100}$  and  $\frac{N}{1000}$  nitric acid at the laboratory temperature for several days, the vessels being frequently shaken, and a few drops of toluol added to each to prevent decomposition. The amount of protein dissolved was estimated by evaporating a part of the solution to dryness on the water bath, with the addition of ammonia to neutralise the free nitric acid, and subtracting from the weight of the residue the weight of ammonium nitrate corresponding to the nitric

acid originally present. The following were the quantities of protein dissolved, in grammes per 100 c.c. of solution :—

Distilled water	Glutenin	Gliadin
$\frac{N}{1000}$ nitric acid	0.0244	0.1056
$\frac{N}{100}$ nitric acid	0.0788	0.7388
$\frac{N}{10}$ nitric acid	0.1028	5.1470
$\frac{N}{10}$ nitric acid	0.0296	0.0032

These results are in agreement with those of Wood and Hardy (*Proc. Roy. Soc.*, 1909, Series B, LXXXI. pp. 38–43), who investigated the action of dilute acids, alkalies and salts upon the physical state of gluten. They found for nitric acid a “critical concentration” of  $\frac{3N}{100}$  at which gluten just retains its coherence. Where gluten is treated with nitric acid below this “critical concentration” it loses its coherence and is dispersed to form an opaque colloidal solution or hydrosol.

The physical properties of gluten are largely due to the gliadin it contains, and the relations of gliadin to acid and alkali are the same as those of gluten.

It appears, therefore, that gliadin forms a hydrosol with extreme ease under the influence of nitric acid, especially at or near a concentration of  $\frac{N}{100}$ , while glutenin is hardly affected.

In the case of the majority of commercially bleached flours, which may contain very minute quantities of nitric acid, the effect on the gliadin will no doubt depend on the amount of neutral or alkaline salts that the flour may contain. Wood and Hardy (*loc. cit.*) have shown that the dispersion of gluten may be completely prevented by the addition of salts.

It was shown above that by reduction of the aqueous extract of a bleached flour with a copper-zinc couple, only 60% of the nitrogen absorbed during bleaching could be recovered as ammonia, while 6 to 7% is absorbed by the fat, leaving about 30% still unaccounted for. It seemed possible that the nitrogen unaccounted for might be present in the form of nitric acid in combination with, or “adsorbed” by, the glutenin or gliadin of the flour. Some experiments were therefore carried out on the reduction of dilute nitric acid solutions containing glutenin and gliadin with a copper-zinc couple, with a view to ascertaining whether the whole of the nitrogen of the nitric acid is recoverable as ammonia under these conditions.

Weighed quantities of each protein were thoroughly mixed with measured amounts of nitric acid of different strengths, and allowed to stand a certain time. The mixtures were then made up to 200 c.c. with ammonia-free distilled water. The liquids were centrifuged and treated with a copper-zinc couple in an incubator at 37° C., a few drops of toluol being added, until no further reaction for nitrites was given. They were then diluted to 500 c.c., one gramme of calcium carbonate added, and the ammonia distilled off and estimated by Nessler's solution. A blank experiment was made at the same time on nitric acid alone.

It was found that with gliadin the whole of the nitric acid could be recovered as ammonia, provided that the amount of gliadin present was not greater than could pass completely into solution. If a greater quantity of gliadin was present than could be dissolved in the liquid, the undissolved portion formed a semi-fluid mass at the bottom of the vessel and retained a part of the nitric acid. In the case of glutenin, which is only very slightly soluble, considerable "adsorption" of nitric acid appears to take place when the protein is treated with  $\frac{N}{100}$  nitric acid, and slight adsorption with  $\frac{N}{1000}$  nitric acid, but none at all with  $\frac{N}{10}$  acid. The following table shows the quantities of protein and nitric acid taken, and the amount of nitrogen recovered as ammonia from the solutions:—

	Duration of contact of protein and acid	Grammes of nitrogen added as nitric acid	Grammes of nitrogen recovered as ammonia
2 c.c. $\text{HNO}_3 \frac{N}{10}$ ... ..	—	·0028	·0028
2 grammes gliadin + 2 c.c. $\text{HNO}_3 \frac{N}{10}$ ...	2 days	·0028	·0027
" " " " " " ...	20 "	·0028	·0028
5 grammes gliadin + 20 c.c. $\text{HNO}_3 \frac{N}{100}$ ...	3 "	·0028	·0015
1 gramme gliadin + 20 c.c. $\text{HNO}_3 \frac{N}{100}$ ...	2 "	·0028	·0028
1 gramme gliadin + 200 c.c. $\text{HNO}_3 \frac{N}{1000}$ ...	2 "	·0028	·0028
2 grammes glutenin + 2 c.c. $\text{HNO}_3 \frac{N}{10}$ ...	2 "	·0028	·0026
" " " " " " ...	20 "	·0028	·0028
5 grammes glutenin + 20 c.c. $\text{HNO}_3 \frac{N}{100}$ ...	3 "	·0028	·0015
2 grammes glutenin + 20 c.c. $\text{HNO}_3 \frac{N}{100}$ ...	2 "	·0028	·0016
2 grammes glutenin + 200 c.c. $\text{HNO}_3 \frac{N}{1000}$ ...	2 "	·0028	·0024
1 gramme gliadin } + 2 c.c. $\text{HNO}_3 \frac{N}{10}$ ...	2 "	·0028	·0028
1 gramme glutenin }	20 "	·0028	·0028
" " " " " " ...			

It would appear, therefore, that both gliadin and glutenin are able under certain conditions to adsorb nitric acid from dilute solution, and this may to some extent account for the failure to recover the whole of the nitrogen introduced during bleaching, by reduction of the aqueous extract of the flour.

#### VIII.—DIGESTION EXPERIMENTS. EFFECT OF NITROGEN PEROXIDE AND OF SODIUM NITRITE ON THE SALIVARY DIGESTION OF STARCH AND FLOUR.

##### (i) *Effect of Sodium Nitrite on the Salivary Digestion of Starch.*

It has been stated by Halliburton (*Journal of Hygiene*, ix, p. 170, 1909), that small quantities of sodium nitrite exercise an inhibitory effect on the salivary digestion of starch, as measured by the time required to reach the achromic point when tested with a solution of iodine. I have not, however, been able to confirm this, and have found on the contrary that within certain limits the addition of minute quantities of sodium nitrite slightly accelerates the action of the saliva, but that a marked inhibition results when the starch has been previously treated with nitrogen peroxide gas.

A 1% solution of soluble starch in distilled water was prepared and to quantities of 200 c.c. were added .04, .02, .01, .005 and 0 grammes of sodium nitrite respectively. The solutions were warmed to 37° C., and 20 c.c. of a 10% solution of saliva added. They were then placed in an incubator at 37° C. and quantities of 20 c.c. withdrawn from each at intervals and immediately added to 30 c.c. of mixed Fehling's solution + 30 c.c. of water. The tubes containing the Fehling's solution were then heated for exactly 20 minutes in a boiling water bath, the liquids filtered, and the precipitates ignited and weighed as cupric oxide. The following table gives the weights of cupric oxide obtained:—

Grammes NaNO <sub>2</sub> per 100 c.c. starch paste	CuO obtained from 20 c.c. after time interval of					
	11 mins.	25 mins.	45 mins.	70 mins.	100 mins.	140 mins.
Nil	·0504	·0861	·1170	·1305	·1408	·1483
·0025	·0508	·0888	·1208	·1347	·1436	·1508
·005	·0525	·0899	·1229	·1345	·1431	·1508
·01	·0554	·0935	·1252	·1364	·1471	·1524
·02	·0601	·0969	·1306	·1404	·1484	·1533

A blank estimation on 20 c.c. of starch paste + the equivalent quantity of saliva solution gave 0·0150 gramme of cupric oxide.

It will be seen from the above results that small quantities of sodium nitrite tend to accelerate the action of the ptyalin of saliva upon starch, the acceleration increasing with the quantity of sodium nitrite present<sup>1</sup>. When larger quantities of sodium nitrite are added, the acceleration increases up to a certain point, after which the rate of digestion becomes irregular. 20 c.c. of a 1% starch solution + 2 c.c. of a 10% solution of saliva were treated with quantities of sodium nitrite varying from .01 gramme to .175 gramme per 100 c.c. of starch solution, and the amount of copper reduced by the solution after one-and-a-half hours determined as in the preceding experiment. The following were the weights of cupric oxide obtained:—

Grammes NaNO <sub>2</sub> per 100 c.c. starch paste	Grammes CuO obtained
0	.0924
.010	.1007
.025	.1067
.040	.1075
.055	.1111
.070	.1100
.085	.1083
.100	.1060
.115	.0900
.130	.0909
.145	.1059
.160	.1037
.175	.0993

(ii) *Effect of Nitrogen Peroxide on the Salivary Digestion of Starch.*

Five grammes of soluble starch were introduced into a glass vessel with ground-in stopper carrying a capillary tube with glass cock, and treated with varying quantities of nitrogen peroxide. One per cent. solutions of the starch were then digested with saliva as in the previous experiments for one-and-a-half hours at 37° C., and the weight of copper reduced by 20 c.c. determined. The solutions were slightly acid to litmus and there was very marked inhibition in all of them; practically no starch hydrolysis occurred in three of the samples. The weights of cupric oxide obtained were as follows:—

c.c. NO <sub>2</sub> per 100 c.c. starch paste	Equivalent in grammes NaNO <sub>2</sub> (corrected)	CuO obtained from 20 c.c. of solution
0	0	.1447
0.8	.0025	.1098
1.6	.005	.0223
3.2	.01	.0202
6.4	.02	.0234

<sup>1</sup> Cf. Wohlgemuth (1908), *Biochem. Zeitschr.* ix. pp. 10-43.

(iii) *Salivary Digestion of Bleached and Unbleached Flour.*

The effect of bleaching upon the salivary digestion of flour was investigated by a polarimetric method. Ten grammes of flour were thoroughly mixed with 200 c.c. of water, and heated on the water bath with constant shaking to 85° C. to gelatinise the starch. The mixture was then cooled to 37° C., and portions of 20 c.c. measured into a series of stoppered bottles, which were placed in an incubator at 37° C. Five c.c. of a 4% solution of saliva were added to each, and at different intervals the bottles were removed from the incubator, and 20 c.c. of a solution of tannic acid added. The tannic acid solution was prepared by dissolving 10 grammes of the acid in 475 c.c. of 50% alcohol and adding 20 c.c. of 25% acetic acid with 1 c.c. of concentrated hydrochloric acid.

After standing overnight the liquids were filtered, and the filtrates examined in a polarimeter. The tannin precipitates the proteins and the unaltered starch and yields a perfectly clear filtrate containing the soluble carbohydrates produced by the action of the ptyalin. The rotation rises at first rapidly owing to the formation of soluble dextrans, and if the reaction is carried to completion a decrease in rotation should subsequently take place owing to the conversion of the dextrans into maltose. Under the particular conditions of the experiment an actual decrease in rotation was not observed. As will be seen from the table below, bleaching exercises a marked inhibitory action on the formation of dextrans. In the bleached samples the rotation appears to reach a higher value eventually than in the unbleached sample. This seems to indicate that the simultaneous formation of maltose with consequent diminution in rotation takes place more rapidly in the unbleached than in the bleached samples.

The actual rotations observed were as follows (200 mm. tube):—

Time in minutes	Unbleached flour	Flour bleached with 100 c.c. NO <sub>2</sub> per kgm.	Flour bleached with 300 c.c. NO <sub>2</sub> per kgm.
0	+0.64°	+0.64°	+0.67°
1	+1.05°	+0.94°	+0.89°
2	+1.27°	+1.18°	+1.03°
3	+1.45°	+1.37°	+1.15°
4	+1.64°	+1.55°	+1.34°
6	+1.89°	+1.86°	+1.55°
9	+2.05°	+2.10°	+1.76°
12	+2.15°	+2.29°	+1.94°
15	+2.23°	+2.35°	+2.10°
18	—	+2.40°	+2.18°
22	+2.33°	+2.48°	+2.30°

## SUMMARY OF RESULTS.

The action of air containing nitrogen peroxide upon flour, in quantities up to 300 c.c. of nitrogen peroxide to one kilogramme of flour, may be summarised as follows:—

I. The golden-yellow tint of the flour is destroyed. Immediately after bleaching no difference in tint due to excess of the bleaching agent could be observed with Lovibond's tintometer, but on keeping for several days the more highly bleached samples became decidedly yellow, while those treated with 30 to 100 c.c. of nitrogen peroxide per kilogramme became still whiter, the maximum of bleaching effect being attained within these limits.

II. The amount of nitrous acid or nitrites present in a freshly bleached flour is approximately proportional to the amount of nitrogen peroxide employed, and corresponds to about 30% of the total nitrogen absorbed, rising to 40% in the more highly bleached samples. After the lapse of several days, the proportion of nitrites present decreases considerably in the higher concentrations, but remains very nearly the same in the more slightly bleached samples.

III. Approximately 60% of the total nitrogen introduced as nitrogen peroxide into the flour during bleaching can be recovered as ammonia a short time after bleaching by reducing the aqueous extract of the flour with a copper-zinc couple, and may be assumed to be present in the flour as nitric and nitrous acids or as nitrates and nitrites. After keeping the bleached flour for some days the amount of nitric acid extracted with cold water decreases. Experiments with pure glutenin and gliadin indicated that in certain circumstances nitric acid may be withdrawn from solution or "adsorbed" by these proteins.

IV. In highly bleached flour a considerable increase in the amounts of soluble proteins and soluble carbohydrates takes place. If one kilogramme of flour is bleached with 300 c.c. of nitrogen peroxide, the amount of soluble nitrogen is doubled. This appears to be due almost entirely to the solubility of gliadin in nitric acid of certain concentrations. The simultaneous increase of soluble carbohydrates would seem to point to an intimate relationship between the gliadin and certain carbohydrates in flour.

V. If highly bleached flour is allowed to stand for some time after bleaching, the oil undergoes very considerable alteration and acquires the characteristics of an oxidised oil. About 6 to 7% of the nitrogen

introduced as nitrogen peroxide during bleaching is absorbed by the oil.

VI. The absorption of nitrogen peroxide by flour does not appear to be accompanied by the production of free nitrogen, nor was any evidence obtained of the formation of diazo-compounds.

VII. Sodium nitrite was found to exert no inhibitory action on the digestion of soluble starch by saliva, but the rate of digestion was greatly retarded if the starch had been previously treated with nitrogen peroxide gas. Bleaching was found to exercise an inhibitory effect on the salivary digestion of flour.