

Ozone Oxidation of Corn Starch

C. D. SZYMANSKI, *Research Department, National Starch & Chemical Corporation, Plainfield, New Jersey*

Synopsis

The oxidation of granular corn starch with ozone under various conditions was investigated. The reaction was found to be influenced by water, with the highest degree of oxidation being obtained at 23°C. in an aqueous solution of acetic acid as the suspending medium. In each medium investigated, approximately 65-75% of the total carbonyls generated were found to be aldehydes. Approximately similar results were obtained when aqueous solutions of the sugar alcohols sorbitol and mannitol were ozonized.

At the turn of the century, Harries conducted fundamental studies on the effect of ozone on some organic compounds and showed that aldoses can be prepared from sugar alcohols via oxidation with ozone.¹ Doree and Healy, using cellulose as the substrate, reported that ozone attacks cellulose in the presence of water to form products that were characterized by high reducing value and comparatively small affinity for methylene blue.² Maximum oxidation of cellulose was observed when the linters contained 45-50% water. Mester and co-workers, using starch and the sugar alcohols sorbitol and mannitol also reported the production of aldehydes with ozone, using the criterion of formazan formation as evidence for aldehyde production.³ The effect of a number of oxidizing agents on starch has been determined, and the concentration of carbonyl and carboxyl groups has recently been reported in aqueous chlorine oxidized samples.⁴

We were interested in introducing relatively large amounts of aldehyde groups into starch by methods other than periodate oxidation.⁵ It was therefore of interest to determine the relative amounts of aldehyde and ketones produced by the ozone oxidation of starch under various conditions, e.g., temperature, solvent, and entrainment gas. Although previous investigators have shown that aldehydes, ketones, and carboxylic acid groups are formed by ozone oxidation of carbohydrates, the fact that no reference to relative proportions of these groups in the oxidation of starch and sugar alcohols was found prompted the publication of these results.

EXPERIMENTAL

Ozonization Procedure

All ozonizations were carried out in a gas washing bottle with a coarse fritted base. Corn starch (in ungelatinized form) was ozonized in the

dry form (10% water) or as a suspension in water or organic solvent. The ozone was produced from a Welsbach T-23 generator and the concentration used as determined iodimetrically was 3.45 vol.-%, 0.015 ft.³/min., unless otherwise specified.⁶

For the sugar alcohols, a 10% aqueous solution was ozonized for 18 hr. and concentrated by evaporation in vacuum. The concentrate was then used for reducing sugar determinations. Ozone generator setting for these reactions was maximum voltage and a flow rate of 0.015 ft.³/min., oxygen being used as the gas source. No attempt was made to determine the ozone concentration of the effluent gas.

Carbonyl Estimation in Ozonized Starch

Total carbonyl in starch was estimated with the hydroxylamine procedure of Gladding and Purves.⁷ Ketone concentrations were determined as residual carbonyl after sodium chlorite oxidation of the aldehydes to carboxyls. Aldehydes were then estimated by difference.

Initially, an attempt was made to determine aldehyde content titrimetrically as carboxyls after sodium chlorite oxidation;⁸ however, no correlation was obtained between the carboxyl determined and the residual carbonyl. This lack of agreement was thought to be due to decarboxylation occurring during the heating required in the analytical procedure.

Carboxyl Estimation in Starch

Two procedures were tried to estimate carboxyl content; the paste titration procedure and the calcium acetate method.⁸ For reasons presently unknown, large differences were obtained when the results of both procedures were compared. These differences rendered the results of the carboxyl analysis questionable.

Carbonyl Estimation in Sugar Alcohol Solutions

The total reducing sugar content was determined colorimetrically using the Somogyi-Nelson arsenomolybdate reagent.⁹ Ketone content was determined with the cysteine-carbazole sulfuric acid method, a color development period of 2 hr. being used.¹⁰ This procedure was found to

TABLE I
Fructose Determination (Interference by Glucose)

Sugar, $\mu\text{g.}$			Optical density at 560 $m\mu$		
Fructose (F)	Glucose (G)	Ratio G/F	160 min.	320 min.	21 hr., 20 min.
4.12	0	0	0.077	0.102	0.133
4.12	269.1	65	0.080	0.118	0.250
20.60	0	0	0.375	0.495	0.665
20.60	149.5	7.25	0.373	0.495	0.720

follow Beer-Lambert Law with 0–20 μg . of fructose, and to be only slightly affected by glucose in relatively high concentrations. Control experiments with a mixture of 20 μg . of fructose and 150 μg . of glucose and fructose alone showed no difference after 2 hr. and only 8% difference in optical density after 21 hr. as measured on a B & L Spectronic 20. These results are presented in Table I. The possible interfering effect of glucose or other aldehyde sugars was further minimized by using less than 20 μg . of total reducing sugars for each analysis.

RESULTS AND DISCUSSION

Factors Affecting the Action of Ozone on Starch

Doree and Healy, examined the effect of a number of variables on cellulose oxidation and reported that the extent of oxidation in cellulose shows large variations with the amount of water used.² Additionally, these authors noted that oxidation of linters moistened with carbon tetrachloride produced lower copper numbers, i.e., lower reducing group content, than that obtained with linters moistened with water. To determine the effect of a number of variables on the ozonization of granular corn starch, the influence of water, some organic solvents, temperature, and some salts was examined.

Effect of Water

The effect of water on the oxidation is presented in Table II. From Table II and Figure 1, it is evident that the degree of oxidation is low when the water content is below 65%, but that it rises rapidly beyond this

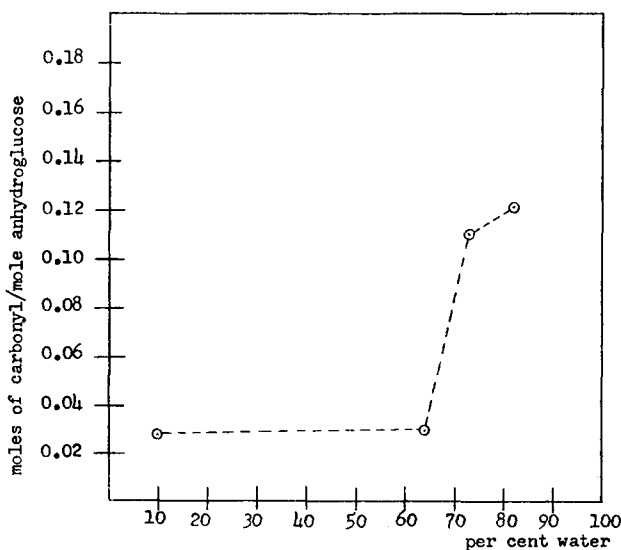


Fig. 1. Influence of water on the extent of oxidation.

TABLE II
Influence of Water on the Extent of Oxidation

H ₂ O, %	Carbonyl, moles/mole anhydroglucose*
10	0.028
64	0.030
73	0.112
82	0.122

* Reaction for 24 hr. at room temperature.

point. Increasing the water content of the reaction slurry from 73 to 82% resulted in only small changes in the level of carbonyl introduced, in contrast to that produced by increasing from 64 to 73%. All subsequent experiments with starch were conducted with a starch slurry containing 73% water. No attempt was made to oxidize anhydrous starch.

The influence of water of the oxidation is complex, in that water can act as a solvent, it hydrates hydroxyl groups and it can also act as a catalyst. This latter effect may be related to the hydration of hydroxyl groups.

Effect of Solvents

Inasmuch as ozone is known to be more soluble in some organic solvents than in water, the effect of a few organic media as suspending agents was examined. From the results presented in Table III it is apparent that increases in carbonyl above that obtained with water were obtained with chloroform, but the most marked increases were observed with solutions of acetic acid-water.

TABLE III
Effect of Temperature and Solvents on Oxidation

Solvent	Carbonyl, moles/mole anhydroglucose		
	0°C.	23°C.	50°C.
Water	0.042	0.112	0.047
Chloroform	0.091	0.125	0.029
Acetic acid-water (95:5)	0.100	0.146	Degraded
Acetic acid-water (75:25)	0.067	0.175	Degraded

Ozonizations in these acidic solvents showed increases in the level of carbonyl produced from 30% to in excess of 50%. The increases in carbonyl content observed in these solvents are most likely the result of a combination of effects, e.g., increased solubility as well as increased stability of the ozone in these solutions. Further, the superiority of the acetic solutions containing higher concentrations of water indicates that with this solvent water is essential for optimum conditions of oxidation. Figure 2 shows graphically the influence of water on the oxidation in acetic acid.

That the increases observed in acetic acid with increasing water concentration were the result of hydrolysis of glycosidic linkages was also con-

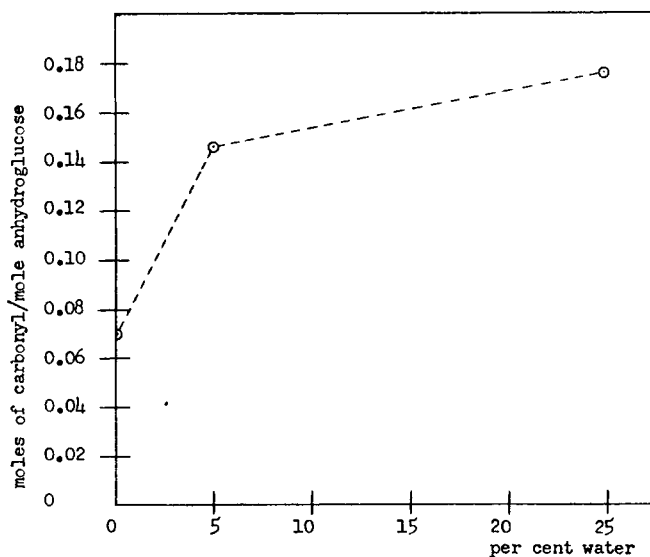


Fig. 2. Influence of water on the extent of oxidation in acetic acid and acetic acid-water solutions.

sidered. Corn starch was slurried in 75:25 and 95:5 acetic acid-water solutions and air was blown through the suspensions for 24 hr. at room temperature. Analysis of the recovered starch showed only 0.0013 and 0.0031 moles of carbonyl produced per mole of anhydroglucose in 95:5 and 75:25 acetic acid-water solutions, respectively. Further, the recovery of starch in each instance was 98%. Thus, the increased oxidation observed in the aqueous acetic acid solutions is not primarily the result of hydrolysis of glycosidic linkages.

Effect of Temperature

The effect of temperature on the oxidation was examined in a few solvents at 0, 23, and 50°C., and these results are presented in Table III. The decrease in carbonyl content observed with an increase in temperature is probably the result of a number of factors. Thus, the increase in the rate of oxidation expected with the higher temperature is counterbalanced by the decreased solubility and increased decomposition of the ozone at the higher temperature. Conversely, the controlling factor at the lower temperature would seem to be the decrease in the rate of oxidation. Corn starch ozonized at the higher temperature in acetic acid water solutions was extensively degraded. This material had a hard, glassy appearance when dried and was recovered in yields of approximately 70%. No attempt was made to analyze this product.

Effect of Salts

Inasmuch as the objective in conducting these investigations was to introduce high levels of carbonyl into corn starch in relatively short times,

the low degree of oxidation obtained was disconcerting. The possibility was considered that the efficiency of the oxidation could be improved if a catalyst was used. Two salts were investigated, ferrous and manganous sulfate, at concentration levels of approximately 3.3% on starch. The catalytic action of these salts on oxidation with oxygen, hydrogen peroxide and permanganate has been reported.^{11,12} The results are presented in Table IV. The manganous salt produced an increase in carbonyl content

TABLE IV
Effect of Some Salts on the Degree of Oxidation

Solvent	Salt	Carbonyl, moles/ mole anhydroglucose
Water	None	0.112
Water	Ferrous sulfate	0.012
Water	Manganous sulfate	0.155

of 38% over that obtained with water. The ferrous salt, on the other hand, showed some inhibition to the oxidation reaction as evidenced by the low carbonyl content obtained.

Effect of Gas Source

In a recent publication on the action of ozone on oxidation, Briner offered evidence which indicated that the rate of "autooxidation" of aldehydes is considerably increased when the oxygen employed is mixed with a low concentration of ozone.¹³ This accelerating action of ozone was observed with a number of compounds, among which are some saturated hydrocarbons and inorganic sulfur derivatives, e.g., sulfurous acid and alkali sulfites and bisulfites, and it therefore seemed of interest to determine whether this effect could be observed with starch. Since all prior experiments were conducted with air as the gas source, the generator was recalibrated for ozone output with oxygen. The concentration of ozone used was equivalent to that applied in experiments in which air was used as the gas source, i.e., 3.45 vol.-%.

TABLE V
Effect of the Gas Source on the Degree of Oxidation

Solvent	Gas source	Ozone concn., vol.-%	Total carbonyl, moles/mole anhydroglucose
Water	Air	3.45	0.112
Water	Oxygen	3.45	0.069
Water	Oxygen	1.6	0.067
Water	Oxygen	0.168	0.052
Chloroform	Oxygen	3.45	0.087
Chloroform	Air	3.45	0.125

The results in Table V show that the oxidation conducted with oxygen as the gas source produces a lesser degree of oxidation than that produced with air as the gas source. This effect was observed not only in water, but also in chloroform. Additionally, it was found that as the concentration of ozone in the gas stream diminished lower oxidation levels were obtained. Thus, the accelerating action of ozone at higher dilutions in oxygen has not been observed in the system applied here. This evidence suggests that the acceleration is inoperative in the oxidation of alcohols.

Aldehyde Content of Oxidized Starches

A number of oxidized starches were analyzed for aldehyde content and the results are presented in Table VI.

TABLE VI

Solvent	Source gas	Total	Total	Total	Carbonyl as aldehyde, %
		carbonyl, moles/mole anhydro-glucose	carbonyl, moles/mole anhydro-glucose	carbonyl after chlorite oxidation, moles/mole anhydro-glucose	
Acetic acid-H ₂ O 95:5	Air	0.146	0.032	0.114	78
Acetic acid-H ₂ O 75:25	"	0.175	0.048	0.127	73
Acetic acid-H ₂ O 75:25 ^a	"	0.201	0.060	0.141	70
Chloroform	"	0.125	0.044	0.081	65
Chloroform ^a	"	0.172	0.045	0.127	74
Water	"	0.122	0.046	0.076	62
Water ^b	"	0.272	0.072	0.200	73
Water	Oxygen	0.069	0.026	0.043	62

^a Ozonized in a column 2.5 × height of the gas washing bottle.

^b 72 hr. experiment; all others 24 hr.

Generally it was found that the starch oxidized with air as the gas source showed a higher aldehyde content than when oxygen was used as the gas source. A portion of this increased aldehyde content with air as the gas source is possibly the result of hydrolysis. Ozone generated from air contains nitrogen pentoxide which, in the presence of water, forms nitric acid. Checking the pH of the starch slurries after the oxidation time was completed, showed pH's of 2.5 and below 1 for oxygen and air, respectively. Thus, hydrolysis of the glycosidic linkages might account for a portion of the aldehydes produced, but the remainder of the difference between oxygen and air is related to the gas source used.

It is unfortunate that neither analytical procedure for carboxyl groups gave reliable results in the author's hands. The decreased aldehyde level

obtained with oxygen as the gas source suggested the possibility that the autooxidation effect was operative at the aldehyde oxidation level and could have been verified as an increase in carboxyl groups, i.e., a decrease in the ratio of aldehyde/carboxyl.

In an attempt to increase the degree of oxidation of starch with ozone, using the same exposure times, starch slurries were suspended in a glass column with a fritted base. The height of the column, 62 cm., was 2.5 times that of the gas washing bottle applied in the other experiments. From Table VI it can be seen that increasing the contact time of the ozone with the starch in this manner produced a significant increase in the degree of oxidation. Table VI also gives the maximum concentration of carbonyls introduced into the starch. Higher levels are undoubtedly possible, but the time required for such oxidation levels under these conditions is prohibitive.

Carboxyl analyses of all of the samples were attempted by using the paste titration method and modified calcium acetate method.⁸ As previously mentioned, it was felt that decarboxylation was occurring during the cooking of the starch in the paste titration procedure, and for this reason the calcium acetate method was tried, since no heating step was involved. However, there was so much discrepancy between the results of both methods that it was felt that in the oxidized starch complicating factors existed which rendered the analyses questionable. Both procedures did show that the concentration of carboxyl groups introduced was low with the highest levels being produced in the 72-hr. experiment.

From these results it is apparent that, in addition to low levels of carboxyl groups, only approximately 65–75% of the total carbonyls generated via ozone oxidation of starch are aldehydes. Thus, the initial objective of introducing high levels of aldehyde into starch has not been realized.

Ozonization of Sugar Alcohols

The sugar alcohols, sorbitol and mannitol, were ozonized in aqueous solution to determine the relative percentage of aldehydes and ketones pro-

TABLE VII
Reducing Sugar Content of Ozonized Sugar Alcohols^a

Alcohol	Total sugars μg./ml. (calculated as glucose)	Ketone, μg./ml. (calculated as fructose)	Total as aldehydes, %
Sorbitol	620	239	61.4
	634	197	68.8
Mannitol	801	264	67.0
	757	272	64.1

^a That the ozonized solutions were mixtures was verified by chromatogramming the solution by paper and thin-layer chromatography. In each case at least four spots were detected.

duced. No attempt was made to determine the effect of varied conditions on the oxidation. On each concentrate, total reducing sugars and ketone content were determined. The results presented in Table VII are evidence that in the oxidation of the sugar alcohols 60-70% of the total carbonyls produced are aldehydes.

The fact that the relative percentage of aldehydes and ketones in the sugar alcohols and starch is approximately the same leads to an interesting deduction. In the starch, as well as in the sugar alcohols, the ratio of primary hydroxyl to secondary hydroxyl groups is 1:2, and the fact that approximately identical levels of aldehyde were obtained seems to indicate that despite the structural differences which are imposed by the starch granule and molecular size, no difference in the oxidizability of the two materials was observed. Thus, it seems that in this instance, the particular configuration peculiar to the starch does not exert any significant effect on the oxidation, e.g., occlusion of OH groups.

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Résumé

On a étudié l'oxydation de l'amidon de blé en grain par l'ozone sous différentes conditions. On a trouvé que la réaction était influencée par l'eau; le degré d'oxydation le plus élevé a été obtenu à 23°C dans une solution aqueuse d'acide acétique servant comme milieu de suspension. Dans chaque milieu étudié on a trouvé que 65-75% des carbonyles formés étaient sous forme d'aldéhydes. Des résultats approximativement semblables ont été obtenus en ozonisant des solutions aqueuses de sorbitol et mannitol.

Zusammenfassung

Die Oxydation granulierter Getriedestärke mit Ozon unter verschiedenen Bedingungen wurde untersucht. Die Reaktion wird von Wasser beeinflusst, u.zw. wurde der höchste Oxydationsgrad bei 23°C in einer wässrigen Lösung von Essigsäure als Suspensionsmedium erreicht. In jedem der untersuchten Medien waren etwa 65–75% der gesamten gebildeten Carbonylgruppen Aldehyde. Ähnliche Befunde ergaben sich bei der Ozonbehandlung wässriger Lösungen der Zuckeralkohole Sorbit und Mannit.

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