

Starch dialdehyde. IV.*

Changes during storage and methods of determination

K. TIHLÁRIK, Š. BATÁRYOVÁ**, K. BABOR, and V. KALÁČ

*Institute of Chemistry, Slovak Academy of Sciences,
809 33 Bratislava*

Received 11 November 1975

The routine methods currently used for determination of percentage of oxidized glucose units in starch dialdehyde (*i.e.* the method based on oxime formation, determination of rapid alkali consumption, formation of corresponding *p*-nitrophenylhydrazone, and the method of additional periodate oxidation) were compared and a new method based on the oxidation of starch dialdehyde sample by iodine in alkaline solution has been developed.

It has been observed that depending upon the length of storage the starch dialdehyde undergoes changes resulting in a decreasing percentage of oxidized glucose units in the product. The relative value of this decrease depends on the method used for determination of the degree of oxidation.

The only method giving adequate information on the number of actually effective aldehyde groups in aged samples of starch dialdehyde is the oximation method.

The decrease in the number of determinable aldehyde groups denoted as "aging" is reversible. The conditions have been found at which practically the original values can be restored.

Сравнением до настоящего времени применяемых методов на определение процента окисленных единиц глюкозы в диальдегиде крахмала (метод основан на образовании оксима, метод быстрого расхода щелочи, метод на основе образования соответствующего *p*-нитрофенилгидразона и метод дополнительного метапериодатного окисления продукта) был разработан новый метод основанный на окислении пробы диальдегида крахмала иодом в щелочной среде.

Было установлено, что в зависимости от времени хранения диальдегида крахмала настают в продукте изменения, которые проявятся в более низких процентах окисленных единиц глюкозы, чем те которые были определены непосредственно после приготовления. Это понижение является при применении отдельных методов определения разным.

Единственный метод, который дает соответствующий образ в данный момент об эффективных альдегидных группах в ранее приготовленных продуктах — метод оксимирования.

Понижение определяемых альдегидных групп обозначенное как «старение» является обратимым. Нашлись условия, при которых можно восстановить их практически на первоначальную величину.

* For Part III see Ref. [1].

** *Present address*: Institute of National Health, Dispensary, 040 00 Košice.

The most important characteristics in evaluation of the quality of starch dialdehyde samples is the degree of oxidation, *i.e.*, the percentage of oxidized glucose units in the product. Several methods, differing both in laboriousness and accuracy have been elaborated for the determination. Regarding the inconsistency of results obtained by different methods we decided to evaluate systematically the existing methods and to develop an exact and simple method that would satisfy especially the requirements of industrial practice.

The most exact method from the theoretical point of view is the reduction of aldehyde groups by sodium borohydride [2, 3]. This method was, however, not employed in the present study since we have found [4] that the oximation method gives comparable results.

The oximation method is based on reaction of aldehyde groups with hydroxylammonium chloride [5] and for this reason it is most suitable for analysis of starch dialdehyde samples showing a high degree of oxidation.

The method of "rapid alkali consumption" [6] is suitable especially for determination of relative differences between the individual samples; it is frequently used in practice when large series of samples are to be compared.

The colorimetric methods are based on the formation of coloured phenylhydrazones as it is *e.g.* in the reaction with *p*-nitrophenylhydrazine [7].

Additional oxidation with periodic acid or its salts enables us to determine the portion of the product that was not oxidized prior to periodate treatment and thus to calculate the original degree of oxidation [3]. The periodate oxidation which is very useful in structural studies, was not tested in the present work.

We have considered the application of oxidation by alkaline iodine as a method for determination of the degree of oxidation in starch dialdehyde. This method has been used long time ago for other purposes in carbohydrate chemistry. With regard to the character of aldehyde groups in starch dialdehyde it was necessary first to test several parameters and to establish the conditions at which the formed carboxyl group is relatively stable, *i.e.* the overoxidation does not take place and the equivalence point can be reliably determined.

The phenomenon called "aging" of starch dialdehyde has not been, so far, described in the literature. The accompanying decrease in the number of effective aldehyde groups can be registered already after several weeks of storage. The detailed study of this problem was initiated mainly by the observation that in the determinations of the degree of oxidation of aged samples of starch dialdehyde higher values were obtained by methods which involved heating of the sample in water suspension (rapid alkali consumption, *p*-nitrophenylhydrazone formation) while the oximation method giving reliable results with freshly prepared samples gave extremely low values, sometimes approaching zero, with aged products.

Experimental

The used samples of starch dialdehyde were prepared according to [8] from maize starch manufactured by Slovak Starch Factories, n.e., Boleráz. One sample was of commercial origin.

DAS 1: freshly prepared sample, content of oxidized glucose units 87.3%.

DAS 2: freshly prepared sample, content of oxidized glucose units 20.6%.

DAS 3: SUMSTAR 190, product of Miles Chemical Co., Elkhart, USA, made in 1964, original content of oxidized glucose units 90.0%.

DAS 4: sample prepared in 1963, original content of oxidized glucose units 92.1%.

DAS 5: sample prepared in 1964, original content of oxidized glucose units 98.3%.

(The values of the degree of oxidation were determined by the oximation method.)

Methods for determination of the degree of oxidation

Oximation method

The procedure is based on the original method of *Gladding* and *Purves* [5] where the sample of starch dialdehyde is shaken with hydroxylammonium chloride in aqueous suspension and the liberated hydrochloric acid is determined by potentiometric titration.

Method of rapid alkali consumption

The determination is performed according to original procedure [6] where the suspension of starch dialdehyde in the solution of sodium hydroxide is heated for 1 min and the amount of consumed alkali is determined.

Hydrazone method

The determination is based on the formation of an intensively coloured product of starch dialdehyde with *p*-nitrophenylhydrazine. The original method of *Mehlretter* and *Wise* [7] has been employed.

Oxidation of starch dialdehyde by alkaline iodine in Na₂CO₃/NaHCO₃ buffer at pH 9.7

The procedure originally employed by *Ellington* and *Purves* [9] was used.

Standard oxidation of starch dialdehyde by alkaline iodine

To 20 mg of highly oxidized starch (or to a proportionally higher amount when the degree of oxidation was lower) 0.05 M iodine solution (5 ml) and 1 M-NaOH (2 ml) were added. The mixture was allowed to stand for 1 hr at room temperature in the dark with occasional stirring. After an addition of 0.2 M-H₂SO₄ (30 ml) the liberated iodine was titrated with 0.05 M-Na₂S₂O₃ using starch gel as indicator. Alternatively, biamprometric method can be used for indication of equivalence point.

Calculation

$$\% \text{ of oxidized glucose units in the sample} = \frac{400(x - y)}{w},$$

where x = ml of 0.05 M iodine solution added before determination,
 y = ml of 0.05 M iodine solution determined after oxidation,
 w = weight of the sample in mg.

Regeneration of ineffective aldehyde groups in aged samples of starch dialdehyde

Aqueous suspension of starch dialdehyde (1 g per 100 ml H₂O) was allowed to stand with occasional stirring:

1. for 16 hrs at room temperature,
2. for 15 min at 90—95°C,
3. for 60 min at 90—95°C.

Results and discussion

The present study deals with several representative samples of starch dialdehyde differing both in the age and the degree of oxidation. All results are given as arithmetic mean values obtained from three determinations.

From among the different methods employed in this study for determination of the degree of oxidation in starch dialdehyde the oximation method was chosen as the referential.

With freshly prepared samples of starch dialdehyde this method gives results which are in best correlation with those obtained by means of sodium borohydride reduction. With aged samples of starch dialdehyde the obtained values were lower due to the "aging". The results obtained by the oximation method give the best information on the decrease in the number of effective aldehyde groups in aged samples of starch dialdehyde which is the criterion of quality and applicability of the product. The conditions of the oximation method are very close to those occurring in the technical application of starch dialdehyde (mild reaction conditions, laboratory temperature) while the other methods are performed under more severe conditions (higher temperature, alkaline pH) which cause partial regeneration of ineffective aldehyde groups in the sample.

The method of rapid alkali consumption is based on Cannizzaro reaction of aldehyde groups in starch dialdehyde. Due to relatively high sensitivity of starch dialdehyde towards alkali, the reaction course and the obtained results depend considerably on the standardization of reaction conditions. Shortening or prolongation of the heating time of the sample (1 min) brings about considerable deviations from the expected results. On the other hand, the method of rapid alkali consumption has an undoubted advantage in its rapidity and for this reason it is suitable for use in the practice for comparative determinations of series of samples under the identical conditions.

The determination of aldehyde groups in starch dialdehyde based on the formation of corresponding hydrazone exhibits all advantages and disadvantages of a spectrophotometric method. Due to its laboriousness and the need of a reference sample of starch dialdehyde with a known degree of oxidation, this method is not suitable for application in the industrial practice. On the other hand, it is well suited for analysis of samples with a low degree of oxidation where it gives more accurate values than the other methods.

In the search for new, precise and simple methods for analysis of starch dialdehyde we tested the determination of aldehyde groups by oxidation with iodine in alkaline solution. The only relevant paper [9] deals with this method only marginally. Starch was oxidized by chromium oxide in acidic solution and an attempt was done to determine the formed aldehyde groups by oxidation with alkaline iodine. Since the described reaction conditions were not suitable for our purposes we had to find a better modification.

The sample of starch dialdehyde with a known degree of oxidation (*DAS 1*) was oxidized by iodine in alkaline solution at pH 9.7 in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer and the effects of the concentration of reagents and time on the obtained results were observed. At three different substrate : iodine molar ratios (1 : 2.5, 1 : 5, and 1 : 7.5)

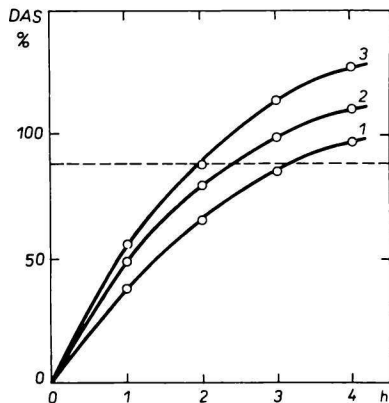


Fig. 1. Oxidation of *DAS 1* sample in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer at pH 9.7 by alkaline iodine.

Dependence of the percentage of determined oxidized glucose units on the reaction time at different substrate : iodine molar ratios.

Molar ratio substrate : iodine : alkali :

1. 1 : 2.5 : 30; 2. 1 : 5 : 30; 3. 1 : 7.5 : 30.

The actual content of oxidized glucose units in the sample was 87.3%.

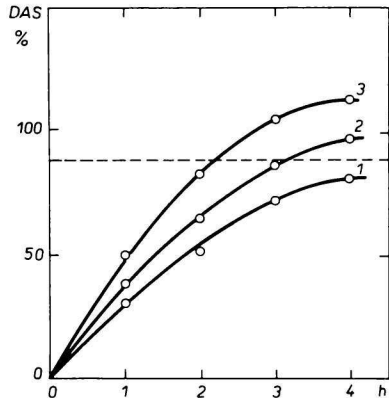


Fig. 2. Oxidation of *DAS 1* sample in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer at pH 9.7 by alkaline iodine.

Dependence of the percentage of determined oxidized glucose units at different substrate : alkali molar ratios on the reaction time.

Molar ratio substrate : iodine : alkali :

1. 1 : 2.5 : 20; 2. 1 : 2.5 : 30; 3. 1 : 2.5 : 40.

The actual content of oxidized glucose units in the sample was 87.3%.

and a constant substrate: alkali ratio (1 : 30) we have found a slow and continuous course of oxidation (Fig. 1), however, without tendency to reach the theoretical value within a suitable reaction time. When substrate: alkali ratio was varied keeping the substrate: iodine molar ratio constant (1 : 2.5), curves of similar shape were obtained (Fig. 2).

The treatment of starch dialdehyde by alkaline iodine in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer at pH 9.7 allows probably a careful oxidation of aldehyde groups under mild conditions; this is, however, not suited for analytical purposes.

The choice of sodium hydroxide concentration was based, with regard to the character of analyzed substrate, on the assumption that the lowest possible substrate to alkali ratio would be the most suitable. This has been proved to be correct when at the molar ratio substrate:iodine:NaOH (1:2.5:20) and 1 hr reaction time the theoretical values were obtained and they did not change considerably during next four hours (Fig. 3). When the concentration of iodine was increased the measured values increased, too, apparently due to overoxidation. Similarly, but to a smaller extent, higher values were obtained also by increasing the proportion of sodium hydroxide in the reaction mixture (Fig. 4). The optimum conditions used in the standard determination are described in Experimental.

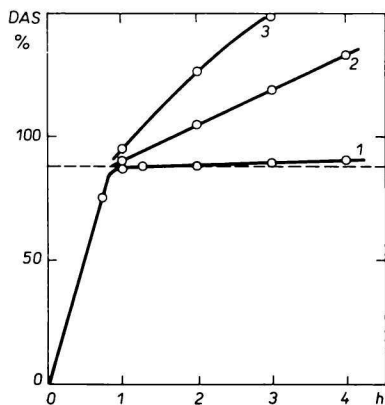


Fig. 3. Oxidation of *DAS 1* sample by alkaline iodine in the presence of NaOH.

Dependence of the percentage of determined oxidized glucose units at different substrate: iodine: alkali ratios on the reaction time.

Molar ratio substrate: iodine: alkali:

1. 1:2.5:20; 2. 1:1.5:20; 3. 1:7.5:20.

The actual content of oxidized glucose units in the sample was 87.3%.

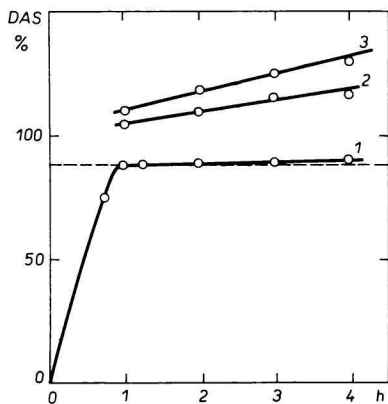


Fig. 4. Oxidation of *DAS 1* sample by alkaline iodine in the presence of NaOH.

Dependence of the percentage of determined oxidized glucose units at different substrate: iodine: alkali ratios on the reaction time.

Molar ratio substrate: iodine: alkali:

1. 1:2.5:20; 2. 1:2.5:30; 3. 1:2.5:40.

The actual content of oxidized glucose units in the sample was 87.3%.

The described procedure was verified with freshly prepared samples of starch dialdehyde and the obtained results were in a good agreement with those obtained by the referential oximation method (Table 1). With freshly prepared sample of starch dialdehyde having a high degree of oxidation (*DAS 1*) only the oxidation by alkaline iodine gave results comparable with those obtained by the referential method. The hydrazone method gave slightly lower values. The great discrepancies were found with the method of rapid alkali consumption. The latter method gave with freshly prepared samples having a low degree of oxidation (*DAS 2*) even less accurate results while the hydrazone method gave results comparable with those obtained by the referential method.

The further three samples of starch dialdehyde (*DAS 3, 4, and 5*) were prepared earlier. They were chosen in order to investigate the effect of long-lasting storage on the determined degree of oxidation and to find possibilities to regenerate ineffective aldehyde groups in these samples. With the latter samples the oximation method gave the lowest results and, with regard to reaction conditions, it gave the best indication on the quality and applicability of the products. The obtained results enable us to predict the character of changes occurring in the course of technical application of the products. The method of rapid alkali consumption, as well as the hydrazone method, gave, in comparison with the oximation method, higher values of the degree of oxidation. However, it is necessary to point out that the procedures of the two latter methods comprise also the heating of the samples in aqueous

Table 1

Percentage of oxidized glucose units in the individual samples
of starch dialdehyde
Comparison of results obtained by different methods

Sample	% of oxidized glucose units (OGU)				
	<i>I</i>	<i>II</i>	<i>III</i>	<i>IV</i>	<i>V</i>
<i>DAS 1</i>	87.3	87.3	76.5	85.7	87.8
<i>DAS 2</i>	20.6	20.6	11.1	20.7	18.9
<i>DAS 3</i>	90.0	5.7	27.3	25.0	54.4
<i>DAS 4</i>	92.1	5.1	50.7	55.2	57.8
<i>DAS 5</i>	98.3	5.6	75.0	75.4	68.1

I — initial percentage of OGU determined by the oximation method immediately after preparation of the sample.

II — oximation method at the time of experiment.

III — rapid alkali consumption method.

IV — hydrazone method.

V — oxidation by alkaline iodine.

suspension thereby causing most probably partial regeneration of ineffective aldehyde groups in starch dialdehyde.

The comparison of results obtained by different methods enabled us to verify the described effect and to suggest the procedure for regeneration of aldehyde groups in aged samples of starch dialdehyde.

Similarly as with the methods described above we can characterize the newly suggested method of oxidation by alkaline iodine. This method gives an adequate information on the free aldehyde groups in freshly prepared samples of starch dialdehyde, however, there proceeds a partial regeneration of aldehyde groups under influence of reaction conditions.

The aging of starch dialdehyde represents a serious problem and its detailed study is stipulated by the technical practice. So far, it has not been confirmed whether the intramolecular or intermolecular interaction of aldehyde groups with primary alcoholic groups takes place. This problem is the subject of a further study.

The observation that the analytical procedures involving heating of the sample in aqueous suspension gave with aged samples higher values than those obtained by the referential method lead us to study the effect of water and heating on the regeneration of ineffective aldehyde groups. The samples were allowed to stand for 16 hrs at room temperature and for 15 and 60 min at 90–95°C with occasional stirring. The aldehyde groups in treated samples were determined by the oximation method, rapid alkali consumption, and the oxidation by alkaline iodine. The obtained results are summarized in Tables 2–4.

The interaction of substrate with water at room temperature did not result in an increase of measured values except in determinations performed by the method of rapid alkali consumption (Table 2).

The heating of freshly prepared samples of starch dialdehyde (*DAS 1* and *2*) in aqueous suspension for 15 min at 90–95°C did not appreciably change the degree of oxidation determined by the oximation method (Table 3). This finding indicates that no degradation, such as the splitting of glycosidic bonds, took place in the course of heating. An increase in the measured values was recorded only when the method of rapid alkali consumption was used. The latter method involves further heating of the sample which affects the degree of oxidation in the sample. In aged samples (*DAS 3, 4, and 5*) the 15 min heating considerably increased the values measured by the oximation as well as by the other methods. The values obtained by other methods were very close to those obtained by the oximation method immediately after the preparation of the given sample (Table 3).

The prolonged heating of the samples in aqueous suspension for 60 min (Table 4) caused a small increase in measured values in freshly prepared samples, which indicates that a prolonged heating of the samples can cause a significant degradation of starch dialdehyde and consequently it can decrease the quality of the product. With aged samples the 60 min heating had approximately the same effect as the 15 min treatment.

Table 2. Influence of 16 hrs standing of starch dialdehyde at room temperature in aqueous suspension on the change of the degree of oxidation

Sample	% of oxidized glucose units determined by									
	oximation method				rapid alkali consumption			oxidation by alkaline iodine		
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>b</i>	<i>c</i>	<i>d</i>
<i>DAS 1</i>	87.3	87.3	88.0	+0.7	76.5	75.1	- 1.4	87.8	87.1	-0.7
<i>DAS 2</i>	20.6	20.6	20.5	-0.1	11.2	27.6	+16.4	18.9	20.9	+2.0
<i>DAS 3</i>	90.0	5.7	5.6	-0.09	27.3	27.3	0	54.4	55.5	+1.1
<i>DAS 4</i>	92.1	5.1	5.1	+0.03	50.7	55.4	+ 4.7	57.8	57.4	-0.4
<i>DAS 5</i>	98.3	5.6	5.5	-0.1	75.0	75.4	+ 0.4	68.1	69.4	+1.3

a) After preparation; b) before regeneration; c) after regeneration; d) effect of regeneration (%).

Table 3. Influence of 15 min heating of starch dialdehyde in aqueous suspension at 90—95°C on the change of the degree of oxidation

Sample	% of oxidized glucose units determined by									
	oximation method				rapid alkali consumption			oxidation by alkaline iodine		
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>b</i>	<i>c</i>	<i>d</i>
<i>DAS 1</i>	87.3	87.3	90.0	+ 2.7	76.5	83.0	+ 6.5	87.8	87.1	- 0.7
<i>DAS 2</i>	20.6	20.6	19.5	- 1.1	11.2	25.5	+14.3	18.9	20.8	+ 1.9
<i>DAS 3</i>	90.0	5.7	89.5	+83.8	27.3	74.7	+47.4	54.4	84.1	+29.7
<i>DAS 4</i>	92.1	5.1	83.1	+78.0	50.7	82.1	+31.4	57.8	85.8	+28.0
<i>DAS 5</i>	98.3	5.6	88.7	+83.1	75.0	80.7	+ 5.7	68.1	94.2	+26.1

a) After preparation; b) before regeneration; c) after regeneration; d) effect of regeneration (%).

Table 4

Influence of 60 min heating of starch dialdehyde in aqueous suspension at 90—95°C
on the change of the degree of oxidation

Sample	% of oxidized glucose units determined by									
	oximation method				rapid alkali consumption			oxidation by alkaline iodine		
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>b</i>	<i>c</i>	<i>d</i>
<i>DAS 1</i>	87.3	87.3	91.3	+ 4.0	76.5	82.7	+ 6.2	87.8	102.4	+14.6
<i>DAS 2</i>	20.6	20.6	19.5	- 1.1	11.2	25.8	+14.6	18.9	19.8	+ 0.9
<i>DAS 3</i>	90.0	5.7	89.5	+83.8	27.3	78.7	+51.4	54.4	97.1	+42.7
<i>DAS 4</i>	92.1	5.1	93.6	+88.5	50.7	83.6	+32.9	57.8	88.8	+31.0
<i>DAS 5</i>	98.3	5.6	91.8	+86.2	75.0	85.1	+10.1	68.1	98.4	+30.3

a) After preparation ; b) before regeneration ; c) after regeneration ; d) effect of regeneration (%).

The analysis of the obtained results gives valuable information important both from the theoretical and practical point of view. The short-time heating of the aged samples of starch dialdehyde in aqueous suspension at 90—95°C leads to regeneration of ineffective aldehyde groups to nearly original values as shown by oximation method. The quality of starch dialdehyde stored for several years can be restored by a relatively simple regeneration whereby the product acquires its initial reactivity.

From the theoretical point of view the aging of starch dialdehyde can be considered as a reversible process that can be affected by the above described procedure.

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Translated by V. Farkaš