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Influence of ascorbic acid on the stability of chitosan solutions

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Abstract

The chitosan solution stability in 2% acetic acid in the presence of different amounts of ascorbic acid (AA) was investigated. It has been shown that already low concentrations of ascorbic acid induce a rapid destruction of the polymer with the formation of water-soluble chitosan fragments at definite stages of the reaction. The elimination of air oxygen from the system considerably inhibits the chitosan destruction. On the other hand, the experiments demonstrate an accelerated oxidation of ascorbic acid in the presence of chitosan. For the initial stages of reactions first order reaction rate constants for the AA destruction can be calculated. It is suggested, that the polymer destruction induce hydroperoxyl radicals, which are formed at reaction of AA with air oxygen. Hydroperoxyl radicals abstract hydrogen from the chitosan or form unstable hydroperoxydes decomposition of which induce splitting of the glycosidic bond in the polymer.

Keywords: Chitosan solutions; Ascorbic acid (AA); Destruction; Oxygen influence

1. Introduction

Aminopolysaccharide chitosan is often used as a food additive for the regulation of some functions of the organism and for confining of some pathological processes. So, it is used for eliminating the organism's fats (Han, Kimura, & Okuda, 1999), lowering the cholesterol level in the organism (Koide, 1998; Muzzarelli, 1998), and heeling some disorders of the digestion system (Kawaguchi, 1998). It has been observed that the physiological activity of chitosan is improved in the presence of ascorbic acid (AA) (Razdan & Petterson, 1994). Chitosan comes in contact with AA also when it is used for the stabilization of fruit juices and wines (Nishihara et al., 1992). It is well known that AA plays the role of an effective antioxidant in biological systems (Bendich et al., 1986). Therefore, it attracts some interest in studies of the influence of AA on the stability of chitosan in AA-containing systems, as well as influence of chitosan on the decomposition rate of ascorbic AA in solution, which is the aim of the present investigation.

2. Experimental

Shrimp chitosan with the deacetylation degree 77% and molecular weight 250 kDa was used for the experiments. The viscosity of 0.4% chitosan solutions in 2% acetic acid was measured by a Ubbelode viscometer at the temperature 20 ± 0.5 °C. Recrystallized AA was introduced into the system in the form of a concentrated solution in water. A part of the solution, immediately after the introduction of AA, was sealed in glass ampoules after exchanging of air to nitrogen. The solution viscosity was measured immediately after breaking the ampoules. The chitosan from the solution in acetic acid was isolated by precipitation with a 5% ammonia solution. Sediments were filtered-off, washed with distilled water, then with ethanol and dried at the temperature 80 °C. The yield of the water-soluble fraction was calculated as a difference between the sediment amount isolated from the original chitosan solution and the solution stored in the presence of AA.

For the quantitative determination of AA in the solution, the permanganometric method was used (Ashworth, 1968).

3. Results

The results of the experiment (Fig. 1) testify that the chitosan solution viscosity decreases very slowly as a result of

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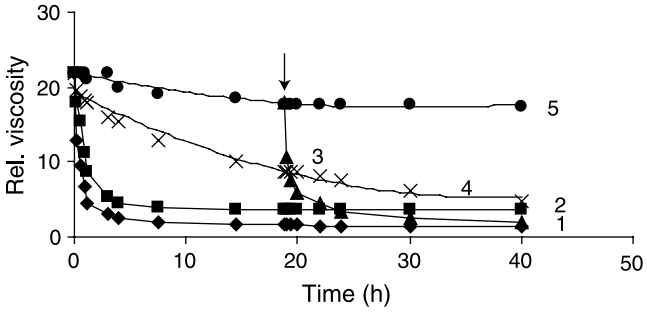


Fig. 1. Change in the viscosity of a 0.4% chitosan solution in 2% acetic acid in the presence of ascorbic acid at the acid concentration 0 (5), 0.1 (2), 0.25 (1) and 2 (3) g/l. 4–0.4% chitosan solution in 2% ascorbic acid. The arrow shows the moment of the introduction of air in sealed ampoules. Storage temperature 20 °C.

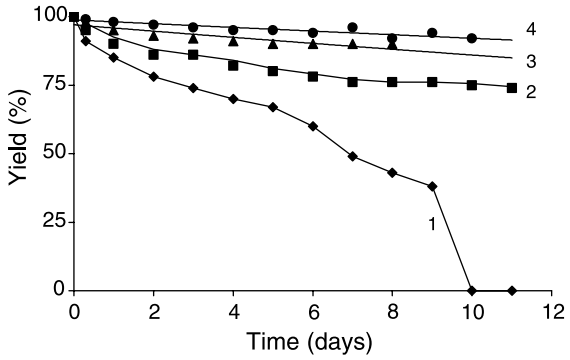


Fig. 2. Yield of the water insoluble fraction of chitosan after it sedimentation from solution in 2% acetic acid at the ascorbic acid concentration in solution 0.1 (4), 0.35 (2) and 2 (1) g/l. 3—ascorbic acid concentration 2 g/l, solution stored in a nitrogen atmosphere. Storage temperature 20 °C.

the oxidative and hydrolytic splitting of the chains during storing (curve 5). The introduction of an already small amount of AA (0.1 g/l) into the solution induces a rapid reduction in the viscosity of the solution (curve 2), as compared with the viscosity change without this additive. An increase in the AA concentration promotes this effect (curve 1). At the same time, if a chitosan solution is prepared in 2% AA, the destruction process proceeds considerably slower than in the presence of small quantities of AA (curve 4). We suppose, that the destruction process proceeds mainly by way of the oxidative cleavage of chitosan macromolecules by air oxygen, while the hydrolytic action is less possible, as the pH of the solution at the introduction of AA varies insignificantly. To demonstrate the influence of air oxygen, a part of the chitosan solution with the introduced AA was stored in sealed ampoules filled with nitrogen. As can be seen from Fig. 1 (curve 3), in the absence of air, the reduction in the solution viscosity proceeds considerably slower, although the influence of air cannot be completely excluded during the filling of ampoules and the viscometer. After the introduction of air in the ampoules (the arrow shows the moment of introduction), the reduction of the solution viscosity proceeds further, with the same rate as shown on curve 1, where air is present all the time.

In the chitosan destruction process, besides the viscosity changes, the chitosan water-soluble fraction (Fig. 2) was determined. At the AA concentration 0.1 g/l, after a 10-day storage at room temperature, approximately 9% of the water-soluble fraction was formed (curve 4). At the AA concentration 0.35 g/l, the water-soluble fraction was already 24%, and, at the acid concentration 2 g/l, after 10 days, practically the whole chitosan was converted into water-soluble fragments and could not be isolated by precipitation with ammonia hydroxide from the solution (curve 1). Simultaneously coloured AA oxidation products were formed. At the same time, in the absence of oxygen and at the AA concentration 2 g/l, only 8% of the water-soluble fraction was formed during a 10-day period. This fact additionally confirmed a significant role of oxygen in the destruction of chitosan.

The above-mentioned results testify that AA in chitosan solutions plays the role of an agent of oxygen transfer from air to chitosan and induces the oxidative splitting of polymer macromolecules. It is strange, taking into account the fact that AA acts as an antioxidant in biological systems. We did not investigate the mechanism of.the chitosan destruction process, but can express our opinion regarding it by using the information available in literature. It has been found (Bendich, Machlin, Scandurra, Barton, & Wayner, 1986), that the oxidation of ascorbat anion (AH^-) in solution in the presence of air is accompanied by the formation of superoxide anion as an intermediate:

$$AH^- + O_2 \rightarrow A^{-\cdot} + O_2^{\cdot -} + H^+$$

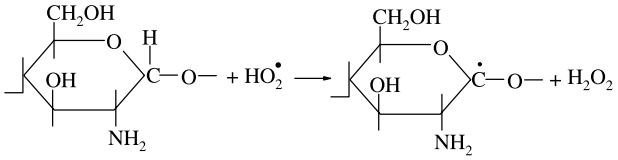
Superoxide anion exists in equilibrium with hydroperoxyl radical:

$$O_2^{\cdot -} + H^+ \leftrightarrow HO_2^{\cdot}$$

The formation of other products, for example, hydrogen peroxide, in the solution is also possible:

$$AH^- + O_2 + H^+ \rightarrow H_2O_2 + A^{\cdot -}$$

Hydroperoxyl radicals can react with polysaccharides by the abstraction of hydrogen from the carbohydrate molecule or by formation of unstable hydroperoxides. The most possible sites for reaction, according to the literature (Timochin & Unskova, 1981) are glycosidic bonds or free aldehyde groups:



The formed radicals are not stable and induce the splitting of the polymer chain at the formation carbonyl or carboxyl groups. The formation of hydroperoxides is also possible by the reaction of the polymer with hydrogen

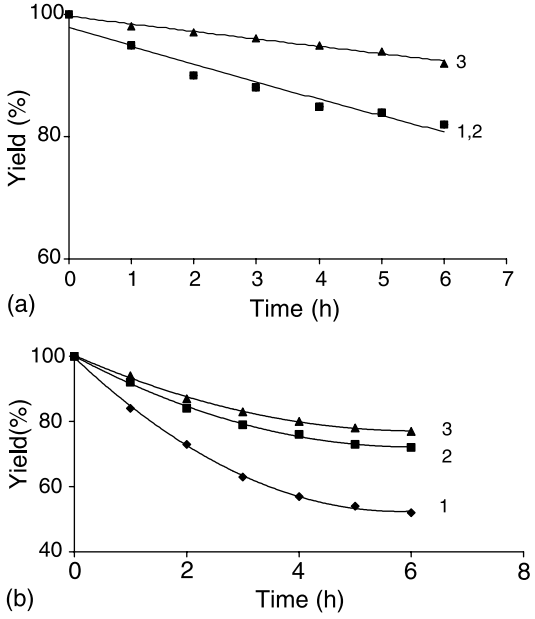


Fig. 3. Change of the ascorbic acid concentration in the solution at storing in the absence (a) and presence (b) of chitosan at the initial ascorbic acid concentration 0.2 (1), 0.35 (2) and 0.5 (3) g/l.

peroxide. It is well known that hydroperoxides are readily formed at room temperature at the addition of hydrogen peroxide to cellulose esters, for example carboxymethyl cellulose (Kubota & Ogiwara, 1978). We suppose that the same is valid for chitosan. We have observed that the addition of small amounts of hydrogen peroxide to chitosan solutions induces a comparatively rapid reduction of the polymer solution viscosity upon storing. However, at equal concentrations of AA and hydrogen peroxide in solution, the former induces a comparatively faster destruction of the polymer in the presence of air. Therefore, in presence of air in some cases AA act as a cooxidant. The acceleration of oxidation reactions by AA has been observed also in studies of yellowing of lignin-containing pulps in presence of oxygen and AA (Schmidt & Heitner, 1997)

It is interesting to note that, at the dissolution of chitosan in a 2% AA solution, the destruction process proceeds considerably slower than in solutions with much lower concentrations of the acid. Obviously, in this case, protection and splitting actions of AA occur simultaneously. Along with the observation of the influence of AA on the chitosan destruction process in solution, some interest is

Table 1
Ascorbic acid oxidation reaction rate constants in a 0.4% chitosan solution at the temperature 20 °C

Concentration of AA in solution (g/l)	Rate constants (K s^{-1} 10^{-5})
0.20	2.34
0.50	1.77
0.20; in the absence of chitosan	1.34

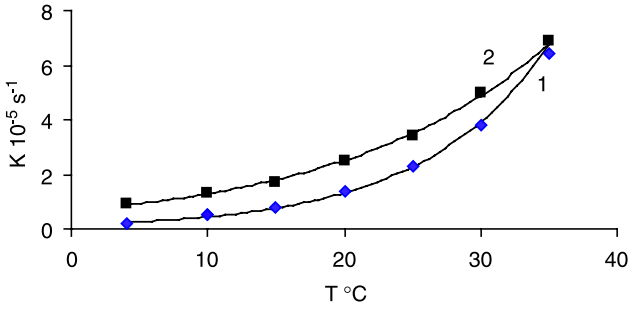


Fig. 4. Dependence of the ascorbic acid oxidation reaction rate constant on temperature in the absence (1) and presence (2) of 0.4% chitosan in the solution. Ascorbic acid concentration in the solution 0.20 g/l.

arisen at the subsequent AA concentration change in the same solution. The instability of AA in solutions in the presence of air is well known (Bendich et al., 1986)). In our experiments (Fig. 3a), a gradual decrease of the AA concentration in a 2% acetic acid solution in time was observed. In the presence of chitosan, the oxidation of AA was considerably accelerated (Fig. 3b), and this effect was dependent on the chitosan-AA proportion in the solution. For the initial periods of the reaction, first order reaction rate constants can be calculated (Table 1).

In the presence of chitosan at the AA concentration 0.2 g/l and room temperature, the rate constant increases approximately 1.5 times. At other temperatures, this proportion may be different (Fig. 4). From the dependence of the rate constant from the temperature, activation energy of the AA oxidation reaction was calculated. The obtained values were 77.2 and 43.8 kJ/mol for the reactions in the absence and presence of 0.4% chitosan in the solution, respectively. It is evident that chitosan diminishes the activation energy of the AA oxidation reaction in the solution. A more detailed elucidation of this reaction is not the aim of the present investigation.

4. Conclusion

It has been shown that ascorbic acid, already at low concentrations at room temperature, induces a comparatively rapid destruction of chitosan in acetic acid solutions with the formation of water-soluble fractions. The air oxygen substantially accelerates this process. At high AA concentrations, this effect is less pronounced than at low concentrations of AA. At the same time, chitosan accelerates the AA oxidation process in the solution and lowers the activation energy of the reaction. AA is supposed to participate in the formation of hydroperoxyl radicals, which attack the chitosan chains. Therefore, if chitosan is introduced in food products containing AA, it is necessary to consider the accelerated destruction of the polymer and the oxidation of AA in such systems.

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