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Degradation of the mucilaginous polysaccharide from *ruredzo (Dicerocaryum zanguebarium)* during autoclaving

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Mucilage was prepared from the leaves of ruredzo *(Dicerocaryum zanguebarium)* with and without heating suspensions of the leaves in water. The mucilage, after purification using copper (II) acetate, was heated in an autoclave at 125°C for up to 60 h. Autoclaving for 1 h released galactose only but autoclaving further, up to 10 h, released xylose in addition to galactose. When samples were autoclaved for over 20 h arabinose was the third monosaccharide released. Continued autoclaving generated fragments, probably disaccharides and oligosaccharides, whose identity has not been determined. There was no difference in the molecular weight of mucilage that was prepared with heating and that in which heating was not used. Heating to near boiling, that is involved in the standard preparation of the mucilage, did not seem to degrade the mucilage. Reducing power in the autoclaved samples, measured in terms of glucose, increased with increased duration of autoclaving.

INTRODUCTION

The leaves of *ruredzo (Dicerocaryum zanguebarium)* produce copious amounts of a slimy mucilage. It is this property that has probably been responsible for the use of *ruredzo* leaves by tribal people in southern Africa in the treatment of measles, cleansing and facilitation of births (Tredgold, 1986). The leaves are also used in the preparation of *derere* which is a dish that is enjoyed partly for its slimy consistency. The plant, which grows as a sprawling creeper in much of southern and central Africa, produces fruit which bears two upward pointing spines from which the plant derives its Latin name (Plowes & Drummond, 1976; Biegel, 1979). Although the structure of the mucilage has not been determined, studies in the authors' laboratory have shown that the mucilage contains galactose, xylose, arabinose and mannose in the ratio 21:19:12:1. Because of the authors' interest in the potential applications of the mucilage, the effect of autoclaving mucilage preparations was studied. In this paper the results of a study of the change in molecular weight of the polymer and the release of monosaccharide residues during autoclaving is reported.

EXPERIMENTAL

Preparation and purification of *ruredzo* mucilage

In the routine procedure small twigs and foreign material were separated from dried leaves which were then ground in a laboratory mill. The ground leaves (200 g) were transferred to a stainless-steel pot containing 5 litres of distilled water. The mixture was heated to near boiling with constant stirring. The hot mixture was allowed to cool to room temperature and then centrifuged for 1 h at 2000 rpm at 4°C in an ICE centrifuge. The centrifugation was repeated until all the suspended material had been removed. When mixtures with very high viscosities resulted, these were diluted by addition of distilled water to facilitate the pelleting of debris. After centrifugation, the first pellet was re-extracted with water and the supernatants pooled.

The clear supernatant was transferred to a beaker and two volumes of 96% ethanol added with constant stirring to precipitate the mucilage. The preparation had a brownish-white fibrous appearance. The mucilage was transferred to another beaker containing 3 litres of distilled water. The mucilage was allowed to

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hydrate and dissolve overnight at 4°C (Dutton & Yang, 1977; Churms *et al.*, 1983).

Mucilage was also prepared from fresh leaves, in this case plants were collected and leaves removed from the stems within 3 h. The leaves were homogenised in cold water (4°C) in a blender and centrifuged at 4°C. Mucilage was then prepared following the routine procedure for dry material. To study the effect of the heating step on the integrity of the polymer, mucilage was prepared without the heating step with all procedures being performed at 4°C.

Fractionation of *ruredzo* mucilage with copper(II) acetate

One volume of copper(II) acetate (7%) was added to a solution of crude *ruredzo* mucilage (1%) and one volume of 96% ethanol added to the mucilage-copper complex with stirring in order to induce precipitation (Jones & Stoodley, 1965). After allowing the precipitate formed to stand for 1 h the mixture was centrifuged and the pellet resuspended in 0·5 M EDTA (Angyal, 1989). The mucilage was recovered from solution by addition of two volumes of ethanol. After being washed three times in 96% ethanol the precipitate was dried in a preheated oven set at 100°C for about 30 min. Instead of precipitating the mucilage from solution dry samples of the mucilage were sometimes obtained by lyophilisation.

Acid hydrolysis

Purified mucilage (1·9 g) was added to 5 mM H_2SO_4 (100 ml) in a conical flask and left overnight to dissolve. The mixture was refluxed for up to 36 h after which time 5 ml aliquots were removed, cooled and neutralised with barium carbonate (Churms & Stephen, 1970). After centrifugation the clear supernatants were transferred to Teflon stoppered bottles and stored at 4°C until required for analysis. After 36 h of hydrolysis in 5 mM acid, the acid concentration was increased to 50 mM and the hydrolysis continued for up to a total of 60 h.

Autoclaving of mucilage dispersion

Purified mucilage (1 g) was added to 100 ml of water and left overnight to hydrate and dissolve. Aliquots were dispensed into screw-cap sample bottles and placed in an autoclave set at 125°C. The samples were autoclaved for up to 60 h. At intervals samples were removed for anlaysis by thin-layer (TLC) gas and gel filtration chromatography. The reducing power of the autoclaved samples was also determined by the dinitrosalicylic acid method (Chaplin, 1986).

For TLC treated samples were analysed for released monosaccharides on Whatman K5 silica gel plates. The solvent system used to develop the plates was ethyl acetate/pyridine/water (10:4:3 v/v) (Churms & Stephen, 1970). After developing plates for 2 h spots were detected using diphenylamine/aniline/orthophosphoric acid reagent (Chaplin, 1986).

For gas chromatography alditol acetates were prepared by adding an equal volume of freshly prepared 0·5 M sodium borohydride to the sample and stirring the mixture continuously for 90 min at room temperature (Supelco Inc., 1985). Glacial acetic acid was added until the bubbling stopped. Methanolic-HCl (3 ml) was added and the solvent removed in a rotary evaporator. The dried samples were placed in a desiccator over phosphorus pentoxide overnight to ensure complete dryness. Dry samples were transferred to reaction vials and 0·2 ml pyridine and 0·4 ml acetic anhydride added. The vials were sealed with Teflon-lined caps and heated at 80°C. Aliquots (1 μl) were injected onto a stainless-steel SP-2330 packed column at 230°C. The gas chromatography of the alditol acetates of monosaccharides released during acid hydrolysis and during autoclaving was used to confirm the identifications made by TLC.

In the gel chromatographic analysis autoclaved samples were dialysed overnight against a gently running stream of tap water. Dialysed samples (0·5 ml) were applied onto a Sepharose 6B column (138 × 3·2 cm) and 3 ml fractions collected on a BioRad Econosystem fraction collector. The molecular weight of the purified mucilage and that of the degradation products were estimated by calibration of the column with dextran T standards from Pharmacia (Uppsala, Sweden). The presence of carbohydrate in the fractions was detected using the phenol-sulphuric acid method (Dubois *et al.*, 1956; Chaplin, 1986).

To determine reducing power 3,5-dinitrosalycilic acid reagent (1 ml) was added to samples (100 μl) and the mixture thoroughly agitated before being heated in a boiling water bath. After 10 min the samples were cooled rapidly and their absorbance at 570 nm measured. Reducing power was expressed in equivalent amounts of glucose (Chaplin, 1986).

Measurement of viscosity

The relative viscosity of solutions of the native mucilage (1%) and of the autoclaved samples was measured at 25°C using an Ostawld-type viscometer.

RESULTS AND DISCUSSION

The yield of mucilage using the standard method of preparation was 14·2%. This decreased to 10·8% when heating was excluded from the preparation procedure. Often the mucilage precipitated from solution as a fibrous mass most of which could be recovered easily by spooling onto a wooden rod or large spatula.

After 1 h of autoclaving only galactose was detected as the free monosaccharide released. Xylose appeared after 3 h of autoclaving. Only these two monomers were detected in samples autoclaved for up to 10 h. Arabinose was detected only in samples that had been autoclaved for over 20 h. Autoclaving for 40 h released, in addition to the monosaccharides, fragments whose nature is yet to be determined but which are probably disaccharides and higher oligosaccharides. That arabinose unexpectedly resisted release from the mucilage is probably a clue to its position in the overall structure of the polymer. Whereas the authors had expected the polymer, because of its high molecular weight, to undergo a measure of degradation upon autoclaving the extent of depolymerisation was much greater than anticipated.

The reducing power of autoclaved samples, expressed in equivalent amounts of glucose, is shown in Fig. 1. As expected, the reducing power increased as monosaccharide residues were released during autoclaving.

Table 1 is a comparison of the products observed during hydrolysis in 5 mM sulphuric acid and autoclaving. As can be seen from the table, the release of galactose by acid hydrolysis required a longer period than by autoclaving. Arabinose was also released much later by acid hydrolysis than by autoclaving. No low-molecular-weight species corresponding to the presumed disaccharides and oligosaccharides were observed in acid hydrolysis. It would appear that autoclaving is a suitable way of producing small fragments for use in structural studies.

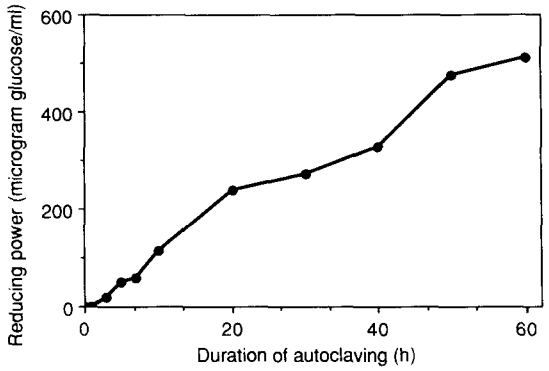

Fig. 1. Reducing power of samples was determined by the dinitrosalicylic acid method and expressed in terms of equivalent amounts of glucose.

There was no appreciable difference between the molecular weight of the mucilage prepared by the routine procedure of heating an aqueous mixture of dried ground leaves to near boiling and that prepared under cold conditions at 4°C. Also, no difference was observed between the molecular weight of the mucilage prepared by the routine procedure and that prepared from fresh leaves under cold conditions. In all cases, the molecular weight of the mucilage was about 500 000 D. It can be concluded that the boiling procedure used at the beginning of isolating mucilage did not degrade the mucilage noticeably. Extensive degradation only occurred when the mucilage samples were autoclaved.

The pattern of degradation of *ruredzo* mucilage during autoclaving is shown in Fig. 2. The native polymer was degraded to form smaller fragments with a dominant component having a molecular weight of about 70 000 D. The relative amount of this dominant component increases up to 20 h of autoclaving after which it decreases as smaller fragments arise from further degradation.

As can be seen in Fig. 3, the viscosity solutions of the mucilage dropped considerably when the solutions were autoclaved for 1 h. Autoclaving mucilage solutions for longer periods led to much less reduction in viscosity.

CONCLUSION

It is clear that *ruredzo* mucilage can be extensively degraded by autoclaving. The degradation released not only the galactose, xylose, arabinose and mannose which had been observed in acid hydrolysis studies but

Table 1. Release of monosaccharide residues during autoclaving of mucilage solutions

Time of treatment (h)	Products that were detected after	
	Acid hydrolysis	Autoclaving
1	None	Galactose
2	None	Galactose
3	None	Galactose and xylose
4	Galactose	Galactose and xylose
6	Galactose and xylose	Galactose and xylose
10	Galactose and xylose	Galactose and xylose
20	Galactose and xylose	Galactose, xylose, arabinose, mannose, unknown 1, and unknown 2
48	Galactose, xylose, and arabinose	As for 20 h
60	As for 48 h	As for 20 h

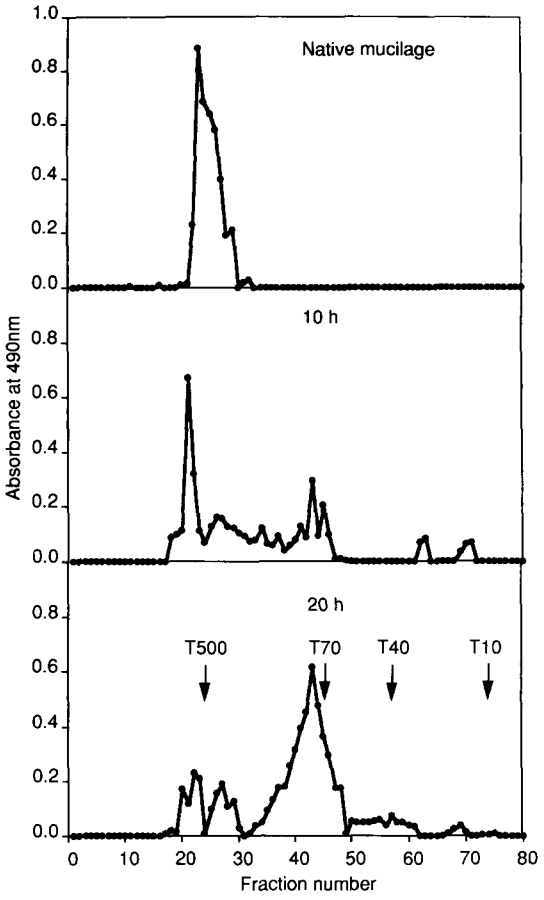

Fig. 2. Samples were autoclaved for up to 60 h and the degradation of polysaccharide monitored by gel filtration on a Sepharose 6B column. The figure shows gel filtration patterns for native mucilage and for samples that had been autoclaved for 10 and 20 h. Carbohydrate in collected fractions (3 ml) was determined by the phenol sulphuric acid method. The arrows indicate the positions corresponding to the shown T dextran standards. The molecular weights of T500, T70, T40, and T10 standards are 500, 70, 40 and 10 kD, respectively.

also fragments that could be disaccharides and oligosaccharides. It appears that autoclaving could be used to generate fragments from *ruredzo* mucilage that are more amenable to analytical structural studies.

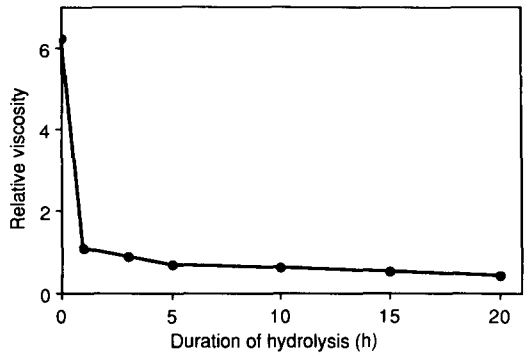

Fig. 3. The viscosity of samples of solutions of mucilage (1%) that had been autoclaved for up to 60 h measured at 25°C using an Ostwald-type viscometer.

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