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The use of cellulases for increasing the sugar content of AIV-silage

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Abstract. AIV silages were treated with cellulases. Five different enzyme preparations were compared. The highest amount of reducing sugar in silage was 190 g/kg (dry basis) and the highest amount of glucose in the press juice 24 g/dm^3. A combination of two enzyme preparations produced more glucose than any enzyme tested alone. Glucose production was approximately proportional to the enzyme concentration. Because of lactic acid fermentation, cellulase alone could not maintain the sugar content at a high level, a preservative being required to inhibit the lactic acid bacteria. No differences were noted in the glucose contents in silages with different dry matter contents (24—29 %).

Introduction

In Finland silage is made mainly by the AIV method, in which the pH is lowered by AIV solutions. Nowadays most often AIV II solution, which contains 80 % formic acid and 2 % phosphoric acid, is used.

In good silages made by the AIV method the lactic acid content is below 1 %, the ammonia content below 0.5 g l and the sugar content above 2 % (HEIKONEN et al. 1978). Rumen microbes need sufficient energy to synthesise protein from ammonia and other soluble nitrogen compounds in feed. In practice it may happen that silages are deficient in energy rather than in protein. Therefore the production of more sugar in silage by cellulolytic enzymes was studied.

In silage experiments cellulases have usually been used to produce sugars for fermentation by lactic acid bacteria. Sometimes the aim has been to improve the digestibility by lowering the fiber content. Only seldom have preservatives been used to stabilize the sugar content.

In earlier investigations, enzymes produced by *Aspergillus* spp., which have hemicellulolytic and proteolytic activity in addition to cellulolytic activity, were used. The organoleptic properties of the treated silages were good, the lactic acid content was higher and the pH lower than in the control silages (Boiko et al. 1967, Ezdakov and Fesjun 1967, Konoplev and Stserbakov 1970).

Henderson and McDonald used formic acid as the preservative and added cellulases produced by *A. niger* (0.4 %) to the herbage. The amount of cellulose in the silage decreased markedly during a period of 61 days. They also compared cellulases produced by *A. niger* and *Trichoderma reesei* and found the latter more efficient. The highest sugar content produced was 153 g/kg DM.

In alfalfa silage the hydrolysis of cellulose increased with successive additions of enzyme (0.1—0.5 %). The maximum hydrolysis was 29 %. The titratable acidity and the content of reducing compounds were greater in enzyme-treated silages (Leatherwood et al. 1963).

Autrey et al. (1975) added a fungal cellulase from *T. reesei* to whole maize. The cellulose content decreased significantly during one year's ensilaging. There was some indication of improved digestibility by cows at the higher levels of cellulase addition, but the differences were not significant. Neither did Olson and Voelker (1961) succeed in improving the digestibility of maize by *A. oryzae* cellulases. By adding cellulase with $CaCO_3$ buffer to sorghum prior to ensiling, McCullough (1964) produced a silage with 20 % less cellulose than the untreated control silage. The digestibility of the former was slightly increased (treated 54.5 %, untreated 42.6 %).

Experimental

Materials and methods

The timothy-clover mixture was obtained from the Viikki Experimental Farm of Helsinki University. Silage was made three times: June 15, July 26 and September 15. The dry matter contents of the silages were 24, 31 and 28 %, respectively. The freshly cut herbage was allowed to dry in the field for 2—5 hours before chopping. The preservative was sprayed onto the herbage from a plastic spray bottle, and the dry enzyme preparation was spread onto the herbage. The silage was packed into polyethene bags, about 500 g per bag. The bags were put into plastic jars (two bags per jar) and a sandbag was placed on the top to compress the silage. The preservative used was AIV II, which was first diluted 1: 6 and then used at the rate of 42 ml/kg. The enzymes used, their manufacturers and activities are shown in Table 1.

The β-glucosidase activity of cellulases was determined by the method of Norkrans (1957) and activity in the digestion of filter paper (FPU) by the method of Mandels et al. (1976). Silage quality was assayed by measuring the pH, determining lactic, propionic, acetic and butyric acids by paper chromatography (Miettinen and Virtanen 1951) and by measurement of ammonia. The hydrolysis of cellulose was followed by determining the reducing sugars (Nelson 1944 and Somogyi 1945) and glucose (GOD-Perid, Boehringer Mannheim GmbH, Mannheim, BRD). The dry matter was determined by drying the samples in aluminium dishes at 80° C overnight.

Table 1. Enzymes used.

Enzyme	β-glucosidase activity, x10^{-3} units	FPU	Supplier
Cellulase S	20	0.21	Société Rapidase, Seclin, France
Hemicellulolytic complex	153	0.07	Société Rapidase, Seclin, France
Maxazyme	153	0.68	Gist-Brocades nv, Delft, Netherlands
Meicelase	163	0.80	Meiji Seika Kaisha Ltd., Osaka, Japan
Onozuka SS	165	0.22	All Japan Biochemicals, Co. Ltd., Shingikancho, Nishinomiya, Japan
VTT cellulase	120	2.0	Technical Research Centre of Finland, Biotechnical Laboratory, Helsinki, Finland.

Results

Comparison of enzymes

The silage was made on June 15. The enzymes used were: Maxazyme, Meicelase and VTT cellulase (0.05 %), Cellulase S and Hemicellulolytic complex (0.11 %).

The greatest increase in the glucose content was obtained with VTT cellulase. The amount of glucose in the press juice of silage treated with it was 24 g/dm^3 after six months of ensilaging (Fig. 1). The amount of reducing sugars was highest in silages treated with Maxazyme or VTT cellulase (Fig. 2). The amount of glucose was 30—65 % of the amount of reducing compounds in the press juice.

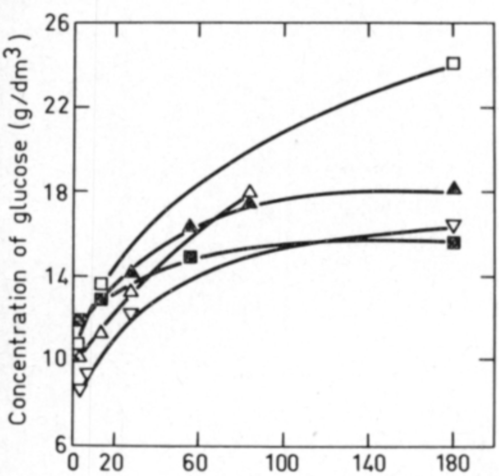

Fig. 1. The concentration of glucose in the press juice of silage with the following cellulase preparations: Cellulase S ▽, Hemicellulolytic complex ■, Maxazyme △, Meicelase ▲ and VTT cellulase □.

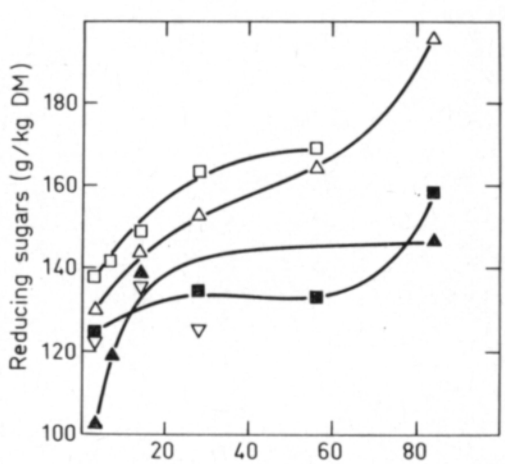

Fig. 2. The content of reducing sugars in silage with Cellulase S ▽, Hemicellulolytic complex ■, Maxazyme △, Meicelase ▲ and VTT cellulase □.

Combinations of enzymes

The silage was made on June 15 and July 26. In addition to VTT cellulase, Onozuka (by weight 1:1, 1:3 and 3:1 June 15; 1.5:1, 3:1 and 6:1 July 26) and Hemicellulolytic complex (1:1 June 15; 1.5:1, 3:1 and 6:1 July 26) were used. The total enzyme concentration was 0.05 %.

The combinations gave greater glucose concentrations than VTT cellulase alone. In the first series of experiments (June 15) only one silage treated with the combination of enzymes (VTT + Onozuka 1: 3) contained less glucose than the silage treated with VTT cellulase alone (Fig. 3). In the second series (July 26) the highest amounts of glucose were obtained with a combination of VTT cellulase and Hemicellulolytic complex. The highest glucose content in the press juice was 17 g/dm^3 (Fig. 4). The lower glucose contents in the second series of experiments were probably due to the higher dry matter content of the silage.

Amount of enzyme

The effect of the amount of enzyme was tested with VTT cellulase and Meicelase, using concentrations of 0.02—0.4 %. The silage with Meicelase was made on June 15 and with VTT cellulase on September 15. A control sample without added enzyme was included in each series.

The amount of glucose formed in 56 days was proportional to the concentration of enzyme, with both VTT cellulase and Meicelase (Fig. 5). The enzymes cannot be intercompared here because of the different raw material.

The silages with highest enzyme concentrations (0.2 and 0.4 %) contained more free water than the control silages.

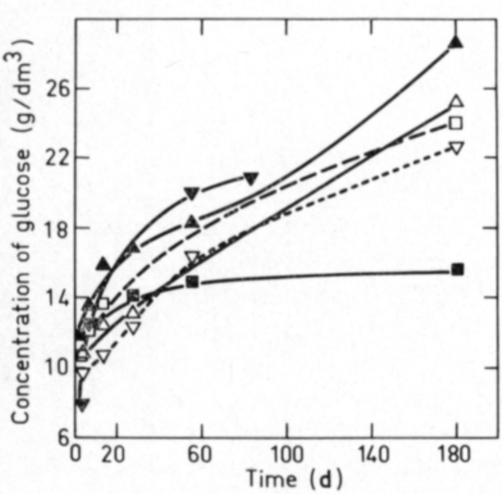

Fig. 3. The concentration of glucose in the press juice of silage, made on June 15, with VTT cellulase □, Hemicellulolytic complex ■, VTT + Hemicellulolytic complex 1: 1 ▲, VTT + Onozuka 1: 1 △, 1: 3 ▽ and 3: 1 ▲.

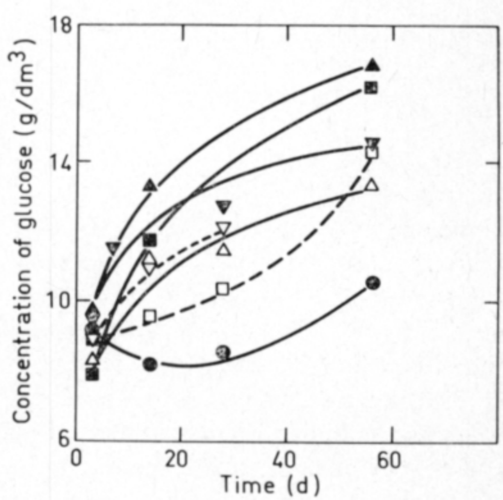

Fig. 4. The concentration of glucose in the press juice of silage, made on July 26, with VTT + Hemicellulolytic complex 1.5: 1 ■, 3: 1 ▲, 6 :1 ▼, VTT + Onozuka 1.5: 1 □, 3: 1 △, 6: 1 ▽ and no enzyme ●.

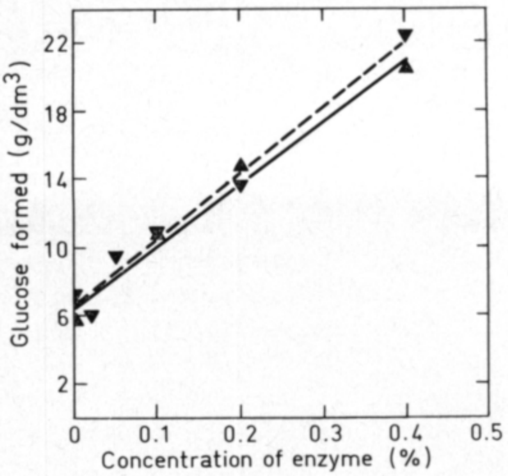

Fig.5. Glucose formed in 56 days with different concentrations of Meicelase (▼) and VTT cellulase (▲).

Use of preservative

The silage was made on June 15. AIV II solution was added at the rate of 0, 5, 6 and 7 ml/kg herbage. The pH of the silage without preservative fell to 4 during a period of one month, while the amount of lactic acid rose to nearly 2 %. After using preservative to lower the pH the concentration of lactic acid remained below 0.2 % throughout the experiment. Without preservative the concentration of glucose fell quickly to below 0.5 %. Cellulases may have formed more sugar in the silage, but lactic acid bacteria fermented it immediately to lactic acid.

Effect of dry matter content

The herbage was dried in air before ensiling. The dry matter contents were 24 %, 27 % and 29 %. There were no significant differences in the amounts of glucose formed in these three silages.

Discussion

Henderson and McDonald reported that the amount of sugar in silage treated with *T. reesei* cellulases increased about 150 % during 175 days of ensilaging. In the experiments reported here the period of ensilaging was shorter; in 84 days the amount of reducing sugar increased about 90 % with the same amount of enzyme (Meicelase 0.4 %). The increase in the amount of glucose was greater, 170 %. The highest amounts of glucose were formed by cellulases from *Trichoderma* spp. (Maxazyme, Meicelase, VTT cellulase.)

The use of cellulases to increase the sugar content of AIV silage made of low-sugar material would be advantageous if the sugar produced by the enzymes were cheaper than that in the usual sugar-containing forages. According to prices of whey powder and molasses the acceptable price for cellulases is 25—120 mk/kg, depending on the enzyme (enzyme protein 50—400 mk/kg). The effect of cellulases on the feeding value of AIV silage, and thus the economics of enzyme treatment, has not yet been evaluated. Recent progress in the cellulase production methods gives rise to the belief that the treatment of silage with enzymes will soon be economically feasible (Linko et al. 1977).

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SELOSTUS

Sellulaasin käyttö AIV-rehun sokeripitoisuuden lisäämiseksi

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Työssä vertailtiin eri sellulaasivalmisteiden vaikutusta selluloosan hydrolyysiin AIV-rehussa, haettiin tarvittava entsyymipitoisuus sekä tutkittiin säilöntäaineen määrän sekä raaka-aineen kuiva-ainepitoisuuden vaikutusta. Parhaiten rehun glukoosipitoisuutta lisäsi VTT:n sellulaasi, puristenesteen glukoosipitoisuus oli lopuksi 24 g/dm^3. Pelkistävien sokereiden määrä kasvoi eniten Maxazymellä ja VTT:n sellulaasilla. VTT:n sellulaasin glukoosintuotantoa voitiin vielä lisätä käyttämällä sen kanssa yhdessä entsyymiä, jonka β-glukosidaasiaktiivisuus oli suuri. Suurimmilla käytetyillä pitoisuuksilla rehun rakenne oli vetisempää ja hajonneempaa kuin kontrollirehujen rakenne. Käytettyjen kuiva-ainepitoisuuksien (24–29 %) välillä ei havaittu eroja syntyneiden sokereiden määrissä. Ilman happoa säilötystä rehusta maitohappobakteerit käyttivät sokerin maitohapoksi, happoa lisättäessä sokeripitoisuus pysyi korkeana.