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BACTERIAL DECOMPOSITION OF GRASS-FRUCTOSAN OF DIFFERENT DEGREES OF POLYMERIZATION

W. KÜHBAUCH and A. KLEEBERGER*

Institut für Grünlandlehre, Technische Universität München, Freising- Weihenstephan

ABSTRACT

The bacterial decomposition of grass fructosans with different degrees of polymerization was monitored *in vitro*. The period required for the microbial decomposition of polymeric fructosans was directly related to the extent of polymerization. It was concluded that there might be a relationship between the digestibility of other organic nutrients of fodder plants and polymerization and that polymerization increases with maturity of the plant.

INTRODUCTION

The variation in the feeding value of grass has been subjected to considerable study. Usually an indirect relationship has been found between the digestibility of total organic substances, and also specific organic substances, and the stage of growth (7, 9). Variability in the composition of different elements of polymeric compounds would be an insufficient explanation for the *status quo* (6, 14). Presumably there is a causal relationship between digestibility and the progressive condensation of highly polymeric organic substances. The base of the stem is the main organ for the storage of reserve carbohydrates in grasses. In temperate zones, grasses accumulate considerable quantities of polymeric fructosan before flowering (8). It is known from earlier investigations that the average extent of polymerization of the fructosans of cocksfoot and timothy is very different (1, 3). So the example of fructosan from grasses would show how extensively the bacterial utilization of such compounds is influenced by different extents of polymerization.

EXPERIMENTAL

Isolation and characterization of fructosan. Fructosan was extracted from the stem base of cocksfoot and from the haplocorm of timothy. The numerical average of the molecular weight of the fructosans was calculated on the basis of the linear relationship that has been found (10) to exist between the reciprocal of the elution time (V_e) and the total bed volume (V_t) and the logarithm of the average molecular weight ($\log_{10} \overline{M}_n$). Constituents of fructosan, fructose and glucose, were determined by the use of enzymes. Both procedures were described in an earlier paper (5). The type of combination and structure of the fructosan chains was determined with the aid of IR-spectrometry: 1–2 mg highly purified fructosan was homogenized with 300 mg potassium bromide and dried at a temperature of 85°C. Directly afterwards a disk was pressed, and the spectrum was recorded with a spectrophotometer, Perkin Elmer model IR-257.

The bacterial decomposition of fructosan. Bacteria with the ability to reduce fructosan were isolated from the juice expressed from grass silage (from a wilted crop, mainly cocksfoot, ryegrass and white clover) using a modified MRS (11) medium containing either fructosan isolated from cocksfoot or timothy as the sole carbohydrate source. Mixed cultures were isolated and cultured on modified MRS agar. Both the isolation and the propagation of isolates on agar were conducted at 30°C under anaerobic conditions, using an atmosphere of 90% N_2 and 10% CO_2. All media were sterilized at 121°C for 15 min.

The nutrient base employed was that of the

*Bakteriologisches Institut, Süddeutsche Versuchs- u. Forschungsanstalt für Milchwirtschaft, Freiseing- Weihenstephan

modified MRS medium, without added dextrose, and in which yeast extract freed from fermentable carbohydrate by a preliminary fermentation with enterobacteria (11) was used. The only source of carbohydrate present in the final medium was fructosan from timothy, fructosan from cocksfoot or the trisaccharide raffinose which were added as filter-sterilized 1% solutions to the modified MRS base to produce a final concentration of 0·1%.

Ten ml quantities of each of the three carbohydrate-defined media were inoculated almost simultaneously with 0·1 ml of a culture of each of the isolates from grass silage. Cultures which had been isolated in the medium containing timothy fructosan were inoculated into the corresponding fructosan medium and into the medium containing raffinose; those isolated in the medium containing cocksfoot fructosan were inoculated to the cocksfoot medium. Similarly all three media were inoculated directly with juice expressed from silage. All these test cultures were incubated at 30°C for 40 h. Uninoculated controls and controls lacking carbohydrate supplements were also prepared. Sufficient replicates were prepared to allow duplicate determinations of the fructosan content at 2-hourly intervals over the 40-h period. A colorimetric procedure was used for fructosan estimation based on arsenic molybdate (12), with a preliminary treatment of samples to remove proteins (4).

Molecular weight and type of fructosan compound. The distribution of the molecular weights of fructosan from timothy and cocksfoot and of the reference substances Dextran 20 and Dextran 10 are shown in Fig. 1. The majority of the fructosans of timothy are seen to lie within the range of molecular weights of Dextran 20, the fructosans of cocksfoot within that of Dextran 10. The numerical average of the molecular weight of the fructosans are given in Table 1.

The relative broad distribution of cocksfoot fructosan with considerable quantities at $V_e/V_t=0{\cdot}49$ and $V_e/V_t=0{\cdot}56$ is very striking. The distribution of the molecular weight of timothy fructosan, however, is very limited, with a distinct maximum at $V_e/V_t=0{\cdot}35$. So there is a very clearly defined average molecular weight of 25,500 for timothy fructosan corresponding to a degree of polymerization (DP) of 157·3. Fructosan from cocksfoot has average molecular weights of 10,600 and 6,200 and DPs of 65·3 and 38·2, respectively.

Both the fructosans appear to be based principally on saccharose, in view of their similarity with results obtained by calculation on the extent of polymerization from the molecular weights after separation through Sephadex G-75 and from the fructose/glucose quotient (Table 1).

Fig. 2 shows the IR-Spectrum with that part in which there is, according to Verstraeten (16), the characteristic field for compounds of ketose between 1000 and 700 cm^{-1}. Fructosans from timothy and cocksfoot have absorption maxima at 822, 856, 875, 924, 946 cm^{-1} and at 822, 856, 875, 924, 948 cm^{-1}, respectively. So they are completely identical and they are in accordance with the typical trend of the 2·6-phlein combination of fructosans (5, 13). So the only difference found between these two fructosans is in their DP.

TABLE 1. Average molecular weight ($\overline{M}_n$) and degree of polymerization (DP) of fructosan of timothy haplocorm and cocksfoot stem base. Reference substance: Dextran 10 and Dextran 20

Origin of fructosan and reference substance	According to gel filtration		According to quotient of constituents fructose : glucose	
	$\overline{M}_n$	(DP)*	DP	$(\overline{M}_n)$†
Timothy haplocorm	25500	(157,3)	155	(25128)
Cocksfoot stem base	10600	(65,3)	52	(8442)
	6200	(38,2)		
Dextran 10	5700	(35,1)	—	—
Dextran 20	15000	(92,5)	—	—

*Calculated from $\overline{M}$; †Calculated from DP.

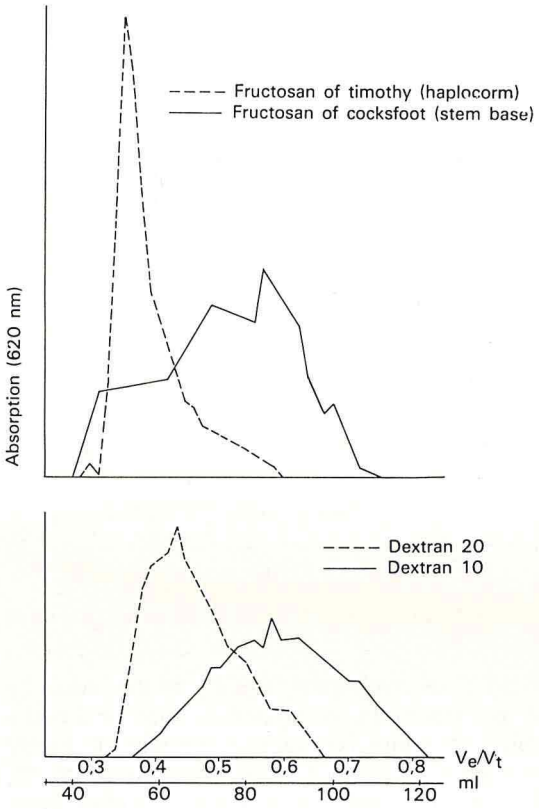

Fig. 1. Distribution of molecular weight from timothy haplocorm and cocksfoot stem base—reference substance: Dextran 10 and Dextran 20.

Bacterial decomposition of fructosan. The culture of bacteria growing under conditions described above were sporeless, katalase-negative, nonmotile bacilli.

Figs 3 and 4 show the decomposition scheme of the different polymeric fructosans of timothy and cocksfoot. Fructosan with DP 157·3 (calculated from $\overline{M}_n$) was reduced by a mixed culture of bacteria isolated from silage juice with not less than a 12 h period of adaptation, whereas fructosan with DP 65·3/38·2 is reduced immediately (Fig. 3).

About 30% of the minor polymeric fructosans were reduced within a period of 8 h. Fructosan with DP 157·3, however, was completely unchanged after this period. Both fructosans were almost completely consumed by the micro-organisms after a period of 20 to 28 h. After 40 h, virtually all of the two fructosans had disappeared. Raffinose was consumed surprisingly slowly.

The mixed cultures of MRS agar maintained had a similar decomposition scheme (Fig. 4). But the difference in the decomposition velocity between the two fructosans was much more apparent. 30% of fructosan of a shorter chain-length was already reduced after 4 h, whereas fructosan of timothy, which is more polymeric, was still unchanged after 8 h. Only a quantity of 10 to 15% was reduced in an adaptation period, which lasts 28 h, whereas the fructosan of cocksfoot had virtually disappeared after 24 h. The long adaptation period with fructosan of timothy was followed by a logarithmic phase of decomposition, in which the complete quantity of fructosan was consumed by the micro-organisms

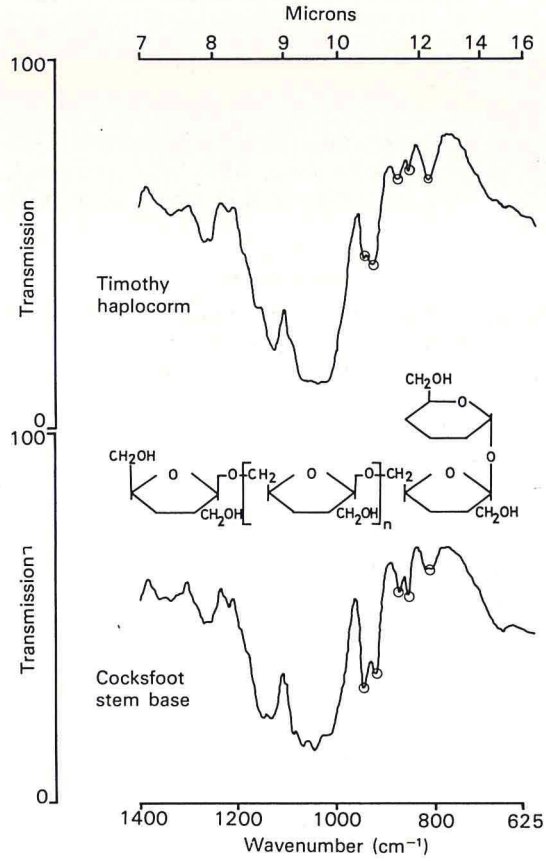

Fig. 2. Infrared spectra of fructosan from timothy haplocorm and from cocksfoot stem base.

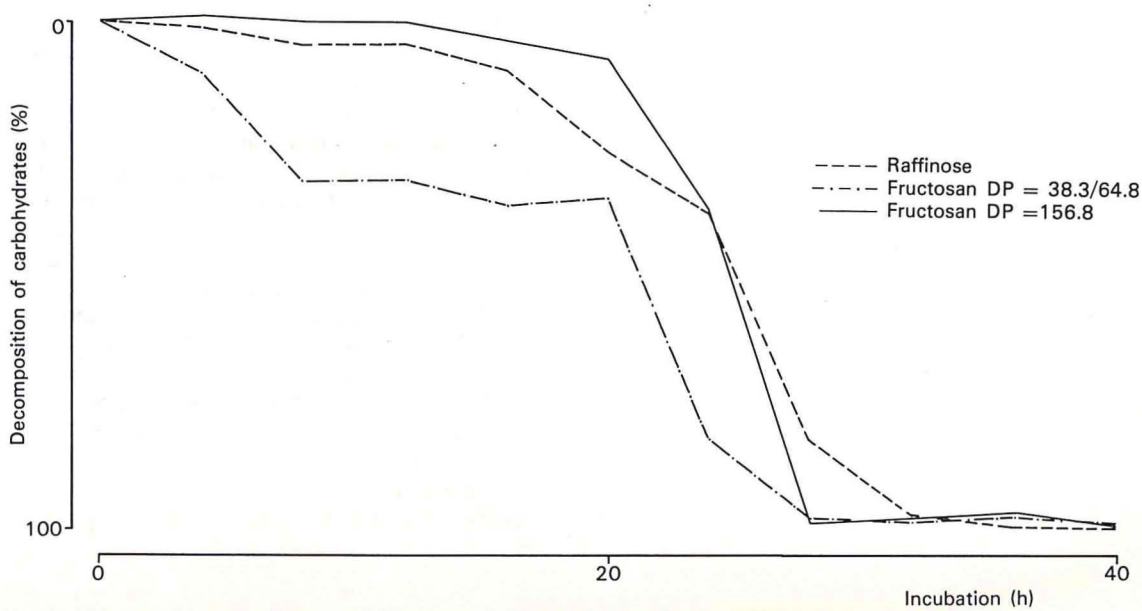

Fig. 3. Decomposition of raffinose and fructosan of different degree of polymerization by bacteria of grass ensilage—direct inoculation.

within 4 h, with only a negligible quantity left. As for raffinose, only about 35% was consumed, even if the incubation period lasted as long as 90 h.

DISCUSSION

The broad distribution of the molecular weights of cocksfoot fructosan which extended down to less than 2000 was undoubtedly a contributory factor in the observed greater rapidity in the reduction of this fructosan, compared to that of timothy which is composed almost entirely of highly polymeric molecules. The difference in the decomposition rates of carbohydrates of different polymeric complexity was not observed in those studies involving direct inoculation with silage juice, indicating that there is a synergistic relationship between component micro-organisms

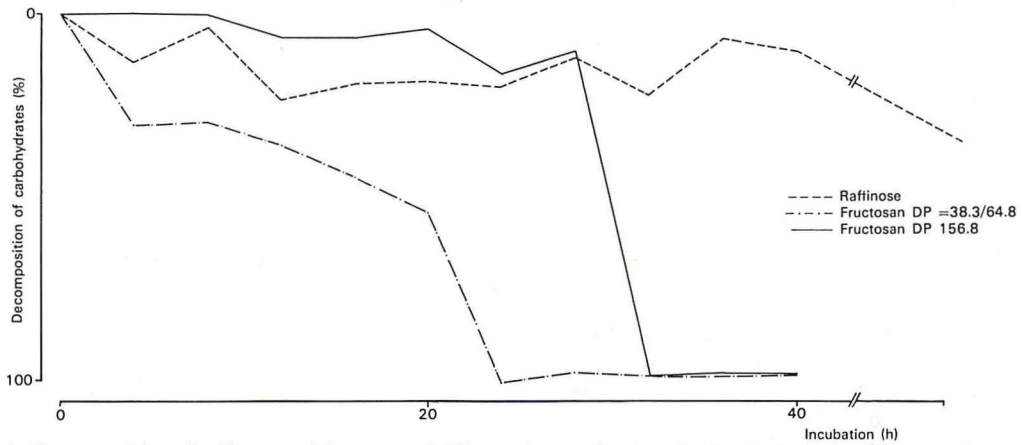

Fig. 4. Decomposition of raffinose and fructosan of different degree of polymerization by bacteria of grass ensilage—cultivated by streaks on solid medium.

in the juice. This results in a much more rapid reduction of all the carbohydrates.

The fact that both fructosans are almost completely reduced within 40 h suggests that the qualitative differences in this type of compound are not observed in the conventional digestive tests in which the incubation period normally extends to 48 h (15). It would appear that this has important ramifications. Certainly, large quantities of highly polymeric carbohydrates agree with ruminants much better than corresponding quantities of monosaccharides, as has already been shown with starch (2). All highly polymeric carbohydrates are presumably particularly effective, if they do not cause hyperacidity in the rumen, in spite of a digestibility which is as high as 100% (6), like the digestibility of fructosan. The widely quoted readiness of ruminants to accept feed might be particularly effective, if a high digestibility coincides with absence of hyperacidity. In addition, the present work illustrates that the degree of polymerization of organic compounds exercises a fundamental influence on their microbial utility, i.e. processes in the rumen are influenced in the same way as, for example, in ensilaging or haymaking.

A similar influence might occur with other types of compounds, for example hemicellulose. No investigation of the fractionated elements of these polymeric carbohydrates has yet explained the decline in digestibility with age of the plant. It is a biological principle that plants compensate for rises in osmotic pressure that would be created by monosaccharides by synthesizing highly polymeric compounds which exert no such pressure. This has been observed in the case of fructosan (3, 4) and has been suggested as being one of the factors responsible for the decline in the digestibility of these substances which increases with the maturity of the plant. The occurrence of intercellular or intracellular inclusions (possibly with lignin) or the increase in the complexity of the branching of the molecules could be another reason.

The insignificant decomposition of the trisaccharide raffinose (Fig. 4) might contradict this theory. But one has to take into consideration the fact that only a small highly specialized part of the microflora was obtained with the aid of streaks on a solid medium, and that the composition of the microflora changes during the fermentation process. Another fact supports this theory, i.e. after inoculation with silage fluid, hardly any of the raffinose was left, whereas after 90 h only 35% of the raffinose was consumed in those samples which were inoculated with micro-organisms which were cultivated by streaks on a solid medium.

In spite of the fact that analogous conclusions can be drawn from silage bacteria, it would be profitable to continue these investigations with rumen liquor. Further investigations should also involve different types of cell-wall constituents.

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