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IN VITRO FERMENTATION ASSESSMENT OF GERMINATION AND RECONSTITUTION PROCESSING FOR DIFFERENT SORGHUM GRAIN TYPES

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SUMMARY

The effects of controlled germination and reconstitution processes on fermentability of sorghum grain were investigated using an *in vitro* fermentation technique. Twenty-two sorghum grain types obtained from the University of Sydney Plant Breeding Institute, Narrabri, NSW Australia, were either dry rolled, reconstituted for 21 d (anaerobic storage) or germinated for 5 d. Grains were then rolled and incubated in buffered rumen liquor, and volatile fatty acid (VFA) production after 5 h was determined. Germination capacity (percent of grains that sprouted after 3 d) and water uptake after soaking for 24 h were also measured to determine their relationship with VFA production from germinated or reconstituted grains. Grain type and processing treatment significantly ($P<0.001$) affected the fermentability of sorghum grain and there was a significant level ($P<0.001$) of interaction between these factors. VFA production ranged from 44-69 mmol/L for germinated grains and 36-55 mmol/L for reconstituted grains, and both treatments gave significantly ($P<0.05$) higher VFA production than VFA production from dry rolled grains (20-28 mmol/L). Fermentability of germinated grains was higher than that of reconstituted grains for all except five grain types. Germination capacity and water uptake were significantly ($P<0.001$) affected by grain type and ranged from 3-97% and 33-41%, respectively. Germination capacity was significantly ($P<0.001$) and negatively correlated with water uptake ($r = -0.81$). VFA production was positively correlated with germination capacity ($r = 0.92$; $P<0.001$) but negatively correlated with water uptake ($r = -0.80$; $P<0.001$). For reconstituted grains, VFA production was neither correlated with germination capacity ($r = -0.06$, $P= 0.80$) nor with water uptake ($r= 0.27$, $P=0.23$). It was concluded from this study that controlled germination processing improved the fermentability of sorghum grain more than the conventional method of reconstitution, and the level of response to germination or reconstitution was dependent on grain type.

Keywords: germination, reconstitution, sorghum, *in vitro* fermentation, water uptake

INTRODUCTION

Unprocessed or minimally processed (dry rolled) sorghum grain is less digestible in cattle when compared to other cereal grains such as barley, oats and wheat. The endosperm of unprocessed sorghum grain contains a tightly packed protein matrix, within which the starch granules are embedded (Rooney and Pflugfelder, 1986). This dense matrix is most abundant in the outer corneous layer of the endosperm and the starch in this part of the endosperm is more resistant to digestive enzymes or bacteria than the starch in the inner floury endosperm, which is more susceptible to degradation (Huntington, 1997). Therefore, to maximize the utilization of sorghum grain by cattle, there is a need for some form of processing to break the protein matrix and make the starch more susceptible to digestion in the rumen and small intestine.

Reconstitution is an effective processing method for improving the digestibility of sorghum grain by cattle. The moisture content of whole grain is increased to at least 30%, after which the grain is stored anaerobically for a period up to 21 d followed by rolling before feeding. The improvement in the digestibility of reconstituted sorghum grains is partly attributed to the initiation of germination, which occurs following water absorption (Simpson *et al.*, 1985). Using a light microscope, Sullins *et al.* (1971) observed a disorganization of the corneous endosperm of reconstituted grain similar to those occurring in germinated grains. Increased solubilization of protein and starch was also reported in sorghum grains that were germinated prior to reconstitution compared to grains that were not germinated (Pflugfelder and Rooney, 1986). During germination, endogenous enzymes present or synthesised in the grain embryo breakdown protein matrix and starch in the endosperm and increase their availability for digestion by digestive enzymes and bacteria. It might therefore, be more beneficial to optimise the conditions of germination for hydrolytic enzyme breakdown of the protein matrix of sorghum grain in order to release the starch for maximum digestion in the rumen and small intestine of cattle.

The response of sorghum grain to reconstitution is variable and can be negative or positive (Hibberd *et al.*, 1986). This variation could be due to the differences in method of reconstitution, the type or cultivar of grain, or the age or storage conditions of the grains. The extent of exposure of grain to aeration prior to anaerobic storage would affect the extent of germination and eventually the response of the grain to reconstitution (Pflugfelder and Rooney, 1986). The compositions of starches and proteins in grains vary but differences in their structures in the endosperm are thought to be responsible for the differences in response to processing of sorghum grain (Rooney and Pflugfelder, 1986). These structural differences would also affect water uptake (Kavitha and Chandrashekar, 1992), which might in turn affect the germination capacity (ability to initiate and complete germination) of the grain. The objectives of this study were therefore (1) to examine the effects of reconstitution and germination processes on *in vitro* fermentation of different sorghum grain types and (2) to determine the relationship between the fermentability of processed sorghum grains and their germination capacity or water uptake.

MATERIALS AND METHODS

Grain and processing treatments

Samples of 22 sorghum grain types were obtained from the University of Sydney Plant Breeding Institute, Narrabri, NSW, Australia. Among these grains, two (Identification numbers 7828 and 7710) were of the waxy-type containing amylopectin starch. Grains were subjected to one of the following treatments: whole grain dry rolled; whole grain soaked in distilled water for 24 h and then stored anaerobically for 21 d (reconstitution) or germinated for 5 d (controlled germination) at 25°C. Immediately after treatment, reconstituted and germinated grains were rolled and dried at 50°C for 24 hours.

In vitro fermentation

A previous study conducted in this laboratory showed that volatile fatty acid VFA production from sorghum grains was closely correlated with starch digestibility (Balogun, unpublished). For this study therefore, the extent of *in vitro* fermentation was determined by measuring the total VFA concentration (mmol/L) after 5 h incubation according to Bird *et al.* (1999), but using smaller samples. Approximately 2 g (air-dry basis) of processed grains were weighed into 50 ml conical flask followed by the addition of 33 ml of buffered rumen liquor (1:3 rumen liquor and McDougall's buffer, McDougall, 1948). Samples were then incubated in a shaking water bath at 39°C for 5 h. Blank samples containing only buffered rumen liquor were also incubated. One millilitre of 3.6M H_2SO_4 was added to stop fermentation, after which liquor samples were taken and VFA concentration determined by gas chromatography (Varian CP 3800 Gas Chromatograph with Varian CP 8400 Autosampler) using isocaproic acid as internal standard (Erwin, 1961). The production of VFA from each grain sample was then calculated by subtracting the initial VFA concentration in the blank samples.

Germination capacity and water uptake

Germination capacity (%) was determined by counting 50 whole grains into petri dishes (9 cm diameter) lined with two layers of filter papers moistened with 4 ml of distilled water. There were three replicates for each grain. The grains were then allowed to germinate for 3 d at 25°C, after which the number of grains that sprouted was counted. For water uptake (%) determination, approximately 5 g of dry, whole grains were soaked in distilled water at 25°C for 24 h. The total moisture after soaking was determined by drying the grain in a force draught oven at 105°C to constant weight.

Statistical analyses

Data on germination capacity, water uptake and VFA production were subjected to analysis of variance using S-Plus statistical software (MathSoft, 1999). Significant differences between means were determined using the Fisher's Least Significant Difference test. Correlation analysis was carried out to determine the relationship between VFA production and germination capacity or water uptake using the S-Plus statistical software (MathSoft, 1999). Results from waxy grains were not included in the correlation analysis because they have endosperm characteristics, which make them to be inherently more digestible than the non-waxy cultivars (Huntington, 1997).

RESULTS

In vitro fermentation

Volatile fatty acid production ranged from 44-69 mmol/L for germinated grains, 36-55 mmol/L for reconstituted grains, and 20-28 mmol/L for dry rolled grains. The ranking of grains from the lowest to the highest VFA production was not similar for germinated, reconstituted or dry rolled grains. Figure 1 shows the increase in VFA production from germinated and reconstituted grains above VFA production from dry rolled grains. Grain type and processing treatment significantly ($P<0.001$) affected VFA production and there was an interaction between these factors ($P<0.001$). The production of VFA from germinated or reconstituted grains was significantly ($P<0.05$) higher than from dry rolled grains, and the level of increase was influenced by grain type. VFA production from germinated grains was significantly ($P<0.05$) higher than that from reconstituted grains except for 5 grains where VFA produced from the grains were similar ($P>0.05$) for both treatments.

Germination capacity and water uptake

Both germination capacity and water uptake were significantly ($P<0.001$) affected by grain type. Germination capacity ranged from 3% to 97% ($P<0.05$; se= 3.66). Water uptake ranged from 34% for grain 7818 to 41% for grain 7828 ($P<0.05$; se= 0.689).

Correlation

VFA production from germinated grains was positively correlated ($P<0.001$) with germination capacity ($r = 0.92$) but negatively correlated with water uptake ($r = -0.80$). There was no significant correlation between VFA productions and the germination capacity ($r = -0.06$; $P=0.80$) or water uptake ($r = 0.27$; $P=0.23$) for reconstituted grains. There was a significant ($P<0.001$) and negative correlation between germination capacity and water uptake ($r = -0.81$).

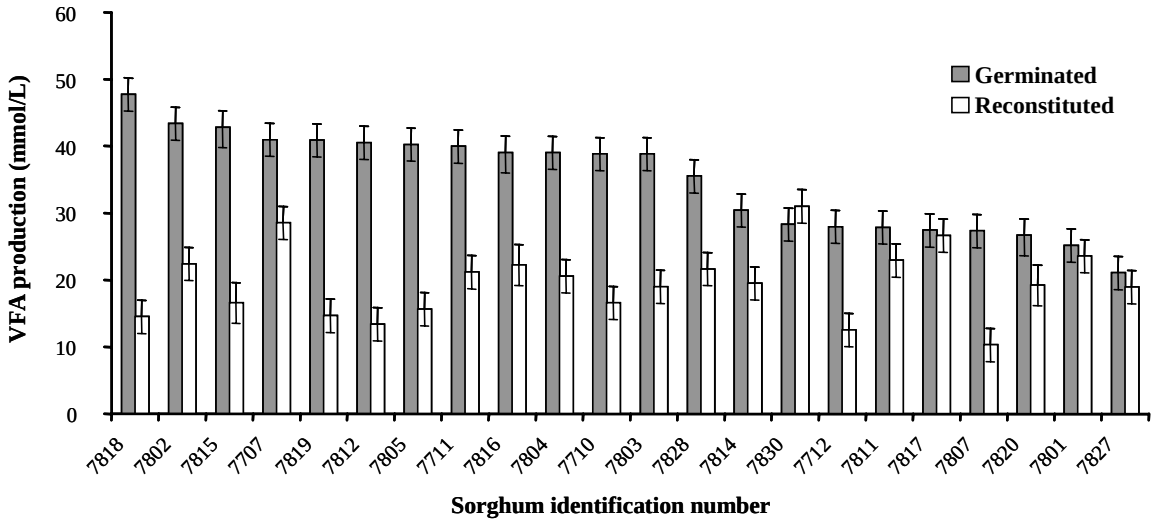

Figure 1. Volatile fatty acid (VFA) production (less VFA production from dry rolled grains) from germinated and reconstituted grains. Grains are arranged in decreasing order of VFA production from germinated grains. Error bars represent standard errors of means. Sample identification numbers are as provided by the University of Sydney Plant Breeding Institute Narrabri.

DISCUSSION

The higher VFA production from fermentation of germinated grains compared to reconstituted grains is consistent with the study of Pflugfelder and Rooney (1986) who reported that germination of sorghum grains prior to reconstitution was responsible for the increased starch and protein solubilization when compared to grains that were not germinated before reconstitution. The synthesis of hydrolytic enzymes and the break down of the protein matrix and starch in the endosperm are optimised during controlled germination process but are inhibited during the anaerobic phase of reconstitution (Simpson *et al.*, 1985). This limited enzyme activity could partly explain the lower improvements of fermentability of sorghum grains by reconstitution compared to germination. The similarity in the fermentability of germinated and reconstituted grains for 5 grain types in this present study could be due to their relatively low germination capacity indicating limited enzyme activities even under aerobic conditions.

The increase in VFA production from germinated grains above that from dry rolled and reconstituted grains ranged from 88 to 226% and -5 to 93%, respectively. This difference in response indicate variation between grain types and is consistent with Hibberd *et al.* (1986) who also reported that different sorghum grain types or varieties responded differently to reconstitution in terms of their *in vitro* dry matter disappearance or gas production. The ranking of grains according to their VFA production did not follow a similar trend for all treatments suggesting that the limiting factors for fermentation of a grain type are different for each processing treatment. The lack of correlation between VFA production from dry rolled, germinated or reconstituted sorghum and the protein or starch content of the grains (data not shown) indicate that the endosperm structure rather than the composition of starch and protein might be the critical factor limiting their digestibility.

Grains with low germination capacity are either inherently low or might have partly lost their germination capacity during storage. The positive and strong correlation between germination capacity and VFA production shows that grains with high germination capacity are likely to respond more positively and strongly to processing by germination than those with low germination capacity. However, despite their poor to medium germination capacity, VFA production from the two waxy grains (7710 and 7828) when germinated were comparable to some of the grains with higher germination capacity. Waxy grains are known to have soft endosperm with loosely packed protein matrix and starch granules, and therefore would require minimal processing to achieve high digestibility (Huntington, 1997). The lack of correlation between germination capacity and the VFA produced from reconstituted grain might be due to the fact that, even though germination is initiated during reconstitution, it is not likely to result in radicle emergence, which was the measurement used to determine germination capacity.

Water absorption is primarily a physical process and the rate and extent of uptake of water by grain would probably be higher for soft endosperm than for hard endosperm grains (Kavitha and Chandrashekar, 1992). The reasons for the negative correlation between water uptake and VFA production from germinated grains are not clearly understood but could be due to the fact that since water uptake is a physical process, grains that did not respond to germination will still absorb water. In fact dead grains will also absorb water. It is therefore, not likely that the extent to which a particular grain type or cultivar absorbs water would influence its response to either germination or reconstitution.

This study has shown that controlled germination for 5 d improved fermentability of sorghum grain compared to dry rolling or reconstitution for 21 d. The extent of improvement in fermentability by either germination or reconstitution process is also influenced by the type or cultivar of the grain. While germination capacity might appear to be a good indicator of the suitability of a sorghum grain for treatment by germination, this is not the case for water uptake.

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