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Comparison of Scanning Electron Microscopic Examination of Oats, Barley and Maize Grains with the Analyzed Degree of Starch Breakdown and Glycaemic Responses in Horses

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ABSTRACT

Size and surface-structure of starch granules and their interconnections influence starch hydrolysis. In native grains, these factors depend mainly from the botanical origin. Previously, it has been demonstrated that microscopic examination of starch granules refers to precaecal starch digestibility. A comparison of the elevated morphological characteristics of starch granules in scanning electron microscopic pictures with the degree of starch breakdown and the glycaemic response in adult horses after feeding a defined meal offers a tool to explain differences in the responsibility to enzyme attack and starch degradation.

Keywords: starch granules, scanning electron microscope, cereal grains, glycaemic response, horse

I. INTRODUCTION

Starch is the main carbohydrate in human and animal nutrition. The nutritional value of starch strongly depends on processing and the state of starch [1]. The glucose release as a source of energy for the body and the timeline of digestion are the major physiological properties of starch [1]. The individual botanical structure of different starch granules influences primarily the small intestinal digestibility in horses [2] and in horses the amylase activity and the capacity for starch digestion in the small intestine is very small [3]. Consequently a high small intestinal digestibility of cereal starch is the precondition for mono-gastric animals to maximize starch utilization [4]. Evidence exists that morphological properties of diverse starch granules according to scanning electron microscopy (SEM) may have a predictive value regarding the small intestinal digestibility of different starch sources in horses [2]. To our knowledge a comparison of SEM-pictures between different starch sources of cereal grain with the aid of proven labor analysis and blood parameters has not been reported before.

Aim was to investigate oats, barley and maize grains by SEM and to compare obtained starch characteristics with the analyzed degree of starch breakdown (DSB) and the glycaemic response to these cereal grains measured previously in adult horses.

II. METHODS AND MATERIAL

Starch granules embedded in surrounding structures deriving from grains of oats (variety 'Melody'), barley (variety 'ACK2927') and maize (variety 'M_002') were visualized by SEM (German Patent and Trademark Office; Brief disclosure for the Patent Application 10 2013 016 050.2) and further analyzed for DSB [5]. Conclusions from this were compared with the glycaemic response during the initial glucose raising period in six horses consuming meals from the same batches of cereal grains (mean of 0.8, 1.0 and 2.0 g starch/kg body weight; area under the glucose curve [AUC_{gluc}] up to 120 min pp. [6]. Prior to SEM, grains were crushed, spread out on a microscope slide, air dried and sputtercoated with gold. So-called secondary electron (SE) pictures were taken to characterize morphologic properties of starch granules and their embedding in surrounding structures.

III. RESULTS AND DISCUSSION

Starch is organized in concentric alterning semi-crystalline and amorphous layers in granules of various sizes within the endosperm [4]. Sizes of the starch granule also may affect digestibility, as the relationship between surface and starch volume, and this contact between substrate and enzyme, decreases as size of granule increases [7]. Cereals with small granules (oats and rice) have greater starch digestibility than maize, wheat and potato with larger granules [8] and show higher enzymatic susceptibility regardless of botanical origin [9]. A high content of small granules with identical magnitude (Fig. 2a-b; Fig. 3a) provide a better contact surface for enzyme attack. A large and smooth surface as well as a very strong, uniform connection (IN, DS) explains the resistance of maize granules (Fig. 1a, 1b) against enzymatic digestion and this corresponds to the lowest DSB and AUC_{gluc} (Table 1).

A certain content of giant granules (GG; 19,1 – 29,1 µm) in oats and barley (Fig. 2b, 3b) may delay the starch degradation but previous studies revealed that starch digestion take place not only on the surface of the starch granule but also in the interior of the granule through channels and amorphous regions [10], [11]. However, this may reduce the dependency of a large surface on rate of starch digestion.

Table 1: DSB, AUC_{gluc} and morphologic characteristics of starch granules from different oat grain varieties and their embedding in surrounding structures

	cereal grain		
	barley	maize	oats
DSB [%]	7.7	5.5	10.5
AUC_{gluc} [mmol/L min^{-1}]	713^{a}	668^{b}	697^{ab}
Morphologic Characteristics of Starch Granules			
BO	x	-	x
GG	x	-	x
DS	-	xxx	x
CS	xx	-	x
IN	x	xx	x

BO, bondings; CS, coverings and/or matrix structures; DS, well defined structures; GG, giant granules; IN, interconnections; -, not-existent; x, weak; xxx, strong; Means with unlike superscripts are significantly different ($P<0.05$).

Furthermore several non-starch components are associated with the starch granule. These components, in the actual study named coverings and/or matrix structures (CS), may also represent a challenge during digestion. A significant portion of lipids were found on the surface of the starch granule [12]. In comparison to maize the surfaces of oat grains and barley are not smooth, coverings and/or matrix structures were noted (Table 1). Assuming that a certain content of the observed coverings are lipids, existing lipid:starch complexes may influence digestion by reducing contact between enzyme and substrate. The quantity of lipid:starch complexes is negatively associated with the extent of swelling (gelatinization), probably due to an increasing hydrophobicity [13]. However, the strong connection and identical magnitude as well the smooth surface of starch granules in maize delay enzyme attack and decrease digestibility in comparison to the loosen connection in oat grains or barley, where enzyme attack seem to be easier or unobstructed.

SEM pictures and from those extracted characteristics (Table, Figures) revealed differences between the grain sources which tended to correspond with conclusions from DSB and AUC_{gluc} (densest visible structure as well as lowest DSB and AUC_{gluc} in maize grain).

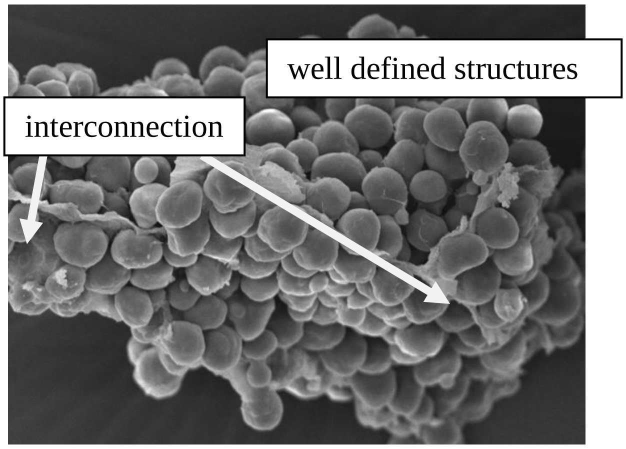

Figure 1a: SEM-picture from maize 'M_008' (x 1000)

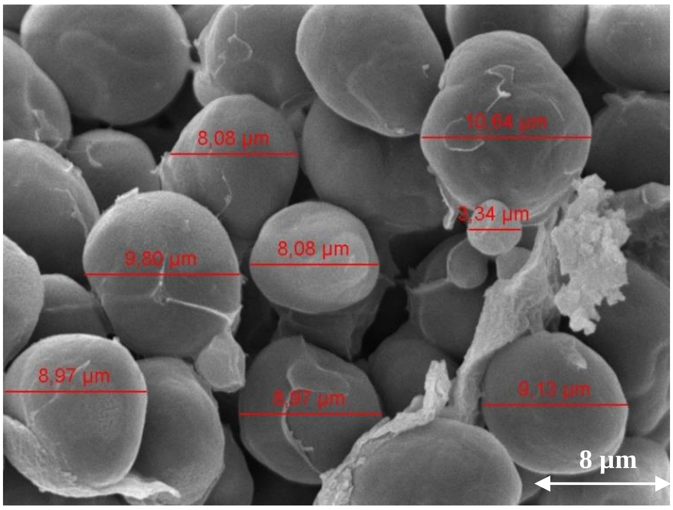

Figure 1b: SEM-picture from maize 'M_008' (x 3000)

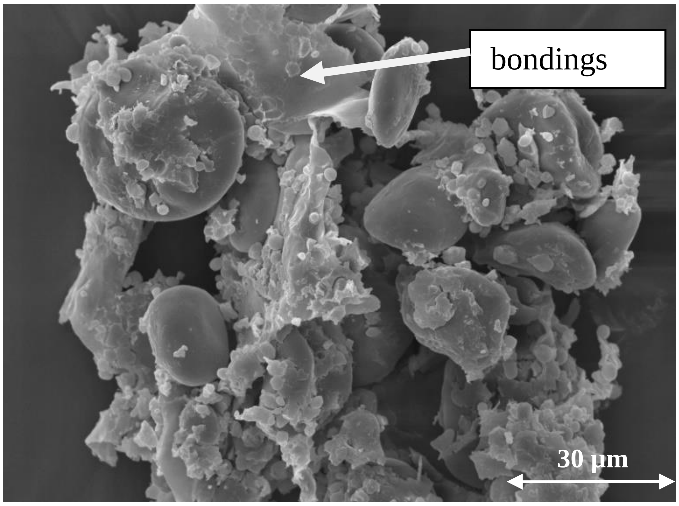

Figure 3a: SEM-picture from barley 'ACK2927' (x 1000)

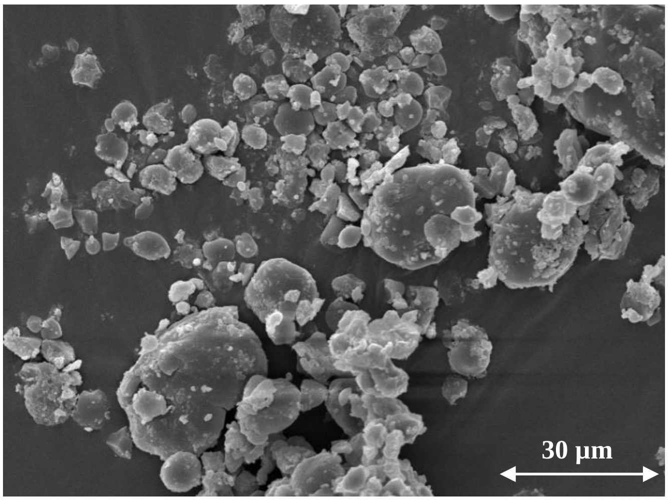

Figure 2a: SEM-picture from oat grains 'Melody' (x 1000)

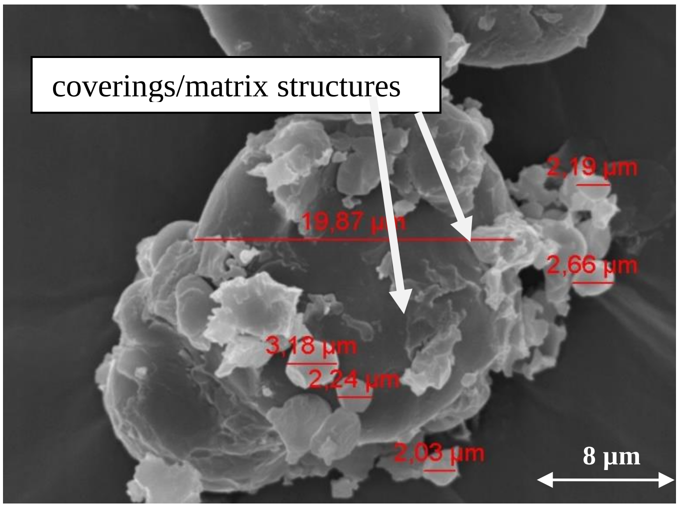

Figure 3b: SEM-picture from barley 'ACK2927' (x 3000)

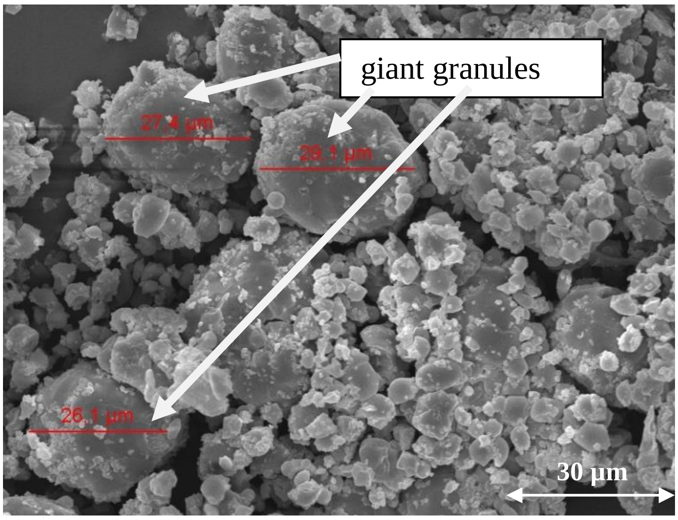

Figure 2b: SEM-picture from oat grains 'Melody' (x 1000)

IV.CONCLUSION

The extent of starch degradation cannot be estimated *via* SE pictures but they may help to explain differences in the responsibility of individual starch sources to enzymatic attack. The SEM may help to estimate degradability by body-own enzymes and thus may offer a tool to select favorable genetic and treatment variants, respectively.

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V. REFERENCES

[1]. Lehmann, U. and Robin, F. (2007) slowly digestible starch – its structure and health implications: a review. Trends in Food Science & Technology 18, 346-355

[2]. Kienzle, E., Pohlenz, J, Radicke, S. (1998): Microscopy of starch digestion in the horse. J. Anim. Physiol. a. Anim. Nutr. 80, 213-216

[3]. Kienzle, E., Pohlenz, J., Radicke, S. (1997): Morphology of starch digestion in the horse. J. Vet. Med. A 44, 207-221

[4]. Svihus, B., Uhlen, A.K., Harstad, O.M. (2005) Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. Animal Feed Science and Technology 122, 303-320

[5]. Naumann C., Bassler R. (2012): Analytical Methods III Book III, Method No. 7.2.6 (VDLUFA)

[6]. Romanowski, K., Vernunft, A., Orgis, A., Gottschalk, J., Einspanier, A., Fürll, M., Zeyner, A. (2013) Effect of feeding different amounts of starch from crushed oats of selected varieties on the glycaemic and insulinaemic response of horses.Proc. Soc. Nutr. Physio. 22, 13

[7]. Manelius, R., Bertof, E. (1996)The effect of Ca2+ions on the alpha-amylolysis of granular starches from oats and waxy-maize. J. Cereal Sci. 24,139-150

[8]. Bednar, G.E., Patil, A.R., Murray, S.M., Grieshop, C.M., Merchen, N.R., Fahey, Jr., G.C. (2001) Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability in vitro in a canine model. J. Nutr. 131, 276-286

[9]. Franco, C.M.L., do Rio Preto, S.J., Ciacco, C.F. (1992). Factors that affect the enzymatic degradation of natural starch granules: effect of the size of granules, Starch/Stärke, 44, 422-426

[10]. Lynn, A., Cochrane, M.P. (1997) A evaluation of confocal microscopy for the study of starch granule enzymatic digestion. Starch 49, 106-111

[11]. Fuwa, H., Sugimoto, Y., Takaya, T. (1979): Scanning electron microscopy of starch granules, with or without amylase attack. Carbohydrate Res. 70, 233-238

[12]. Baldwin, P.M., Melia, C.D., Davies, M.C. (1997) The surface chemistry of starch granules studied by time-of-flight secondary ion mass spectrometry. J. Cereal Sci. 26, 329-346

[13]. Vasanthan, T. and Bhatty, R.S. (1996). Physicochemical properties of small and large granule starches of waxy, regular and high amylose barleys. Cereal Chem. 73, 199-207