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PREDICTING RESISTANT STARCH AND RESISTANT STARCH TYPE 1 FROM PARTICLE SIZE DISTRIBUTION IN RAW- MILLED BARLEY GRAINS

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ABSTRACT

Resistant starch (RS) is well accepted as a complex carbohydrate which is resistant to digestion in the small intestine by alpha amylase with potential health and nutritional benefits. Resistant starch type 1 (RS1) is physically enclosed form of the starch and represents a major fraction of raw starch in raw milled grains. Quantifying both RS and RS1 in milled raw grains is necessary to determine its fate in the digestive system. The objective of this study is to predict RS and RS1 in raw milled barley grains by knowing particle size distribution after milling. Obtained different particle size in raw milled barley grains were fractionated by using sieves with different sizes (0.125mm, 0.250mm, 0.50mm, 1.0mm, 1.70 mm, and 2.8mm) and pan. Starch in each segregated particle size was *in vitro* digested for 120 minutes to determine extent of RS and RS1. As expected, this study showed that RS and RS1 increased with increasing particle size. This study revealed the existent of excellent correlation (coefficient of determination (R^2) = 0.99) between extent of RS1 (y) for raw grains and reciprocal of particle size (x) after segregation by sieving process (y = -19.09x + 59.90). Furthermore, excellent linear correlation (R^2=0.98) is existed between reciprocal of starch digestibility (i.e. 1/ (100-RS)) and uncooked particles size (x) after segregation by sieving process (y =100x-0.015). It can be concluded that both RS and RS1 are perfectly correlated with different uncooked particle size obtained by sieving analysis. Incorporation of predetermined particle size distribution in both animal and human diets can predicate the extent of RS and RS1 with potential health and nutritional benefits.

Keywords: resistant starch, amylase, particle size, barley.

1. INTRODUCTION

Resistant starch (RS) is a complex structure of carbohydrate that is relatively resistant to digestion in the small intestine by alpha amylase (Maier *et al*., 2017). Quantity of RS is of a great importance in animal and human nutrition as it determines the extent of energy delivery after digestion (Black, 2000; Wong and Louie, 2016). Once RS entered large intestine, it is exposed to fermentation by the action of microbes and converted to volatile fatty acids that possess several health benefits (Gao *et al.*, 2009; Louis *et al.*, 2009; Willis *et al.,* 2016). Furthermore, presence of RS has been reported to possess several industrial benefits such as its role as a texture modifier in baked products, crisping agent, and as a functional ingredient in other foods such as expansion (Sajilata *et al.,* 2006). In human nutrition, RS is classified into five main categories depending on its structure and the reason behind starch resistant to breakdown by alpha amylase enzyme have been reported in literature (Englyst *et al.* 1992; Sajilata *et al*., 2006; Ai *et al*., 2013; Birt *et al.*, 2013). The first category is resistant starch type 1 (RS1) which is defined as physically enclosed form of the starch (such as starch found in whole grains or partly milled grains). Secondly, resistant starch type 2 (RS2) which is characterized by a tight packaged granular starch and is relatively dehydrated (such as ungelatinized starch). Resistant starch type 3 (RS3) represents retrograded starch formed after hydrothermal treatments (involving mainly amylose). Fourthly, resistant starch type 4 (RS4) represent modified starches obtained by chemical treatments by either cross-linking or by adding chemical derivatives. Finally, resistant starch type 5 (RS5) which represent starch that interacts with lipids, amylose and long branch chains of amylopectin that form single-helical complexes with fatty acids and fatty alcohols after cooking treatment. From above, it can be clearly seen that in the absence of any hydrothermal treatments, RS1 and RS2 seems to be the main components of RS such as found in raw milled grains.

Knowing the distribution of particles size in ground grains and their respective digestibility, it is possible to determine the critical particle size above which grain fragments resist digestion (Al-Rabadi *et al.*, 2012). However, amounts of RS and RS1 were not quantified in milled barley grains. The objective of this study is to quantify and predict the amount of RS and RS1 from measuring the distribution of particle size of raw milled barley grains.

2. MATERIALS AND METHOD

2.1 Particle size segregation

Barley grains were coarsely milled using 4 mm hammer mill screen size to generate wide distribution of particle size. Segregated different particle size of milled barley grains were obtained by using the arrangement of seven analytical sieves (4.7, 2.8, 1.7, 1.0, 0.50, 0.250, and 0.125 mm) and a pan (Endecotts Ltd, London, U.K.), after mechanical shacking with a sieve shaker (Endecotts Shaker, ExTech Pty. Ltd., Victoria, Australia). To estimate the average particle size of milled fraction retained on each sieve, particle size was calculated by taking the

average size of sieve (average size of the sieve where material retained on and the sieve where the materials passed through). For example, average particle size of particles retained on sieve size 0.5mm is 0.75 mm (i.e measured by taking the average of both sieve sizes 0.50mm and 1.0mm). The estimated particle size retained on pan is 0.045 mm (Al-Rabadi *et al*., 2009). Segregated particles retained on each sieve including pan was reserved in a nylon bag and stored at 4 ˚C until further analysis.

2.2 Starch content and In vitro starch digestibility determinations

In vitro starch digestibility of segregated particles (i.e eight sizes) retained on each sieve was measured according to the procedure described by Al-Rabadi *et al*. (2009). The concentrations of released glucose were measured after 120 minutes to determine the amount of resistant starch type 1 (RS1) (Englyst *et al.* 1992). Zero hour of digestion was defined at the time when alpha amylase was added. The percentage of starch digestibility of each particle size level and enzyme incubation time was analyzed separately in a randomized order and in duplicate. Starch content for each segregated particle size was analyzed as described previously by Al- Rabadi *et al.* (2009). The percentage of starch digestibility is represented as amount of digested starch to the total amount of starch in each particle size and calculated on dry matter basis. RS and RS1 were calculated as reported by Englyst et al. (1992) as the following:

$RS = TS - S_{120}$

Where:

TS = Total starch content of investigated particle size.

S_{120} = Starch digested by alpha amylase after 120 minutes for investigated particle size.

$RS_1 = S_{120} - S_{120(a)}$

Where:

$S_{120(a)}$ = Starch digested by alpha amylase after 120 minutes for particles passed sieve 0.250mm.

2.3 Dry mater analysis

Dry matter was determined by remained weight after drying ground material in an oven at temperature 135 ˚C for a period of 3 hours (Al-Rabadi *et al*., 2009).

2.4 Scanning Electron Microscopy (SEM)

Images of different particle size were taken by using Scanning Electron Microscopy according to the procedure described by Al-Rabadi (2014).

2.5 Statistical analysis

A completely randomized design was used in this experiment to evaluate the effect of segregated particle size on RS and RS1. Data for RS and RS1 were represented as means ± standard deviation in duplicate measurements. Multiple comparisons of means were conducted by using least significant difference (LSD) method. Statistical differences at $\alpha = 0.05$ were used using SAS software programs (v.9.1, SAS Institute, Cary, NC). For the sake of simplicity, simple linear regression analysis was used to determine the correlation between particle size and determined amount of RS and RS1 by using Excel sheet (Microsoft, 2007).

3. RESULTS

The effect of barley particle size after segregation by sieving process on extent of RS and RS1 is shown in Table-1. Percentage of RS increased by increasing particle size; being lowest for average grain particle size 0.045mm and 0.188 mm (40.96 and 42.58%, respectively) and was highest for average grain particle size 3.78mm (97.4%). Similarly, percentage of RS1 increased by increasing particle size; being lowest for average particle size 0.188 mm (10.01%) and was highest for average particles size 3.78mm (54.81%). Excellent linear fitting ($y = -19.09x + 59.90$; $R^2 = 0.990$) is existed between extent of RS1 for raw grains and x (reciprocal of particle size, after segregation by sieving process) as shown in Figure-1. This study also revealed that the presence of excellent linear fitting (Figure-2) between reciprocal of starch digestibility (i.e. 1/ digestible starch or 1/(100-RS)) and uncooked particles size after being segregated by sieving analysis ($y=100x-0.015$, $R^2=0.98$).

Table-1. Effect of segregated particle size by sieving analysis on RS and RS1 in barley grains (means represented ± SD).

Sieve size (mm)	Average particle size (mm)	RS1 (%)	RS (%)
Pan	0.045	-*	$40.96^{d} \pm 7.87$
0.125	0.1875	-*	$42.58^{d} \pm 2.26$
0.250	0.375	$10.01^{d} \pm 3.10$**	$52.64^{c} \pm 3.16$
0.5	0.75	$31.38^{c} \pm 1.64$	$73.97^{b} \pm 1.64$
1.0	1.35	$46.87^{b} \pm 1.01$	$89.46^{a} \pm 1.01$
1.7	2.25	$52.32^{a} \pm 0.11$	$94.91^{a} \pm 0.11$
2.8	3.78	$54.81^{a} \pm 0.46$	$97.40^{a} \pm 0.46$
P value		<0.001	<0.001

* excluded from analysis.
** Values within a single column with different superscripts differ significantly (P < 0.05).

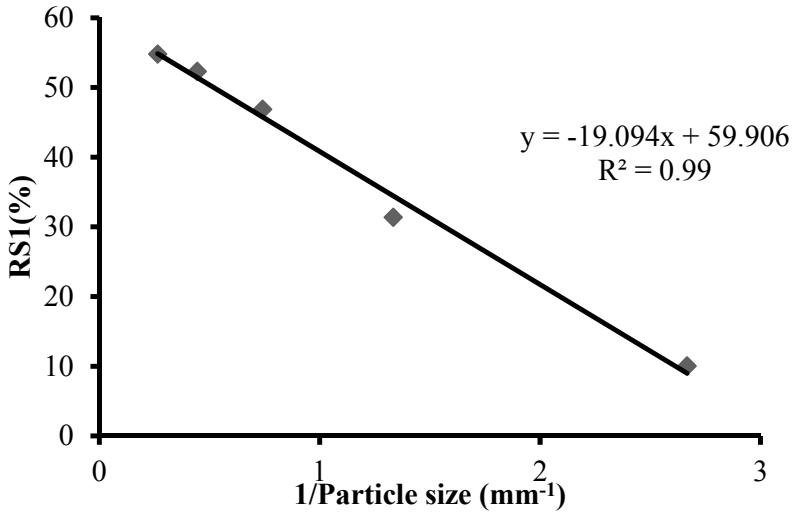

Figure-1. The association between the reciprocals of particle size and RS1 obtained by conventional regression analysis.

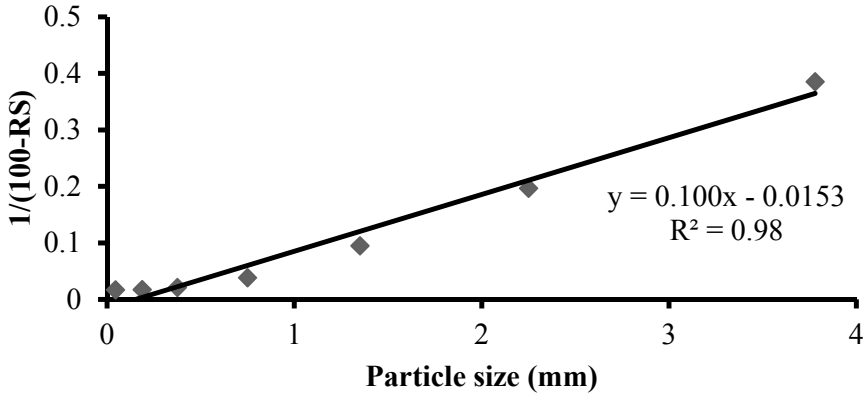

Figure-2. The association between particle size and the reciprocals of digestible starch (1/(100-RS) obtained by conventional regression analysis.

4. DISCUSSIONS

Similar extent of RS for both average particle sizes 0.045mm and 0.188 mm suggest that particles passed through sieve size 0.250 mm is considered the cutoff point that determine the extent of RS1 for raw/uncooked milled barley grains. Single endosperm cells in grains are in a range of 0.05 – 0.1 mm (Al-Rabadi et al., 2009). Thus, starch granules in grain fragment greater than 0.5 mm will be mainly surrounded by undamaged cell walls or grain husk (Figure 3) whereas grain particles passed 0.25 mm sieve would be likely to have damaged or cracked cells with uncovered intra-cellular components (Figure 4). The outcome of this experiment is in agreement with study reported by Englust et al. (1992) who suggested that whole grains milled to pass 0.20 mm sieve size is the cut-off point to determine the extent of RS1 for raw /uncooked grains after *in vitro* starch digestion by amylase for 120 minutes. Thus, all grain particles that passed 0.25mm sieve was omitted from the measuring the relationship between grain fragment particle size and RS1.

Excellent linear fitting ($y = -19.09x + 59.90$; $R^2 = 0.990$) is existed between extent of RS1 for raw grains and reciprocal of particle size (Figure 1). This excellent correlation showed that with the increase in raw grain particle size, extent of RS1 increases. Particle size have been reported to be the most important factor that affect starch digestibility in milled raw barley grains (Al-Rabadi *et al*., 2012). Previous study showed that the main factor that affects amylase diffusion is the passage through barley grains fragment endosperm (Al-Rabadi, 2009) and that alpha amylase

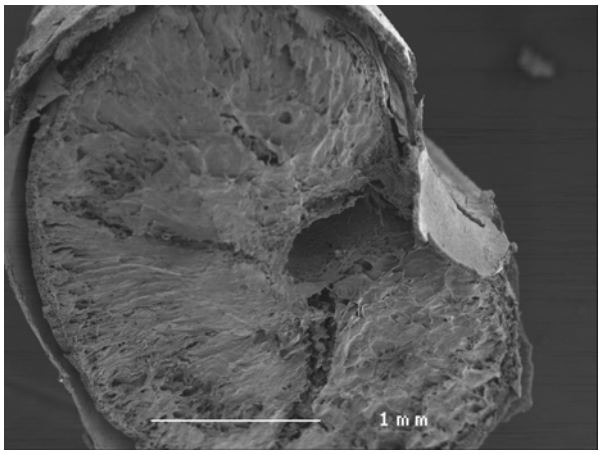

Figure-3. Cross section of half broken barley grain showing grain endosperm is surrounded by husk.

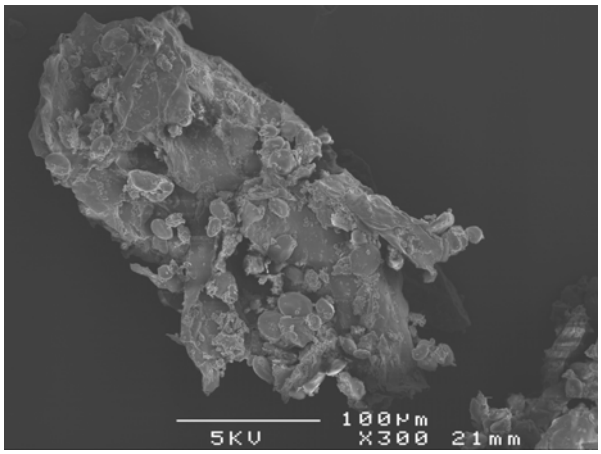

Figure-4. Grain particles passed sieve size 0.25 mm showing uncovered starch granules.

cannot penetrate/diffuse through barley husk (Al-Rabadi 2014). When x is close to zero (i.e, when grain particle size is whole grain), RS1 approaches 59% suggesting that whole barley grain respond greatly to milling process (i.e. reduction of particle size). Different particle size of barley grains after being segregated by sieving analysis has been reported to possess different chemical composition (Al-Rabadi 2013). However, different chemical composition did not affect relationship between grain fragment particle size and RS1 excellent linear fitting (Figure-1) in this study suggest that chemical composition of different grains fractions has no effect on RS1. Furthermore, excellent linear fitting (Figure-2) may suggest that RS1 for ground uncooked samples could be predicted by knowing the weight distribution of particle size of milled grains. in feed industry, different milling machines produce different particle size heterogeneity. Hammer mill is the mostly used equipment for milling grains in animal feed industry (Amerah *et al*., 2007), however, hammer mill has been reported to produce wide distribution of particle size (Douglas *et al*., 1990). Removing of large particles of hammer milled barley grains followed by regrinding them to reduce their size accomplished the benefit of improving animal performance (Al-Omari *et al*., 2014; Al-Rabadi *et al*., 2017) possibly by reducing amount of RS in coarse milled grain fractions. In corn grains, reducing particle size from 0.9 to 0.3 mm have been reported to more than double the surface area of milled grains (Healy *et al*., 1994).

By definition, digestible starch is equal to total starch excluding RS. Thus, RS can be obtained by the excellent linear fitting between reciprocal of total starch excluding resistant starch (i.e. 1/ (100-RS) and uncooked particles size after being segregated by sieving analysis (($y=100x-0.015$, $R^2=0.98$); Figure-2). *In vitro* starch digestibility of weighted average values based on fraction yields were not different from values gained from non-fractionated ground barley grains at similar digestion times (Al-Rabadi *et al*., 2012). These results may suggest that RS of weighted average values based on fraction yields

may not differ from values gained from non-fractionated ground barley grains at similar digestion times.

RS have been reported to be fermented in the large intestine by microflora providing volatile fatty acids (Champ, 2004). Fermentation of RS in lower tract has been reported to possess positive influence on animal and human health (Sajilata *et al*., 2006). For quantifying RS *in vivo*, animals in certain cases have to be killed to determine physiological and anatomical effects of RS in host. Current outcomes in this study may provide an assist to quantify RS reaching lower tract of digestive system with lower animal sacrifices. However, digestibility of uncooked starches *in vivo* in different animals must be compared with care because of differences in amylases activity in different animal species (Birt *et al*., 2013). For example, pancreatic α-amylase activity is higher in rats compared with that in pigs (Sugimoto et al., 1980; Birt *et al*., 2013). Further research must be conducted to study the suitability of applying linear regression analysis to predicate quantity of RS and RS1 through knowing the distribution in other raw milled grains.

5. CONCLUSIONS

It can be concluded that both RS and RS1 in uncooked barley grains are well correlated with particle size obtained by sieving analysis. Furthermore, the outcomes of this study showed that the distribution of particle size is a major factor that determines quantity of RS and RS1 in response of barley grains to milling process. Manipulating RS through particle size distribution of uncooked barley grains can be modified to achieve particular nutritional or industrial objectives.

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