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## ORIGINAL ARTICLE

# Particle size Heterogeneity helps in Quantifying resistant starch and resistant starch type 1 in raw- milled sorghum grains

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**ABSTRACT**
*Particle size distribution in raw milled grains can be manipulated to assist in determining the faith of resistance starch (RS) in host. Particle size heterogeneity can also mimic chewing extent which will determine physical acceptability of starch by alpha amylase. This work aims at establishing a predictability measure to quantify the amount of RS and resistance starch type 1 (RS1) by using various particle sizes of milled raw grains. The results of this study show that different sorghum particle size (ranging from very fine particles (0.045mm) to half broken grain (2.8 mm)) has a significant impact on the percentage of RS (ranged from 75.5 to 98.28%). This experiment also showed perfect linear association ($R^2$=0.98) between the reciprocal of starch digestibility (i.e y= 1/ (100-RS)) and the uncooked particles size (x), as illustrated by the (y = 0.142x + 0.009, $R^2$ = 0.98). Furthermore, excellent linear association ($R^2$ = 0.985) between extent of RS1 and the reciprocal of raw grain particle size (x), as described by the best fit graph (y = -5.512x + 19.39, $R^2$=0.985). It can be concluded from this study that both RS and RS1 of raw sorghum grains after milling/chewing are associated with the particle size. The manipulation of the distribution of the grain particle sizes and their relevant RS in feed/food can assist in supplying appropriate quantity of RS with implications on maintaining the host's health and performance.*
**Keywords:** *Sorghum, Starch digestibility, Resistant starch, Correlation*

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## INTRODUCTION

Resistant starch (RS) is considered as a prebiotic that is metabolized by microbes found in large intestine, where they are fermented mainly into short-chain fatty acids which directly or indirectly affect the health [1,2]. The RS has been reported to assist body to reduce risk exposure of obesity via different mechanisms such as synthesis and secretion of leptin and adiponectin, and improvement in intestinal microflora [3,4]. In human nutrition, the recommended amount of RS has been suggested to be 6 g per meal [5]. The global trend in animal nutrition is to reduce dependency on antibiotics in animal feed, and hence the presence of RS is of significance [6]. Feed enrichment with resistant starch is a good alternative for using antibiotic in animal feed. Resistant starch type 1 (RS1) is one of RS types which is synthesized in the endosperm of cereal grains or seeds where starch granules are surrounded by protein matrix and cell wall material which are not accessible by alpha amylase [1]. Several strategies that focus on changing grain composition (i.e increasing both fibre and RS) in order to increase fermentation of carbohydrates to achieve health benefits in the host such as grain breeding programs, mutagenesis and TILLING (Targeting Induced Local Lesions in Genomes), and Transgenesis [7]. However, these strategies can be time consuming and/or may not be satisfactory to include genetically modified grains in the food chain by the relevant regulatory authorities. This work aims at investigating various particle sizes of milled raw grains in an attempt to predict and quantify the values of RS and RS1. The predetermined particle size distribution in raw milled grains can be controlled to assist in determining the faith of the RS in the host. Furthermore, the impact of raw grain particle size after milling on RS can be used to mimic the influence of chewing extent which will determine the physical acceptability of starch by alpha amylase enclosed within firm structure [8]. In this study, sorghum was taken as the grain model for investigation.

**MATERIAL AND METHODS**

*Grain milling and particle size separation*

Sorghum grains were milled using a hammer mill which is known to produce a wide range of particle sizes; that range vary from fine to half broken grains. Different sorghum grains fragments obtained after milling was isolated by sieving analysis using mechanical shake. The Sieving sizes used for particle separation/segregation are 4.0, 2.8, 1.7, 1.0, 0.5, 0.25, and 0.125 mm including pan. Particles retained on pan were estimated to have an average particle size of 0.045mm [9]. Particles retained on each sieve was stored in nylon bags and kept refrigerated for starch digestibility analysis.

*Resistant starch measurement*

Sorghum grain fragments retained by each sieve were analyzed for starch and *in vitro* starch digestibility according to the procedure described by Al-Rabadi [9]. Based on *in vitro* starch digestibility measurements (concentrations of released glucose after 120 minutes), and total starch content in each sample both resistant starch (RS) and resistant starch type 1 (RS1) were calculated as reported by Englyst [8].

*Dry matter measurement*

Dry matter content was analyzed according to the procedure described by Al-Rabadi [9].

*Scanning Electron Microscopy (SEM)*

Scanning Electron Microscopy images of different particle sizes were taken according to the method demonstrated by Al-Rabadi [10].

*Statistical analysis*

Data for both RS and RS1 were represented as means ± SD of duplicate measurements. A complete randomized design was used to investigate the effect of the particle size on both RS and RS1. Multiple comparisons of means were performed by least significant difference at $\alpha = 0.05$ by using SAS program (v.9.1, SAS Institute, Cary, NC). For data fitting, a simple linear regression analysis utilizing Excel (Microsoft, 2007) was used to determine the association between the particle sizes and both RS and RS1.

**RESULTS AND DISCUSSION**

The result of this study showed that sorghum particle size has a significant ($P<0.05$) impact on the percentage of both RS and RS1 (Table 1).

Particles of finest size retained on the pan has a high percentage of resistant starch (more than 75%). Particles retained on pan that have passed through sieve size 0. 25 mm are composed of damaged cell walls and showing exposed starch granules (Figure 1). Grain particles that passed a hammer mill screen of size 0.2 mm have been reported to distinguish alpha amylase accessible particle [8]. However, the presence of protein matrix that surrounds starch granules may reduce the starch digestibility after 2 hours of digestion by alpha amylase (even in the presence of pepsin enzyme which is used in *in vitro* assay in this study) (Figure 2). In sorghum, the arrangements of protein granules that form the protein matrix encapsulating starch granules (Figure 2) are composed of kafirin. Kafirin is considered to be the main component in sorghum protein which is known to have a low digestibility because of it is hydrophobicity [11,12] as shown in Figure (3).

In sorghum, the rate of starch digestion has been reported to be lower in sorghum grains compared to barley grains for the same particle size levels [9]. In the case of the finest particles ( i.e particles passed sieve size 0.125mm and have been retained on the pan) (Table 1), only quarter of starch is accessible by alpha amylase. In a study involved analysis of 49 different sorghum genotypes, RS mean values ranged from 31% to 65.7% of sorghum flour on dry basis [13]. On the other hand, negligible quantity of starch is accessible by alpha amylase for the particles that were retained by sieve size 2.8 mm (mainly composed of half broken grains surrounded by seed coat) as shown in Figure (4). The correlation analysis showed that the reciprocal of starch digestibility (y) (or reciprocal of 100-RS) is highly correlated with the particle size (x), according to the equation: $y=0.142x+0.009$; $R^2=0.98$) (Figure 5).

Figure 6 show excellent correlation between RS1 (y) and reciprocal of particle size (x), with the following linear regression $y=-5.512x + 19.32$, $R^2=0.985$. The intercept in Figure 6 represents the maximum percentage of RS1=19.39% that can be obtained from the coarse sorghum particles when the value of the variable x defined as (1/particle size ) is close to zero.

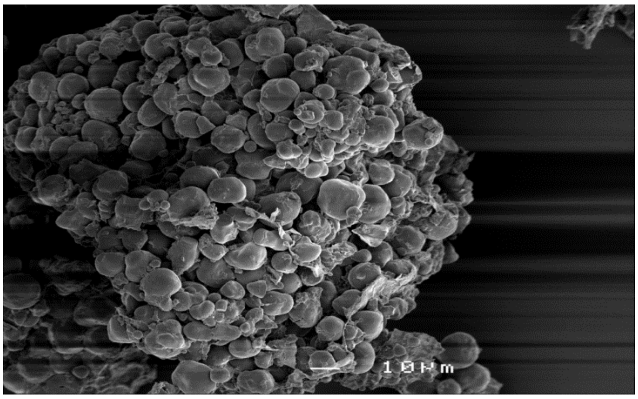


Figure 1. SEM of milled sorghum particle retained on sieve size 0.125mm showing uncovered starch granules.

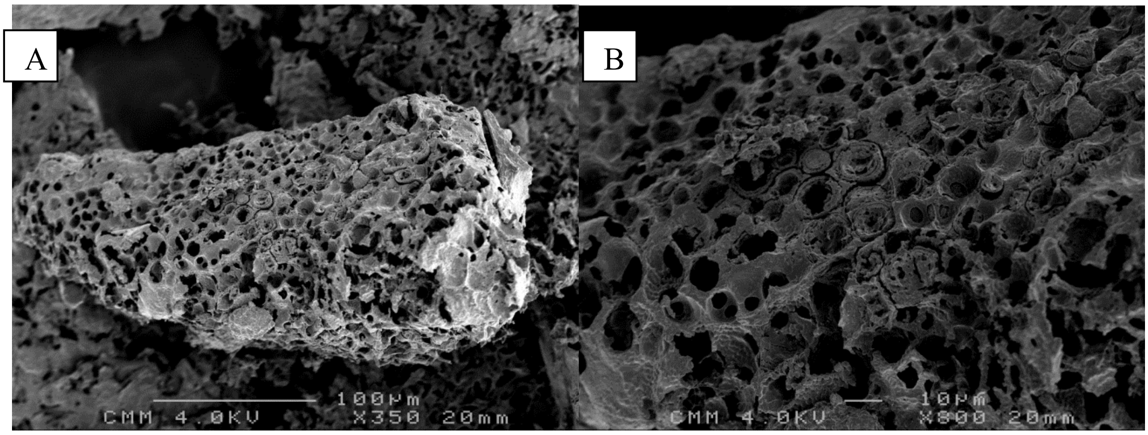


Figure 2. Particles retained on sieve size 0.25mm after 2 hours of digestion at low (A) and high (B) magnification levels. Starch digestion starts in the middle of the starch granule and away from the protein matrix.

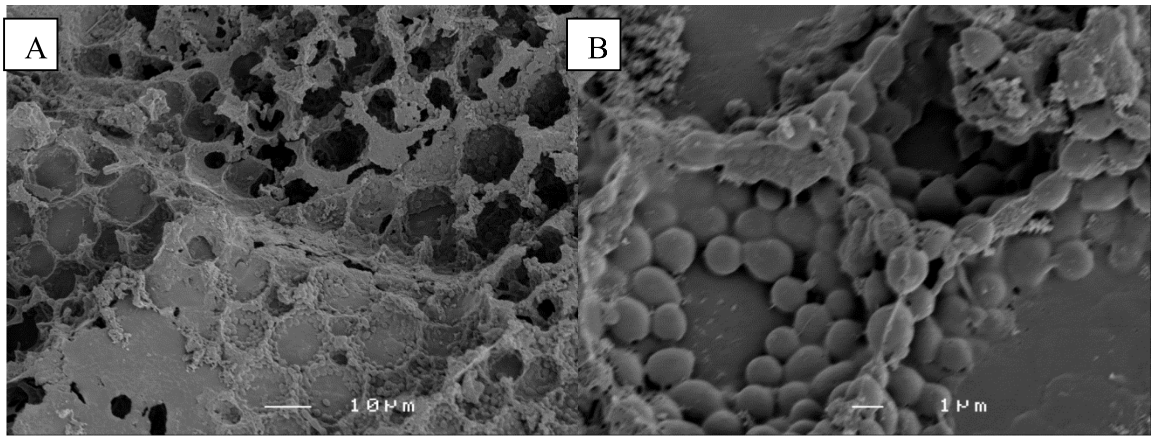


Figure 3. SEM showing intact protein granules/matrix after 24 hours digestion at low (A) and high (B) magnification.

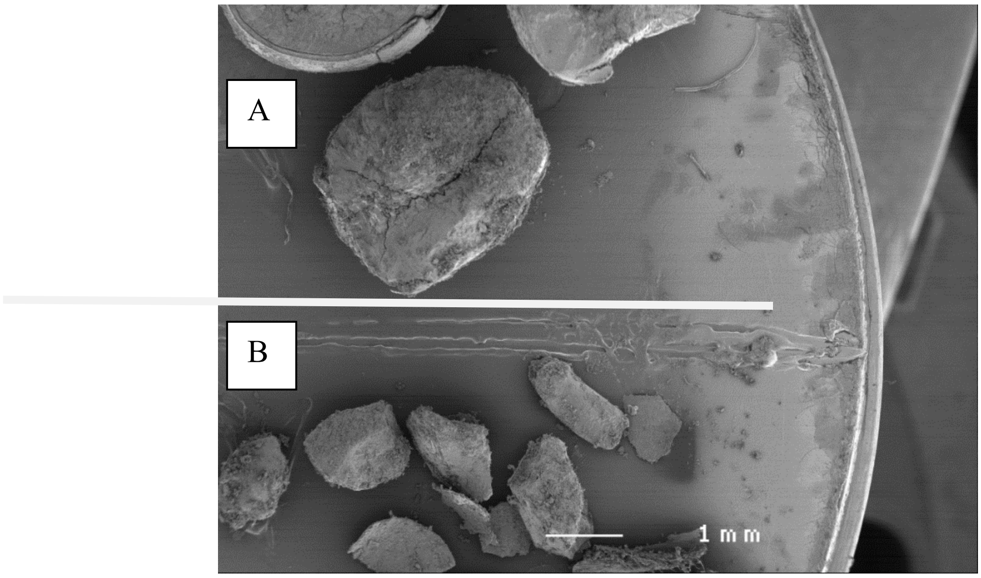

Figure 4. SEM for milled sorghum grains retained on sieve sizes 2.8 mm (A) and 1 mm(B).

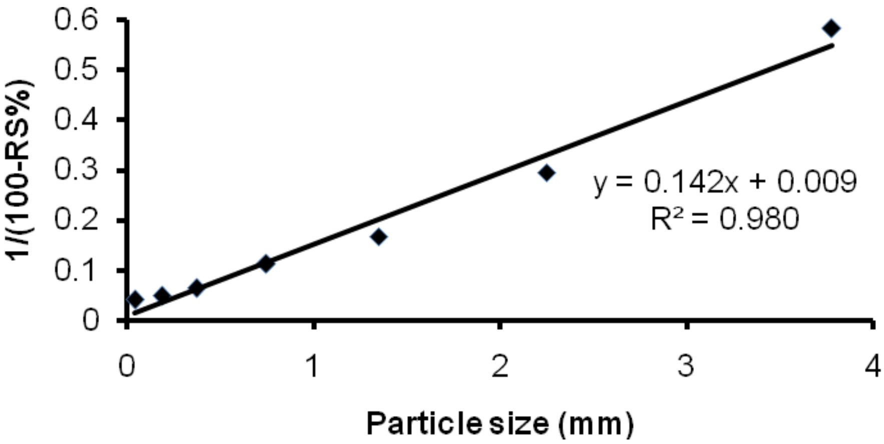

Figure 5. Correlation between reciprocal of digested starch (i.e 1/(100-RS%)) and the particle size (mm)

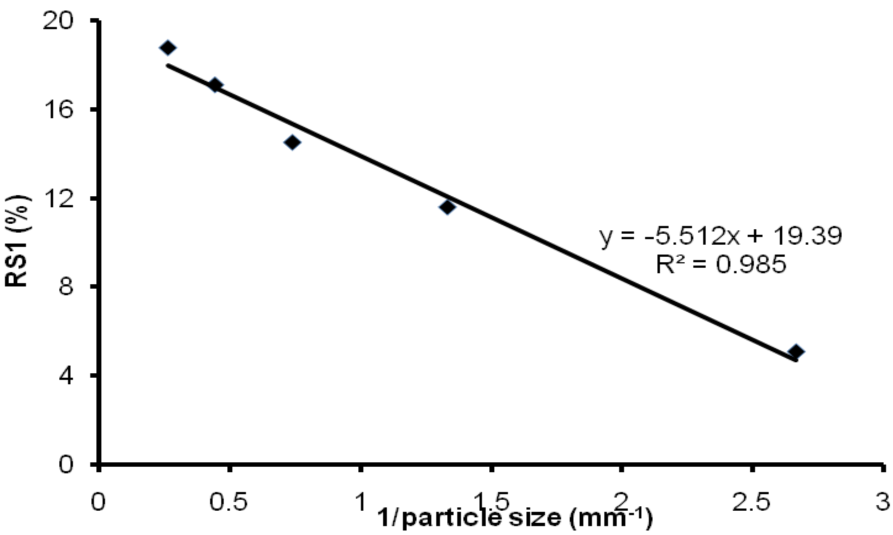

Figure 6. Correlation between the resistant starch type RS1 and the reciprocal of the particle size.

 

Table1. Effect of particle size on RS and RS1 in sorghum particle size after segregation by sieving analysis (means represented ± SD[a]).

| Sieve size | Average particle size[b] | RS | RS1 |
|---|---|---|---|
| pan | 0.045 | 75.51f ± 2.32 | - |
| 0.125 | 0.1875 | 79.53e ± 0.65 | - |
| 0.250 | 0.375 | 84.59d ±0.74 | 5.05d ± 0.74 |
| 0.5 | 0.75 | 91.1c ± 0.50 | 11.56c ± 0.50 |
| 1.0 | 1.35 | 94.02b ± 0.67 | 14.48b ±0.67 |
| 1.7 | 2.25 | 96.60a ± 0.98 | 17.07a ±0.97 |
| 2.8[c] | 3.78 | 98.28a ± 0.10 | 18.74a ±.0.10 |
| P value | | <0.001 | <0.001 |

[a] Values with different superscript within same column differ significantly[b]. Average particle size was calculated by taking the average size of two sieves where particles retained in between.[c] No particles retained on sieve size 4.0mm.

These results suggest that the response of sorghum grains to starch digestion by the milling process is low or ineffective in maximizing starch digestibility. However, sever hydrothermal treatment such as the extrusion of different particle sizes of sorghum grains have been reported to increase the extent of starch digestibility (i.e reducing the extent of RS) [9]. In sorghum, the rate of starch digestion has been reported to be lower in raw milled sorghum grains compared to raw milled barley grains [9]. This has been attributed to that diffusion coefficient for alpha amylase is lower in milled sorghum grains compared to milled barley grains [9]. RS present in raw milled grains is of greater importance in animal nutrition than in human nutrition as substantial animal diets are offered in raw/unprocessed from. Most of the processed animal diets are offered in pelleted form where extent of starch gelatinization dose not exceeds 20% of the total starch [14] leaving RS1 as the main fraction among other RS types. Several studies reported that the pelleting process increases starch digestibility by inducing gelatinization, however, size reduction in pelleted feed during the pellet-pressing process through the die is overlooked. Wolf [15] reported that significant reduction in particle size after the pelleting process when wet sieving analysis was conducted. Accordingly, the particle size analysis after the pelleting process can give a better insight to influence of pelleting process on RS. It has been suggested that improved animal growth performance can be achieved by the selection of the aproppriate particle size distributions, as different particle sizes provide a broad range of starch fractional digestion rates [16]. Higher digestion rates of starch can ensure more efficient use of starch through complete digestion in the small intestine rather than fermentation in the large intestine [17]. While increasing starch digestibility in milled grains is important to increase energy delivery in animals, the presence of RS is very useful for animal and human health [18]. The fermentation of glucose in the large intestine produces less energy than the glucose digested in the small intestine by 10-15% [19]. Furthermore, the metabolites produced by fermentation process of RS are mainly composed of short-chain-volatile-fatty acids (acetate, propionate and butyrate). These metabolites have been reported to change the gene expression that modifies the cell proliferation and differentiation of the digestive system in addition to the regulation of genes expression which assists in controlling apoptosis [7]. The intake of RS diet has been reported to enhance the lower tract health by stimulating mucin secretion and controlling unwanted protein fermentation [18]. Similar to non-starch polysaccaride, RS have been reported to slow down gastric emptying and thus can decrease the absorption rate of glucose [20], however, the fermentation of RS when compared to other types of dietary fibre has been reported to produce more butyrate [21]. Among other fermented RS metabolities, butyrate is of special interest for being the main energy source for the colonic cells and useful in the prevention of colorectal cancer [7] by enhancing healing mechanisms in damaged DNA [22].

It can be concluded from this study that sorghum is an excellent source of RS. The RS and RS1 of milled/chewed raw sorghum grains are well associated with the particle size. The incorporation of the particle size distribution and the relevant RS in feed/food formulation software can assist in supplying appropriate quantity of RS with implications for maintaining animal/human health and performance.

## REFERENCES


1. Birt, D. F., Boylston, T., Hendrich, S., Jane, J. L., Hollis, J., Li, L., McClelland. J., Moore, S., Phillips, G. J., Rowling, M., Schalinske, K., Scott, M. P. & Whitley, E. M. (2013). Resistant starch: promise for improving human health. Adv. Nutri., 4: 587–601.
2. Scott, K. P., Gratz, S. W., Sheridan, P. O., Flint, H. J. & Duncan, S. H. (2013). The influence of diet on the gut microbiota. Pharmacol. Res., 69:52–60.


 

3. Shen, R. L., Zhang, W. L., Dong, J. L. Ren, G.X. & Chen, M. (2015). Sorghum resistant starch reduces adiposity in high-fat diet-induced overweight and obese rats via mechanisms involving adipokines and intestinal flora. Food Agr. Immunol., 26: 120–130
4. Schönhofen, A., Zhang, X. & Dubcovsky, J. (2017). Combined mutations in five wheat Starch Branching Enzyme II genes improve resistant starch but affect grain yield and bread-making quality. J. Cereal Sci., 75: 165-174
5. Murphy, M.M., Douglass, J.S., Birkett, A. (2008). Resistant starch intakes in the United States. J. Am. Diet. Assoc., 108: 67 – 78.
6. Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: impacts on human health. Clin. Microbiol. Rev., 24: 718–733.
7. Lafiandra, D., Riccardi, G. and Shewry, P. R. (2014). Improving cereal grain carbohydrates for diet and health. J. Cereal Sci., 59: 312–326.
8. Englyst, H. N., Kingman, S. M. and Cummings, J. H. (1992). Classification and measurements of nutritionally important starch fractions. Eur. J. Clin. Nutr., 46 (Suppl. 2): 33-50.
9. Al-Rabadi, G. J. (2009). Cereal processing, particle size distribution and Starch digestibility in animal feed. School of Animal Science. Queensland University. Brisbane. Australia. PhD thesis.
10. Al-Rabadi, G. J. (2014). Microscopic analysis of *in vitro* digested milled barley grains : influence of particle size heterogeneity. Jordan J. Biolog Sci., 7: 199-203.
11. Belton, P. S., Delgadillo, I., Halford, N. G. & Shewry, P. R. (2006). Kafirin structure and functionality. J. Cereal Sci., 44: 272–286.
12. Liu. S. Y., Fox G., Khoddami, A., Neilson, K. A., Truong, H. H., Moss, A. F. & Selle, P. H. (2015). Sorghum grain: A Conundrum for Chicken-Meat Production. Agric., 5:1224-1251.
13. Teixeira, N. C., Queiroz, V. A., Rocha, M. C., Amorim, A. C., Soares, T. O., Monteiro, M. A., de Menezes, C. B., Schaffert, R. E., Garcia, M. A & Junqueira, R. G. (2016). Resistant starch content among several sorghum (Sorghum bicolor) genotypes and the effect of heat treatment on resistant starch retention in two genotypes. Food Chem., 197:291-296
14. 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. Anim. Feed Sci. Tech., 122: 303-320.
15. Wolf, P., Rust, P., & Kamphues, J. (2010). How to assess particle size distribution in diets for pigs?. Livest. Sci., 133: 78–80
16. Al-Rabadi, G. J., Torley, P., Williams, B. A., Bryden, W. L., & Gidley, M. J. (2012). Particle size heterogeneity in milled barley and sorghum grains: Effects on physico-chemical properties and starch digestibility. J. Cereal Sci., 56: 396-403.
17. Sun, T., Laerke, H. N., Jorgensen, H. & Knudsen, K. E. B. (2006). The effect of extrusion cooking of different starch sources on the in vitro and in vivo digestibility in growing pigs. Anim. Feed Sci. Tech., 131: 66-85.
18. Zhou, L., Fang, L., Sun, Y., Su, Y. & Zhu, W. (2017). Effects of a diet high in resistant starch on fermentation end-products of protein and mucin secretion in the colons of pigs. Starch – Stärke 69: 1-7
19. Black, J. L. (2000). Bioavailability: the energy component of a ration for monogastric animals. In: Moughan, P.J., M. Verstegen, W. A. and Visser-Reyneveld, M.I. Editors, Feed Evaluation—Principals and Practice, Wageningen Pers, Wageningen, The Netherlands, pp. 133–152.
20. Bodinham, C. L., Frost, G. S. & Robertson, M. D. (2010). Acute ingestion of resistant starch reduces food intake in healthy adults. Br. J. Nutr., 103:917–922.
21. Topping, D. L. & Clifton P. M. (2001). Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol. Rev., 81:1031–1064.
22. Topping, D. L., Bajka B. H., Bird, A.R., Clarke, J. M., Cobiac, L., Conlon, M. A., Morell M. K. & Toden, S. (2008). Resistant starches as a vehicle for delivering health benefits to the human large bowel. Microb. Ecol. Health Dis. 20: 103–108.