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Original Article

Effect of lintnerization (acid hydrolysis) on resistant starch levels and prebiotic properties of high carbohydrate foods: A meta-analysis study

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Abstract

Lintnerization (acid hydrolysis) is a chemical modification technique widely used to increase in the resistant starch levels in carbohydrate foods. However, this technique has different effects on each type of high-carbohydrate diet. This study aimed to analyze by type carbohydrate foods in which the levels of resistant starch are increased by lintnerization. This study used 26 articles following the PRISMA method. The data were analyzed by Effect Size Hedges'd (standardized mean difference/SMD) and confidence interval (CI) using OpenMEE software. The results showed that the lintnerization of food significantly increased levels of resistant starch and prebiotic properties (SMD 8.813; 95% CI: 6.566 to 11.060; $p<0.001$). In conclusion, this study confirmed that linearization had a significant effect with a 95% confidence level in increasing the levels of resistant starch and prebiotic properties of high-carbohydrate foods.

Keywords: lintnerization, meta-analysis, modified starch, prebiotic properties, resistant starch

1. Introduction

Starch consists of linear chains of amylose (20-30%) and branched structural units of amylopectin (70-80%) (Na, Jeong, Park, & Lee, 2021; Navaf *et al*., 2021). Starch is the primary carbohydrate storage in plants and is considered the second largest biomass component after cellulose. According to *in vitro* starch digestibility, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992).

The rate of starch digestibility is influenced by various factors, such as the amylose/amylopectin ratio (Raza *et al*., 2021), the interaction of starch with proteins and lipids (Ciardullo, Donner, Thompson, & Liu, 2019), and by various modifications done using physical methods, chemistry, and enzymes. Among the modification procedures, lintnerization (acid hydrolysis) can produce many linear amylose starch chains, which resist the hydrolysis by digestive enzymes due to the formation of solid crystals (Zhou *et al*., 2019).

The slow acid hydrolysis technique aims to increase the short-chain amylose fraction with low molecular weight, resulting from the degradation of the long-chain amylose fraction at the branching points of "α" -1,6-amylopectin chains (Wang, Jiang, Guo, Zheng, & Zhang, 2021; Yeum *et al*., 2020;). Borries-Medrano, Jaime-Fonseca, Aguilar-Mendez, and Garcia-Cruz (2018) added that if the short-chain amylose fraction is increased, the amylose fraction that contributes to retrogradation increases. This causes the process of forming RS 3 to improve and impacts the decrease in digestibility. The formation of amylose fraction with linear structure will facilitate the formation of cross-links by hydrogen bonds to form a very compact amylose structure (Wu, Sun, Zhang, & Zhong, 2020).

The acid hydrolysis can cause physical changes in the starch. Acid hydrolysis can cleave the branching points of

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the “α” -1.6 bonds and produce short linear “α” -1.4 glucan bonds, which accompanies the reform of the double helix structure at temperatures below the melting temperature (Wang, Liu, Zhang, & Chen, 2020; Zheng *et al*., 2021). Several studies have shown that high acid concentrations and short branching times are suitable for making SDS and RS from waxy rice or corn starch, whereas long branching times are beneficial for RS formation (Xiao *et al*., 2021; Zeng *et al*., 2020).

Lintnerization of carbohydrate foods can affect their physicochemical constitution, such as total sugar content, and total starch in its amylose and amylopectin levels. Chemical modification by strong acid hydrolysis changes the structure and amount of short chain amylose to increase RS levels. Xiao *et al*. (2021) reported that acid treatment and debranching increased granule motility allowing interaction with water, leading to the mobility of amylopectin to facilitate gelatinization and retrogradation. Amylopectin is easily affected structurally by physical, chemical, and enzymatic treatments (Wu *et al*., 2020; Xie *et al*., 2021; Ye *et al*., 2019).

Acid hydrolysis can be performed by suspending flour in an acid solution with a concentration of 36-40% and heating it at a temperature below the starch gelatinization temperature, 40-60ºC, with stirring during the incubation stage (Raza *et al*., 2021). Strong acids will hydrolyze glycosidic bonds, causing chain shortening and amylose molecular weight to become lower (Oh, Seo, Park, Kim, & Baik, 2022).

Acid hydrolysis is carried out using strong acids. Mapengo, Ray, and Emmambux (2021) indicated that in the early stages of acid modification, the amount of amylose or short chain linear fraction increased, indicating that the acid also hydrolyzes the accessible part of amylopectin (Martínez-Ortiz *et al*., 2017). Mei, Zhou, Jin, Xu, and Chen (2015) reported in their research that hydrolyzing corn flour with 0.1 N HCl for 6 hours caused an increase in RS 3 levels to 13.8-14.9%. The acid hydrolysis of banana flour is slowly carried out using strong acids such as HCl and H2SO4 (Na *et al*., 2021; Navaf *et al*., 2021).

Resistant starch (RS) is divided into five categories based on the natural presence of starch in food and the way it is made (Borenstein, Hedges, Hinggins, & Rothstein, 2009; Martinez-Ortiz *et al*., 2017; Wu *et al*., 2020). RS type 1 is a naturally occurring starch physically trapped in plant cells and the matrix in starch-rich foods, especially in legumes and cereals (Martinez-Ortiz *et al*., 2017). Type 2 resistant starch is starch granules that are naturally resistant to -amylase and are generally crystalline granules. RS2 in immature bananas and in raw potato and corn starch was found to have high amylose content (Zhou *et al*., 2019). RS 3 is the most resistant starch fraction, mainly in the form of retrograded amylose formed during gelatinized starch cooling and at room temperature.

Type 4 resistant starch is chemically resistant starch which has new chemical bonds (-1,4-glycosidic and -1,6-glycosidic) due to chemical treatment such as with the new trimeta salt which forms a phosphate ester bridge between two starch molecules (Zeng *et al*., 2019). RS type 5 through the formation of starch interacts with lipids, resulting in amylose forming a single helical complex with fatty acids and fatty alcohols. Tu *et al*. (2021) reported that linear chains of starch in a helical structure will form complexes with fatty acids in the helical cavity, so that the bonds make it difficult to hydrolyze by amylase enzymes.

Lintnerization uses a strong acid such as hydrochloric acid. Mei *et al*. (2015) reported that in the early stages of the acid modification process, the amount of amylose or linear short chain fraction increased, indicating that the acid hydrolyzes the accessible moiety of amylopectin (Navaf *et al*., 2021). Sudheesh *et al*. (2020) reported in their research that lintnerization of corn flour with 0.1 N HCl for 6 hours caused an increase in RS 3 levels to 13.8-14.9%. However, several studies using lintnerization in food resulted in different properties and levels of resistant starch. Meta-analyses may provide up-to-date information on improving properties and levels of resistant starch in lintnerized carbohydrate foods. Hence, this study aimed to determine the types of carbohydrate foods that have significantly increased levels of resistant starch and prebiotic properties induced by acid hydrolysis.

2. Materials and Methods

2.1 Materials and tools

The materials used in this meta-analysis are research articles from reputable and accredited international publications from various online databases such as ScienceDirect, Wiley Online Library, Taylor & Francis online, Springer Link, and Google Scholar. The tools used were Mendeley software (version 1.19.8 (2020), Zotero (version 5.0.97 (2021)), OpenMEE software (version 10.10 (2020)), and Microsoft Excel (version 16.53 (2019). The literature was curated using Mendeley and Zotero, while Microsoft Excel and OpenMEE were used to analyse the data.

The entire data were then processed in the OpenMEE worksheet to determine the effect size, heterogeneity/inconsistency (I^2) and p-value. Effect sizes from each study were re-analyzed using OpenMEE to determine the combined effect size with a 95% confidence interval (CI) at a significance level of 0.05.

2.2 References search strategy

The analysis and selection of literature were carried out by following the rules of preferred reporting items for systematic reviews and meta-analyses (PRISMA), a series of analytical processes to select the required literature. PRISMA rules are used to facilitate reporting in the research journal selection process. The library selection is divided into several stages: selection based on title and duplication, selection based on abstract, selection based on method, and full-text selection. The literature was searched through the web server databases ScienceDirect, Wiley Online Library, Taylor & Francis Online, Springer Link, and Google Scholar, using the keywords “acid hydrolysis, resistant starch, foodstuff”. The three keywords were combined using the Boolean operator “and” and then a further selection by year of article publication (2012-2022) was done to narrow the search.

2.3 Study references selection

The selection of research literature was based on the screening of titles and abstracts. Then, it was analysed further using the inclusion and exclusion criteria specified. The inclusion criteria are the selection of a reputable and internationally accredited library. The selected study is also primary data research published in the last ten years (2012-2022). The selected literature contains data on levels of resistant starch before and after acid hydrolysis treatment and is limited to research using acid hydrolysis techniques. Exclusion criteria were the study using starch processing methods, analysis of prebiotic properties, and additional treatment methods other than acid hydrolysis treatment (other treatments).

2.4 Data collection

The research data on selected references, by using Zotero's assistance, were transferred to a Microsoft Excel worksheet. Data were recorded on the author's name, year of publication, food ingredients, the mean and standard deviation of control and experimental resistant starch levels, and the number of replicates.

2.5 Statistical analysis

The data were analyzed using the Hedges'd effect (Standardized Mean Difference/SMD) with a 95% confidence interval to investigate the effect sizes in the data using OpenMEE software (Wallace *et al*., 2013). The data collected from the selected papers were the mean, standard deviation or standard error, and the number of repeated attempts. SMD with a corresponding 95% CI, was pooled using a random-effects model. Higgins and Thompson (2016) reported that the exploration of heterogeneity across studies was carried out using the index I^2 (I^2 > 50% indicates good heterogeneity). The moderator variables for analysis of sub-groups were food ingredients and prebiotic properties. Meta-analysis was carried out using OpenMEE software with the output in forest plots for analysis.

3. Results and Discussion

3.1 Library selection

There were 8,535 scientific papers obtained from selected reference search strategies on websites of reputable international journals. The references were entered into the Zotero software to remove duplicates and get 732 references. Then, further screening was based on the abstracts leaving 203 references. The following screening eliminated 177 references because they did not analyze prebiotic properties and did not include complete quantitative data in the form of resistant starch content before and after acid hydrolysis. Reference selection was also based on being published in a reputable international journal. The selected literature was also limited to primary data research published in the last ten years (2012-2022) and to studies using acid hydrolysis techniques. A total of 26 complete references were used as relevant material in the meta-analysis, as shown in Figure 1.

Data analysis of indigestible starch content from each selected reference had 26 data records. Table 1 summarizes the data for each study. The effect size is the Hedges'd (Standardized Mean Difference/SMD) value to analyze the treatment effects that have a relationship.

3.2 Data analysis

Lintnerization (acid hydrolysis) can increase the RS content. Increasing the degree of debranching will give the linear amylose chains more opportunities to form a perfect crystal structure, thus leading to the formation of more RS. Various studies have been conducted to find the effects of acid hydrolysis on the formation of resistant starch in cassava, potato, corn, and rice starch (Shaikh, Ali, Mustafa, & Hasnain, 2019; Xiao *et al*., 2021).

The results of the meta-analysis in forest plot data using OpenMEE (Figure 2) showed that there was a significant effect in the acid hydrolysis process on increasing levels of resistant starch, with a combined SMD effect of 8.813 with 95% CI (6.566 to 11.060) $p<0.001$ and the heterogeneity value (I^2) was categorized as high, namely 80.88. The meta-analysis used Continuous Random-Effects Model to assess differences between studies. So, the heterogeneity was used to indicate the diversity between the analyzed studies. Wallace *et al*. (2013) explained that a meta-analysis study can be said to be good if it has a heterogeneity close to 100%. The higher the heterogeneity between studies, the more heterogeneous and diverse are the data in the cited studies.

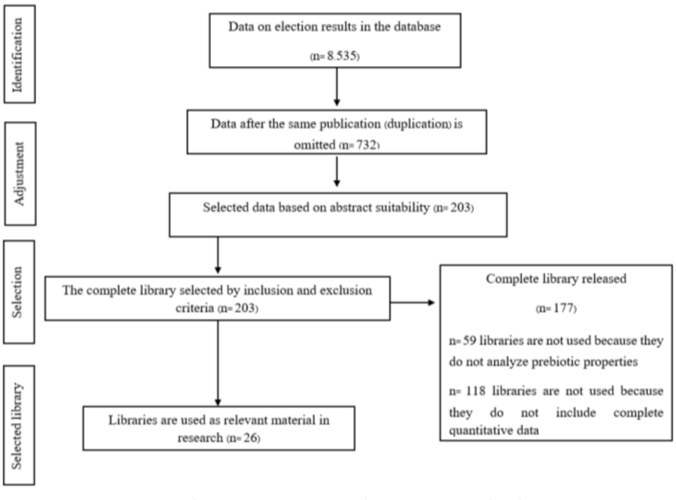


Figure 1. The process of selecting literature/study articles for further meta-analysis.

Table 1. Data on changes in levels of resistant starch in carbohydrate foods

No.	Foods sample	Control (native) %	Experiment (treated starch) %	Resistant starch level (%)	Reference
1	Rice	8.01	26.95	18.94	Lu *et al.* (2021)
2	Maize	2.49	23.33	20.84	Mapengo and Emmambux (2020)
3	Maize	9	17	8	Mapengo *et al.* (2021)
4	Tubers	4.48	29.27	24.79	Martinez-Ortiz, Vargas-Torres, Roman-Gutierrez and Chavarria-Hernandez (2017)
5	Cassava	4.56	48.37	43.81	Mei *et al.* (2015)
6	Potato	17.20	69.27	52.07	Na *et al.* (2021)
7	Palm	38.79	49.11	10.32	Navaf *et al.* (2021)
8	Corn	7.51	80.25	72.74	Oh, Seo, Park, Kim and Baik (2021)
9	Tubers	28.57	41.73	13.16	Raza *et al.* (2021)
10	Corn	5.13	85.36	80.23	Shaikh, Ali, Mustafa and Hasnain (2019)
11	Kithul	17.33	54.67	37.34	Sudneesh *et al.* (2020)
12	Maize	7.67	15.79	8.12	Sun, Hong, Gu, Cheng and Li (2019a)
13	Maize	6.46	77.33	70.87	Sun, Hong, Gu, Cheng, Li and Li (2019b)
14	Lotus	4.05	12.77	8.72	Tu, Ou, Zheng, Zhang, Zheng and Zeng (2021)
15	Corn	4.85	10.96	6.11	Borries-Medrano *et al.* (2018)
16	Lotus	14.15	59.2	45.05	Wang, Jiang, Gou, Zheng and Zhang (2021)
17	Corn	13.10	33.28	20.18	Wang, Liu, Zhang and Chen (2020)
18	Canna	18.50	83.67	65.17	Wu *et al.* (2020)
19	Corn	19.33	80.33	61	Xiao *et al.* (2021)
20	Pea	4.53	67.29	62.76	Zhou *et al.* (2019)
21	Barley	9.85	16.61	6.76	Xie, Zhung, Liu, Fan, Zhang, Qin and Liu (2021)
22	Rice	37.50	90.33	52.83	Ye, Luo, Huang, Chen, Liu and McClements (2019)
23	Potato	25.88	93.83	67.95	Yeum *et al.* (2020)
24	Wheat	18.63	56.67	38.04	Zeng *et al.* (2020)
25	Maize	6.4	44.80	38.4	Zeng, Li, Zhao, Chen, Yu and Liu (2019)
26	Rice	9.5	26.33	16.83	Zheng, Yin, Kong, Chen, Enbo and Liu (2021)

Mean of Control (native) % (n= 26); 50.80%
Mean of Experiment (treated starch) % (n= 26); 191.49%
Mean of Resistant starch level (%) (n= 26); 140.68%

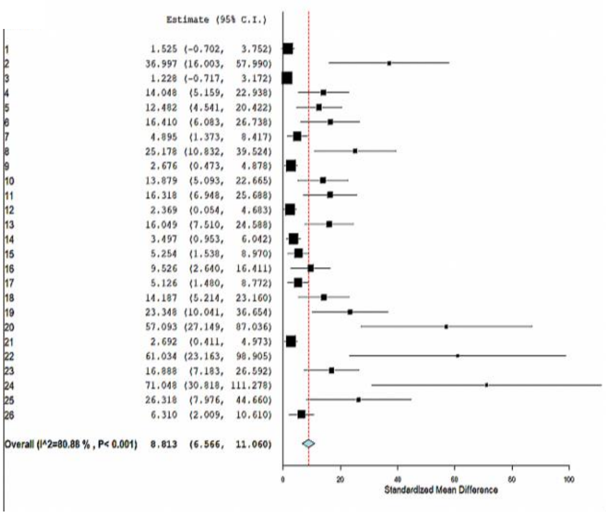


Figure 2. Forest plot of the results from meta-analysis of all data

The effect size data in Figure 2 provide general information on results of the meta-analysis to support the hypothetical association of acid hydrolysis and an increased resistant starch level. There are 4 data records on carbohydrate foods whose resistant starch content did not increase significantly, namely tubers, lotus, barley in China, and maize in South Africa. 2 data records experienced a decrease in RS levels, namely rice food located in China in the research of Lu *et al*. (2021) and corn in South Africa in the analysis of Mapengo and Emmambux (2020). The locations are an essential factor in the presence of natural starch in carbohydrate foods, because of influences on physicochemical characteristics of the soil where the food grows, and also the weather cycle in the area is very influential affecting nutrients in the ground and air (Mei *et al*., 2015; Navaf *et al*., 2021). Signs of decreased levels of resistant starch were associated with the degradation of RS1 and RS2 after acid hydrolysis treatment of corn, rice, and barley (Sun *et al*., 2019a; Sun *et al*., 2019b; Wu *et al*., 2020).

3.3 Association of carbohydrate food type with increased resistance starch levels

Table 1 presents 26 research records on 26 carbohydrate foods with increased levels of resistant starch after acid hydrolysis. A total of 50.80% of the average control data for resistant starch from 26 research studies reported increased levels of resistant starch (Table 1).

A total of 13 carbohydrate foods studied using the acid hydrolysis gave varying results as regards increasing the levels of resistant starch. Acid hydrolysis can increase levels of resistant starch by generating short chain amylose fraction with low molecular weight, from the degradation of long chain amylose at the α-1,6-glycosidic branching points of the amylopectin chain (Yeum *et al*., 2020).

Figure 3 presents the results of data analysis in a forest plot from, on the effects by type of carbohydrate food. The forest plot shows that high-carbohydrate foods have a significant effect in increased levels of resistant starch with an SMD of 8.813 with 95% CI (6.566 to 11.060) p <0.001 and the heterogeneity (I^2) was categorized as high, namely 80.88. The forest plot for the carbohydrate food sub-group indicates that, in general, the acid hydrolysis for all foods significantly increased the levels of resistant starch.

3.4 The effect of carbohydrate food on prebiotic properties

Further studies were conducted to determine the effects of the type of carbohydrate food on the prebiotic properties. Food products in the form of flour that are processed physically, chemically, and enzymatically have a high content of resistant starch. Starchy foods with high resistant starch content can have improved prebiotic properties because they are more challenging to digest in the gastrointestinal tract (colon). The effect of prebiotics as a sole carbon source is to increase the viability of probiotics in absolute terms, dependent on the concentration of prebiotics and their selectivity against pathogenic bacteria in the gut (Vardakou *et al*., 2008). Lu *et al*. (2021) reported that prebiotic products are intended for all food ingredients that cannot be digested and hydrolyzed in the digestive tract. Prebiotics can carry out a selective stimulus process based on the growth and activity of several beneficial probiotic bacteria in the colon (Ciardullo *et al*., 2019). Each test of prebiotic properties is divided into the starch composition (SC), amylose interaction (Al), the viability of lactic acid bacteria (VBAL), and viability of *Enteropathogenic Escherichia coli* (VEPEC). The literature was analyzed and the forest plot of data can be seen in Figure 4.

The forest plot shows that carbohydrate foods with an SMD of 8.813 with 95% CI (6.566 to 11.060) p<0.001 had a significant effect on prebiotic properties. The effect sizes of food ingredients mean that the carbohydrate food has a proportional effect on the prebiotic properties. Borenstein *et al*. (2009) reported that the effect size describes the average in each meta-analysis study that can be distributed.

Food has good prebiotic properties if it is metabolized selectively by probiotic bacteria such as *Lactobacillus plantarum*, *L. acidophilus*, and *Bifidobacterium* but is not metabolized selectively by pathogenic bacteria such as EPEC (Mei *et al*., 2015; Lu *et al*., 2021; Na *et al*., 2021). Type of carbohydrate food significantly affect its prebiotic properties with an SMD of 8.813 with 95% CI (6.566-11.060) p<0.001, as shown in the forest plot.

4. Conclusions

This meta-analysis study concluded that lintnerization (acid hydrolysis) significantly increases the levels of resistant starch and the prebiotic properties of high-carbohydrate foods with a 95% confidence level. The acid hydrolysis of carbohydrate foods is recommended for increasing the levels of resistant starch, and can be used in home or factory scale. Various starchy foods have different physicochemical components, differing in amylose to amylopectin ratio and their degrees of polymerization, so further laboratory-scale research is needed to improve resistant starch content by acid hydrolysis treatments.

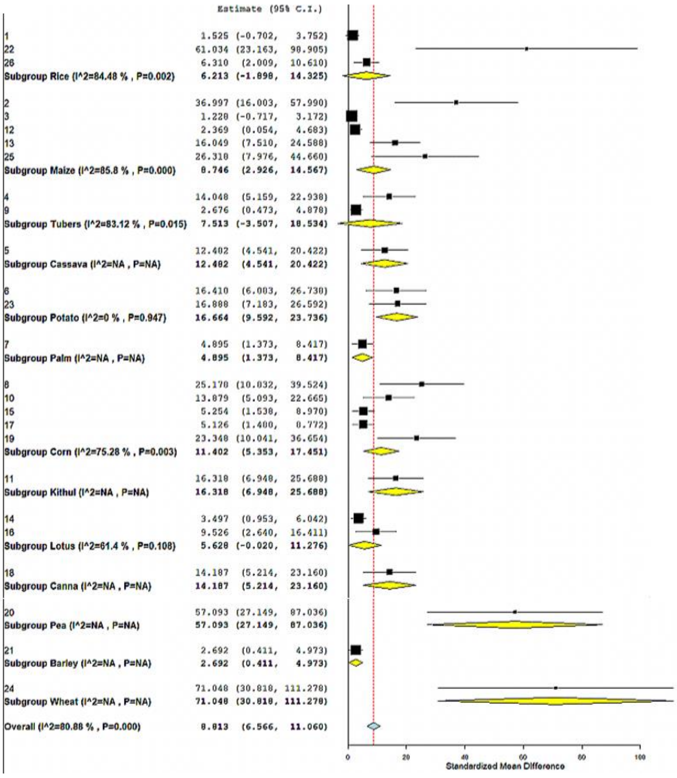
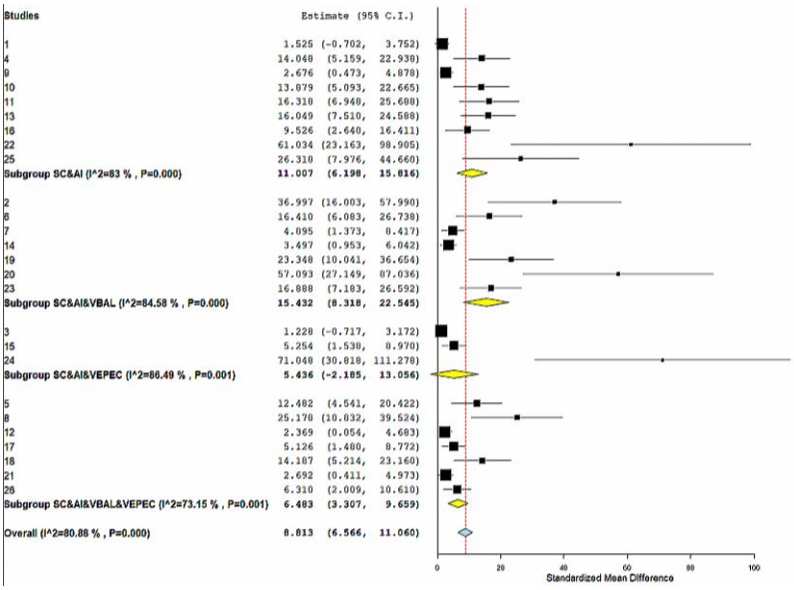


Figure 3. Forest plot of the results from meta-analysis of effects by type of carbohydrate food on increasing resistant starch levels



Information:

SC: Starch Composition	AI: Amylose Interaction
VBAL: Viability BAL	VEPEC: Viability EPEC

Figure 4. Forest plot of the results from meta-analysis of effects of carbohydrate food on prebiotic properties

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