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# EFFECT OF AUTOCLAVING-COOLING ON RESISTANT STARCH CONTENT AND PREBIOTIC PROPERTIES OF HIGH CARBOHYDRATE FOODS: META-ANALYSIS STUDY

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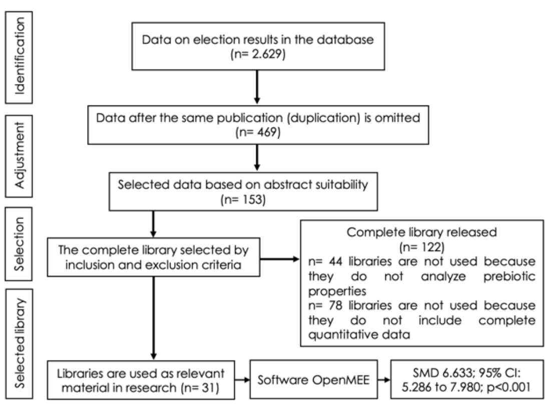
## Graphical abstract




## Abstract

Autoclaving-cooling is a starch physical modification technique widely used to analyze the increase in resistant starch levels in foodstuffs. However, this technique has a different effect on each high-carbohydrate diet. This study investigates the type of carbohydrate food to increase the levels of resistant starch through the autoclaving-cooling process. This study used 31 articles using the PRISMA method. Data were analyzed with Effect Size Hedges'd (standardized mean difference/SMD) and confidence interval (CI) using OpenMEE software. The results showed that the autoclaving-cooling method had a significant effect on increasing levels of resistant starch and prebiotic properties (SMD 6.633; 95% CI: 5.286 to 7.980; p<0.001). In conclusion, this study confirmed that the autoclaving-cooling method had a significant effect with a 95% confidence level in increasing the levels of resistant starch and prebiotic properties of high-carbohydrate foods.

*Keywords*: Autoclaving-cooling, meta-analysis, modified starch, prebiotic properties, resistant starch



## 1.0 INTRODUCTION

Starch consists of a long linear chain of amylose that branch into structural units, which are the main energy source in the human diet [1]. Resistant starch (RS) is a starch fraction with short linear chain amylose which cannot be digested by digestive enzymes and is resistant to gastric acid so that it can reach the large intestine to be fermented by probiotic bacteria [1]. Increased levels of resistant starch (RS) in high-carbohydrate foods aim to improve prebiotic properties and reduce the glycemic index and digestibility of starch to amylase enzymes [2]. Resistant starch has physiological effects that benefit health, such as colon cancer prevention, and has hypoglycemic hypocholesterolemic effects [2].

The principle of physical modification, in general, is by heating. Physical modification treatments include extrusion, pre-cooking, steam cooking, microwave irradiation, roasting, and hydrothermal treatment. Most of the physical modification methods mentioned can increase the levels of resistant starch [3]. Another physical modification to improve resistant starch content and prebiotic properties in foodstuff is autoclaving-cooling. The autoclaving-cooling technique combines gelatinization and starch retrogradation [4, 5, 6]. The autoclaving-cooling method causes retrogradation of the amylose fraction, where the resistant starch (RS) content is proportional to the amylose content in foodstuffs [7]. The starch retrogradation process can cause recrystallization and increase resistant starch formation. Crystallization occurs due to forming a new double helix between the amylose molecules. The double helix structure will form an enlargement (aggregation) with the double helix on other amylose molecules through hydrogen bonds to form crystallites [8].

Generally, it was reported that autoclaving-cooling decreased the peak viscosity and breaking viscosity, increased the gelatinization temperature, and decreased the swelling capacity of starch granules [9]. Zailani et al. [6] and Lader et al. [10] reported that using three cycles in the autoclave-cooling technique could increase the highest RS level with some changes during the starch modification process, the formation of amylose-lipid complexes and a decrease in the enzymatic hydrolysis of starch. Starch gelatinization occurs, which results in the loss of birefringence properties of starch granules due to excess water and heating at a certain time and temperature so that the starch granules swell and cannot return to their original condition (irreversible), resulting in a decrease in starch digestibility [11]. Decreased starch digestibility, increased crystallinity, and increased interactions between starch chains by forming double helix bonds will increase RS3 levels [12].

The autoclaving-cooling treatment on starch digestibility has been studied extensively by Ovando-Martinez *et al.* [13] and Lee *et al.* [14]. Generally, autoclaving-cooling causes retrogradation of amylose fraction over a certain period, affecting starch digestibility. However, several studies using autoclaving-cooling in food resulted in different properties and levels of resistant starch. This meta-analysis study contains the latest information on increasing the prebiotic properties and contents of resistant starch in foodstuffs using autoclaving-cooling. Hence, this study aimed to investigate the type of carbohydrate food that has a significant effect on increasing levels of resistant starch and its prebiotic properties through the autoclaving-cooling technique.

## 2.0 METHODOLOGY

### 2.1 Materials and Tools

The materials used in this meta-analysis are research articles from reputable and accredited international publications from various online database web servers such as Science Direct, Wiley Online Library, Taylor & Francis Online, Springer Link, and Google Scholars. The tools used are Mendeley software (version 1.19.8 (2020), Zotero (version 5.0.97(2021)), OpenMEE software (version 10.10 (2020)), Microsoft Excel (version 16.53 (2019). The literature studies were selected using Mendeley and Zotero, while Microsoft Excel and OpenMEE were used to analyze the data.

### 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, abstract, selection based on method, and full-text selection. The literature was searched through the web server database Science Direct, Wiley Online Library, Taylor & Francis Online, Springer Link, and Google Scholars, using the keywords "autoclaving-cooling, resistant starch, foodstuff." The three keywords are combined using a Boolean operator with the expression "and" and then added a selection in the year of article publication (2018-2022) 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 is analyzed to determine its suitability with the specified inclusion and exclusion criteria. The inclusion criteria are the selection of a reputable and internationally accredited library. The selected research is also primary data published in the last ten years (2018-2022). The selected literature has data on resistant starch levels before autoclaving-cooling (Untreated) and after autoclaving-cooling (autoclaving-cooling-treated) modifications and is limited to research using autoclaving-cooling techniques. Exclusion criteria were studying research results using starch processing methods, analysis of prebiotic properties, and additional treatment methods other than autoclaving-cooling (Microwave-cooling and annealing).

### 2.4 Data Collection

The research data from the journal webserver's selected references using Zotero's assistance was transferred to a Microsoft Excel worksheet. Data were collected based 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

Data were analyzed using Hedges'd effect (Standardized Mean Difference/SMD) with a 95% confidence interval to determine Effect Size data using OpenMEE software developed by Wallace *et al.* [15]. The data collected from the selected journals are the mean, standard deviation or standard error, and the number of repeated attempts. SMD with a corresponding 95% CI, pooled using a random-effects model. Higgins *et al*. [16] reported that the exploration of heterogeneity across studies was carried out using an index of I^2 (I^2>50% indicates good heterogeneity). The moderator variables for analysis for the sub-groups were food ingredients and prebiotic properties. Meta-analysis was conducted using OpenMEE software with the output in forest plots for analysis.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Library Selection

The libraries obtained from the selection process in the database are 2.629 references. The references were entered into the Zotero software to remove the same duplicate libraries and get 469 references. Then, it proceeds with the selection based on the abstracts obtained from 153 references. The results of the following choice eliminated 122 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 autoclaving-cooling. References selection is also based on literature published in reputable international journals. The selected literature is also the result of primary data research published in the last ten years (2013-2022) and is limited to studies using autoclaving-cooling techniques. A total of 31 complete references were used as relevant material in the meta-analysis study, as shown in Figure 1.

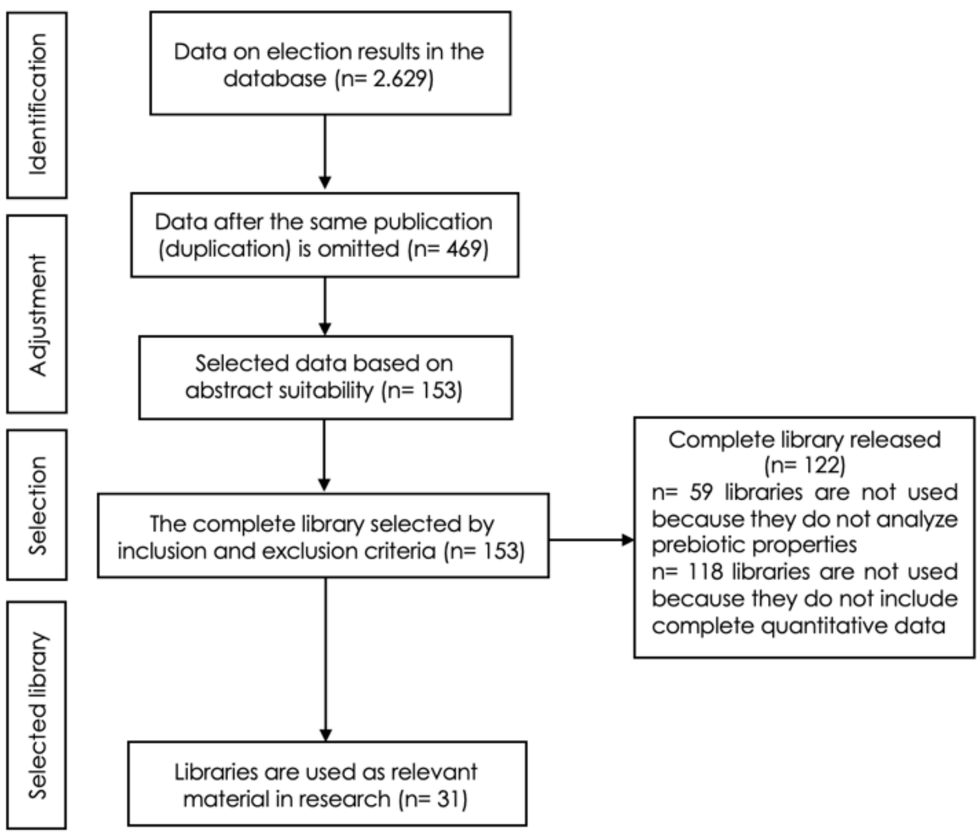



**Figure 1** The process of selecting literature/study articles for further meta-analysis

### 3.2 Data Analysis

Data analysis of resistant starch content from each selected reference was obtained as many as 31 data. Table 1 summarizes the data for each study. The data is then processed on the OpenMEE worksheet to determine the effect size, heterogeneity/ inconsistency value (I^2), and p-value. Effect Size values from each study were re-analyzed using OpenMEE to determine the combined effect measurement value with a 95% confidence interval (CI) with a significance level of 0.05. The effect Size value is the Hedges'd value (Standardized Mean Difference/SMD) to analyze the effect of treatment that has a relationship.

**Table 1** Data on changes in levels of resistant starch in foodstuffs

| Foodstuffs | Material specific information | Control (%) | After modification (%) | Content Change (%) | References |
|---|---|---|---|---|---|
| Potato | West Bengal, India | 14.57 | 36.18 | 21.61 | [17] |
| Banana | Pei-Chiao in Taiwan | 46.34 | 49.45 | 3.11 | [18] |
| Pea | Canada | 13.30 | 38.17 | 24.87 | [19] |
| Bean varian Nata | Fujian, China | 17.18 | 36.02 | 18.84 | [20] |
| Bean varian Karo | Nochów, Poland | 15.07 | 60.04 | 44.97 | [21] |
| Oat | North Dakota, USA | 18.69 | 76.66 | 57.97 | [13] |
| Potato | Tamil Nadu, India | 14.40 | 64.31 | 49.91 | [22] |
| Glutinous Rice | Shanghai, China | 7.15 | 70.91 | 63.76 | [23] |
| Millet | China | 14.50 | 80.70 | 66.2 | [24] |
| Maize | USA | 17.92 | 47.64 | 29.72 | [25] |
| Corn | Toluca, Mexico | 2.55 | 71.04 | 68.49 | [26] |
| Rice S-7250 | Seoul, Korea | 0.48 | 26.70 | 26.22 | [12] |
| Bean | China | 18.61 | 46.74 | 28.13 | [27] |
| Lianhe Rice | Anhui, China | 5.00 | 82.75 | 77.75 | [28] |
| Rice | Jiangxi, China | 14.12 | 35.70 | 21.58 | [29] |
| Corn | Beijing, China | 11.76 | 41.74 | 29.98 | [30] |
| Bean | West Bengal, India | 6.44 | 81.68 | 75.24 | [31] |
| Rice | Jingzhou, China | 11.88 | 16.02 | 4.14 | [32] |
| Corn | Guizhou, China | 4.37 | 80.42 | 76.05 | [33] |
| Rice | Taipei, Taiwan | 8.36 | 40.80 | 32.44 | [34] |
| Bean | East Java, Indonesia | 5.01 | 79.21 | 74.2 | [35] |
| Maize | Morelos, Mexico | 14.28 | 50.52 | 36.24 | [36] |
| Banxia | Beijing, China | 31.29 | 77.00 | 45.71 | [37] |
| Rice | Xihe in Gansu | 16.46 | 39.39 | 22.93 | [38] |
| Rice | Srinagar, India | 2.70 | 13.06 | 10.36 | [39] |
| Corn | Jiangsu, China | 6.29 | 43.73 | 37.44 | [40] |
| Pea | Quer ́etaro, Mexico | 6.08 | 35.08 | 29 | [41] |
| Sorghum | Chengdu, China | 1.97 | 20.03 | 18.06 | [42] |
| Corn | Karachi, Pakistan | 16.56 | 64.28 | 47.72 | [43] |
| Pea | Jiangsu, China | 15.38 | 84.19 | 68.81 | [44] |
| Potato | Weifang, China | 3.53 | 86.27 | 82.74 | [45] |

Note:
The average levels of control resistant starch (n= 31); 12.33%.
The average content of resistant starch after modification (n= 31); 54.03%.
The average increase in resistant starch (n= 31); 41,75%.

The autoclaving-cooling technique can increase the RS content. Repeated autoclaving-cooling modification can increase the amylose fraction by retrogradation, thereby affecting the gelatinization characteristics of starch which is the main characteristic of type 3 resistant starch (RS) [9]. RS 3 was the most resistant starch fraction, mainly in the form of retrograde amylose formed during the cooling of gelatinized starch and at room temperature. Studies on increased levels of RS in other foods have been reported by Mutlu *et al*. [46] showed an increase in the RS content of corn flour by 2% after three autoclave cooling cycles. Lu *et al*. [12] reported that the autoclaving-cooling technique in peanut flour can increase the RS content to 8 times that of natural peanut flour. The formation of RS 3 through recrystallization and retrogradation processes is the main cause of increasing levels of resistant starch in foodstuffs [7].

The results of the meta-analysis of forest plots data using OpenMEE (Figure 2) showed that the autoclaving-cooling process had a significant effect on increasing levels of resistant starch, with a combined effect value of 6.633 with a 95% CI (5.286 to 7.980) $p<0.001$ and a heterogeneity value (I^ 2) included in the high category, namely 79.2. The meta-analysis used Continuous Random-Effects Model analysis to see the differences between one study and another. Thus, the interpretation of heterogeneity values was used to interpret the variance between the analyzed studies. Wallace *et al.* [15] explained that a meta-analysis study could be said to be good if it has a heterogeneity value close to 100%. The higher the heterogeneity value between studies, the more heterogeneous and can represent the diversity of data for each study.

The effect size data in Figure 2 provide general information for the meta-analysis results to support the theory of the relationship between modified acid hydrolysis techniques and the associated increased levels of resistant starch. There are 2 data on foodstuffs whose resistant starch content did not experience a significant increase, namely bananas from the research of Liao *et al.* [18] and pea research by Lu *et al.* [12]. Signs of decreased levels of resistant starch are related to the degradation process of RS1 and RS2 after modification.

### 3.3 The Effect of Different Carbohydrate Foodstuffs on Increasing Resistance Starch Levels

Table 1 presents 31 research data reporting 31 foodstuffs that experienced increased levels of resistant starch after the modified autoclaving-cooling technique was applied. As many as 41.75% of research data reported increased resistant starch levels. A total of 11 types of carbohydrate foods were used in this study. Each study gave different results for increasing levels of resistant starch before and after the autoclave cooling technique modification. According to Yang *et al*. [33], differences in the increased levels of resistant starch were caused by differences in amylose and amylopectin levels in foodstuffs. Liu *et al*. [45] added that in addition to amylose content and amylopectin-modified starch, it was also influenced by several factors such as reducing sugar content, starch digestibility, starch composition, fiber content, starch gelatinization, and starch retrogradation.

The modification technique of waxy corn starch with autoclaving-cooling, a combination of acid hydrolysis and autoclaving-cooling, a combination of debranching and autoclaving-cooling and a combination of acid hydrolysis, debranching and autoclaving-cooling can increase the degree of polymerization (DP) 25-30 which contributes to the formation of RS 3 [47]. Liang *et al*. [48] reported that waxy corn starch has the most common DP range of 20-35. Retrograded amylose is more stable to heat, highly complex, and resistant to amylase enzymes. Autoclaving-cooling technique has disadvantages, including less efficiency because it takes longer production time, more incredible heat energy, and high production costs [49]. The drawbacks of the autoclaving-cooling technique that can be overcome by pre-treatment carried out are acid hydrolysis, debranching of pullulanase enzymes for severance of amylopectin, and fermentation by lactic acid bacteria (LAB) [2].

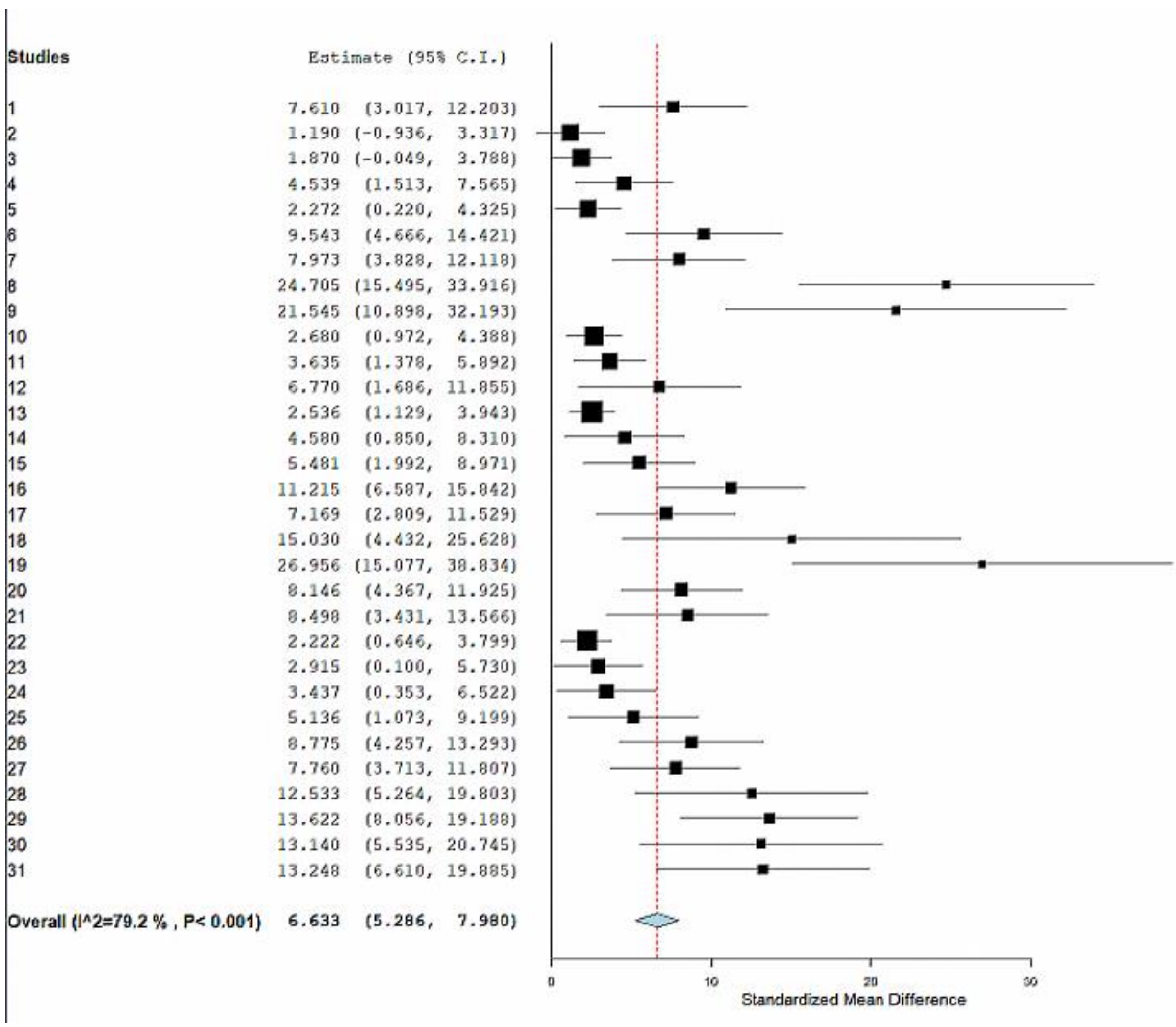



**Figure 2** Forest plot of the results of the meta-analysis study of all data

The results of the data analysis of the forest plots on the effect of different types of carbohydrate foods are presented in Figure 3. The results of the forest plots show that carbohydrate foods significantly increase the levels of resistant starch with an SMD effect value of 6.633 with 95% CI (5.286 to 7.980) p<0.001 and a value of heterogeneity (I^2) were included in the high category, namely 79.2. The forest plots for the carbohydrate food subgroup data stated that, in general, the autoclaving-cooling technique for all foods had a significant effect on increasing levels of resistant starch.

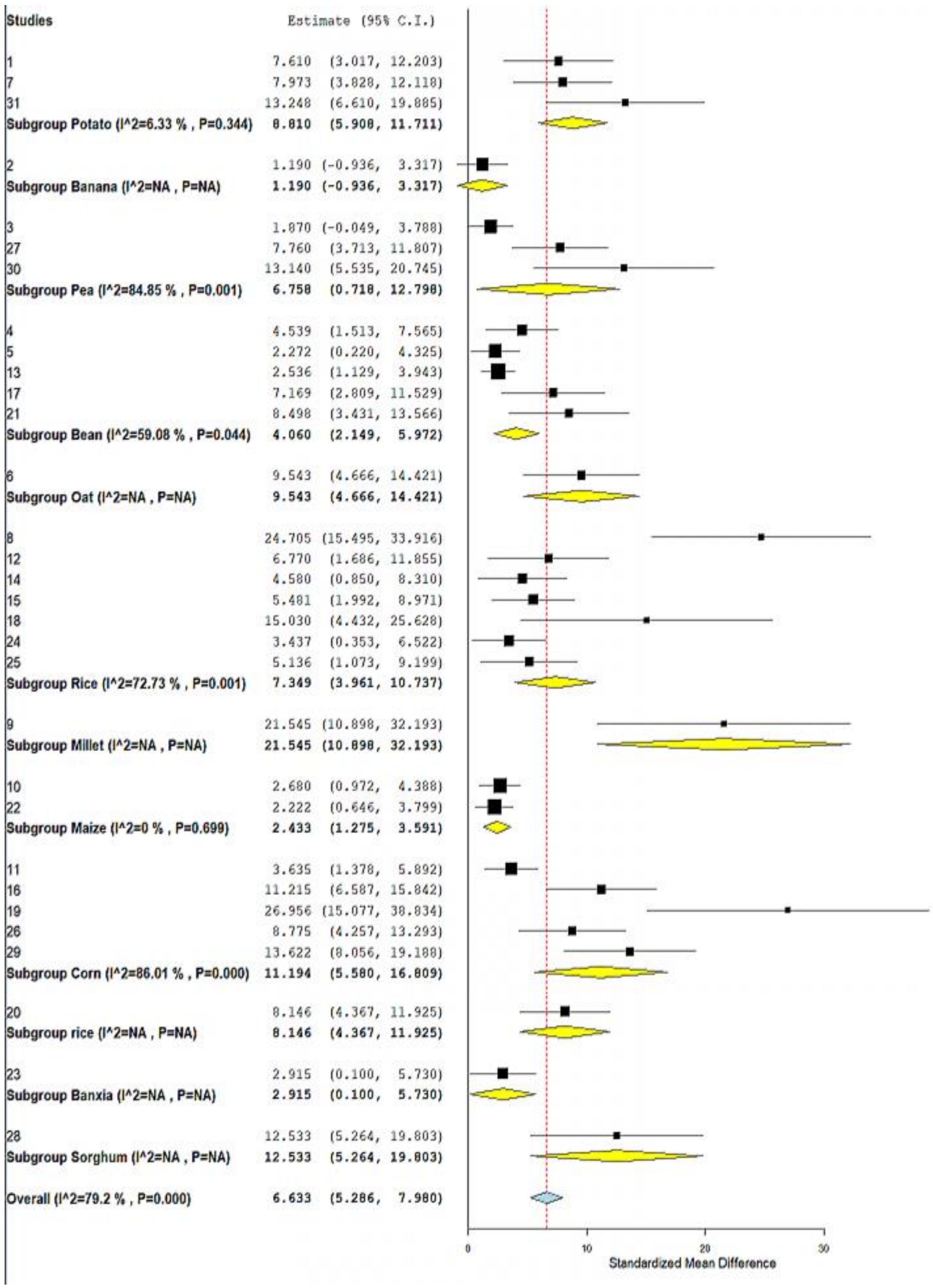
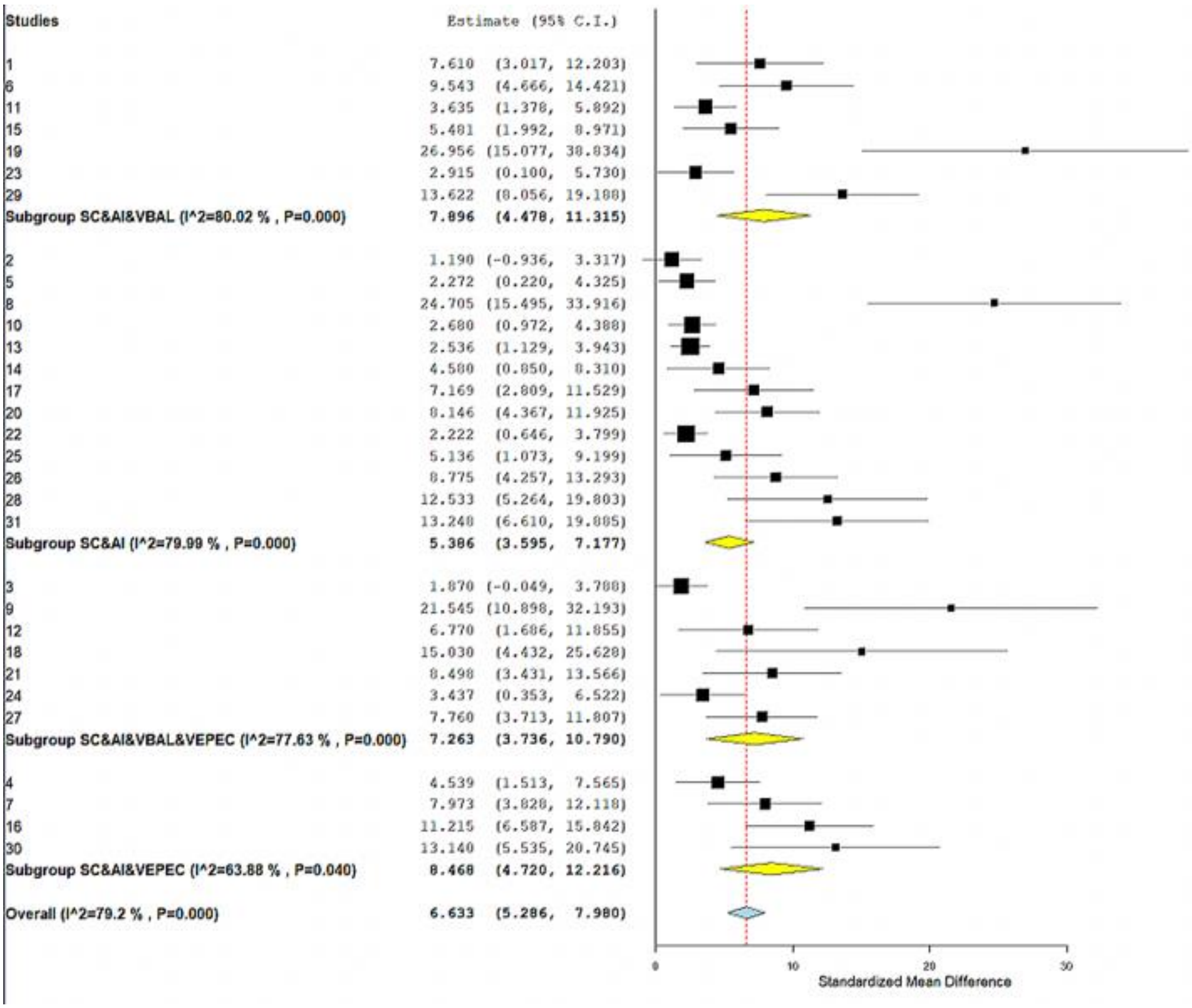



**Figure 3** Forest plot of the results of the meta-analysis of the study of the effect of different types of carbohydrate foods on increasing resistance starch levels

### 3.4 The Effect of Carbohydrate Foods on Prebiotic Properties

Further studies were conducted to determine how the type of carbohydrate food affects the prebiotic properties. Each test of prebiotic properties is divided into the starch composition (SC), amylose interaction (AI), the viability of lactic acid bacteria (VBAL), viability of *Enteropathogenic Escherichia coli* (VEPEC) in the literature are analyzed and obtained forest plot data can be seen in Figure 4.




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

**Figure 4** Forest plot of the results of the meta-analysis of the study of the effect of carbohydrate foods on prebiotic properties

Based on the results of the forest plot, carbohydrate food with an SMD effect value of 6.633 with 95% CI (5.286 to 7.980) $p<0.001$ has a significant effect on prebiotic properties with an SMD effect value of $p<0.001$. The effect size value of food ingredients has the same effect as the effect size on prebiotic properties, meaning that carbohydrate foods have a proportional effect on prebiotic properties. Resistant starch is the fraction of starch that digestive enzymes cannot digest. Resistant to stomach acid that reaches the large intestine and is fermented by probiotic bacteria [12]. Resistant starch is the fraction of starch that cannot be digested in the small intestine. It has physiological effects that benefit health, such as colon cancer prevention and hypoglycemic and hypocholesterolemic effects [22]. Borenstein *et al.* [50] reported that the effect size value describes the average of 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 by pathogenic bacteria such as EPEC [6, 10, 12]. Based on the results in the forest plot, the type of carbohydrate food significantly affected the prebiotic properties with an SMD effect value of 6.633 with a 95% CI (5.286 to 7.980) $p<0.001$. Autoclaving cooling can increase the gelatinization temperature, increase the viscosity of starch paste, and increase the tendency of the retrogradation process which causes foods to have low digestibility [13]. Foods with low digestibility, good for diabetics, or those who are on a low-calorie diet. The characteristics of modified forage feed are influenced by several factors, including the type of feed and pH [23].

## 4.0 CONCLUSION

This meta-analysis study concluded that the autoclaving-cooling treatment had a significant effect with a 95% confidence level in increasing the levels of resistant starch and improving the prebiotic properties of high-carbohydrate foods.

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