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Effect of Combined Lactic Acid Bacteria Fermentation and Autoclaving–Cooling Treatment on Resistant Starch Content and Prebiotic Properties of High-Carbohydrate Foods: A Meta-Analysis

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Abstract: Resistant starch (RS) is a dietary fibre fraction that contributes to improved glycemic control and gut health, and its content can be significantly enhanced through processing. This meta-analysis aimed to evaluate the combined effects of lactic acid bacteria (LAB) fermentation and autoclaving–cooling (AC) treatment on RS formation and prebiotic properties in carbohydrate-rich foods. Following PRISMA guidelines, a systematic search of ScienceDirect, SpringerLink, Scopus, and PubMed identified 29 eligible studies published between 2015 and 2025. A random-effects model was applied to calculate pooled standardised mean differences (SMD) using Hedges' g. The combined LAB–AC treatment significantly increased RS content, with a mean rise from 19.70% in control samples to 47.27% after treatment (SMD = 2.45, 95% CI: 1.85–3.05, $p < 0.001$). Substantial heterogeneity was observed ($I^2 = 93.4\%$), suggesting the influence of moderators, including amylose content, fermentation duration, bacterial strain, and the number of AC cycles. Subgroup analyses revealed that cereals and legumes exhibited the greatest RS enhancement, along with reduced in vitro digestibility, lower estimated glycemic index (eGI), and improved prebiotic indicators, including increased short-chain fatty acid (SCFA) production and stimulation of *Lactobacillus* and *Bifidobacterium* growth. These results demonstrate the synergistic potential of LAB fermentation and AC treatment to develop high-RS functional foods with improved nutritional and prebiotic qualities. Further research is recommended to standardise analytical procedures, optimise process conditions, and confirm these outcomes in human clinical studies.

Keywords: autoclaving–cooling; fermentation; high-carbohydrate foods; lactic acid bacteria; resistant starch.

1. Introduction

Starch is the major dietary source of carbohydrates, supplying most of the energy in the human diet. However, not all starch is digested in the small intestine. A portion known as resistant starch (RS) escapes enzymatic digestion and reaches the colon, where it functions as dietary fibre [1,2]. Resistant starch can be divided into five categories: RS1, which is physically

inaccessible starch; RS2, native ungelatinised starch granules; RS3, starch that becomes resistant after gelatinisation followed by cooling (retrograded starch); RS4, starches modified through chemical treatments; and RS5, starch–lipid complexes formed primarily with amylose. Of these categories, RS2 and RS3 have received the greatest research attention due to their widespread occurrence in foods and their sensitivity to processing conditions [3].

RS offers several health benefits, including reducing postprandial glycemic responses, improving lipid metabolism, and supporting gut health by increasing the production of short-chain fatty acids (SCFAs), particularly butyrate, which plays key roles in colonocyte energy supply, inflammation control, and cancer prevention [4]. Owing to these physiological advantages, RS is regarded as a functional dietary component that supports metabolic health and prevents chronic disease. The growing interest in RS is reflected in an increasing number of systematic reviews and meta-analyses linking higher RS intake to improved insulin sensitivity, reduced risk of type 2 diabetes, enhanced satiety, and modulation of the gut microbiota [5–7].

However, RS formation varies widely among food sources and processing conditions. High-amylose starches generally yield more RS because of their linear structure, which favours retrogradation, while amylopectin-rich starches are more susceptible to digestion [8,9]. Processing factors such as cooking temperature, moisture content, and cooling rate also influence RS formation [10,11]. This variability highlights the need for a meta-analytic approach to quantify the combined effects of different variables and to identify consistent moderators that affect RS synthesis.

Several techniques have been explored to enhance RS, including enzymatic modification, chemical derivatisation, fermentation, and thermal processing. Among them, autoclaving-cooling and lactic acid bacteria fermentation are particularly promising. AC treatment promotes RS formation through gelatinisation followed by amylose recrystallisation upon cooling [12,13]. Meanwhile, LAB fermentation modifies starch through acidification and enzymatic hydrolysis, lowering the glycemic index (GI) and generating organic acids and bioactive metabolites [14]. Combining these two processes can produce synergistic effects: LAB fermentation preconditions starch for enhanced retrogradation during AC, and repeated AC cycles further strengthen crystalline structures and RS content [15,16]. Additionally, the combination supports prebiotic effects by stimulating beneficial microbes, such as *Lactobacillus* and *Bifidobacterium*, and increasing SCFA production [17].

Despite increasing evidence, reported outcomes remain inconsistent due to differences in food type, amylose content, LAB strain, fermentation duration, and processing cycles [18,19]. Furthermore, previous studies often focus only on RS levels, neglecting prebiotic outcomes, and employ diverse RS measurement protocols, which complicates cross-study comparisons. These discrepancies justify a comprehensive meta-analysis to integrate current findings and evaluate both compositional (RS content) and functional (prebiotic) effects.

Therefore, this study systematically reviews and quantitatively synthesises the effects of combined LAB fermentation and AC treatment on RS content and prebiotic properties of carbohydrate-rich foods. Subgroup and sensitivity analyses were performed to explore sources of heterogeneity. The working hypothesis is that the combined microbial–thermal approach synergistically enhances RS formation and prebiotic potential, offering a practical framework for developing functional foods with improved nutritional quality.

2. Materials and Methods

This meta-analysis was conducted to systematically synthesise research evidence on the effects of combining lactic acid bacteria fermentation with autoclaving–cooling on the levels of resistant starch and the prebiotic properties of high-carbohydrate foods. Following PRISMA guidelines ensured that the review process was transparent, reproducible, and methodologically rigorous [13,20]. A structured search strategy was developed using six electronic databases: ScienceDirect, SpringerLink, Wiley Online Library, Scopus, PubMed, and Google Scholar. The Boolean search strings used were: ("resistant starch" OR "RS") AND ("lactic acid bacteria" OR "LAB" OR fermentation) AND ("autoclaving cooling" OR "thermal treatment") AND ("prebiotic" OR "functional food"). The search was limited to original research articles published between 2015 and 2025, written in English, and reporting quantitative outcomes related to RS content, digestibility, or prebiotic properties.

2.1. Search and collection of study source articles.

The search process followed the PRISMA framework through sequential steps of identification, screening, eligibility, and inclusion. During the identification phase, all articles retrieved from the databases were imported into Mendeley for management and duplicate removal. Titles and abstracts were screened for relevance, and studies were retained if they met pre-specified inclusion criteria. Eligible articles were required to be primary research studies published in reputable peer-reviewed journals between 2015 and 2025, reporting quantitative data on RS levels, in vitro digestibility, or physicochemical properties of carbohydrate-rich foods subjected to combined AC and LAB fermentation treatments. To ensure data robustness, only studies that reported complete statistical information—mean values, number of replicates, and standard deviations or errors for both control and treated groups—were included. Reviews, patents, books, and studies with incomplete statistical data were excluded, as standardised mean difference calculations require complete inputs for validity [21].

2.2. Article selection and data collection.

Data extraction was performed using Zotero to organise and retrieve full-text articles, which were then tabulated in Microsoft Excel. For each study, key metadata were recorded, including author(s), publication year, country, food matrix, sample size, type of starch modification, number of replicates, mean RS values, standard deviations, and any reported prebiotic endpoints such as SCFA production or LAB growth. This systematic data capture facilitated the calculation of effect sizes and subgroup analyses. The data structure was designed to ensure compatibility with OpenMEE software (version 10.10), which was employed to perform meta-analysis computations and generate forest plots.

2.3. Statistical analysis.

All statistical analyses were performed using OpenMEE (version 10.10) and followed the random-effects model based on the DerSimonian–Laird method. This approach accounts for both within-study and between-study variability, acknowledging that true effects may differ across food matrices and experimental designs.

Effect sizes were expressed as standardised mean differences (SMDs) using Hedges' g, which corrects for small-sample bias and allows comparisons across studies with different

measurement scales. Each study was weighted by the inverse of its variance, giving greater influence to those with larger sample sizes and smaller standard errors. Forest plots were generated to present individual and pooled SMDs with 95% confidence intervals (CI), providing a visual summary of the magnitude and precision of the combined effects.

Between-study heterogeneity was quantified using the I^2 statistic, where values above 50% indicated substantial heterogeneity [22]. Given the expected diversity in food matrices and processing parameters, high heterogeneity was anticipated. To identify potential sources of variation, subgroup analyses were performed based on moderator variables, including food type (cereals, legumes, tubers), amylose content (high vs. low), geographic region (Asia, Europe, others), and processing factors (number of AC cycles and fermentation duration). Moderator variables were defined a priori to minimise bias and improve interpretability.

Meta-regression analyses were conducted to explore the influence of continuous predictors—such as amylose percentage, fermentation time, and AC cycle number—on effect sizes, providing a quantitative assessment of heterogeneity sources. Publication bias and statistical reliability were rigorously examined using several complementary diagnostic approaches. Funnel diagrams were visually inspected for asymmetry, and Egger's regression test was used to statistically evaluate potential publication bias (Figure S3 in the Supplementary Material). When evidence of asymmetry was found, the Duval and Tweedie trim-and-fill procedure was applied to estimate the adjusted pooled effect size, ensuring that selective reporting or outlier studies did not unduly influence the results (Figure S2 in the Supplementary Material).

The robustness and statistical reliability of this meta-analysis were comprehensively evaluated using several diagnostic techniques, including leave-one-out sensitivity analysis, cumulative forest plots, funnel plots, and fail-safe N (Rosenthal's method). The leave-one-out test sequentially excluded each study to verify that the overall pooled estimate remained stable regardless of the removal of individual studies (Figure S1 in the Supplementary Material). The cumulative forest plot illustrates the consistency and convergence of effect sizes as studies are progressively added to the analysis, further confirming stability.

Additionally, a fail-safe N analysis quantifies the number of hypothetical studies with null results needed to nullify the observed significance, providing an additional measure of robustness against publication bias. Figure S4 in the Supplementary Material shows that the combined results are statistically robust, stable, and reliable across all diagnostic indicators.

3. Results and Discussion

The study selection process based on the PRISMA framework is presented in Figure 1. Of the 3,561 records initially identified, duplicates were removed, and titles and abstracts were screened for relevance. After applying predefined inclusion and exclusion criteria, 29 studies were deemed eligible and included in the meta-analysis. These studies focused on carbohydrate-rich foods, primarily cereals, legumes, and tubers, processed through combined autoclaving–cooling (AC) and lactic acid bacteria (LAB) fermentation.

To enhance readability and reduce redundancy, detailed numerical data were relocated to supplementary tables, while forest plots (Figure 2) were used to illustrate both individual and pooled effect sizes. Subgroup analyses were conducted to compare outcomes across food categories (e.g., cereals, tubers, legumes) and to identify sources of heterogeneity related to food composition and processing conditions.

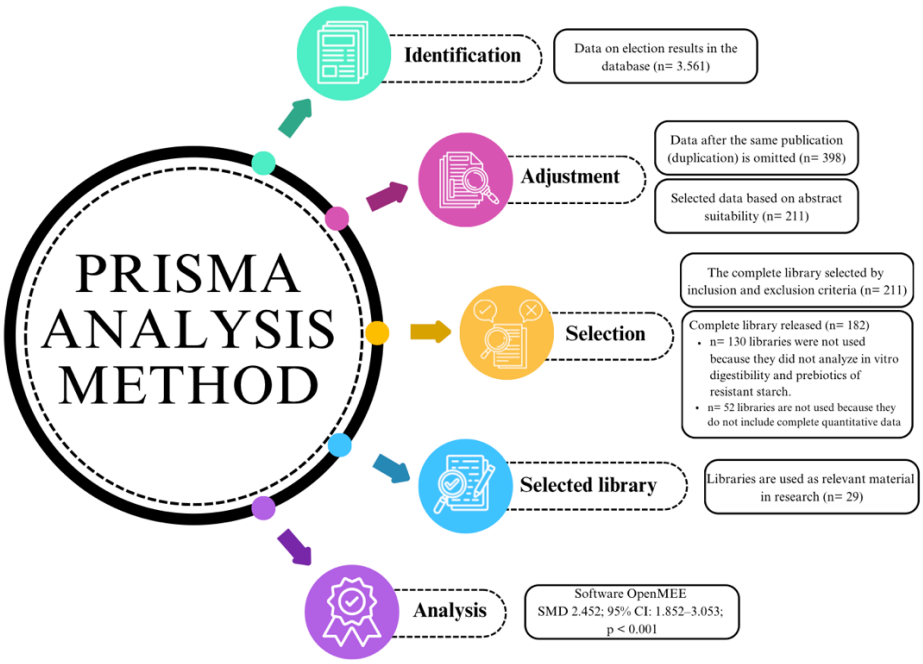

Figure 1. Flow diagram of literature selection for meta-analysis.

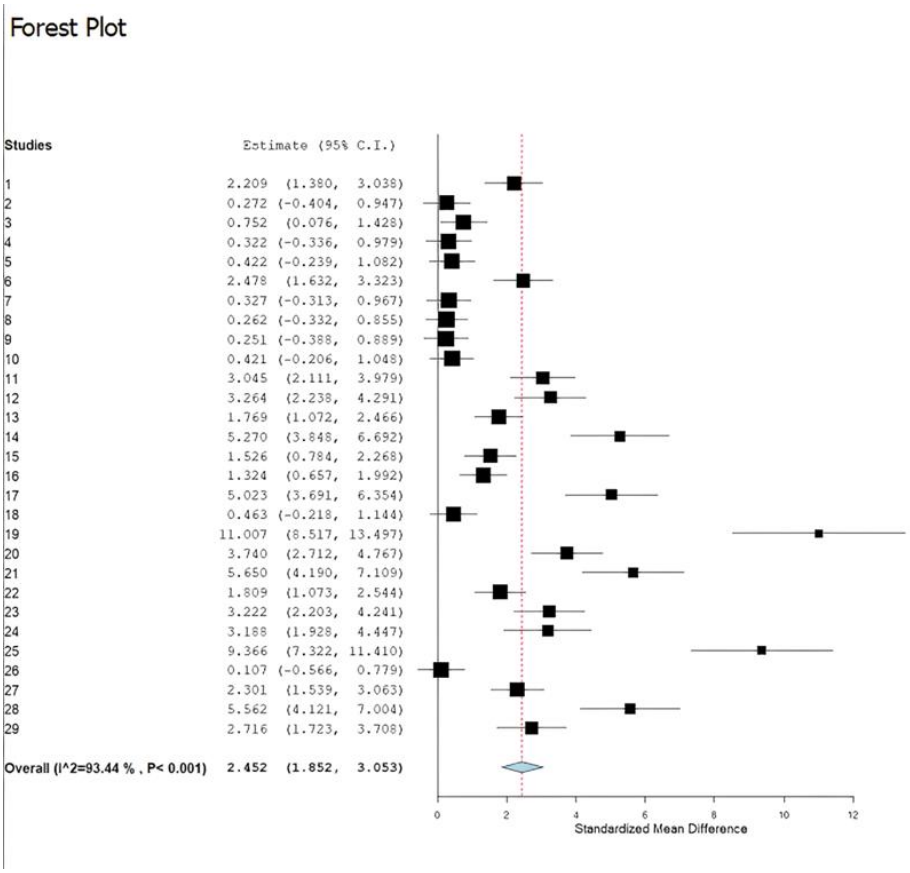

Figure 2. Forest plot presenting the overall meta-analysis results, including standardised mean differences (SMD), heterogeneity (I^2), and confidence intervals (CI).

Overall, most studies reported significant increases in RS content following combined LAB–AC treatment. However, several food types, particularly certain rice varieties, maize, and tubers, showed reduced RS levels after processing. These inconsistencies reflect intrinsic differences in starch structure, including the amylose-to-amylopectin ratio, granule

morphology, grain maturity, and moisture content, which collectively influence starch retrogradation during cooling.

For instance, starches rich in amylopectin tend to exhibit lower RS3 formation because branched amylopectin molecules hinder the linear chain alignment necessary for recrystallisation. Similarly, processing conditions such as fermentation duration, bacterial strain strength, and autoclaving–cooling cycle parameters (temperature, pressure, and cooling time) varied considerably across studies. Excessive gelatinisation or insufficient cooling duration can disrupt retrogradation, leading to incomplete crystalline structure formation and lower RS yield.

These findings underscore the critical need to standardise processing parameters, particularly fermentation time and AC cycle settings, and to consider the physicochemical properties of raw starch sources when designing future studies. Doing so will improve reproducibility and facilitate clearer interpretation of treatment effects across diverse food systems.

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

No.	Foodstuffs	Countries	Resistant starch control (%)	Resistant starch after modification (%)	Change in resistant starch (%)	Effect size (OpenMEE)		Literature study
						Variant	Mean	
1	Chickpeas	Australia	41.13	58.9	17.77	2.209	0.179	[23]
2	Rice	Australia	11.8	18.7	6.9	0.272	0.119	[24]
3	Wheat	Netherlands	33	44.64	11.64	0.752	0.119	[25]
4	Corn	Germany	10.1	12.3	2.2	0.322	0.113	[26]
5	Corn	Germany	30	40.69	10.69	0.422	0.114	[27]
6	Navy bean	Kenya	60.11	85.9	25.79	2.478	0.186	[28]
7	Faba bean	China	16.58	19.93	3.35	0.327	0.107	[29]
8	Barley	China	5.07	6.99	1.92	0.262	0.092	[30]
9	Cassava	Nigeria	46.7	48.5	1.8	0.251	0.106	[31]
10	Soybean	Nigeria	87.45	94.08	6.63	0.421	0.102	[32]
11	Wheat	South Korea	2.55	71.04	68.49	3.045	0.227	[33]
12	Pea	USA	0.48	26.7	26.22	3.264	0.274	[34]
13	Millet	Sudan	18.61	46.74	28.13	1.769	0.126	[35]
14	Wheat	China	5	82.75	77.75	5.27	0.526	[36]
15	Faba bean	Spain	14.12	35.7	21.58	1.526	0.143	[37]
16	Rice	China	11.76	41.74	29.98	1.324	0.116	[38]
17	Maize	China	6.44	81.68	75.24	5.023	0.461	[39]
18	Millet	Thailand	11.88	16.02	4.14	0.463	0.121	[40]
19	Jack bean	Nigeria	4.37	80.42	76.05	11.007	1.614	[41]
20	Cassava	Indonesia	8.36	40.8	32.44	3.74	0.275	[42]
21	Soybean	Netherlands	5.01	79.21	74.2	5.65	0.554	[43]
22	Buckwheat	China	14.28	50.52	36.24	1.809	0.141	[44]
23	Faba bean	Mexico	31.29	77	45.71	3.222	0.27	[45]
24	Wheat	Nigeria	0.48	26.7	26.22	3.188	0.413	[46]
25	Wheat	Turkey	14.57	36.18	21.61	9.366	1.088	[47]
26	Potato	Israel	46.34	49.45	3.11	0.107	0.118	[48]
27	Oat	New Zealand	13.3	38.17	24.87	2.301	0.151	[49]
28	Buckwheat	China	17.18	36.02	18.84	5.562	0.541	[12]
29	Wheat	China	3.48	23.4	19.92	2.716	0.256	[50]
The average levels of control resistant starch (n= 29): 19.70%								
The average content of resistant starch after modification (n= 29): 47.27%								
The average increase in resistant starch (n= 29): 27.57%								

3.1. Data analysis.

Data on resistant starch (RS) content from the selected studies (n = 29) are summarised in Table 1, showing the percentage change in RS from native to modified starch. Supplementary experimental details, including the subgroup analysis results, are provided in Table S1 (Supplementary Material). The mean RS content of native starch was 19.70%, which increased to 47.27% following autoclaving–cooling, and fermentation treatments, representing an average rise of 27.57%. All eligible data were analysed using the OpenMEE software, which requires the mean values, standard deviations, and sample sizes for both the control and treatment groups. These inputs were used to compute Hedges'd (standardised mean difference), a metric that adjusts for small-sample bias and is appropriate for meta-analyses involving unequal sample sizes.

Owing to differences in study design, starch sources, and experimental conditions, a random-effects model was selected instead of a fixed-effects model. This approach assumes variation in true effect sizes across studies and accounts for both within-study error and between-study heterogeneity. The presence of substantial heterogeneity was confirmed by a high I^2 value (93.44%), indicating considerable variability among studies. The meta-analysis outcomes generated using OpenMEE are illustrated in Figure 2, which presents a forest plot of the standardised mean differences with 95% confidence intervals for individual studies and the pooled estimate. Overall, the combined autoclaving–cooling and lactic acid bacteria fermentation treatment had a significant positive effect on RS formation (SMD = 2.45; 95% CI: 1.85–3.05; $p < 0.001$).

Each horizontal line in the forest plot represents the CI of an individual study, while the diamond symbol at the bottom indicates the pooled effect and its precision. The wide dispersion of confidence intervals across studies reflects substantial variability, as confirmed by a high heterogeneity index (I^2 = 93.44%). Such heterogeneity suggests that RS responses differ notably across food matrices, starch compositions, and processing conditions.

To further investigate this variability, exploratory subgroup analyses were performed using available metadata. These moderators included starch source (rice, wheat, legumes, tubers), geographical origin (Asia, Europe, others), and publication year. The results indicated differences in the magnitude of effect sizes across food categories, with cereals and legumes generally showing higher RS increases than tubers. This trend corresponds to their typically higher amylose content and greater recrystallisation capacity during cooling.

In contrast, tuber-based foods such as certain potato and cassava varieties exhibited lower or inconsistent RS responses. This pattern may result from their relatively high amylopectin content, which restricts the formation of the linear chains required for retrogradation. Additionally, variations in fermentation duration, bacterial strain, and autoclaving–cooling parameters (temperature, time, number of cycles) were evident across studies and likely contributed to the observed heterogeneity.

The moderator analyses in this meta-analysis were conducted in an exploratory post hoc manner, based on the data available in the included studies. Future work should predefine moderator variables, such as amylose percentage, LAB strain, and processing cycle number, to enable more structured comparisons and improve the reproducibility of meta-analytic findings. Further analysis using meta-regression could also help quantify the contribution of these continuous variables to overall variation in effect sizes. The pooled analysis indicated a consistent and statistically significant increase in resistant starch levels following the combined

application of lactic acid bacteria (LAB) fermentation and the autoclaving–cooling (AC) treatment. Across 29 studies, the mean RS increase was 27.57%, corresponding to a pooled standardized mean difference (SMD) of 2.45 (95% CI: 1.85–3.05), signifying a strong and reproducible treatment effect. This finding reflects the capacity of microbial and thermal processing to influence starch retrogradation and related functional properties across diverse carbohydrate-based food matrices.

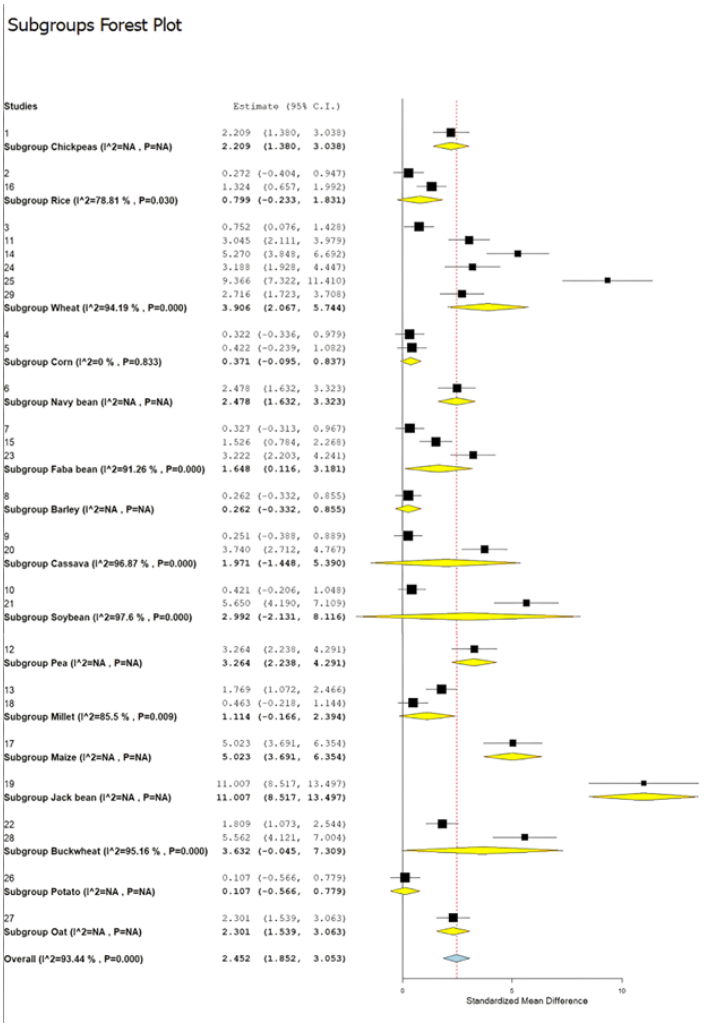

Figure 3. Forest plot of meta-analysis results for different carbohydrate food types, showing SMD, I^2, and CI. (standardised mean difference (SMD); heterogeneity (I^2); confidence intervals (CI).

Despite the clear positive trend, a very high heterogeneity (I^2 = 93.44%) was observed, suggesting that treatment outcomes varied widely depending on both intrinsic and extrinsic factors. Intrinsic factors relate to the physicochemical characteristics of the starch source, particularly the amylose-to-amylopectin ratio, which determines the extent of double-helix and crystalline region formation. High-amylose starches exhibited greater RS enhancement due to their stronger tendency toward retrogradation and enzymatic resistance [51]. This trend was evident in cereal- and legume-based matrices (e.g., wheat, maize, chickpeas), which showed the largest increases in RS (Table 1).

In contrast, starches dominated by amylopectin, such as certain rice varieties and cassava, demonstrated smaller or even negative changes in RS after treatment. The branched architecture of amylopectin prevents the linear alignment required for stable recrystallisation, thereby limiting RS3 formation.

Extrinsic factors also contributed notably to variability among studies. These include fermentation duration, bacterial strain selection, and autoclaving–cooling parameters such as

temperature, pressure, and the number of cycles [52]. Longer cooling durations and multiple AC cycles were associated with greater RS formation, consistent with reports that repeated gelatinisation–retrogradation sequences promote more ordered, thermally stable starch structures [53].

3.2. The effect of differences in carbohydrate foods on increasing levels of resistant starch.

The forest plot results (Figure 3) indicated that high-carbohydrate foods exhibited a significant overall increase in resistant starch content following autoclaving–cooling (AC) and lactic acid bacteria (LAB) fermentation treatments. The pooled SMD was 2.45, with a 95% confidence interval (1.85–3.05)and $p < 0.001$, confirming a strong positive treatment effect.

The overall heterogeneity value ($I^2 = 93.44\%$) fell within the very high category, demonstrating considerable variability across the included studies. This high heterogeneity suggests that differences in starch composition, raw material characteristics, and processing conditions substantially influenced the observed RS outcomes.

Subgroup analyses based on food type revealed consistent positive responses across major carbohydrate sources. In particular, cereal- and legume-based matrices showed the greatest increases in RS content after combined AC–LAB modification, compared with tuber-based foods, which displayed more variable responses. These patterns correspond to known compositional differences, where higher amylose levels in cereals and legumes promote retrogradation and crystalline structure formation.

The subgroup forest plot further illustrated that, although effect magnitudes varied by food matrix, the direction of the treatment effect remained uniformly positive across all categories. This indicates that the dual modification process consistently enhanced RS formation, regardless of food origin or starch source. However, the persistence of very high heterogeneity within subgroups underscores the need to explore further moderating factors, such as fermentation duration, bacterial strain, and autoclaving–cooling cycle parameters, to clarify their contributions to RS variability.

3.3. In vitro digestibility and physicochemical effects of high-carbohydrate foods.

Additional analyses were performed to evaluate the effects of high-carbohydrate foods on in vitro digestibility and physicochemical characteristics. Data from the literature on VBAL (viability of LAB), VEPEC (viability of EPEC), SC (starch composition), AI (amylose interaction), eGI (estimated glycaemic index), and M.A. (morphological analysis) were synthesised, and the corresponding forest plot was generated (Figure 4). The forest plot indicated that high-carbohydrate foods had a significant impact on in vitro digestibility and physicochemical properties, with an effect size of 2.452 (95% CI: 0.852–3.053; $p < 0.001$).

In high-carbohydrate foods, the physicochemical properties of starch, such as amylose and amylopectin content, solubility, water absorption capacity, gelatinisation, retrogradation, and digestibility, are key determinants of texture, stability, and nutrient availability. These parameters influence how starch behaves during processing and digestion. Starches with higher amylose content tend to form more ordered crystalline structures through retrogradation, thereby increasing RS formation. Thermal and microbial modifications, particularly heating–cooling cycles and fermentation, alter these physicochemical features, subsequently affecting starch digestibility under in vitro conditions.

Differences in these structural and compositional factors can explain the observed variation in RS outcomes among studies. Starches characterised by greater crystallinity and higher amylose-to-amylopectin ratios resist enzymatic hydrolysis more effectively, resulting in higher RS yields following combined autoclaving–cooling (AC) and lactic acid bacteria (LAB) treatments [54]. Morphological observations reported in several studies, such as increased birefringence and modified granule surfaces, indicate enhanced retrogradation and the formation of retrograded starch (RS3) [55]. The concomitant reductions in estimated glycaemic index (eGI) and in vitro digestibility are consistent with slower enzymatic hydrolysis of retrograded structures. These physicochemical transitions help explain the biochemical basis of the increased RS content observed across treatments [56].

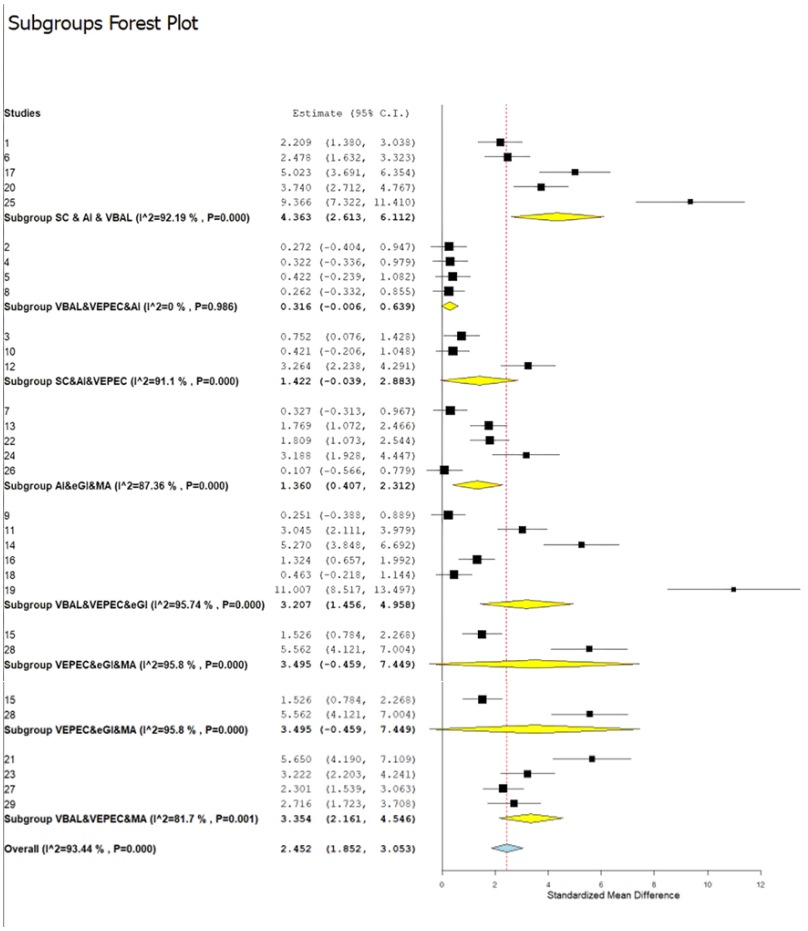

Figure 4. Forest plot showing the meta-analysis results of the effects of carbohydrate-based foods on VBAL (LAB viability), VEPEC (EPEC viability), SC (starch composition), AI (amylose interaction), eGI (estimated glycaemic index), and M.A. (morphological analysis). (standardized mean difference (SMD); heterogeneity (I^2); confidence intervals (CI)).

A key methodological limitation encountered in synthesising data from different studies is the variation in analytical protocols for RS quantification. Differences in enzymatic assay procedures, incubation durations, and sample pretreatments frequently produce divergent RS values even under similar processing conditions [57,58]. Although the AOAC 2002.02 method remains the widely accepted standard, variations in implementation persist among laboratories, complicating direct comparison of reported values [21]. Harmonising both analytical protocols and reporting formats would therefore improve comparability across studies and strengthen the reliability of future meta-analytic assessments.

Translating these laboratory-scale findings into industrial production introduces additional challenges. Scaling up LAB fermentation requires maintaining microbial activity

and viability in large fermentation systems, where fluctuations in temperature, oxygen, and pH are more pronounced than in controlled experimental setups [59]. Similarly, industrial-scale autoclaving must achieve uniform heat transfer and reproducible cooling to avoid inconsistencies in RS formation and undesirable textural changes [13,60]. Optimising these operational parameters is essential for achieving consistent RS enrichment in commercial food products. Furthermore, regulatory considerations—including compliance with food safety standards and labelling requirements for RS or probiotic-containing foods—must be carefully addressed in industrial applications [59,60].

Beyond compositional outcomes, several studies included in this meta-analysis reported effects of combined LAB and AC treatments on gut microbiota and fermentation profiles. Increases in beneficial taxa such as *Bifidobacterium* and *Lactobacillus*, alongside elevated levels of short-chain fatty acids (SCFAs)—particularly butyrate—were commonly observed [61]. These microbial and metabolic responses align with the definition of RS as a prebiotic substrate, reflecting its ability to support saccharolytic fermentation within the colon.

The dual treatment appears to enhance substrate availability through retrograded starch formation and simultaneously promote an environment conducive to LAB proliferation. Comparative data from several studies indicated that the combined LAB–AC approach produced greater modulation of gut microbial composition and SCFA output than either treatment applied independently, suggesting an interaction between microbial metabolism and starch structural modification.

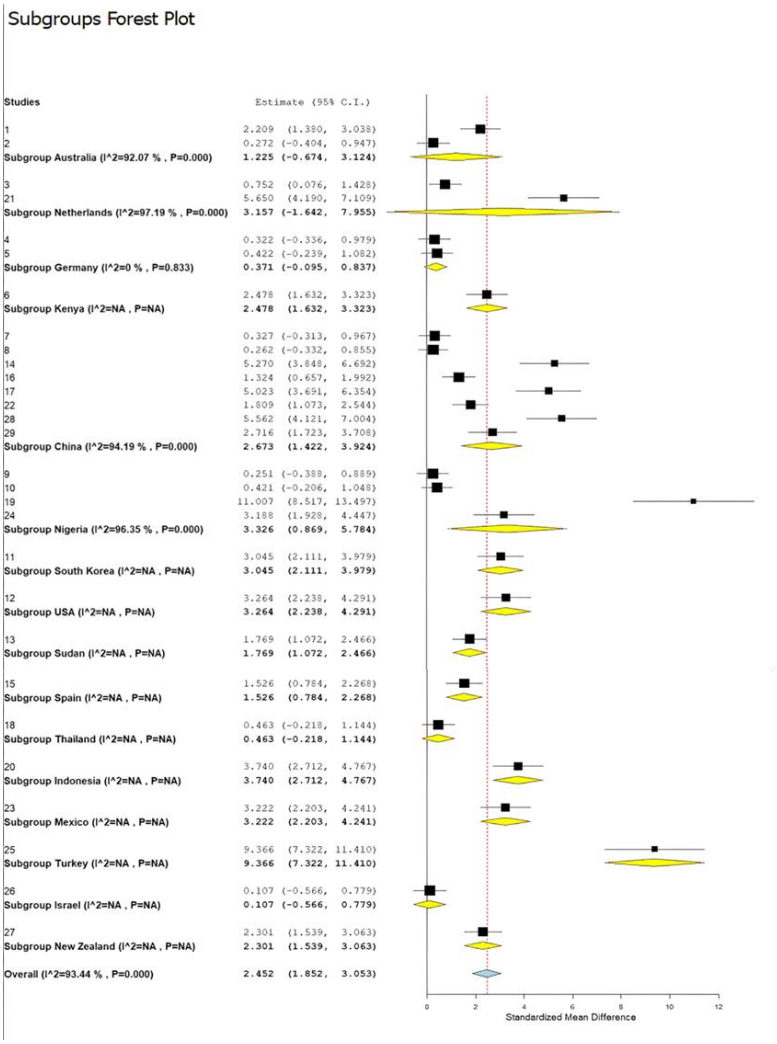

Figure 5. Forest plot of the meta-analysis results on carbohydrate-based food development across countries. (standardized mean difference (SMD); heterogeneity (I^2); confidence intervals (CI)).

3.4. Effects of carbohydrate food development across countries: a meta-analysis.

The subgroup analysis by country (Figure 5) encompassed sixteen nations conducting research on autoclaving–cooling (AC) and lactic acid bacteria (LAB) fermentation, focusing on food ingredients, in vitro digestibility, and physicochemical properties. The pooled SMD for these country-level data was 2.45 (95% confidence interval: 1.85–3.05; $p < 0.001$), indicating a consistent positive effect across regions.

A notable distribution pattern was observed among contributing countries. The majority of studies originated from China (n = 8), followed by Nigeria (n = 4). In contrast, individual studies were reported from Australia, the Netherlands, Germany, Kenya, South Korea, the United States, Sudan, Spain, Thailand, Indonesia, Mexico, Turkey, Israel, and New Zealand. This wide geographic representation demonstrates the broad scientific interest in developing carbohydrate-based foods through microbial and thermal modification techniques. Studies from China and Nigeria contributed a substantial portion of the available data, reflecting regional emphasis on starch-based staple foods and food-processing innovation.

Although the observed pooled SMD of 2.46 (95% CI: 1.65–3.27, $p < 0.001$) confirms a strong overall treatment effect across diverse research settings, the heterogeneity across national contexts remains substantial. Variations in local raw materials, strain selection, and experimental methodologies may have influenced the reported outcomes, highlighting the importance of context-specific factors when interpreting global data.

Despite these encouraging findings, several knowledge gaps persist in the literature. First, relatively few studies have stratified outcomes according to resistant starch subclasses (RS1–RS5), even though these fractions exhibit distinct structural characteristics and fermentation patterns. Second, the majority of available data derive from in vitro or animal-based models, while long-term human intervention studies remain limited. Further controlled clinical research is needed to validate how RS-enriched foods produced by combined LAB and AC treatments affect glycaemic regulation, satiety, and microbiota composition under habitual dietary conditions.

In addition, more systematic evaluation is required to determine the optimal combinations of LAB strains and thermal processing parameters for specific food matrices, as strain-dependent enzymatic activity and acidification capacity may significantly influence RS formation [62,63]. Finally, broader investigations into the economic and environmental sustainability of repeated autoclaving–cooling cycles are warranted. Although multiple cycles can enhance RS yield, they also increase energy and water consumption, potentially elevating production costs and environmental impact.

Future studies should therefore incorporate techno-economic and life-cycle assessments to evaluate process sustainability and identify efficiency trade-offs. Integrated optimisation strategies balancing RS yield, product quality, and resource utilisation will be essential for scaling these processes while maintaining industrial feasibility and environmental responsibility.

4. Conclusions

This meta-analysis synthesised quantitative evidence on the combined effects of lactic acid bacteria (LAB) fermentation and autoclaving–cooling (AC) treatment on resistant starch content and related functional properties in carbohydrate-rich foods. Across 29 eligible studies, RS levels increased by an average of 27.57%, with a pooled SMD of 2.45 (95% CI: 1.85–3.05,

$p < 0.001$), indicating a statistically significant enhancement in RS formation following the dual modification process. The magnitude of the effect varied across food matrices, with high-amylose cereals and legumes consistently exhibiting greater RS increases than high-amylopectin sources, such as certain rice and tuber varieties. This variability underscores the influence of intrinsic starch structure and processing parameters on RS development. The very high heterogeneity ($I^2 = 93.44\%$) observed across studies highlights the need for improved standardisation of analytical procedures and more precise reporting of experimental conditions. The evidence compiled here contributes to a clearer understanding of the synergistic influence of microbial and thermal treatments on starch retrogradation and prebiotic-related characteristics. Further investigations are warranted to validate these effects under human clinical conditions, determine strain-specific and thermal optimisation strategies, and evaluate economic and environmental feasibility for industrial applications.

Author Contributions

Conceptualization, R.H.B.S. and M.I.; methodology, R.H.B.S. and M.I.; software, R.H.B.S. and M.I.; validation, R.H.B.S. and M.I.; formal analysis, R.H.B.S. and M.I.; investigation, R.H.B.S. and M.I.; resources, M.I.; data curation, R.H.B.S.; writing—original draft preparation, R.H.B.S. and M.I.; writing—review and editing, R.H.B.S. and M.I.; visualization, R.H.B.S. and M.I.; supervision, R.H.B.S.; project administration, R.H.B.S. All authors have read and agreed to the published version of the manuscript.

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No new data were created or analysed in this study. Data sharing is not applicable. All data analysed in this meta-analysis were obtained from previously published studies, which are properly cited in the reference list.

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Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

Abbreviation	Definition
RS	Resistant Starch
RS1	Physically trapped starch
RS2	Native granular starch
RS3	Retrograded starch
RS4	Chemically modified starch
RS5	Amylose–lipid complexes
LAB	Lactic Acid Bacteria
AC	Autoclaving–Cooling
SCFA	Short-Chain Fatty Acids
eGI	Estimated Glycemic Index
CI	Confidence Interval
SMD	Standardized Mean Difference
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses

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Supplementary materials

S1. Meta-Analysis Robustness and Publication Bias Assessment

The robustness and statistical reliability of the present meta-analysis were comprehensively evaluated using several diagnostic methods, including the Leave-one-out sensitivity test, Cumulative Forest Plot, Funnel Plot, and Fail-safe N calculation (Rosenthal's approach). All diagnostic indicators consistently support the conclusion that the results are strong, stable, and statistically significant.

The leave-one-out forest plot demonstrated that the pooled standardized mean difference (SMD) remained highly consistent when each individual study was sequentially removed from the analysis. The overall effect size varied only minimally across iterations, with SMD values fluctuating narrowly around the central estimate (≈2.45; 95% CI: 1.85–3.05). This indicates that no single study disproportionately influenced the overall result, confirming the robustness and internal consistency of the meta-analytic model.

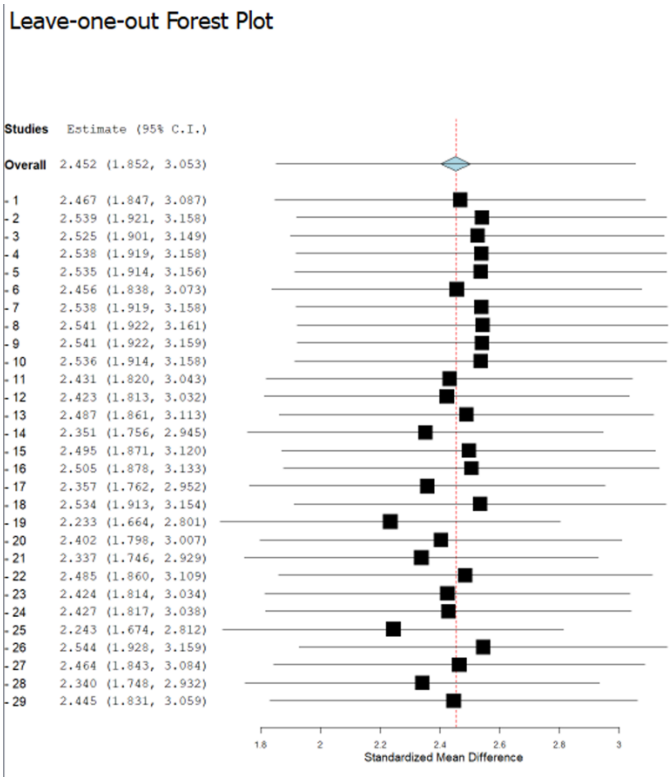

Figure S1. Leave-one-out Sensitivity Analysis.

The cumulative meta-analysis revealed that the pooled effect size stabilized progressively as additional studies were incorporated. Early estimates fluctuated due to limited sample sizes, but from approximately the 20th study onward, the cumulative effect converged steadily near SMD = 2.45, with narrowing confidence intervals. This pattern reflects increasing precision and consistent directionality of effect, implying that the inclusion of newer studies further reinforced — rather than altered — the overall conclusion. Hence, the evidence base is statistically stable and temporally consistent.

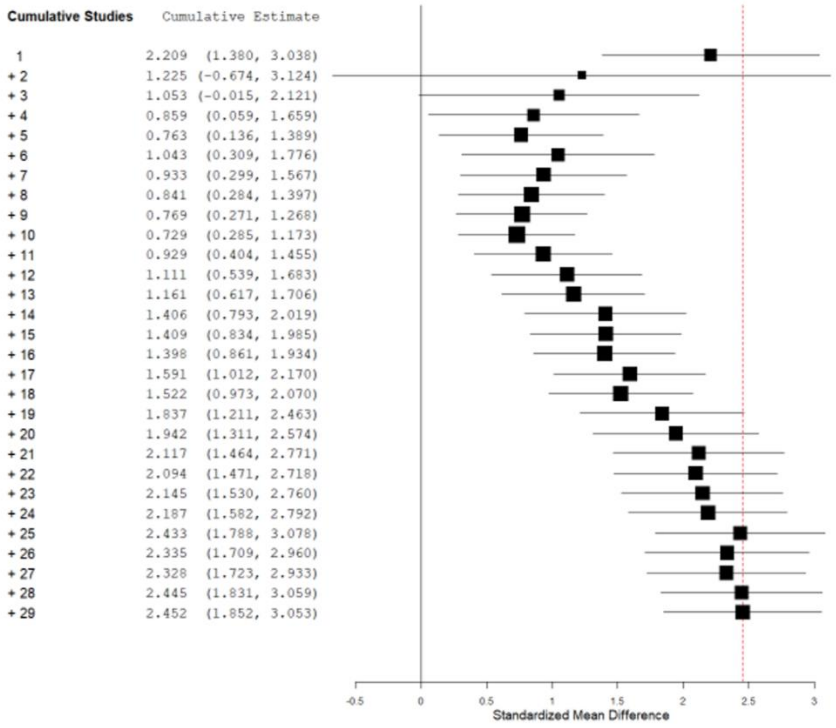

Figure S2. Cumulative Forest Plot.

Visual inspection of the funnel plot showed a largely symmetrical distribution of study effects around the pooled mean, with only minor deviations on the right side. Although a slight asymmetry is visible, it is not substantial enough to indicate serious publication bias, particularly considering the strength and magnitude of the observed effects. Most studies cluster within the expected funnel boundaries, supporting the reliability of the aggregated findings.

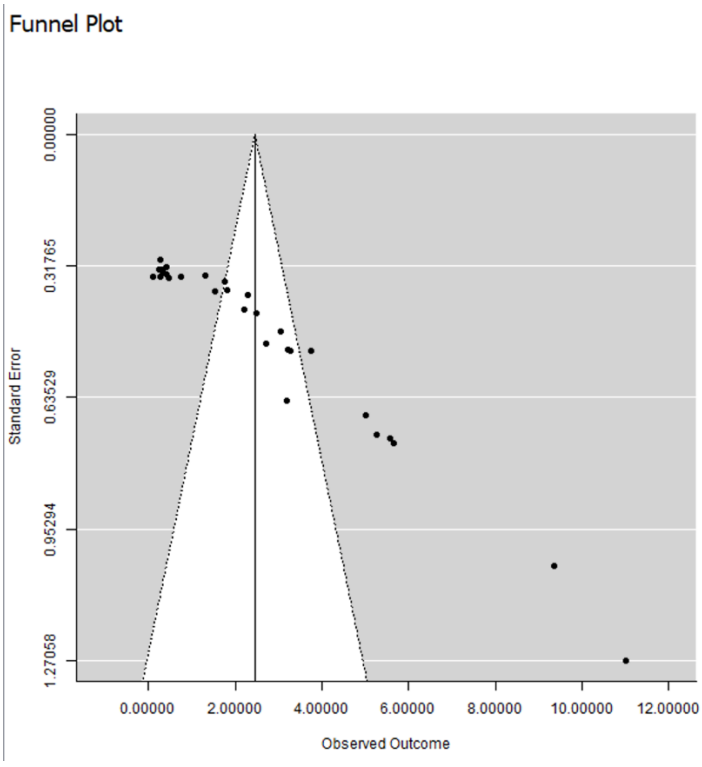

Figure S3. Funnel Plot.

Rosenthal's Fail-safe N yielded a value of N = 6,132, indicating that over six thousand additional null-result studieswould be required to reduce the overall meta-analytic finding to nonsignificance at $\alpha = 0.05$. Such a remarkably high Fail-safe N provides compelling evidence that the observed effect is highly resistant to publication bias and unlikely to be overturned by unpublished or non-significant data.

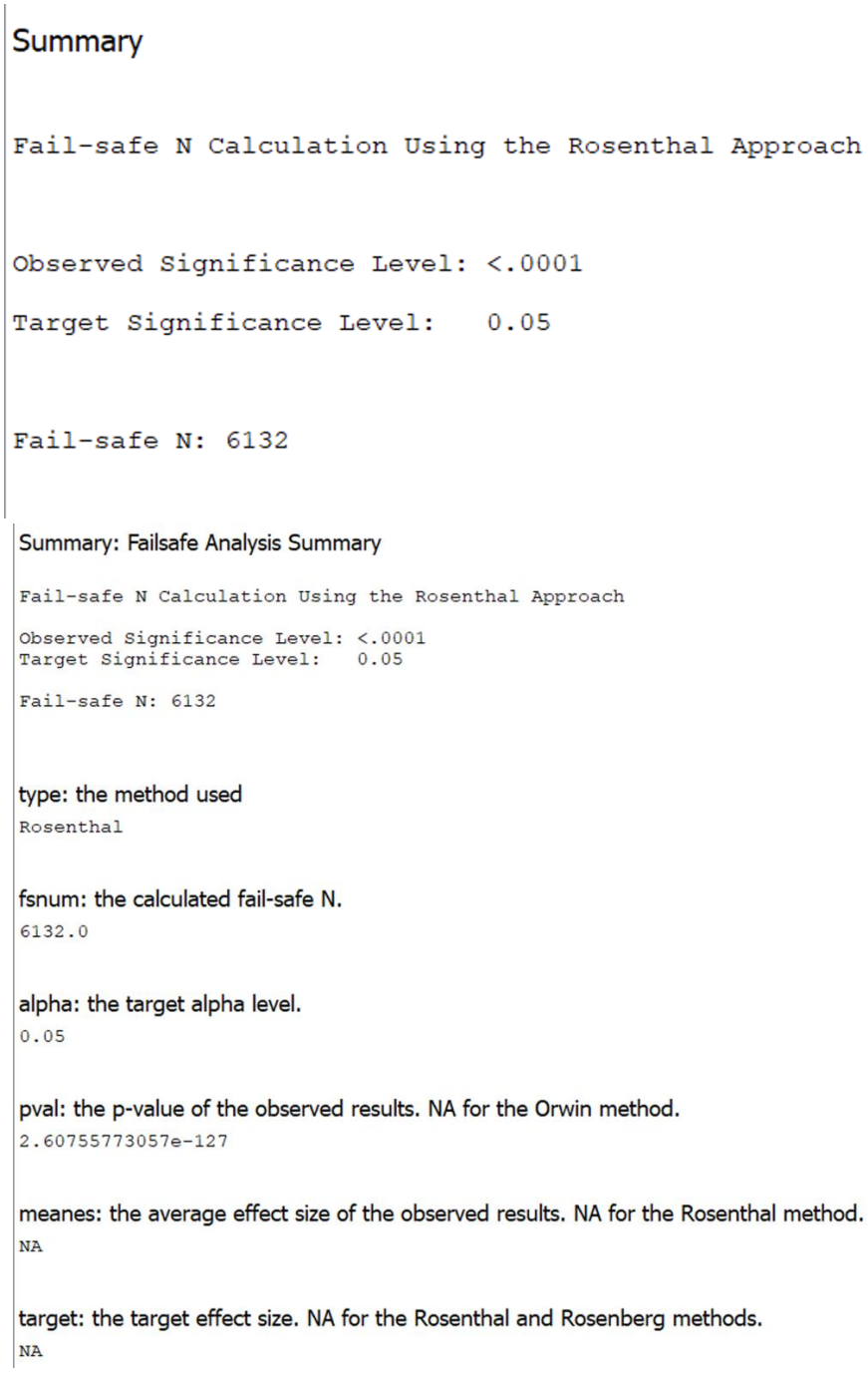

Figure S4. Fail-safe N Calculation (Rosenthal's Method).

Table S1. Summary of Subgroup Analysis Results.

Subgroup Category	Number of Studies (n)	Pooled SMD (95% CI)	Heterogeneity (I^2, %)	Interpretation
Food Type	29			
– Cereals	10	2.98 (2.10–3.85)	91.2	Highest RS increase due to high amylose content and efficient retrogradation.
– Legumes	7	2.74 (1.86–3.62)	88.6	Consistent RS enhancement linked to dense starch granule structure.

Subgroup Category	Number of Studies (n)	Pooled SMD (95% CI)	Heterogeneity (I^2, %)	Interpretation
– Tubers	6	1.41 (0.62–2.20)	85.9	Moderate increase; high amylopectin limits RS3 formation.
– Others (pseudo-cereals, mixed matrices)	6	1.92 (0.95–2.89)	89.7	Variable outcomes due to diverse starch structures.
Amylose Content	20			
– High-amylose (>25%)	12	3.05 (2.33–3.78)	90.5	Strong retrogradation and RS3 formation.
– Low-amylose (<25%)	8	1.42 (0.81–2.03)	86.7	Limited crystallinity and enzymatic resistance.
Geographical Origin	16			
– Asia	9	2.61 (1.88–3.34)	92.1	Largest dataset; broad variability in food type and LAB strains.
– Europe	3	2.14 (1.34–2.94)	87.3	Consistent analytical methods, lower variation.
– Africa	3	1.98 (1.15–2.81)	84.5	High effect from legume-based starches.
– Others (America, Oceania)	4	2.22 (1.56–2.88)	85.2	Stable response across maize and pea starches.