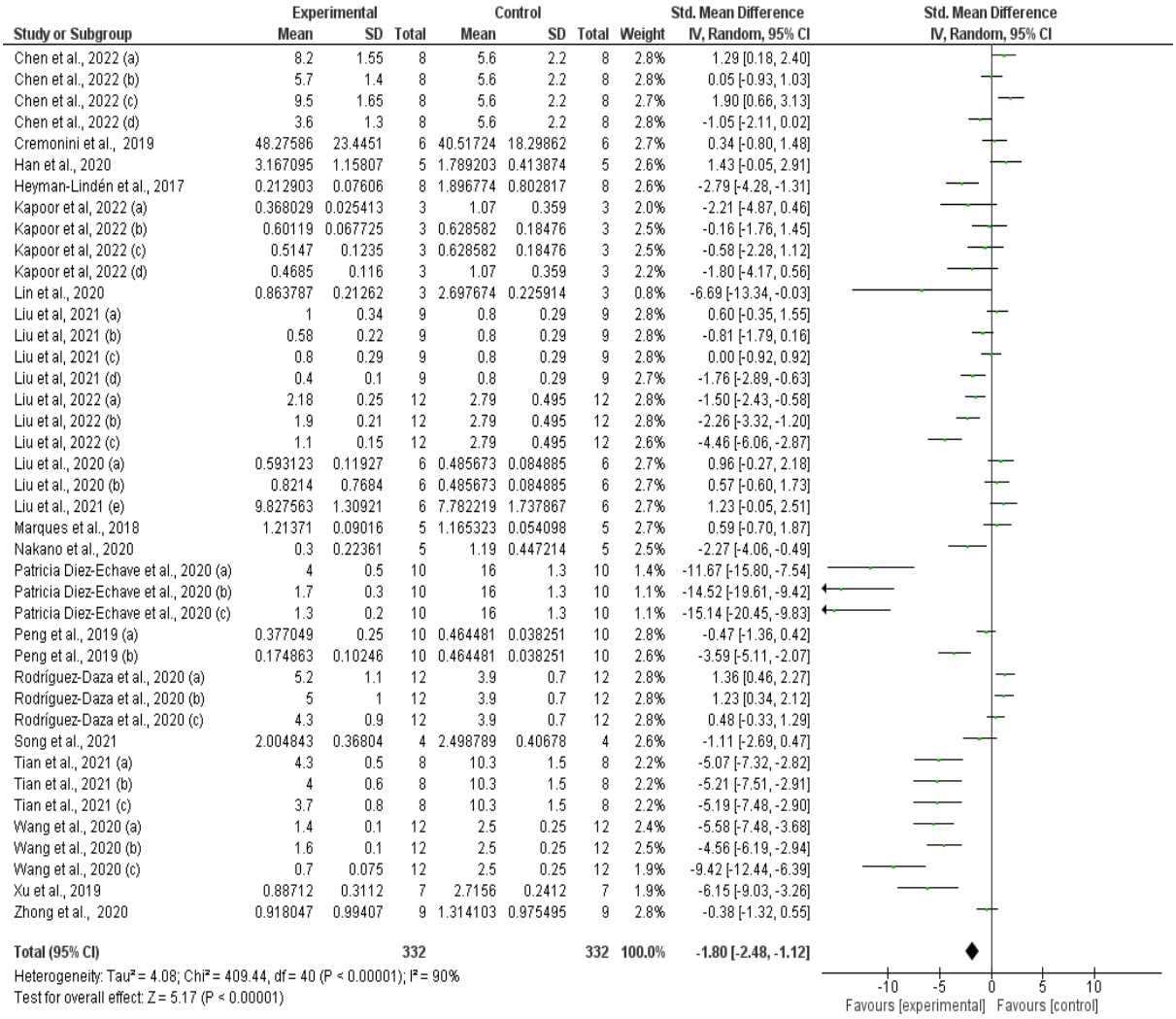
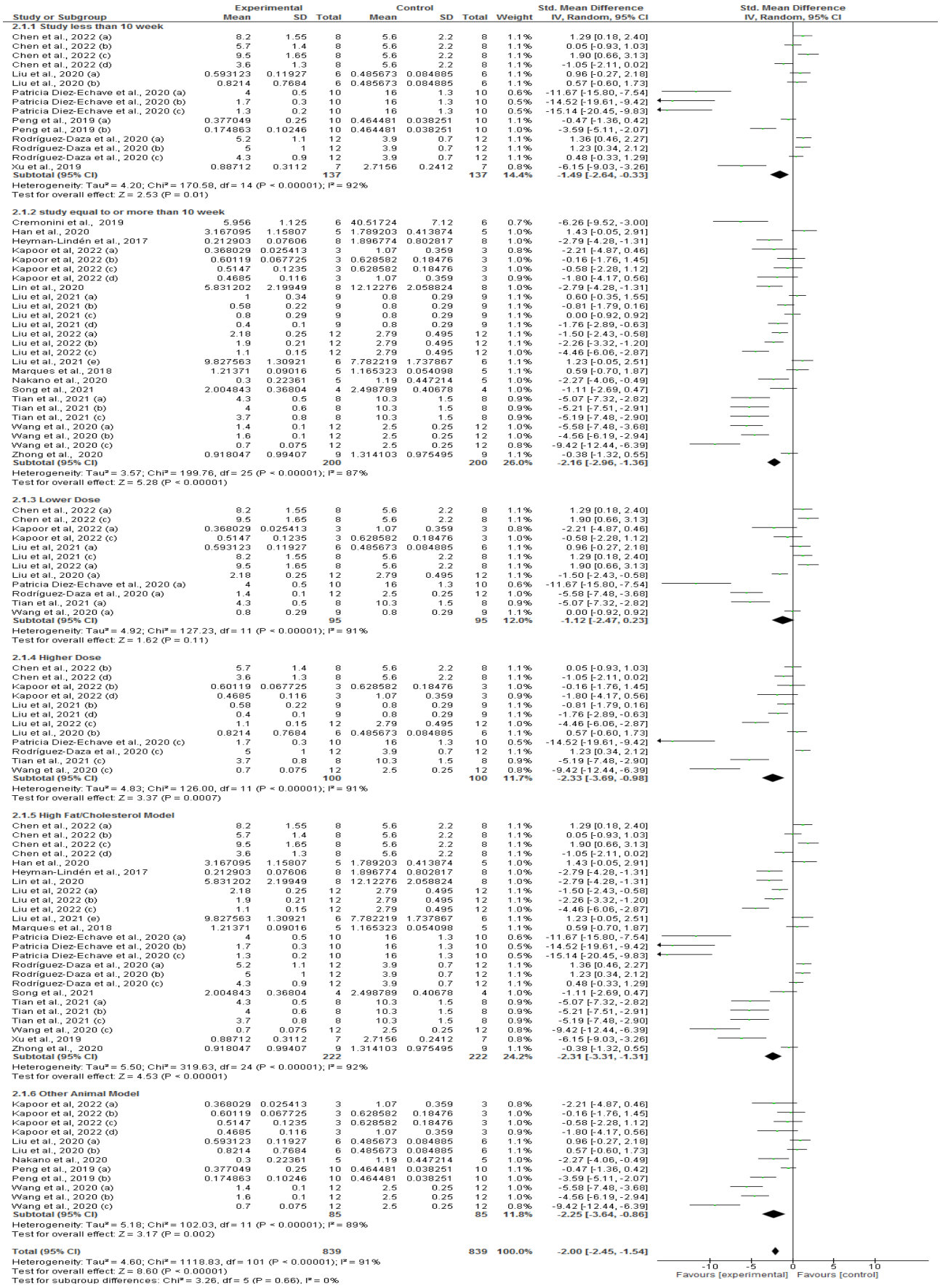
Effect of anthocyanins on gut health markers, *Firmicutes-Bacteroidetes* ratio and short-chain fatty acids: A systematic review via meta-analysis

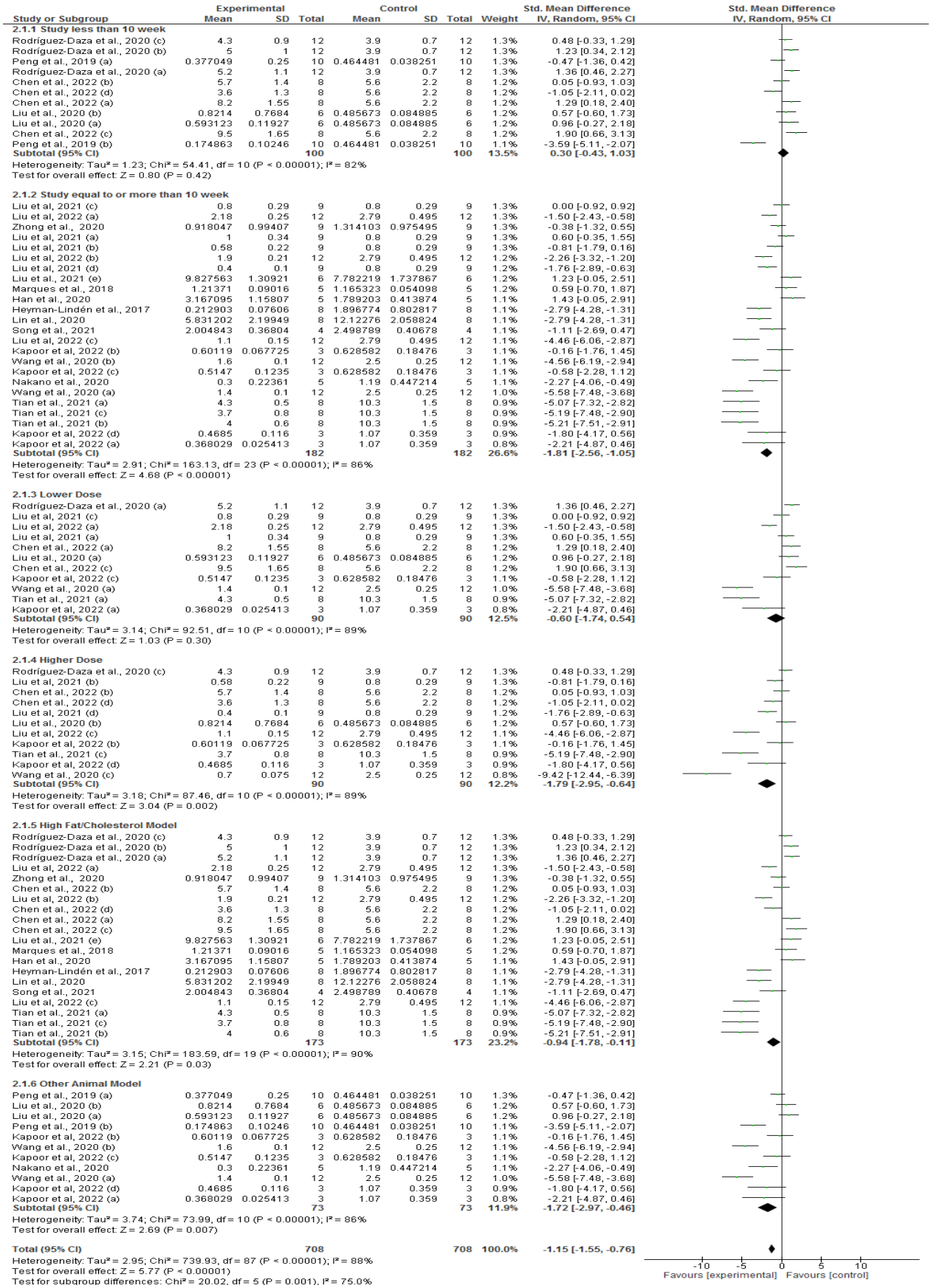
Payal Kapoor



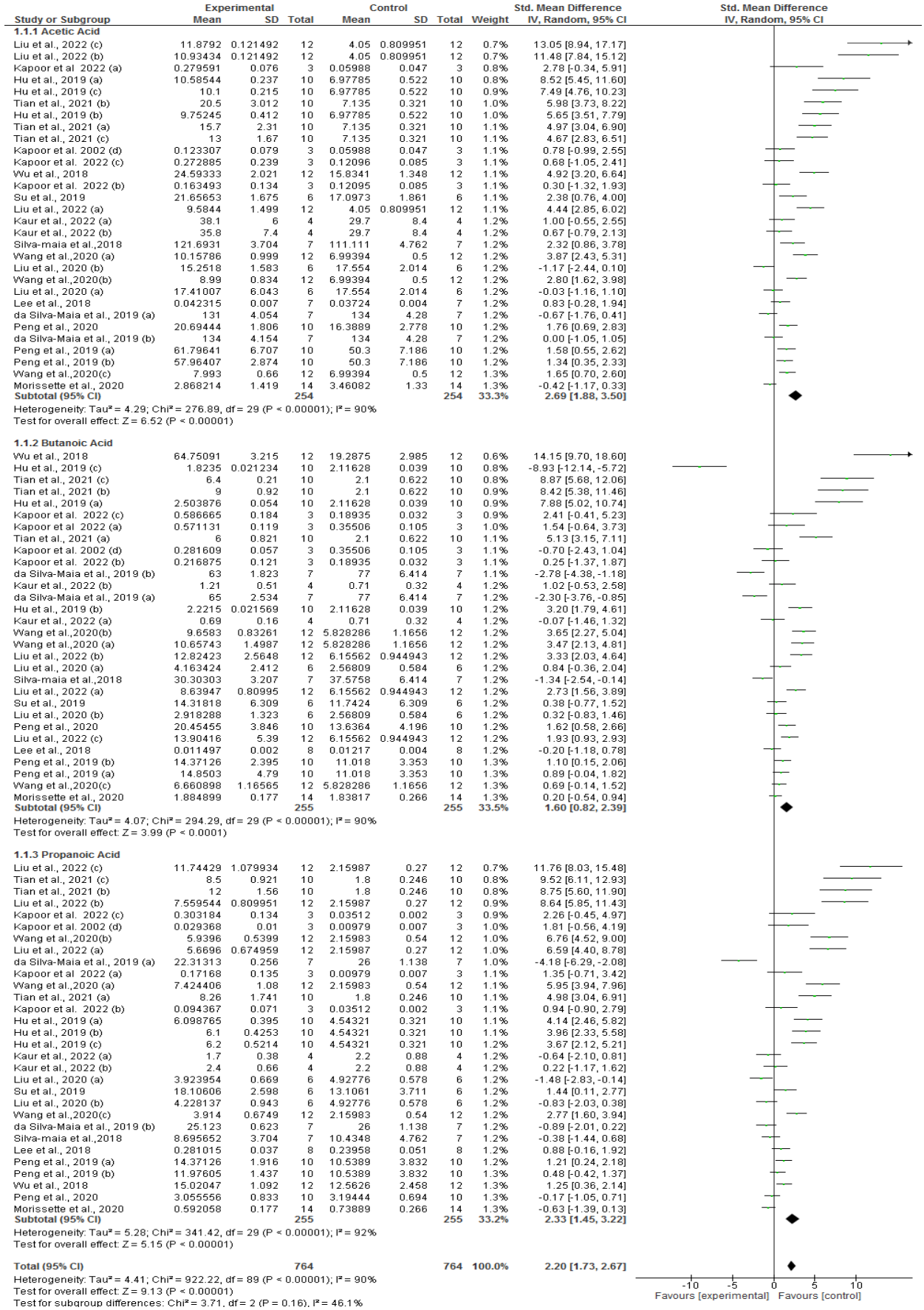
S Figure 1: Forest plot of studies investigating the effect of anthocyanin supplementation on the *Firmicutes* to *Bacteroidetes* ratio (Fir/Bac). Pooled effect estimates (diamonds) for Fir/Bac are shown. Values are standardized mean differences with 95% CIs determined with the use of random-effects models. Heterogeneity was quantified by I^2, inverse variance and standardised mean difference (SMD).



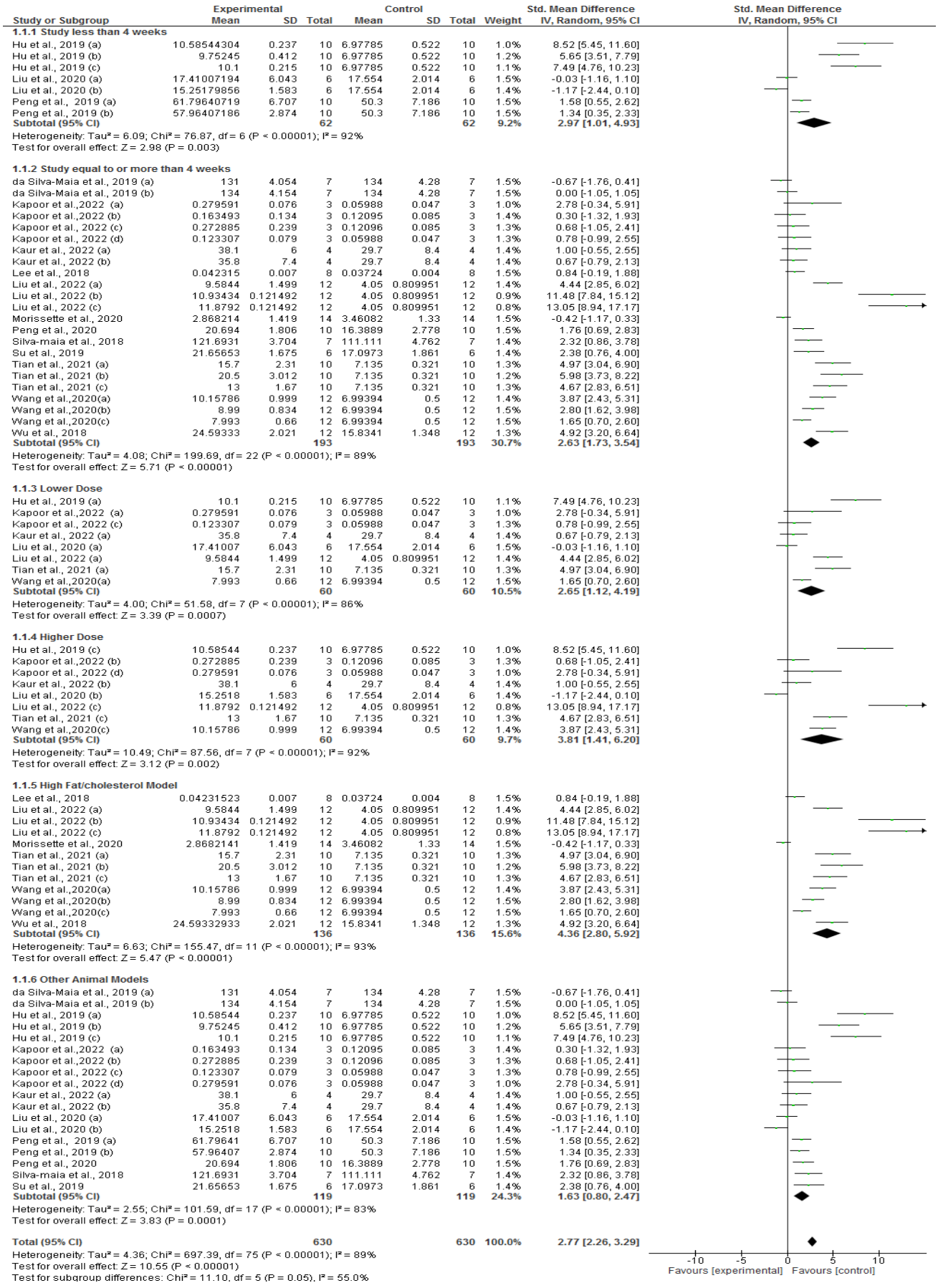
S Figure 2: Forest plot of the sub-group analysis to study effect of anthocyanin supplementation on the *Firmicutes* to *Bacteroidetes* ratio (Fir/Bac). Sub-grouping was on the basis of duration of the study, dose of the anthocyanin given and disease status of the subjects (animal models). Pooled effect estimates (diamonds) for Fir/Bac are shown. Values are standardized mean differences with 95% CIs determined with the use of random-effects models. Heterogeneity was quantified by I^2, inverse variance and standardised mean difference (SMD).



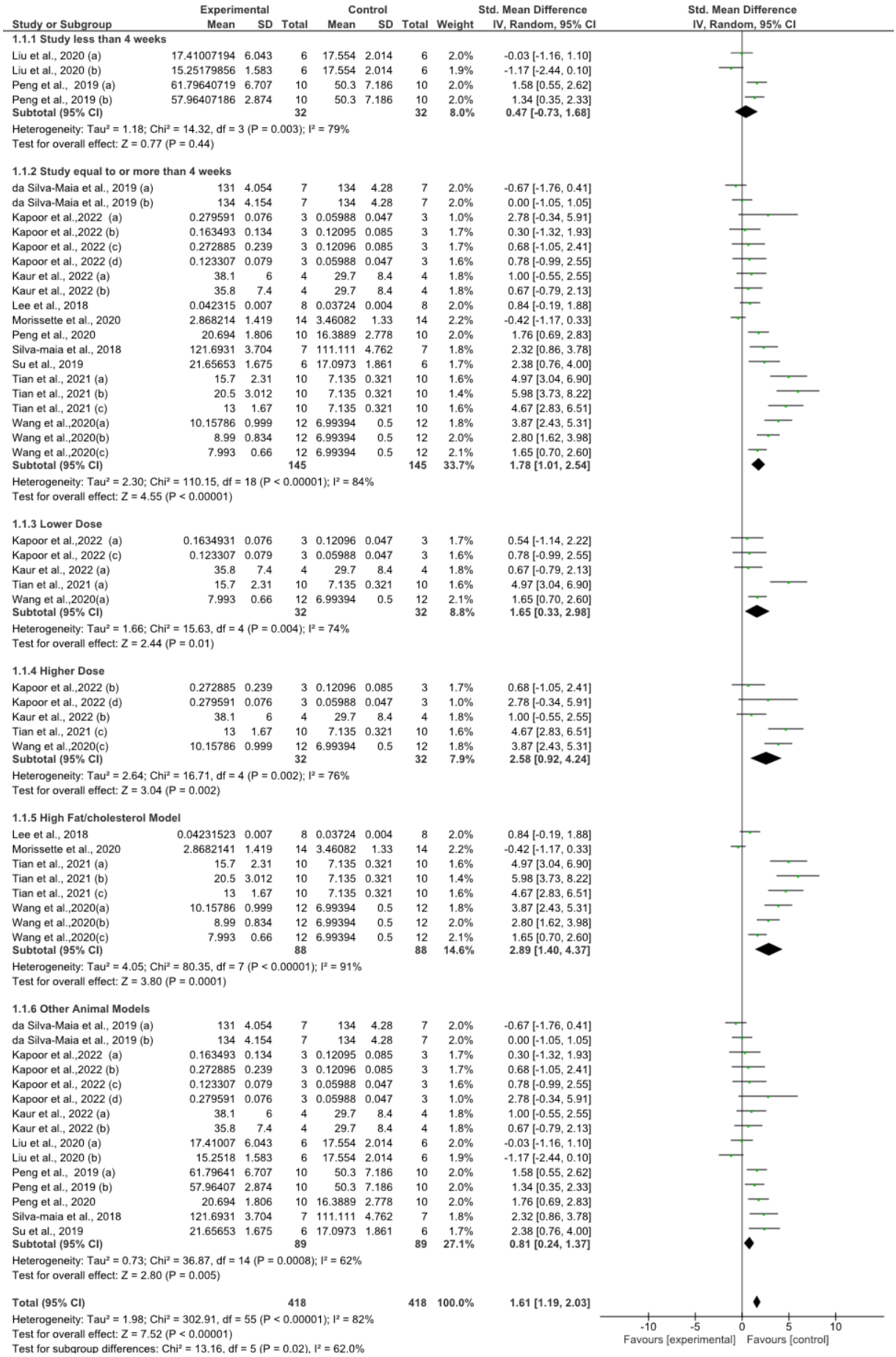
S Figure 3: Forest plot of the sub-group analysis after elimination of high-influencer studies, for understanding the effect of anthocyanin supplementation on the *Firmicutes* to *Bacteroidetes* ratio (Fir/Bac).Sub-grouping was on the basis of duration of the study, dose of the anthocyanin given and disease status of the subjects (animal models). Pooled effect estimates (diamonds) for Fir/Bac are shown. Values are standardized mean differences with 95% CIs determined with the use of random-effects models. Heterogeneity was quantified by I^2, inverse variance and standardised mean difference (SMD).



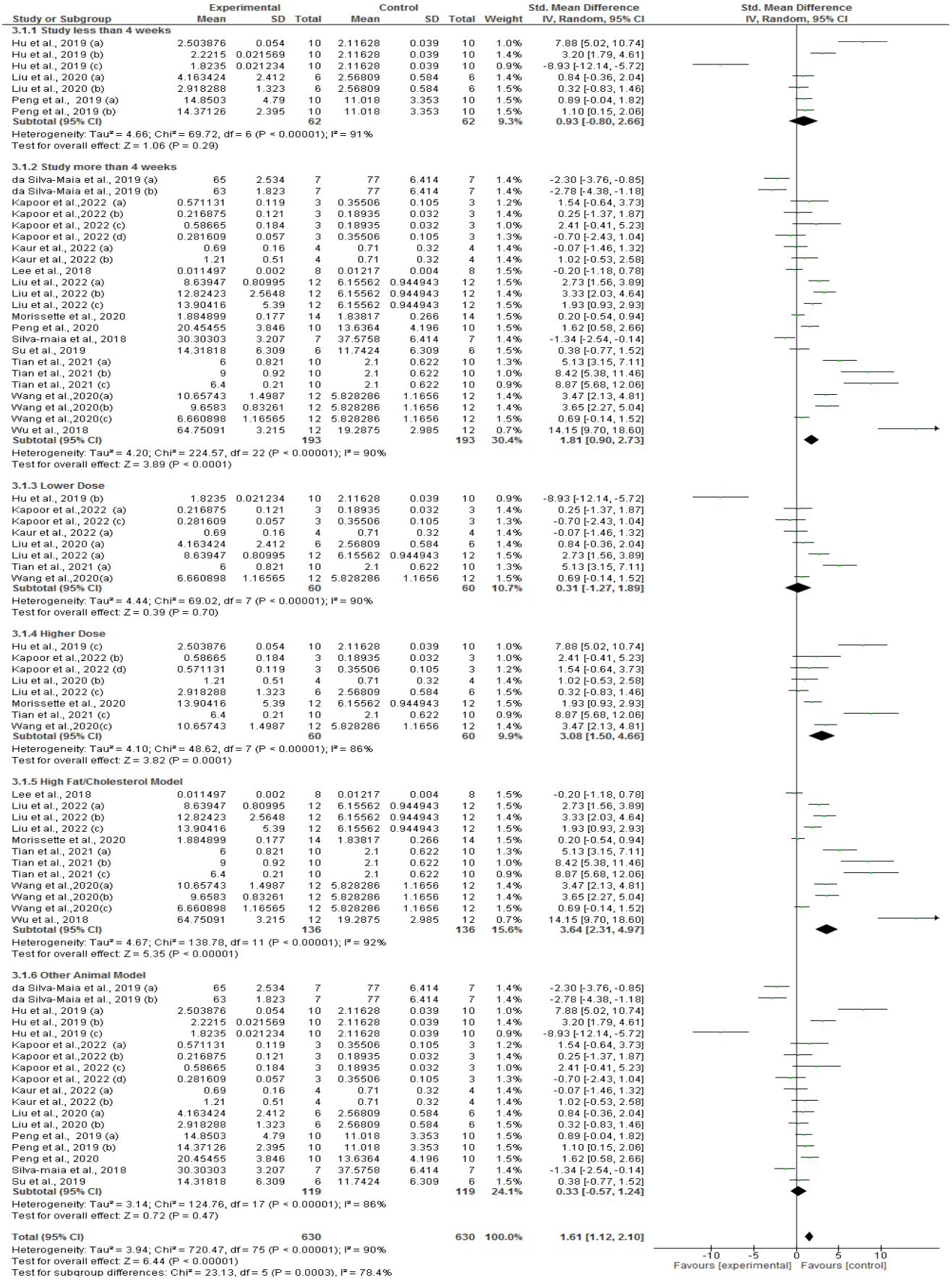
S Figure 4: Forest plot of studies investigating the sub-grouped based effect of anthocyanin supplementation on the SCFA profile. Sub-grouping was on the basis of proportion of acetic acid, butanoic acid and propionic acid. Pooled effect estimates are shown by diamonds. Values are standardized mean differences with 95% CIs determined with the use of random-effects models. Heterogeneity was quantified by I^2, inverse variance and standardised mean difference (SMD).



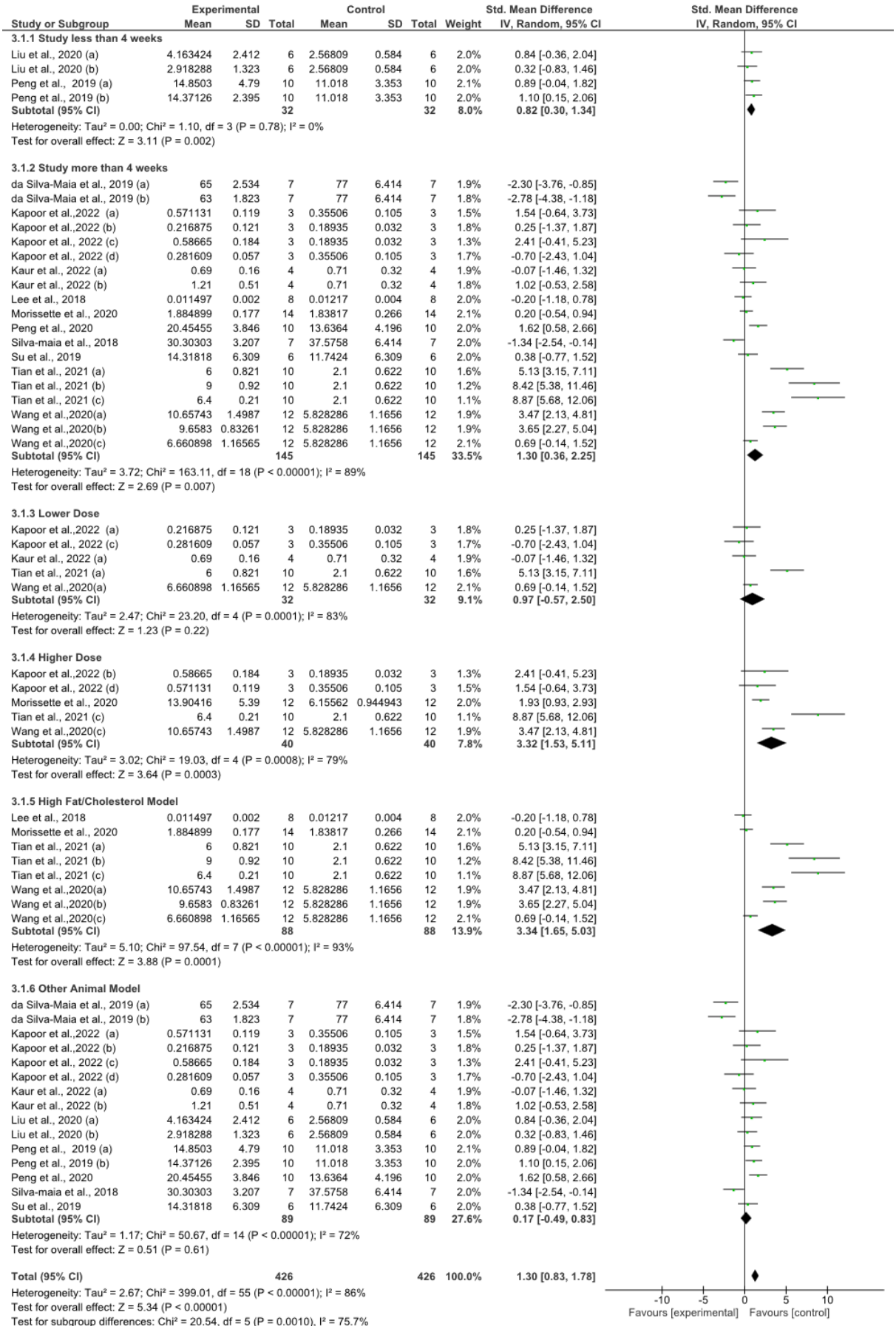
S Figure 5: Forest plot of the sub-group analysis to study effect of anthocyanin supplementation on the acetic acid, SCFA. Sub-grouping was on the basis of duration of the study, dose of the anthocyanin given and disease status of the subjects (animal models). Values are standardized mean differences with 95% CIs determined with the use of random-effects models. Heterogeneity was quantified by I^2, inverse variance and standardised mean difference (SMD).



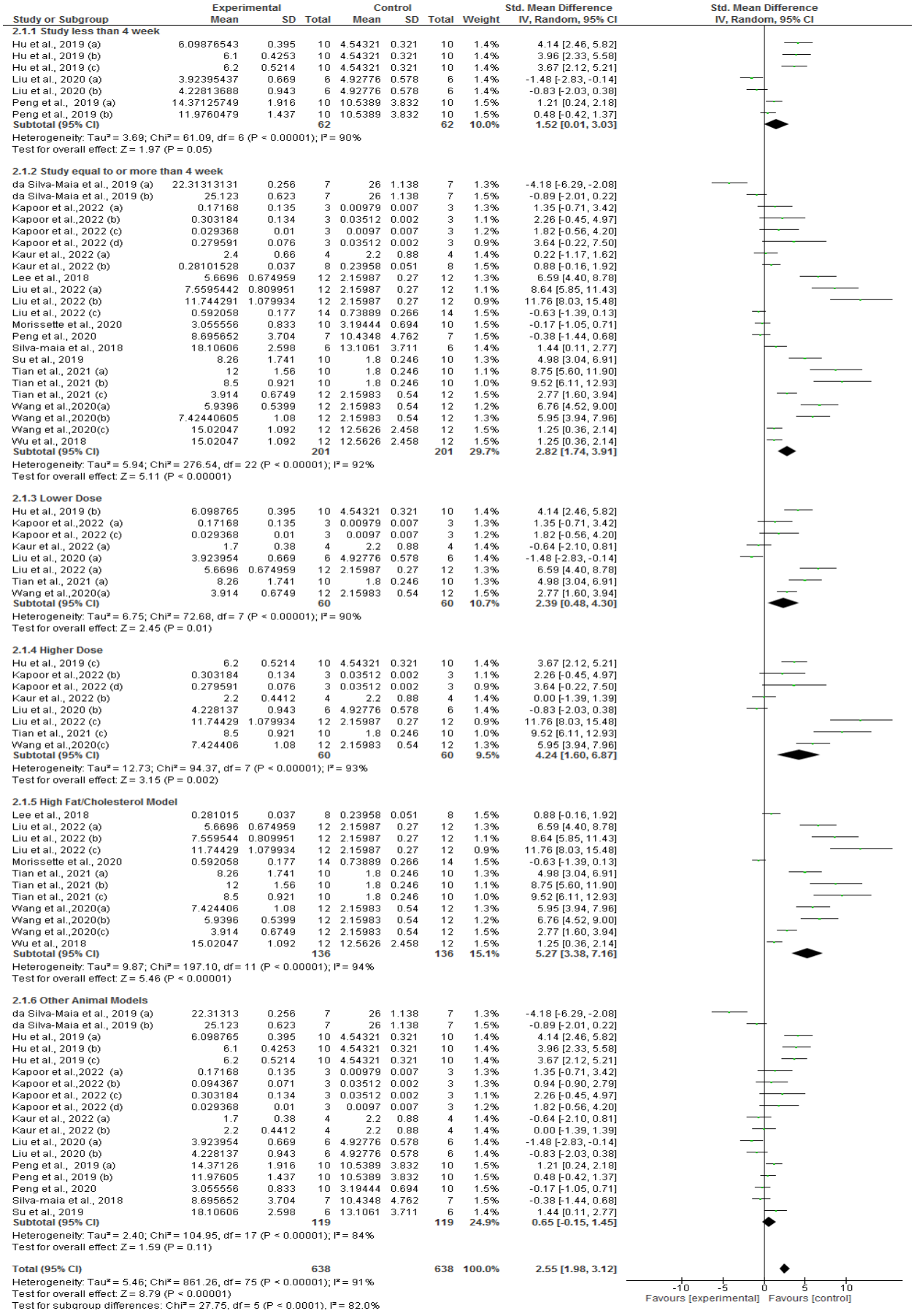
S Figure 6: Forest plot of the sub-group analysis after elimination of high-influencer studies, for understanding the effect of anthocyanin supplementation on the acetic acid, SCFA type. Sub-grouping was on the basis of duration of the study, dose of the anthocyanin given and disease status of the subjects (animal models). Values are standardized mean differences with 95% CIs determined with the use of random-effects models. Heterogeneity was quantified by I^2, inverse variance and standardised mean difference (SMD).



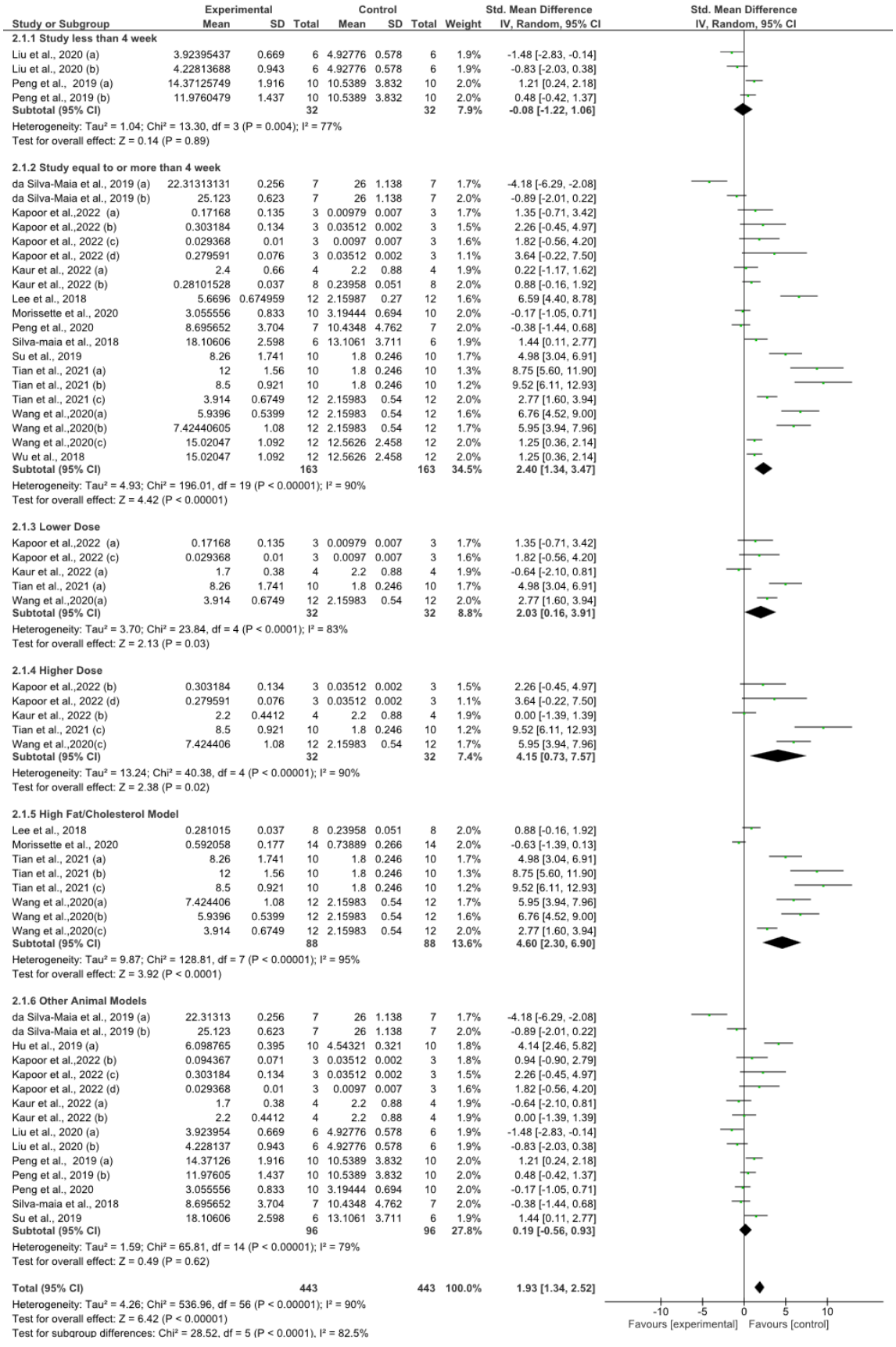
S Figure 7: Forest plot of the sub-group analysis to study effect of anthocyanin supplementation on the butanoic acid, SCFA type. Sub-grouping was on the basis of duration of the study, dose of the anthocyanin given and disease status of the subjects (animal models). Values are standardized mean differences with 95% CIs determined with the use of random-effects models. Heterogeneity was quantified by I^2, inverse variance and standardised mean difference (SMD).



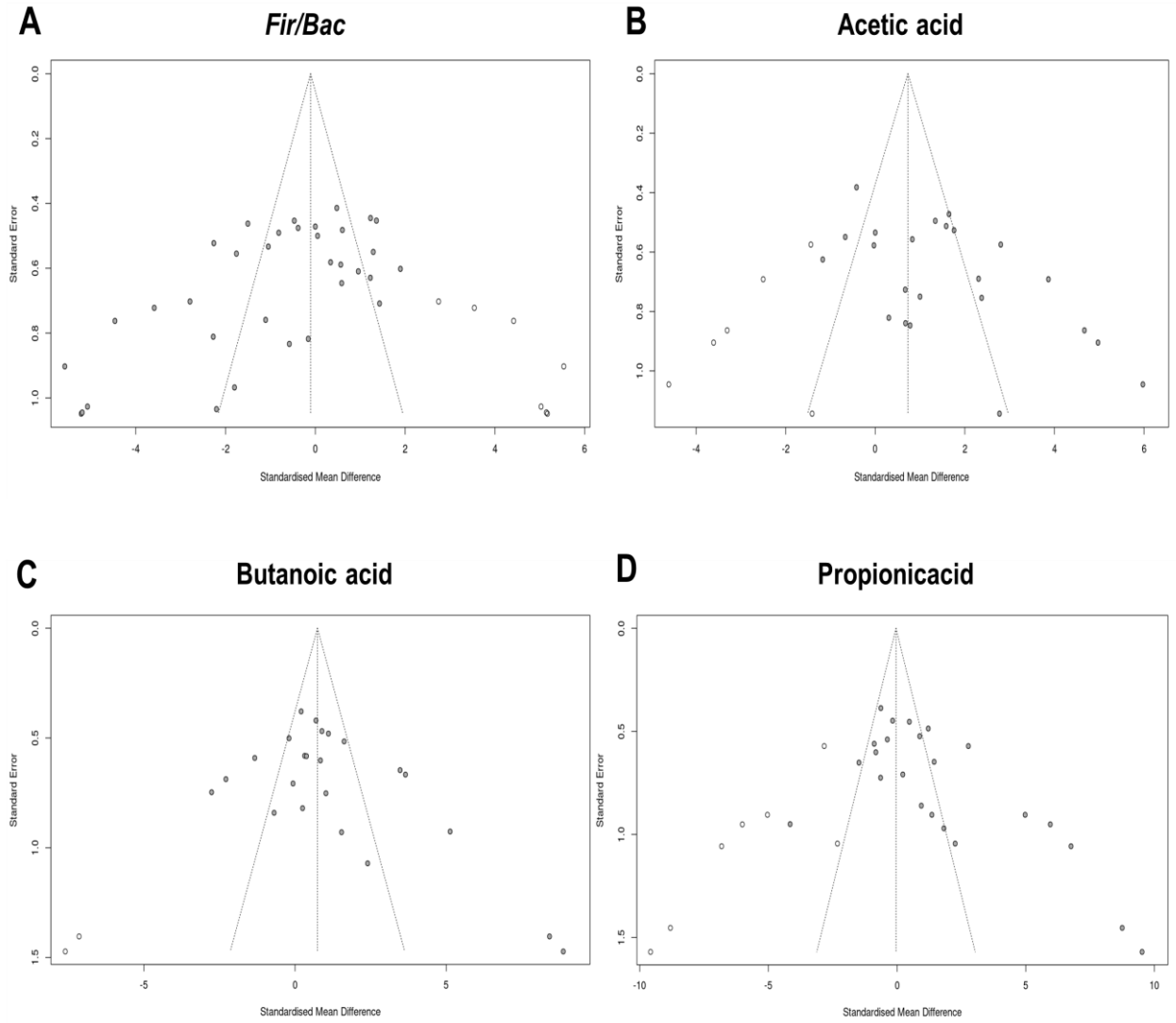
S Figure 8: Forest plot of the sub-group analysis after elimination of high-influencer studies, for understanding the effect of anthocyanin supplementation on the butanoic acid, SCFA type. Sub-grouping was on the basis of duration of the study, dose of the anthocyanin given and disease status of the subjects (animal models). Values are standardized mean differences with 95% CIs determined with the use of random-effects models. Heterogeneity was quantified by I^2, inverse variance and standardised mean difference (SMD).



S Figure 9: Forest plot of the sub-group analysis to study effect of anthocyanin supplementation on the propionic acid, SCFA type. Sub-grouping was on the basis of duration of the study, dose of the anthocyanin given and disease status of the subjects (animal models). Values are standardized mean differences with 95% CIs determined with the use of random-effects models. Heterogeneity was quantified by I^2, inverse variance and standardised mean difference (SMD).

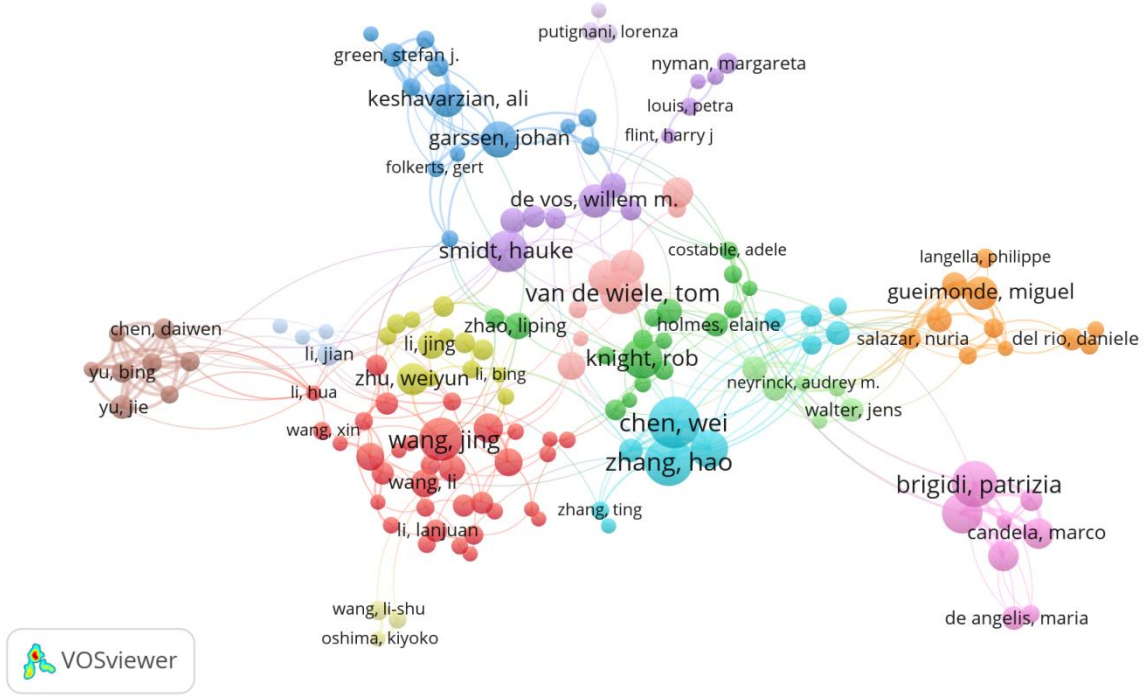


S Figure 10: Forest plot of the sub-group analysis after elimination of high-influencer studies, for understanding the effect of anthocyanin supplementation on the propionic acid, SCFA type. Sub-grouping was on the basis of duration of the study, dose of the anthocyanin given and disease status of the subjects (animal models). Values are standardized mean differences with 95% CIs determined with the use of random-effects models. Heterogeneity was quantified by I^2, inverse variance and standardised mean difference (SMD).

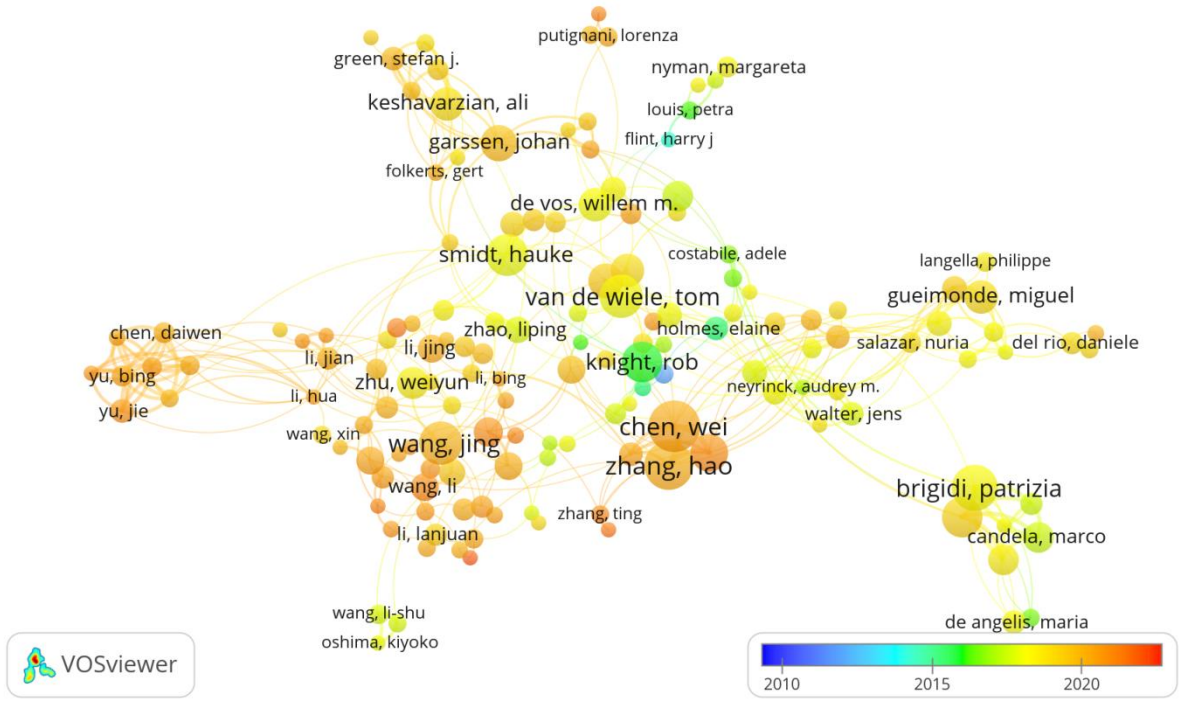


S Figure 11: **Funnel plots for publication bias assessment(A)** For *Fir/Bac*using data from 41 studies. **(B)**For Acetic acid using data from 29 studies. **(C)** For Butanoic acid using data from 25 studies. **(D)**For Propionic acid using data from 30 studies.
The plot was created by putting the standard error on the y-axis and SMD on the x-axis

A

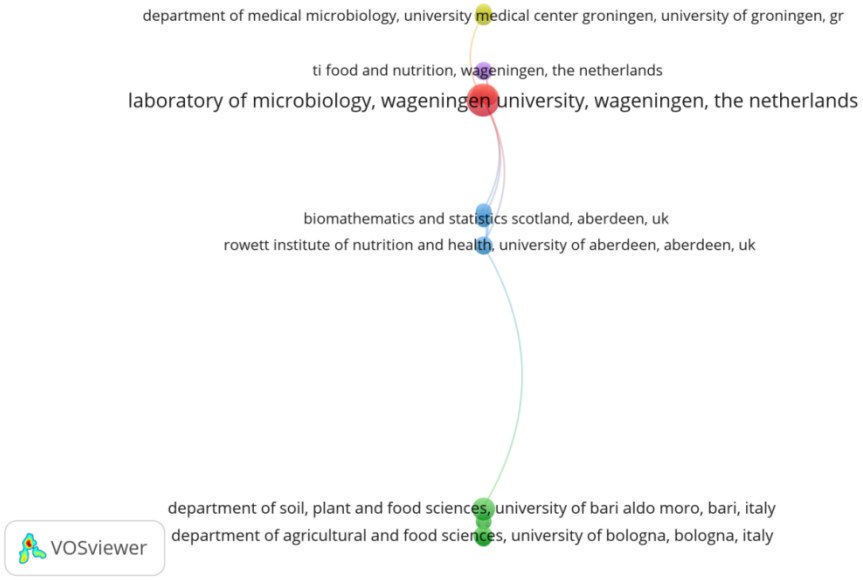


B

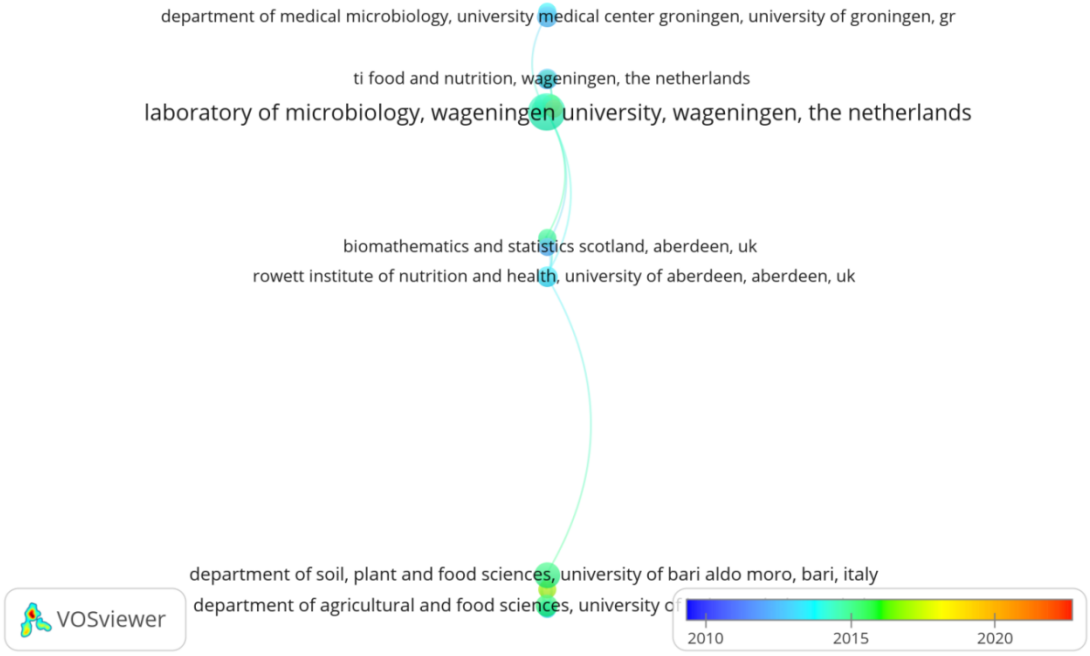


S Figure 12: Bibliographic coupling of leading researchers in anthocyanin, gut microbiota and SCFA research (A) Network visualization **(B)** Overlay visualization (year wise)

A



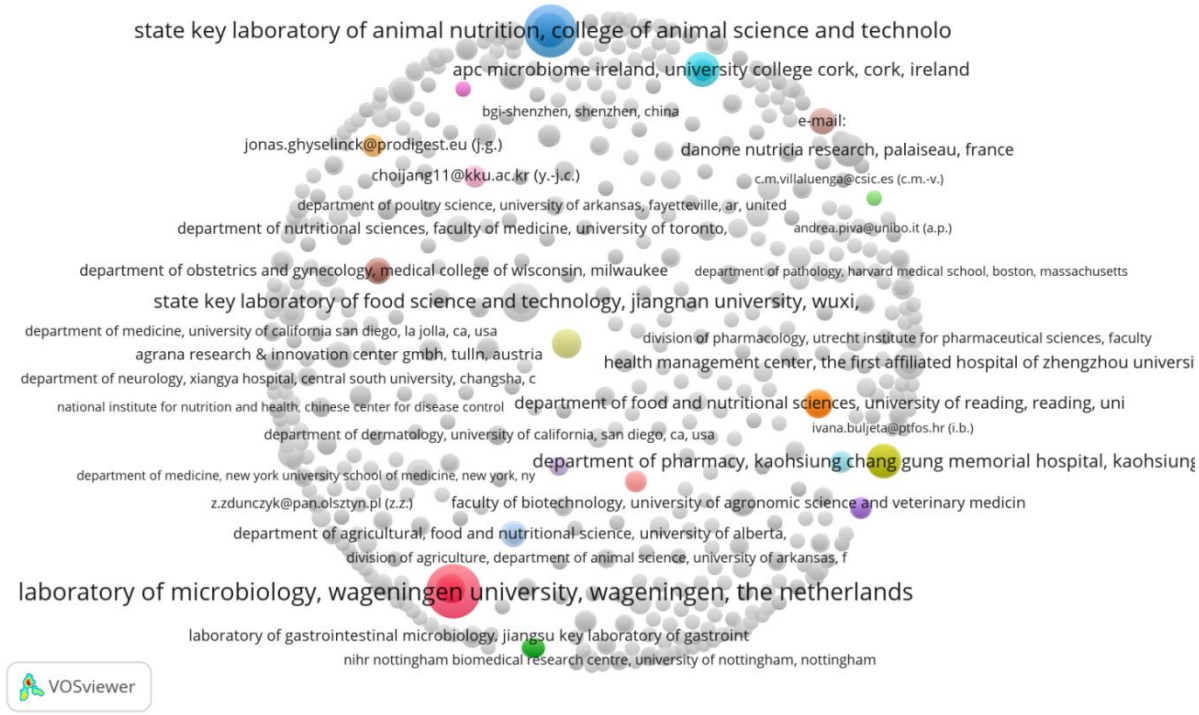
B



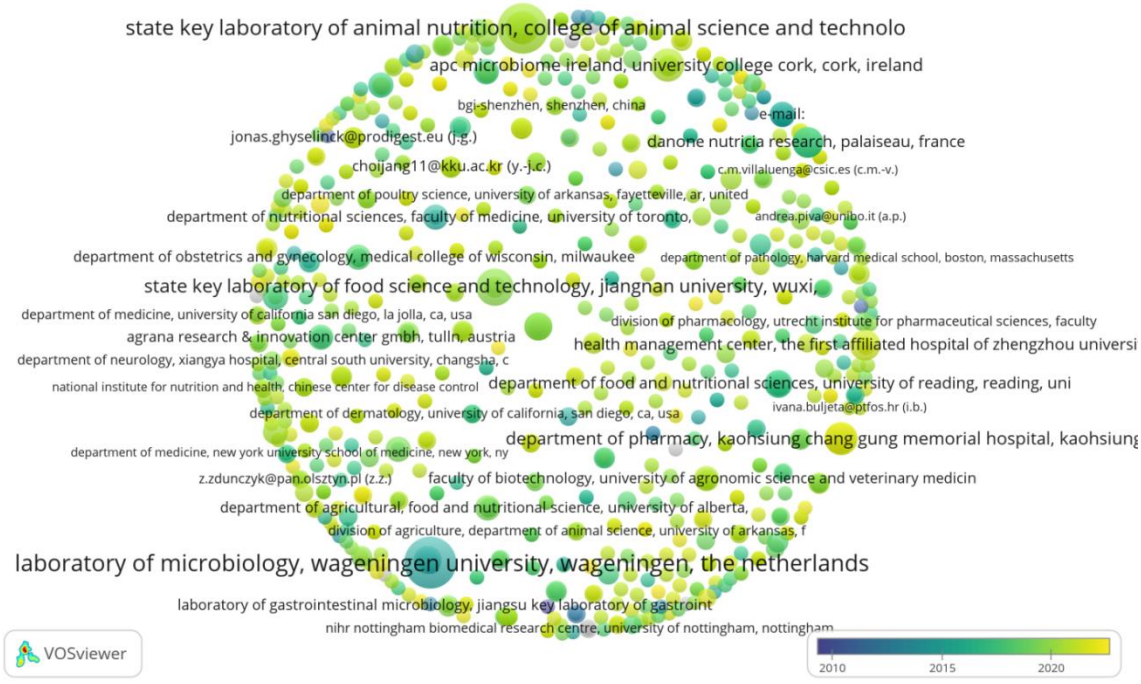
S Figure 13: Institutional collaborations for anthocyanin, gut microbiota and SCFA research

(A) Network visualization (B) Overlay visualization (year wise)

A



B



S Figure 14: Independent institutional collaborations for anthocyanin, gut microbiota and SCFA research (A) Network visualization **(B)** Overlay visualization (year wise)

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page no. 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Page no. 1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Page no. 2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page no. 3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Page no. 3 -4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page no. 1, 18-25 (Table 1 &2)
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Page no. 3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page no. 3 & 4 4 reviewers (each 2 worked independently)
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	4 reviewers (each 2 worked independently) Online tools like Webplotdigitizer used to extract data from bar graphs
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Page no. 4 (Data extraction)
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Page no. 3-4 Study with missing or unclear information removed
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Page no. 5
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	SMD (standardised mean difference)
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page no. 18-25 (Table 1 & 2)

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Supplementary data 1& 2
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Supplementary data 1& 2
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Page no. 5 (statistical analysis), RevMen 5.4 and R script (meta-package)
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	subgroup analysis, meta-regression
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Studies with wider cumulative intervals were removed
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Begg's and Egger's regression asymmetry tests and Trim-Fill correction method
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	pooled effect estimate from a meta-analysis was used for assessing the certainty assesment
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	-NA-
Study characteristics	17	Cite each included study and present its characteristics.	Page no. 18-25 (Table 1 & 2)
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Page no. 8 (Table 8 & Supplemental Figure 11)
Results of	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its	Page no. 6-8

Section and Topic	Item #	Checklist item	Location where item is reported
individual studies		precision (e.g. confidence/credible interval), ideally using structured tables or plots.	(Figure 2 & 3, Table 3-7, Supplemental Figure 1 -10)
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Table 1 & 2
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Page no. 6-8 (Figure 2 & 3, Table 3-7, Supplemental Figure 1 -10)
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Page no. 6-8 (Figure 2 & 3, Table 3-7, Supplemental Figure 1 -10)
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Page no. 6-8 (Figure 2 & 3, Table 3-7, Supplemental Figure 1 -10)
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Page no. 8 (Table 8 & Supplemental Figure 11)
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Figure 2 & 3
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Page no. 9-10
	23b	Discuss any limitations of the evidence included in the review.	Page no. 9-10
	23c	Discuss any limitations of the review processes used.	Page no. 9-10
	23d	Discuss implications of the results for practice, policy, and future research.	Page no. 9-11
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Not registered
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	PRISMA protocol used
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	-NA-
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	No Financial Support
Competing interests	26	Declare any competing interests of review authors.	Authors have no conflict of interest

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Supplemental Files in the form of tables and figures

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71.doi: 10.1136/bmj.n71

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