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Meta-analysis of the Effect of Garlic on Microbial Count in Meat Products

Meta-analisis Aktivitas Antimikroba Bawang Putih pada Produk Daging

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Abstract. Garlic (*Allium sativum* L.) is a natural ingredient used for microbiological control in meat products. This study aimed to analyze the effect of garlic addition on microbial count reduction in meat products through meta-analysis. The meta-analysis was conducted using 304 extracted data points from 10 articles, analyzed with the Hedges' d method. The results showed that the addition of garlic significantly reduced microbial counts (p<0.05) in three types of meat products: raw meatballs (minced mutton), ground beef, and raw chicken meat. Among the different forms of garlic tested, freeze-dried powder, oven-dried powder, and microencapsulated essential oil were the most effective in reducing microbial counts. Garlic treatment led to a reduction in seven types of tested microbes, except for *Staphylococcus aureus*. The highest microbial reductions were observed for *Listeria monocytogenes* (-6.626 to -2.009 $\log_{10}$ CFU/g), sulfite-reducing anaerobes (-4.521 to -2.193 $\log_{10}$ CFU/g), and *Escherichia coli* (-2.771 to -1.225 $\log_{10}$ CFU/g).

Keywords: antimicrobial, bacteria, garlic, meat product, meta-analysis

Abstrak. *Bawang putih (Allium sativum L.) merupakan bahan alami yang digunakan untuk pengendalian mikrobiologis pada produk daging. Penelitian ini bertujuan untuk menganalisis pengaruh penambahan bawang putih terhadap penurunan jumlah mikroba dalam produk daging melalui meta-analisis. Meta-analisis dilakukan terhadap 304 data yang diekstraksi dari 10 artikel dan dianalisis menggunakan metode Hedges' d. Hasil penelitian menunjukkan bahwa penambahan bawang putih secara signifikan mengurangi jumlah mikroba (p<0,05) pada tiga jenis produk daging: bakso mentah (daging domba cincang), daging sapi giling, dan daging ayam mentah. Bentuk bawang putih yang paling efektif dalam menurunkan jumlah mikroba dibandingkan bentuk lainnya adalah bubuk hasil pengeringan beku, bubuk hasil pengeringan oven, dan minyak esensial dalam bentuk mikroenkapsulasi. Penambahan bawang putih pada produk daging terbukti dapat menurunkan jumlah tujuh jenis mikroba yang diuji, kecuali Staphylococcus aureus. Penurunan jumlah mikroba tertinggi ditemukan pada Listeria monocytogenes (-6,626 hingga -2,009* $\log_{10}$ *CFU/g), anaerob sulfite-reducing (-4,521 hingga -2,193* $\log_{10}$ *CFU/g), dan Escherichia coli (-2,771 hingga -1,225* $\log_{10}$ *CFU/g).*

Kata kunci: *antimikroba, bakteri, bawang putih, produk daging, meta-analisis*

Practical Application: This study is expected to provide information to the meat processing industry regarding the effects of adding various forms of garlic on reducing microbes in meat products. The results of this study can also add scientific references related to the use of meta-analysis on microbiological quality and microbial control of food products.

INTRODUCTION

Meat is an ideal growth medium for microorganisms because it contains nitrogen and carbohydrates, which can be fermented by microbes. Fresh meat has a high moisture content (72–75%) and pH 5.3–6.5, provide suitable condition for microbial growth (Hernando *et al*. 2015; Indriyani *et al*. 2019). Meat stored at freezer temperature (-18 °C) has a high water and nutrient content. A study by Hussein *et al*. (2020) showed that fresh meats such as buffalo, lamb, beef, and chicken meat stored at freezer temperature for 1 month had a water content of 72.59%, 73.15%, 73.19%, and 73.88%, respectively; while the protein content of each meat was 20.22%, 19.01%, 20.36%, and 18.98% in buffalo, lamb, beef, and chicken meat, respectively. Meat and meat products may contain microorganisms such as *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *C. perfringens*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus*, and *Yersinia enterocolitica* which can cause foodborne diseases (Pal *et al*. 2018). *E. coli*, *Listeria*, and *Salmonella* can grow on raw meat, ready-to-eat meat, and packaged meat products (Li *et*

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al. 2020). A study by Hussein *et al*. (2020) showed that several types of fresh meat contained different total microbes in each meat, namely $2×10^5$ CFU/g in buffalo meat, $2.5×10^5$ CFU/g in lamb meat, $5×10^5$ CFU/g in beef, and $3×10^5$ CFU/g in chicken meat. Further studies showed that buffalo meat, lamb, beef, and chicken meats contain coliforms of $4×10^4$ CFU/g, $5×10^4$ CFU/g, $7×10^4$ CFU/g, and $9×10^4$ CFU/g, respectively (Hussein *et al*. 2020).

In Indonesia, Soepranianondo *et al*. (2019) reported that out of 40 fresh beef samples from slaughterhouses in East Java, 32.5% contained *E. coli* and 20% contained *S. aureus*; while 2.5% of samples contained *Salmonella* spp. From 10 slaughterhouses, the average of microbial count on fresh beef samples were as follows: total plate count of $2.6×10^5$ CFU/g, *S. aureus* of 41.58 CFU/g, and *E. coli* of 27.03 CFU/g (Soepranianondo *et al.* 2019). Research by Al-Thubaiti *et al.* (2021) on processed meat products showed that 39.5% of hamburger samples in Jeddah (15 out of 38 samples) were contaminated by *Salmonella*. The hamburger samples contained 204.3 CFU/mL of mold and yeast; total plate count (total aerobic mesophiles) of 69.5 CFU/mL, coliforms of 16.2 CFU/mL, and *E. coli* of 10.0 CFU/mL (Al-Thubaiti *et al.* 2021).

Processing and storage of meat products should be carried out properly to control microbial growth. Natural ingredients are widely used as antimicrobial agents for meat products. Review by Yu *et al.* (2021) stated that several natural ingredients such as thyme leaves (*Thymus vulgaris* L.), oregano, and cinnamon were able to inhibit several types of microbes in various meat products. A concentration of 1% thyme leaf essential oil inhibited *E. coli* in hamburgers stored at 4 °C; as much as 3 mL of oregano essential oil inhibited *L. monocytogenes*, *Salmonella* Enteritidis, and *E. coli* in beef dried at 55 °C for 6 hours; and 5% of cinnamon essential oil inhibited *L. monocytogenes* in ground beef stored at -18 to 8 °C (Yu *et al*. 2021). In addition to these ingredients, natural ingredients commonly used in the processing of meat products that can add flavor and act as antimicrobials is garlic (*A. sativum* L.).

In garlic, the bioactive component that functions as an antimicrobial agent is allicin (diallyl thiosulfinate) (Figure 1). Alliinase enzyme in garlic will converts alliin to allicin when garlic is crushed or cut. This compound can inhibit DNA gyrase activity in *E. coli*, *in vitro* (Reiter *et al*. 2019). Various studies have shown that garlic can reduce the number of microbes in meat products. The addition of garlic paste to raw chicken meat stored at refrigerator temperature (4±1 °C) for 1, 3, 5, 7, and 9 days significantly reduced the number of total plates, coliforms, molds and yeasts compared to the control (without the addition of garlic) (Singh *et al*. 2014). The addition of 1% garlic essential oil to minced beef with a storage temperature of 4 °C reduced the number of *S. aureus* and *E. coli* (Saad *et al*. 2019). Study of Mahros *et al*. (2021) showed that the addition of garlic significantly reduced more than 3 $\log_{10}$ CFU/g of aerobic microbes in ground beef stored at 4 °C for 15 days.

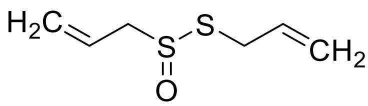

Figure 1. Allicin chemical structure

Various studies have suggested that the addition of garlic in different extract forms to different meat products has different effects on reducing the number of microbes (Aydin *et al*. 2007; Najjaa *et al*. 2020; Yang *et al*. 2011). There are studies that show a significant decrease in the number of microbes in meat products with the addition of garlic, but there are also reports of an insignificant decrease in the number of microbes. Based on this and the absence of a meta-analysis on the effect of garlic addition on reducing microbes in meat products, a comprehensive scientific study using meta-analysis is needed. Meta-analysis can produce a more comprehensive picture of most of the study results (Gurevitch *et al*. 2018). This study aims to analyze the effect of garlic addition on reducing the number of microbes in meat products using meta-analysis towards various studies that have been conducted. The results of this analysis are expected to provide new scientific references for the microbiological quality and microbial control of meat products.

MATERIALS AND METHODS

Materials

This meta-analysis used research articles published in reputable and accredited international and national journals in databases such as PubMed, Science Direct, Scopus, Springer, Research Gate, Wiley Online Library, and Google Scholar. The tools used in this meta-analysis were Microsoft Excel [version 15.0.4420.1017 (2013)], Mendeley (version 1.19.4), Publish or Perish (version 8.2.3944.8118), and OpenMEE.

Methods

Articles used in the analysis were selected following the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines (Page *et al*. 2021). The research questions were formulated using the Population, Intervention, Comparison, Outcome (PICO) method. The population is the subject given treatment, intervention is the treatment given, comparison is the control, and outcome is the response to the influence of the treatment given (Cook and West

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2012). Articles were searched in the Pubmed, Science Direct, Scopus, Springer, Research Gate, Wiley Online Library, and Google Scholar databases with the keywords "garlic", "antimicrobial", "garlic extract", "powder dried garlic", "meat", and "poultry" using Boolean operators (AND, OR, and NOT). The next research procedures are study of searching and selecting articles, data collection, and statistical analysis (Nabilah *et al*. 2022).

Search and selection of studies

Articles were searched using the advanced search features of each database by entering the specified keywords. Each database had a different search strategy; therefore, the search strategy was adjusted for each database. The articles used in the analysis went through a series of selection stages, namely, the identification stage, and the article suitability assessment stage based on the inclusion and exclusion criteria. Inclusion and exclusion criteria are determined to determine the scope of the research question (Swift and Wampold 2018). The inclusion criteria in this analysis were research articles published in the last 25 years (1998–2023) and published by reputable international journals indexed by Scopus or national journals accredited by Sinta 2 at a minimum. In addition, the article must contain data on the types of microbes in meat products and statistical data required in the analysis, namely, the number of repetitions, averages, and standard deviation values or standard errors from the negative control group (meat products before garlic addition) and experiments (meat products after garlic addition). The exclusion criteria were articles that did not meet the inclusion criteria and did not have the statistical values required in the analysis. Selected studies that went through a series of stages of selection and identification, removal of duplication, assessment of the appropriateness of titles and abstracts, selection of articles that did not contain statistical data, and statistical analysis of selected articles are shown in Figure 2.

Data collection

Data from the selected articles were then extracted into a database using Microsoft Excel. Two types of data were extracted, article identity and research data (Aji *et al*. 2022). The identity of the extracted articles included the name of the journal, year of publication, type of article, author's name, research title, and research location. The extracted data included types of meat products, forms of garlic, and types of test microbes, as well as the required statistical data (mean value, standard deviation, and number of repetitions from the experimental and negative control classes).

Statistical analysis

Data analysis was performed statistically using OpenMEE software with the Hedges'd method (Wallace *et al.* 2017). Hedges'd method was used to calculate the effect size value regardless of the heterogeneity of the number of samples, measurement units, and statistical test results (Palupi *et al*. 2012). The output of the effect size in the form of standardized mean difference (SMD) was used to determine the magnitude of the difference between the meat products added with garlic and the controls. If there was a significant difference (heterogeneity) between the synthesized studies, the meta-analysis was performed using the random-effect model (Hamman *et al.* 2018).

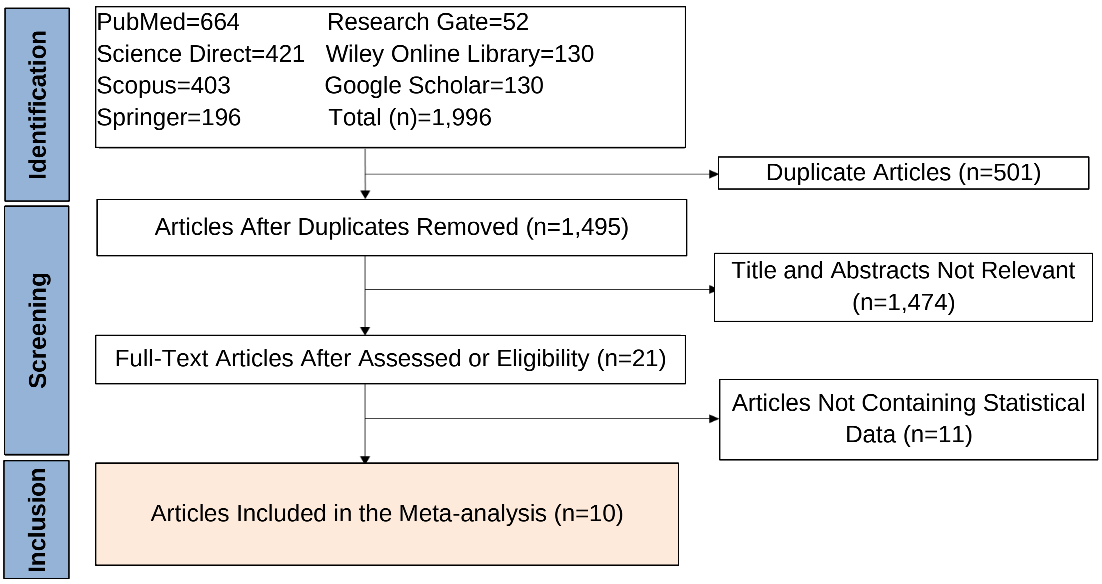

Figure 2. Flowchart of article selection in meta-analysis

The effect size was described using 95% confidence interval (CI). A CI value that does not reach a zero effect size indicates that the effect size is significant (Palupi *et al*. 2012). In this analysis, heterogeneity of the study was calculated using the I^2 statistical value. If the study heterogeneity value was quite high (>50%), a moderator variable analysis or subgroup analysis was performed (Afandi *et al*. 2021). To determine the strength of evidence (robustness) of the analysis results, Rosenthal's fail-safe-number (Nft) test was carried out, calculated using OpenMEE software.

RESULTS AND DISCUSSION

Data selection results

The source of the meta-analysis was seven journal databases, and 1,996 articles was obtained. After removing duplicate articles, 1,495 articles were obtained. Furthermore, articles were re-selected based on their titles and abstracts. At this stage, 21 articles were in accordance with the research topic. Of the 21 articles obtained, 11 articles were excluded because they did not have the complete statistical data required. Based on the selection results, 10 articles (published from 2007 to 2020) were analyzed using meta-analysis (Figure 2). The data used from 10 articles, namely 304 data, were analyzed to study the effect of adding garlic to meat products.

Research on the effect of garlic addition on reducing microbes in meat products was conducted in India (Singh *et al*. 2014), Iran (Hosseini and Shabani 2013; Nejad *et al*. 2014; Sadaghiani *et al*. 2019), Korea (Yang *et al*. 2011), North Macedonia (Kuzelov *et al*. 2017), Egypt (Saad *et al*. 2019), Spain (Linares *et al*. 2013), Tunisia (Najjaa *et al*. 2020), and Turkey (Aydin *et al*. 2007). Based on the 10 articles analyzed, there were 8 types of meat products, 10 forms of garlic, and 9 types of test microbes. The types of meat products used in this analysis were hamburger, ground beef, raw meatball/minced mutton meat, raw chicken meat, vacuumed minced pork meat, irradiated raw ground beef, dry sausage from wine-marinated pork, and fresh minced beef (Table 1).

The forms of garlic used in antimicrobial testing of meat products were garlic water extract, crushed fresh garlic, freeze-dried garlic powder, oven-dried garlic powder, garlic essential oil microcapsules, garlic paste, unspecified extract, fresh minced garlic (semi-liquid), garlic juice, and garlic essential oil (Table 2). The types of microbes used in this study were 9 types, namely total plate count, *S. aureus*, coliforms, *Staphylococcus/ Micrococcus*, yeasts and molds, *E. coli*, sulfite reducing anaerobes, *Salmonella* spp., and *L. monocytogenes*.

Effect of garlic addition to meat products

Table 3 shows the overall effect size/standardized mean difference (SMD), which had a negative value (-1.177), indicating that the treatment of garlic addition to meat products significantly reduced the number of test microbes ($p<0.05$) with a confidence interval range of -1.337 to -1.017. The antimicrobial activity of garlic is likely due to the presence of organosulfur compounds called allicin (diallyl disulfide and diallyl trisulfide) (Mahros *et al*. 2021). Allicin is a reactive sulfur species (RSS) compound that can cross cell membranes. This active compound can oxidize thiol groups in glutathione and cysteine residues in proteins, thereby disrupting DNA gyrase activity in bacteria (Reiter *et al*. 2019; Nadeem *et al*. 2022).

Table 1. Types of meat products used in the meta-analysis

No	Meat Products	Information
1	Hamburger	Hamburgers prepared under laboratory conditions (Hosseini and Shabani 2013; Nejad *et al.* 2014)
2	Ground beef	• 10 g of ground beef in 90 ml buffered peptone water (BPW) was homogenized for 2 min (Najjaa *et al*. 2020). • Meat was ground with a 3 mm plate twice (Aydin *et al*. 2007; Yang *et al*. 2011) • Commercially available (Sadaghiani *et al*. 2019)
3	Raw meatball/minced mutton meat	Commercially available (Aydin *et al.* 2007)
4	Raw chicken meat	The meat minced twice using 6 mm and 4 mm grinding plates (Singh *et al.* 2014)
5	Vacuumed minced pork meat	Minced meat (without salt) was prepared according to the procedures for the minced meat, preparation of minced meat, and meat products in Republic of Macedonia (Kuzelov *et al*. 2017)
6	Irradiated raw ground beef	Ground beef was aerobically packaged and then irradiated at 0 or 2.5 kGy using a linear accelerator facility (Circe IIIR; Thomson CSF Linac, St. Aubin, France) with an energy of 10 MeV and a power of 5.6 kW at 22 °C. The average dose rate was 74.1 kGy/min. The samples were irradiated for 30 min. Raw meat samples were stored in a cold storage room at 4 °C (Yang *et al*. 2011)
7	Dry sausage from wine-marinated pork	Commercially available (Linares *et al.* 2013)
8	Fresh minced beef	Commercially available (Saad *et al.* 2019)

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Table 2. Forms of garlic applied to meat products

No	Garlic forms	Information
1	Garlic water extract	Garlic was peeled, washed with sterile distilled water, then mixed with 200 mL of isotonic sodium chloride solution (0.9%/10 g in 90 mL) under sterile conditions, then filtered and stored at -20 °C (Hosseini and Shabani 2013; Nejad *et al*. 2014)
2	Crushed fresh garlic	Garlic was cleaned and crushed using a sterile mortar (Aydin *et al*. 2007)
3	Freeze-dried garlic powder	Garlic was cut into small pieces using a knife and frozen at -80 °C for 24 hours. The frozen samples were then transferred to a freeze dryer (CHRIST Alpha 1–4 LSC) using a pressure of 0.94 mbar and a temperature of -5 °C for 72 hours. The final moisture content of the powder obtained by freeze drying was 6.3 ± 0.1% (Najjaa *et al*. 2020)
4	Oven-dried garlic powder	Fresh garlic samples were cut into small pieces with a knife and dried in an oven at 50 °C for 5 hours. The final moisture content of the powder obtained by convection oven drying was 6.7±0.5% (Najjaa *et al*. 2020)
5	Garlic essential oil microcapsules	Essential oil was obtained by steam distillation (30 kg of *A. sativum* was distilled for 4 hours at 80 °C), the obtained oil was dried with anhydrous sodium sulfate and stored in a dark vial at 4 °C for use in encapsulation. The microcapsules were oil in water (O/W) emulsion. The microcapsule was coated with an aqueous solution of maltodextrin (MDX) (70% W/V) and gum Arabic (AG) (30% W/V). MDX and AG were previously dissolved in distilled water at 50 °C and stirred using a heater-stirrer, then left for 12 hours at room temperature. Garlic essential oil (concentrations of 5, 10, 15, and 20%) was added to the coating suspension using an Ultraturrax homogenizer at 24,000 rpm for 30 minutes. The final microencapsulation efficiency of the emulsion used was more than 70% (Najjaa *et al*. 2020)
6	Garlic paste	Garlic was ground using a grinder (Inalsa Maxie plus, 07120219, Inalsa Technologies, New Delhi, India) (Singh *et al*. 2014)
7	Extract (not specifically stated)	Commercially available (Kuzelov *et al.* 2017)
8	Fresh minced garlic (semi liquid)	Garlic was ground into a semi-liquid form using a Kitchen Aid food processor (Model KSM 90; Kitchen Aid Inc., St. Joseph, Mich., USA) (Yang *et al*. 2011)
9	Garlic juice	Garlic was peeled and washed using a household-scale centrifugal juicer (Kenwood Chef-AT641, Maia, Portugal) (Linares *et al*. 2013)
10	Garlic essential oil	Commercially available at Elgamhoria Co., Sharkia, Egypt (Saad *et al*. 2019)

Table 3. Overall effect size analysis of the effect of garlic addition on meat products

Parameter		N	SMD [95% CI]	*P-value*	I^2(%)
Overall		304	-1.177[-1.337;-1.017]	<0.001	54.37
Meat products	Hamburger	24	-0.176[-0.528;0.176]	0.328	0,00
	Ground beef	213	-1.269[-1.469;-1.069]	<0.001	55,72
	Raw meatball/minced mutton meat	30	-3.313[-4.166;-2.459]	<0.001	70,14
	Raw chicken meat	15	-1.093[-1.445;-0.741]	<0.001	16,95
	Vacuumed minced pork meat	9	-0.504[-1.054;0.045]	0.072	0,00
	Irradiated raw ground beef	2	-0.003[-0.985;0.979]	0.995	0,00
	Dry sausage from wine-marinated pork	1	-1.039[-2.515;0.438]	NA	NA
	Fresh minced beef	10	-0.220[-0.729;0.289]	0.397	0,00
Garlic forms	Garlic water extract	32	-0.619[-1.089;-0.148]	0.010	47,41
	Crushed fresh garlic	160	-1.064[-1.244;-0.884]	<0.001	34,61
	Freeze-dried garlic powder	12	-3.276[-5.016;-1.536]	<0.001	81,40
	Oven-dried garlic powder	12	-2.929[-4.365;-1.493]	<0.001	76,12
	Garlic essential oil microcapsules	48	-2.539[-3.210;-1.868]	<0.001	74,80
	Garlic paste	15	-1.093[-1.445;-0.741]	<0.001	16,95
	Extract (unspecified)	9	-0.504[-1.054;0.045]	0.072	0,00
	Fresh minced garlic (semi-liquid)	5	0.258[-0.367;0.884]	0.418	0,00
	Garlic juice	1	-1.039[-2.515;-0.438]	NA	NA
	Garlic essential oil	10	-0.220[-0.729;0.289]	0.397	0,00
Test microbes	*Staphylococcus aureus*	20	-0.242[-0.604;0.119]	0.189	0,00
	Total plate count	91	-1.389[-1.692;-1.087]	<0.001	56,70
	Coliforms	47	-0.863[-1.110;-0.615]	<0.001	0,00
	Staphylococcus/Micrococcus	32	-0.714[-1.017;-0.410]	<0.001	0,00
	Yeasts and molds	52	-0.946[-1.287;-0.605]	<0.001	47,09
	Escherichia coli	29	-1.998[-2.771;-1.225]	<0.001	73,01
	Sulfite-reducing anaerobes	24	-3.357[-4.521;-2.193]	<0.001	79,21
	Salmonella spp.	1	-1.039[-2.515;-0.438]	NA	NA
	Listeria monocytogenes	8	-4.318[-6.626;-2.009]	<0.001	76,22

Note: N=number of studies, SMD=standardized mean difference, 95% CI=confidence interval SMD (lower limit; upper limit), I^2=percent variation, NS=not specified, NA=not available

The heterogeneity test (I^2) was carried out on the parameters of the type of meat product, the form of garlic, and the type of test microbes, which produced a heterogeneity value (I^2) of 54.37%. This heterogeneity value was quite high for each parameter, at more than 50% (Afandi *et al*. 2021). The resulting heterogeneity value indicates that there were variations in the antimicrobial activity of garlic; therefore, further subgroup analysis was needed.

The effect of different types of meat products on the antimicrobial activity of garlic

The effect of the moderator variable of meat product type on the ability of garlic to reduce the number of microbes ($\log_{10}$ CFU/g) is presented in Table 3. The result of the subgroup analysis of the meat product types in the form of forest plots is shown in Figure 3. The addition of garlic resulted in a decrease in the number of microbes in three out of eight types of meat products. A decrease in the number of microbes was observed in ground beef, raw meatball/minced mutton meat, and raw chicken meat. Raw meatball/minced mutton meat showed a higher decrease in microbial number (SMD: -3.313; 95% CI: -4.166 to -2.459) compared to other types of meat products with the lowest negative effect size value and a confidence interval range (95% CI) that did not intersect the *x*-axis = 0 (Figure 3). The decrease in the microbial count in ground beef (SMD: -1.269; 95% CI: -1.469 to -1.069) was similar to that in raw chicken meat (SMD: -1.093; 95% CI: -1.445 to -0.741) because it had a negative effect size value and overlapping confidence intervals (95% CI).

The results of the analysis showed that raw meatball/minced mutton meat products showed a greater decrease in the microbial number compared to other types of meat products. The condition of the meat, which was raw and processed into meatballs by mincing, was thought to increase the microbial number in this type of meat product. When garlic was added, the decrease in the number of microbes in this meat product was greater than that in other meat products because the initial number of microbes was already higher. According to Orpin *et al*. (2019), fresh meat has the potential to be overgrown by microorganisms because it provides excellent nutrition for the growth of many microorganisms, especially harmful microbes that can cause infections in humans. Several types of microbes, especially pathogens, were found in raw meat samples (beef, lamb, and chicken), namely *S. aureus* (24.2%), *E. coli* (17.7%), *Klebsiella pneumoniae* (16.1%), *Proteus vulgaris* (12.9%), *B. cereus* (12.9%), *S. epidermidis* (11.3%), and *Proteus mirabilis* (4.9%) (Oluwatobi *et al*. 2021). In this study, ground beef products showed the second-highest reduction in microbial counts.

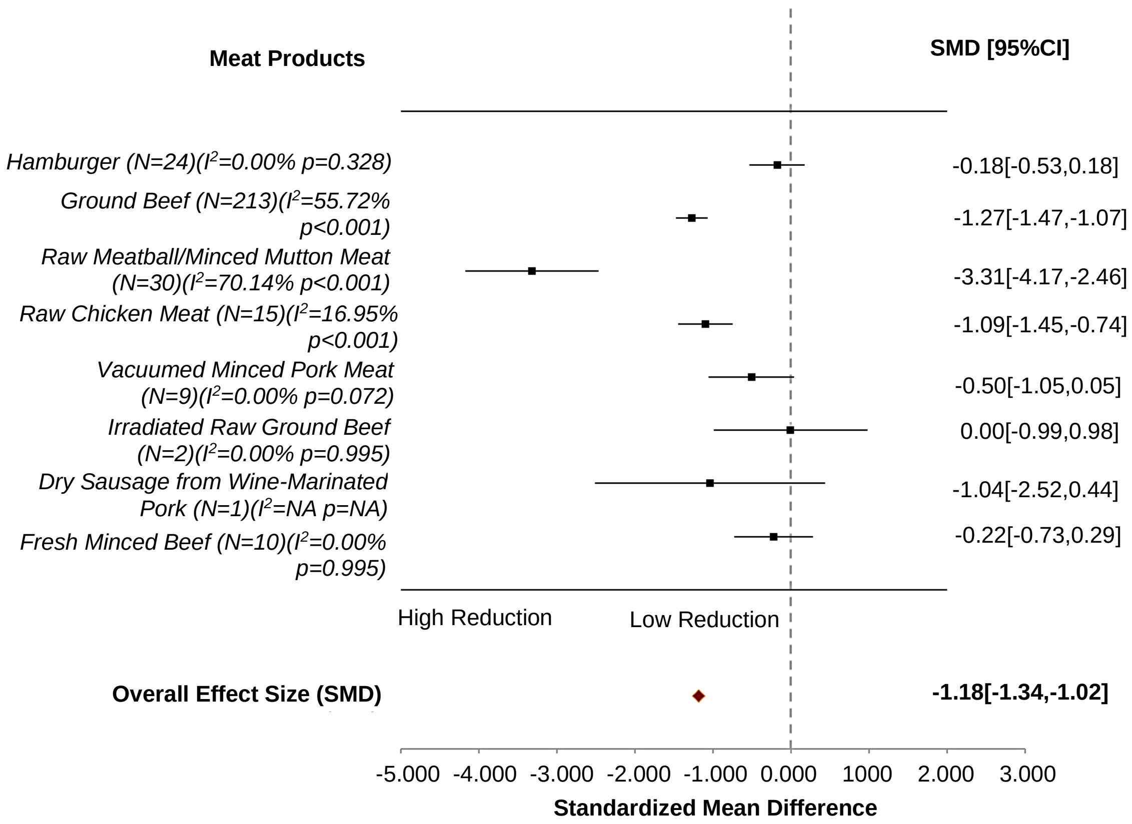

Figure 3. Forest plot of subgroup analysis on meat products

Another factor that can affect the reduction in the number of microbes in raw meatball/minced mutton meat product was the form of garlic used. Based on the results of this meta-analysis, of the six forms of garlic added to ground beef (crushed fresh garlic, freeze-dried garlic powder, oven-dried garlic powder, garlic essential oil microcapsules, fresh minced garlic, and garlic water extract), there were three forms of garlic that were effective in reducing the number of microbes, namely freeze-dried garlic powder, oven-dried garlic powder, and garlic essential oil microcapsules. The reduction in the number of microbes in raw chicken meat, which was higher than that in other products, might be influenced by the type of meat product (raw) and the form of garlic added, namely garlic paste, which showed better inhibitory activity than garlic water extract and crushed fresh garlic.

Meat products that showed a decrease in the number of microbes but were not significant were hamburgers, vacuum minced pork meat, irradiated raw ground beef, dry sausage from wine-marinated pork, and fresh minced beef (Table 3). Microbes in the hamburger showed a decrease, but this was not significant. The decrease in the number of microbes in the hamburger could be caused by the form of garlic added to meat product. Antimicrobial compounds can damage microbial cell membranes, thereby inhibiting microbial growth (Yamamura *et al*. 2021). Based on a meta-analysis study, hamburger added with garlic water extract had a lower activity than freeze-dried garlic powder, oven-dried garlic powder, and garlic essential oil microcapsules. Dry sausage from wine-marinated pork showed a decrease in the number of microbes, but the significance could not be determined due to limited data. The addition of garlic could reduced the number of microbes in fresh minced beef, but this was not significant, possibly because the concentration of garlic being added was low (Saad *et al*. 2019).

The decrease in the number of microbes in irradiated raw ground beef after the addition of garlic was the lowest compared to that in other meat products. A study by Yang *et al*. (2011) on fresh beef and irradiated meat by ionizing radiation at a storage temperature of 4 °C before the addition of garlic showed that the number of microbes in fresh meat was higher than that in irradiated meat. Irradiation is known as "cold sterilization" and can cause damage to microbial DNA (Chellaiah *et al.* 2020). The use of ionizing radiation can reduce the number of microbes in food products (Arapcheska *et al.* 2020).

The results of the analysis showed that the irradiated raw ground beef had the lowest effect size value, indicating that the decrease in the number of microbes in irradiated raw ground meat products after adding garlic was lower than that in other meat products. This is likely because some microbes are in a lethal injury condition, yet still alive when they are grown in media. Irradiation treatment of meat before garlic addition is thought to have caused the microbes in the meat to be in an injured condition, so that the effect of adding garlic on the decrease in the number of microbes was not significant. The number of microbes due to irradiation treatment is likely to decrease, but in this analysis, the initial number of microbes was still counted (control group of irradiated meat products before adding garlic) so that the decrease in the number of microbes after adding garlic could be analyzed. Pathogenic microbes that are very sensitive to ionizing radiation treatment are *E. coli*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, and *Campylobacter* spp (Belbe and Tofana 2010).

The effect of different forms of garlic on microbial count

Subgroup analysis was conducted on the data of microbial numbers in meat products with the addition of garlic in 10 different forms. The forest plot results showed that six forms of garlic significantly reduced the number of test microbes: garlic water extract, crushed fresh garlic, freeze-dried garlic powder, oven-dried garlic powder, garlic essential oil microcapsules, and garlic paste (Figure 4). The forms of garlic that significantly reduced the number of test microbes ($p < 0.05$) were garlic water extract (SMD: -0.619; 95% CI: -1.089 to -0.148), crushed fresh garlic (SMD: -1.064; 95% CI: -1.244 to -0.884). freeze-dried garlic powder (SMD: -3.276; 95% CI: -5.016 to -1.536), oven-dried garlic powder (SMD: -2.929; 95% CI: -4.365 to -1.493), garlic essential oil microcapsules (SMD: -2.539; 95% CI: -3.210 to -1.868), and garlic paste (SMD: -1.093; 95% CI: -1.445 to -0.741) (Table 3). The form of garlic that reduced the microbial number more than other extract forms were freeze-dried garlic powder, oven-dried garlic powder, and garlic essential oil microcapsules because they had the lowest negative effect size values and overlapping confidence intervals (95% CI). These three forms of garlic were obtained without high temperature heating (Aydin *et al*. 2007; Hosseini and Shabani 2013; Nejad *et al*. 2014; Singh *et al*. 2014); therefore, it is likely to affect bioactive compounds that can reduce microbes in meat products.

Strika *et al*. (2017) showed that crushed fresh garlic can inhibit test microbes better than garlic processed by heating (boiled). The active chemical components in fresh garlic are allegedly better than those in garlic processed by heat. Garlic water extract was obtained from the process without heating, namely by mixing garlic that had been added with distilled water at the specified concentrations, and then filtered using Whatman filter paper. The extract was obtained and ready to use (Huzaifa *et al*. 2014). The study of Huzaifa *et al*. (2014) showed that phytochemical screening of garlic water extract resulted in active components that could inhibit pathogenic microbes, namely flavonoids (0.05 g/100 g); saponins (0.24 g/100 g); alkaloids (0.12 g/100 g); tannins (2.52 g/100 g); and cardiac glycosides (1.88 g/100 g).

The forms of garlic extracts that reduced the number of microbes in meat products but were not significant were extracts (not specifically mentioned) (SMD: -0.504; 95% CI: -1.054 to 0.045), garlic juice (SMD: -1.039; 95% CI: -2.515 to -0.438), and garlic essential oil (SMD: -0.220; 95% CI: -0.729 to 0.289). The form of garlic that did not show a decrease in the number of microbes in meat products was fresh minced garlic (semi-liquid) (SMD: 0.258; 95% CI: -0.367 to 0.884) (Table 3). The form of fresh minced garlic (semi-liquid) did not show a decrease in the number of microbes in meat products, possibly due to the effect of irradiation treatment on meat used in the study by Yang *et al*. (2011).

In this meta-analysis study, garlic essential oil reduced the number of microbes but not significantly. This was likely influenced by the low concentration of garlic essential oil used in the test (1%); therefore, it was not significant in reducing microbes in meat products. Saad *et al.* (2019) showed that the addition of 1% garlic essential oil can reduce the number of *S. aureus* and *E. coli* in fresh minced beef, but not significantly, because an increase in bacterial number was also observed after the treatment. Similar results were reported by Atia *et al.* (2022), which stated that garlic essential oil added to beef steak at a storage temperature of 4 °C can reduce the number of total plate counts, coliforms, and *S. aureus* microbes at 24, 48, and 72 hours of treatment, but over a longer period of time, the number of each microbe increased so that the decrease in the number of microbes produced was not significant.

The effect of different types of microbes on the antimicrobial activity of garlic

The results of subgroup analysis (Figure 5) showed that the addition of garlic significantly reduce 7 types of test microbes in meat products, namely total plate count, coliforms, *Staphylococcus/Micrococcus*, molds and yeasts, *Escherichia coli*, sulfite-reducing anaerobes, and *L. monocytogenes* (Figure 5). One type of microbe, *Salmonella* spp., also showed a decrease, but limited data made it impossible to determine its significance. The three types of bacteria in meat products with the highest inhibition due to the addition of garlic were *L. monocytogenes* (SMD: -4.318; 95% CI: -6.626 to -2.009), sulfite-reducing anaerobes (SMD: -3.357; 95% CI: -4.521 to -2.193), and *E. coli* (SMD: -1.998; 95% CI: -2.771 to -1.225). A decrease in *S. aureus* number (SMD: -0.242; 95% CI: -0.604 to 0.119) was observed, but was not significant (p>0.05) (Table 3).

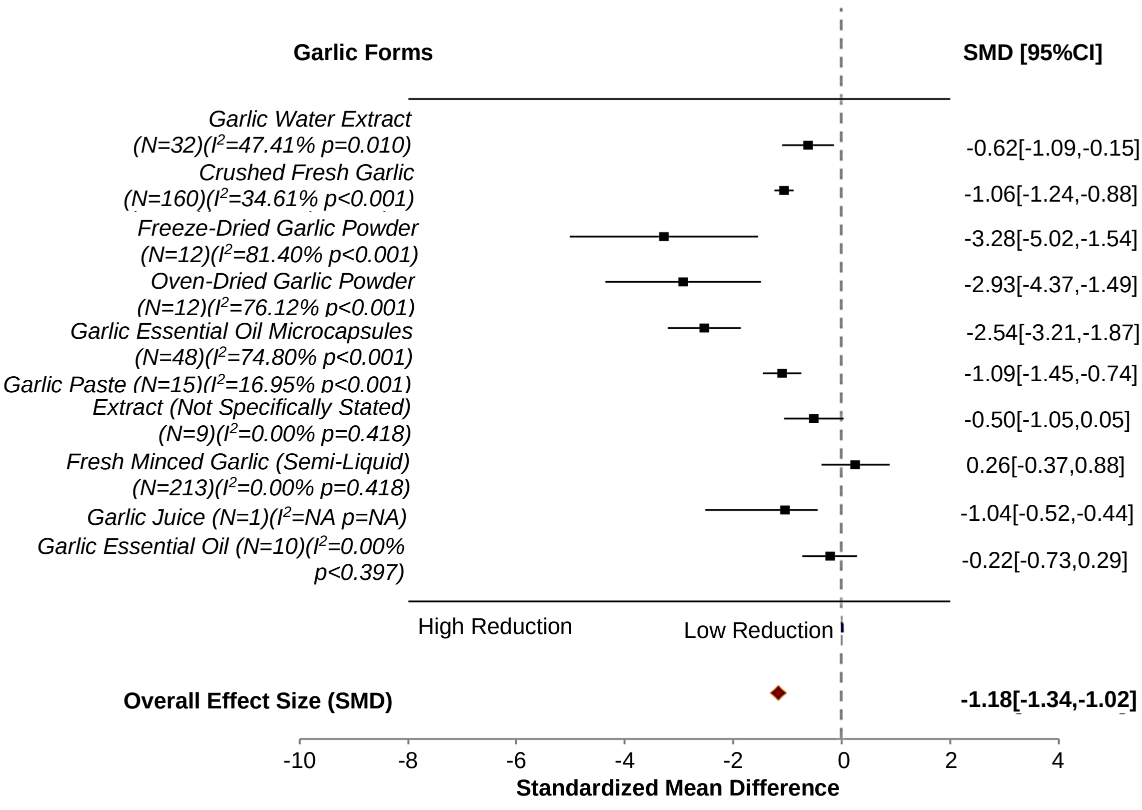

Figure 4. Forest plot of subgroup analysis on garlic forms

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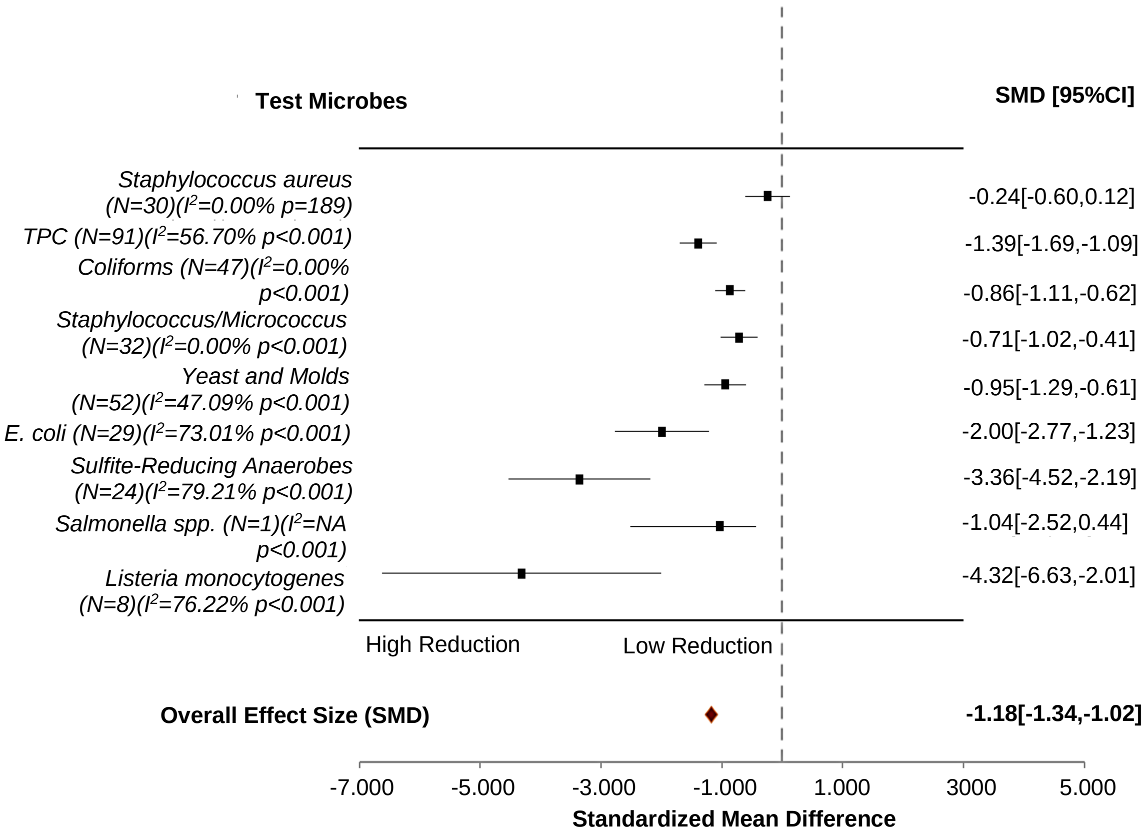

Figure 5. Forest plot of subgroup analysis on microbial types

The bioactive compound suspected as an antimicrobial in garlic is allicin. Allicin is an organosulfur compound that is included in reactive sulfur species (RSS) and is a non-proteinogenic amino acid alliin (S-allyl-L-cysteine sulfoxide) compound (Borlinghaus *et al*. 2014; Reiter *et al*. 2019). This compound is not found in garlic but is formed when garlic is crushed. Allicin is the result of the conversion of alliin by the allinase enzyme when garlic undergoes destruction (Londhe *et al*. 2011). Allicin functions by inhibiting enzymes with thiol groups in bacteria, thereby disrupting RNA synthesis. Allicin disrupts bacterial intracellular metabolism by blocking acetyl-CoA formation. Disruption of bacterial cell membrane formation is caused by allicin-derivative compounds (Ankri and Mirelman 1999; O'Gara *et al*. 2000). A review by Hasrianda and Setiarto (2022) stated that allicin in garlic can inhibit the growth of several Gram-positive and Gram-negative bacteria. The Gram-positive bacteria that can be inhibited by the allicin compound are *Bacillus* spp., *Streptococcus* spp., methicillin sensitive *S. aureus* NDRC 12732, and methicillin resistant *S. aureus* (clinical isolates), while the types of Gram-negative bacteria that can be inhibited are *Salmonella* Typhimurium, *Agrobacterium tumefaciens*, *E. coli* K12, *Pseudomonas syringae* (various pathovars), and *Vibrio cholerae* (Hasrianda and Setiarto 2022).

Rosenthal's fail-safe-number result

The number of studies (N) used in this analysis was 304 studies; therefore, the 5N+10 value was 1,530. The Nft value obtained in this study was 60,136. In the Rosenthal test, the Nft value was > 5N+10, indicating that the tendency/possibility of publication bias was minimal, thus providing evidence of a strong meta-analysis model (Fragkos *et al*. 2014; Palupi *et al*. 2012). The Nft value of this study was greater than 5N+10; therefore, the meta-analysis was robust against the possibility of publication bias. The Rosenthal test is used to represent the number of unpublished results that are not significant so that they affect the test results to be insignificant. If the fail-safe N value is large (Nft > 5N+10), then the analysis results can be considered robust against publication bias because a large number of statistically insignificant results are likely to be absent (Nakagawa *et al*. 2021).

CONCLUSION

The forms of garlic that are effective in significantly reducing the number of microbes in meat products are freeze-dried garlic powder, oven-dried garlic powder, and garlic essential oil microcapsules. The reduction in microbial count was higher in raw meatball/minced mutton meat (between -4.166 $\log_{10}$ CFU/g and -2.459 $\log_{10}$ CFU/g). *Listeria monocyto-*

genes, sulfite-reducing anaerobes, and *Escherichia coli* were inhibited the most by the addition of garlic. The reduction in the number of each test microbe was as follows: *L. monocytogenes* by -6.626 to -2.009 $\log_{10}$ CFU/g; sulfite-reducing anaerobes by -4.521 to -2.193 $\log_{10}$ CFU/g; and *E. coli* by -2.771 to -1.225 $\log_{10}$ CFU/g.

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