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Effects of lactic acid bacteria and coagulase-negative staphylococci on dry-fermented sausage quality and safety: systematic review and meta-analysis

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Abstract

The meta-analysis aim was to confirm and quantifying the influence of starter cultures on microbiological and physical-chemical parameters of dry-fermented sausages at the end fermentation stage. The literature search yielded 1194 citations, and 77 studies with 178 experiments were eligible and included in the meta-analysis, a random-effects model was used to estimate the pooled weighted mean difference (MD) with 95% confidence interval (CI).The use of starter culture in dry-fermented sausages significantly reduced pH (MD: -0.364; CI: -0.414; -0.319), moisture (MD: -1.443; CI: -1.931; -0.955), a_w (MD: -0.011; CI: -0.017; -0.006), *Enterobacteriaceae* count (MD: -1.119; CI: -1.293; -0.945), yeasts and molds count (MD: -0.351; CI: -0.691; -0.084), and increased color component a* (MD: 0.859; CI: 0.266 ;1.452), color component L* (MD: 1.288; CI: 0.433; 2.143), LAB count (MD: 0.981; CI: 0.696 ;1.267), Staphylococci count (MD: 0.484; CI: 0.293; 0.675) and TVC (MD: 0.529; CI: 0.098; 0.959). The results of the sub-analysis suggest that the addition of LAB and LAB/CNS inocula have a greater effect on the physico-chemical and microbiological parameters studied in this work. In the meta-regression analysis, a positive linear relationship was found in starter culture sausages in comparison with control batch between LAB count and the dose of starter culture added, and in the pH and *Enterobacteriaceae* count with the passage of fermentation days. In contrast, a negative linear relationship was found between redness and increased casing diameter of the sausages. Therefore, our work shows impact that addition of starter cultures has on safety and quality of dry-fermented sausages.

Keywords: Dry-sausages, Meta-analysis, Coagulase Negative Staphylococci, Lactic acid bacteria, starter culture, food safety and quality.

1. Introduction

Fermented meat products have been consumed for centuries throughout the world and they constitute one of the most important types of food. Among them, dry-fermented sausage is characterized by its raw, preserved and unrefrigerated consumption and also by its vast shelf life. In addition, it has valuable organoleptic characteristics, including its typical red color, consistency, aroma and flavor (Kumar et al., 2017).

The manufacture of dry-fermented sausages differs substantially from one country to another and there are even differences between regions within the same country (Boumaiza et al., 2021). Currently, it is known that during the fermentation process the microbiota present in the meat plays a decisive role, where fermenting microorganisms participate in the microbiological stability of the fermented food, thus contributing to its organoleptic properties (Domínguez et al., 2016).

Starter cultures are defined as an individual or mixed living microorganisms, added at known concentrations to lead the fermentation process (Laranjo et al., 2019). Dry-fermented sausages can be manufactured without their assistance, although their inclusion can help ensure safety, standardize product features (including flavor and color), decrease the fermentation period, and produce the same quality product year-round in any climate zone. However, well-adapted and qualified presumption of safety (QPS) strains must be included, and the establishment of the starter culture must be checked to guarantee the quality and quantity of the cultures in order to obtain the expected performance (Leroy et al., 2019). Moreover, there is currently a trend to use indigenous starter cultures, which promote traditional product identity and unique characteristics by improving quality and safety, while maintaining the typical sensory attributes of these products (Palavecino et al., 2021). A great advantage of using indigenous starter cultures is that they are very well adapted to a particular product meat, precisely because they are

microorganisms selected from there indigenous microbiota, which provides the typicity of the dry fermented sausage.

Nowadays, starter groups that combine cultures of lactic acid bacteria (LAB), Gram-positive Catalase-positive Cocci (GCC+) (mainly non-pathogenic Coagulase-Negative Staphylococci, CNS), yeasts and molds are employed in the meat industry since they are the main microorganisms present in spontaneous fermentation of meat products (Laranjo et al., 2017). The bacteria are responsible for the microbial reactions that occur during meat fermentation, such as catalase activity, acidification and bacteriocin production. Molds are inoculated in high quantities on the surface of sausages. Their contribution to the final characteristics of these products may be related to their ability to ferment different sugars, to degrade peroxides and amino acids, and to their lipolytic activity (Najjari et al., 2020).

The most commonly used bacterial commercial starter cultures for the development of dry sausages were *Lactiplantibacillus plantarum*, *Latilactobacillus curvatus*, *Lacticaseibacillus casei*, *Lactiplantibacillus pentosus, Pediococcus pentosaceus* and *Pediococcus acidilactici*, belonging to the LAB, and some staphylococcal species, such as *Staphylococcus xylosus* and *Staphylococcus carnosus* (Lorenzo et al., 2014; Mejri et al., 2016).

Starters composed of LAB strains produce lactic acid that acts on meat proteins, modifying their water retention capacity, and thus contributing to the texture, moisture, flavor and aroma of the products while acting on their microbiological safety. On the other hand, Coagulase-Negative Staphylococci (CNS) have a fundamental role in the development of sausage organoleptic properties through the reduction of nitrates, giving them their characteristic red color, lipolytic and proteolytic activity (Li et al., 2022; Xiao et al., 2020).

Many articles claim that the addition of starter culture in dry-fermented sausages provides safety and quality based on the study of certain factors, such as pH and pathogen

count/detection. Therefore, it is important to collect all published information on the subject, in order to know the magnitude to which these factors affect dry- fermented sausages. For this purpose, statistical techniques are used to allow a quantitative evaluation of the results obtained to date. Hence, it is extremely relevant to carry out a meta-analysis.

This study aimed to perform a systematic review and meta-analysis to assess the effect of the addition of starter culture on the quality and safety of dry-fermented sausages and studied the microbiological and physical-chemical parameters.

2. Materials and methods

This systematic review was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Page et al., 2021). The research question, which was used as a guide for data search and eligibility, was: “Is there evidence from the literature that the addition of starter culture (lactic acid bacteria or coagulase-negative staphylococci) to dry-fermented sausages can modify their quality and safety characteristics compared with untreated sausages?; How much can the starter culture modify these characteristics?; Is the effect of the addition of starter culture related to the meat matrix used in the production of sausages?; Is the effect of the addition of starter culture in sausages related to the type of bacterial inoculum used?”.

2.1. Search strategy

An exhaustive systematic literature search was conducted for articles in the Scopus, Google Scholar, Scielo, Pubmed and ScienceDirect databases, unrestricted by language and published from 1980 to June 2023. Search terms included the words “sausages” AND “starter” AND “culture” AND (“fermented” OR “cured” OR “dry”). Studies were primarily evaluated, and the articles that met the a priori inclusion criteria were selected. Initially,

titles and abstracts were reviewed and certain research articles were removed due to irrelevance. Two reviewers independently selected eligible primary studies (María Ángeles Stegmayer and Noelí Estefanía Sirini), and any discrepancies and disagreement were resolved by consensus or discussion with a third reviewer (Lorena Soto).

2.2. Criteria for study selection

The full texts of the research articles accepted in the first screening were evaluated in the next step. The studies included in this systematic review and meta-analysis were selected based on the following criteria: randomized and controlled trials published in peer-reviewed journals between 1980 and June 2023. Dry-fermented sausages stuffed in natural or artificial casings with a diameter between 22 and 100 mm are included. No exclusion due to type of meat used in the formulation were made. To assess the effect of the addition of starter cultures on the quality and safety of sausages, studies with information on physico-chemical and microbiological parameters were chosen. Studies should report the mean value with measures of variance of physicochemical parameters: pH, water activity (a_w), moisture (M), color; and microbiological parameters: lactic acid bacteria count (LAB), total viable count (TVC), Staphylococci count (St), *Enterobacteriaceae* count (Et) and yeasts and molds count (Y-M) (Table S1 and S2). Assorted reviews, duplicate reports, and uncontrolled studies (dry-sausages without starter culture) were excluded. The term "study" refers to a scientific article that can involve one or more experiments (each experiment being a controlled one to compare the aggregate of LAB and/or CNS *vs*. control in dry-sausages). Experiments that indicated the use of a commercial starter but did not specify the starter strains were excluded. Experiments using yeasts or molds as a starter culture were also excluded.

2.3. Outcomes and definitions

Inoculation with LAB and/or CNS was analyzed as a tool that may enhance fermentation parameters, the microbiological composition, and the safety of dry-sausages. Data referring to the response variables were extracted on day 7 ± 3 days of sausage preparation, this being the end of ripening or fermentation. When the study included more than one inoculation strain in different experimental groups, each inoculated group was compared to the non-inoculated group separately.

2.4. Data extraction

From each research report, the article publication data (the author's last name, publication year, and country of author's origin), sample size, means and variations (standard deviation, SEM or VC) of the following response variables were extracted: pH, moisture (g/100 g of dry matter), a_w, color (L*, b* and a* component), LAB, TVC and yeasts-molds, Staphylococci and *Enterobacteriaceae* counts (log CFU per g). For each study, the methodology used to achieve the results was assessed; however, scores were not used to exclude studies (Lean et al., 2009).

The PlotDigitizer 2.5.0 software was used to extract data from the chosen studies that presented only graphs (Jelicic Kadic et al., 2016). Extracted data were compared with two reviewer authors, with any discrepancies being resolved by consensus.

Other relevant information extracted from the research articles was the type of meat included in the sausage, type of bacterial inoculum composition, type of inoculum (mono or multi-strain), starter species included, concentration of starter culture, and casing diameter. This information was incorporated in the meta-analysis with the aim of explaining the heterogeneity for random effect estimates across experiments and research articles.

2.5. Statistical and subgroups analysis

Statistical analysis was done using Comprehensive Meta-Analysis version 2.2 (2011). Due to continuous variables being analyzed, the effect measure used to present the results was the mean difference (MD) between the starter culture and control treatments with 95% confidence intervals using a random-effects model. In this model, the true effect could vary from experiment to experiment; between-experiment variability (true heterogeneity), as well as sampling error, are included (Borenstein et al., 2009). Heterogeneity among studies was assessed using the DerSimonian and the Laird test (Q-statistic). The degree of heterogeneity was quantified with the inconsistency index, I^2- statistic (Higgins & Thompson, 2002).

To evaluate the sources of heterogeneity, *a priori* subgroup analyses were planned depending on factors that could potentially influence the physicochemical and microbiological parameters of dry-fermented sausages: (1) type of bacterial inoculum composition (LAB, CNS or LAB/CNS), (2) type of inoculum (mono-strain *vs*. multi-strain), (3) the starter species strain used (with *Lactobacillus* spp. (species included in the genus formerly called *Lactobacillus* spp. or species included in the *Lactobacillaeae* family without the genus *Pediococcus* spp.) with *L. plantarum*, with *L. sakei*, with *Pediococcus* spp.; with *P. acidilactici*; with *Staphylococcus* spp.; with *S. carnosus*, and with *S. xylosus*), (4) the lean meat used in the preparation of sausages (pork, beef, among others), (5) with or without superficial yeasts or molds and (6) with or without nitrate/ite salts.

Additionally, a meta-regression analysis was performed to explore the sources of heterogeneity in the treatment effects. Meta-regression allows for evaluating the relationship between the casing diameter used in the preparation of the sausages, the dose of the starter culture in each experiment, and the sampling day as covariates, while the pH, moisture, a_w, color, TVC, LAB, Staphylococci, yeasts and molds, and *Enterobacteriaceae* are evaluated as outcomes. In this work, the seventh day of sausage processing was defined to extract information on physicochemical and microbiological

parameters, although several studies had information from days close to this one, which made it possible to perform a meta-regression with the sampling day. On the other hand, the starter culture dose was defined as the sum of the CFU/g of each strain in the case of the multi-strain inoculum.

2.6. Risk of bias assessment

An adjusted rank correlation test using the Egger method (Egger et al., 1997) and the Begg's test (Begg and Mazumdar, 1994) was performed in combination with funnel plots to analyze the publication bias. In those cases where any evidence of bias was observed (P< 0.01), the 'trim' and 'fill' method (Duval & Tweedie, 2000) was applied to estimate the quantity and magnitude of missing and resultant unbiased effect size.

3. Results

3.1. *Excluded studies*

The literature search yielded 1194 scientific papers on dry-fermented sausages and starter culture. Of the studies identified at the beginning of the systematic reviews, 871 were excluded on the basis of publication type: duplicate reports (n= 323), reviews (n= 38), probiotic topics (n= 20), isolation (n= 30), semi-dry sausages (n= 18), conference papers (n= 23), simulation model studies (n= 8), studies with added pathogens (n= 58), corrigendum studies (n= 7), thesis (n= 1), book chapters (n= 47), and studies in dry-fermented sausages for other purposes (n= 315) were disregarded.

Two hundred and eighty-four studies that passed initial screening were rejected due to a lack of statistical information for conducting a meta-analysis (n= 42), studies without control treatment (n= 115), and studies with non-useful sampling data (n= 50) (**Figure 1**). Finally, 77 studies and 178 experiments fulfilled the acceptability criteria to be included in this systematic review and meta-analysis.

3.2. Overview of included studies

The 77 studies eligible for inclusion in the current systematic review are presented in **Table S1,** and a detailed description is presented in **Table S2**. Most of the research papers reviewed did not evaluate the starter effect over all the parameters under study. Consequently, the number of studies included in the meta-analysis differed in each variable considered.

Of the screened studies, 38 were conducted before 2015 and the remaining 39 after 2015. Twenty-nine studies were performed in China, sixteen in Spain, ten in Italy, seven in Tunisia, three in Turkey and Japan, two in Portugal and Serbia, and one in Romania, Croatia, Korea, Brazil and Argentina.

Of all the experiments with a starter culture (n= 178), the largest proportion used a multi-strain inoculum (98 experiments). In addition, most of the experiments were composed of combined LAB/CNS inocula (98 experiments), followed by inocula composed of LAB in 67 experiments, and finally, there were 13 experiments with CNS composite inocula. **Figure 2** shows the most commonly used LAB and CNS species as starter cultures in dry-fermented sausages. Also, the majority of the experiments were sausages made from pork (n=144), followed by sausages made from beef (n=10), mutton (n=10), turkey (n=6), camel (n=5), poultry (n=2) and horse (n=1).

3.3. Physicochemical and microbiological parameters and analysis subgroups

The effects of the addition of starter culture in dry-fermented sausages on the physicochemical and microbiological parameters at the end of the ripening across studies are shown in **Table 1**. In the pooled estimate, the addition of starter culture decreased the pH, moisture, a_w, *Enterobacteriaceae* count ($P< 0.001$ in all cases) and yeast and molds count ($P= 0.01$) but increased the LAB, Staphylococci count ($P< 0.001$ in both cases) and TVC, color component a* and L* ($P= 0.015$, $P= 0.004$ and $P< 0.001$) of the sausages

compared to controls in the pooled mean difference random-effects model. In addition, the use of starter culture did not modify the color component b* (P= 0.124). Significant heterogeneity (I^2 statistic> 60%) was observed in all response variables. Hence, subgroups were assessed in order to find sources of variability.

In accordance with the subgroup analysis of the pH variable shown in **Figure 3a**, pH decreased in experiments with mono- and multi-strain starter culture (P< 0.001). In turn, pH decreased in experiments with LAB, LAB/CNS, and CNS starter cultures (P< 0.001, P< 0.001 and P= 0.008, respectively). The effect on pH was greater when LAB was used as culture starter since the pH value was the lowest in this case. Considering the type of meat used, subgroup analysis indicated that the pH decreased in sausages with pork, poultry, mutton, beef and camel meat (n=116, P< 0.001; n=3, P= 0.04; n=10, P< 0.001; n=7, P=0.04

; n=2, P< 0.001, respectively), however with turkey and horse meat the pH did not change (n= 3, P= 0.55 and n=1, P= 0.19, respectively). Among the starter species strains used, it was observed in the subgroup analysis that the pH decreased significantly due to inoculation with *Lactobacillus* spp. (P< 0.001), *Staphylococcus* spp. (P<0.001) and *Pediococcus* spp. (P< 0.001). Finally, it was observed that inoculation with *Lactobacillus* spp. had a greater effect on pH than without *Lactobacillus* spp. inoculation.

Regarding moisture, the sub-analysis showed that the use of mono- and multi-strain starter culture decreased moisture compared to the control (**Figure 3b**). When studying the type of inoculum added, no differences in moisture were found when using LAB and CNS, but moisture decreased significantly when using LAB/CNS type inoculum as starter culture (P= 0.011). The sausages with camel and beef meat showed no difference in moisture content compared to sausages without the addition of starter culture (n= 1, P= 0.13 and n=1, P= 0.35, respectively). On the other hand, the other types of meat showed lower moisture content with respect to the control (P< 0.001). The use of *Lactobacillus*

spp., *Pediococcus* spp. and *Staphylococcus* spp. strains decreased the moisture content of the sausages compared to the control group.

Water activity decreased when using mono and multi-strains (**Figure 3c**), although when differentiating the inoculum composition, it was found that the use of starter culture with LAB and the inocula with LAB/CNS caused the decrease in a_w, but the use of the inoculum with CNS did not present a difference in the a_w value in comparison to the control group (without starter culture). In addition, the use of the *Lactobacillus* spp. in the inoculum was important for the decrease in a_w, since when the strain with *Lactobacillus* spp. was not used a_w did not differ as compared to the control group. The sausages with turkey and beef meat showed no difference in a_w value compared to sausages without the addition of starter culture (n= 3, *P*= 1 and n=4, *P*= 0.3, respectively). On the other hand, the other types of meat showed lower a_w value with respect to the control (n= 69, *P*< 0.01 for pork meat and n=9, *P*< 0.01 for mutton meat).

The use of starter culture in sausages increased the color component a* in experiments using mono-strain inocula (n= 24, *P*< 0.001), LAB (n=23, *P*< 0.001) and CNS inoculum (n= 2, *P*< 0.001) (**Figure 3d**). In contrast, an increase of the estimate point in the color component a* was observed when LAB/CNS was used as inoculum with respect to the control group, but the confidence interval includes zero (n= 13, *P*= 0.17). The sausages with inoculum containing *Lactobacillus* spp. showed an increase in color a* with respect to the control (n= 34, *P*= 0.025), but the increase was greater when *Lactobacillus* spp. was not used in the inoculum (n=4, *P*< 0.001). In particular, the use of *Latilactobacillus sakei* in the starter culture resulted in an increase in color a*, while this increase was lower when it was not used. However, *Lactiplantibacillus plantarum* showed an opposite effect, not differing from the control when used but increasing the color a* when *L. plantarum* was not used. The addition or not of *Staphylococcus* spp. as starter culture resulted in an increase of the color component a* with respect to the control group. In addition, a sub-analysis of

the color component a* was performed according to the addition or not of nitrate/ite salts in the preparation of the sausages, and an increase in color was observed with respect to the control in both cases (P< 0.001 with nitrate/ite salts and P= 0.033 without nitrate/ite salts). Regarding the type of meat, the subgroup analysis indicated that pork, mutton and poultry meat showed an increase in the color component a* compared to the control batch (P< 0.001, P= 0.012 and P= 0.028, respectively). In contrast, sausages with beef and camel meat showed no difference in this property compared to the control batch (P= 0.96 and P= 0.57, respectively).

Continuing with the subgroup analysis in terms of physicochemical properties, the use of starter culture in sausages increased the L* color component in the experiments using mono-strain (n=24, P< 0.001), LAB inoculum (n=23, P< 0.001), pork meat (n=26, P< 0.001) and with inocula of *Lactobacillus* spp. (n=34, P< 0.001) (**Figure 3e**). In sausages with mutton, beef and camel meat, the addition of the starter culture did not change the colour component L* (n= 4, P= 0.97, n=4, P= 0.95; n=2, P= 0.098). On the other hand, the color component L* decreased when using inoculums with CNS, although this point case has an n of 2 and P= 0.005.

With respect to microbiological parameters, the total viable count increased in the experiments with LAB, CNS and LAB/CNS strain (n= 30, P= 0.032; n= 7, P= 0.03 and n=42, P< 0.001, respectively) (**Figure 4a**). Concerning the species used in the starter culture, the addiction of *Lactobacillus* spp. and *Staphylococcus* spp. showed an increase in TVC (P< 0.001 in both cases), but the experiments with *Pediococcus* spp. showed no differences concerning the control (P= 0.22). As for the type of meat, an increase in TVC was observed in the experiments using pork, camel and poultry meat, while a decrease was observed in those studies using turkey meat (n=6, P= 0.025).

A higher LAB count was observed in all subgroup analyses (**Figure 4b**), except in the turkey and horse meat experiments (n= 6, P< 0.001 and n=1, P< 0.001, respectively).

Staphylococci counts increased with the addition of multi-strain inocula (n= 83, *P*< 0.001) and LAB/CNS inocula (n= 79, *P*< 0.001). On the contrary, when using mono-strain inocula (n= 33, *P*= 0.11), it was observed that the point estimate of Staphylococci count decreased, although the confidence interval indicates that no differences were reported with respect to the control group. Specifically, in terms of mono-strains, it was observed that when using CNS as an inoculum the Staphylococci count increased with respect to the control group, while it decreased when using LAB despite the confidence interval including zero in both cases.

Regarding the species of the strains, a great difference was observed in Staphylococci counts when using or not using *Staphylococcus* spp. in the inocula. This count increased when *Staphylococcus* spp. were used (n= 87, *P*< 0.001), but decreased markedly when *Staphylococcus* spp. were not added (n= 29, *P*= 0.002). Therefore, these results indicate that the use of *Staphylococcus* spp. in the multi-strain inoculum or in LAB/CNS was key to the increase in Staphylococci counts. On the other hand, an increase in the Staphylococci count was noticed with respect to the control group when *Lactobacillus* spp.was used or not in the inoculum, although the increase in this count was greater when this strain was not used in the starter culture.

Enterobacteriaceae counts are lower in sausages with starter culture regardless of the subgroup analysis performed (*P*< 0.001). When CNS inoculum was used, a decrease in *Enterobacteriaceae* counts was noticed, but the confidence interval includes zero (n= 6, *P*= 0.17) (**Figure 4d**). In addition, differences were found when using starter culture with *Lactobacillus* spp. , since the *Enterobacteriaceae* counts decreased in the experiments with inocula with *Lactobacillus* spp. (n= 65, *P*< 0.001), and the count increased when it was not used (n=12, *P*= 0.1).

Finally, **Figure 4e** shows the sub-analysis performed for yeasts and molds count. Moulds and yeast count are observed to decrease when LAB are used. (n=12 and *P*< 0.001). The

figure also shows the effect on moulds and yeasts count when surface mould was used on sausages. Thus, using surface mold showed a small increase in the point estimate for yeasts and molds count over the control group, but the confidence interval includes zero (n=3 and *P*= 0.469). In contrast, not using surface mold showed a decrease in molds and yeasts count (n=29 and *P*< 0.001). These results show that the number of experiments in which bacterial starter cultures with surface molds were used was very few (n=3).

Based on the results from the meta-regression analysis (**Table 2**), interactions were found between the dose of the starter culture and color component b* (*P*= 0.016), color component L* (P<0.001), LAB (*P*= 0.01), and yeasts and molds count (*P*= 0.01). It was observed that the addition of higher doses of starter culture to the sausages increased the concentration of LAB and yeasts and molds count. On the other hand, it was observed that higher doses of starter culture decreased the b* and L* color components.

The casing diameter of the sausages was associated with color component a* (*P*< 0.001), moisture (*P*= 0.002), and a_w (*P*= 0.004). The larger the sausage casing diameter, the greater the difference between sausages with and without starter in a_w and moisture. However, the color component a* decreases when the casing diameter is larger.

Finally, the sampling day was associated with pH (*P*= 0.046), moisture (*P*< 0.001), component color L* (P< 0.001), LAB count (*P*= 0.017), *Enterobacteriaceae* count (*P*= 0.002) and Staphylococci count (*P*< 0.001) in the meta-regression (**Table 2**). The increase in days of fermentation increases the difference between the control and treated groups in the pH, moisture, *Enterobacteriaceae*, and Staphylococci parameters. On the contrary, it decreases the difference in LAB and component color L* parameters.

Meta-regression analysis was also performed according to the percentage of nitrate/nitrite salts added in the sausage formulation, with no interaction with the color component a* being observed (Intercept: 0.77; Slope: 0.07; *P*= 0.98).

As part of this study, the Egger's regression test, the Begg and Mazumdar's rank correlation test, and the fail-safe N method were used to find publication bias in the included studies for each of the eleven parameters assessed. The results are summarized in **Table 3**. There was a publication bias for the L* color component; however, the great number of scientific articles included in this meta-analysis provides valid results beyond the potential bias. This evidence of publication bias requires that these results be interpreted with caution.

4. Discussion

During the production of dry-fermented sausages, two maturation phases can be distinguished, fermentation and drying, considering that the fermentation phase occurs during the first days and then the drying phase begins. This work was focused on studying the changes given at the end of fermentation, which occurs approximately 7 days after the sausages are produced, although this may depend on certain characteristics such as the diameter of the sausages and the relative humidity to which they were subjected. During the maturation of the dry-fermented sausage, microbiological, biochemical, and sensory changes occur that are closely related to the activity of the dominant microorganisms present in the matrix of the sausage (Essid & Hassouna, 2013; Liu et al., 2023).

This quantitative meta-analysis of data from randomized controlled experiments displayed that the addiction of starter culture with LAB, CNS, and LAB/CNS strains lowered pH in the pooled estimate. In many studies, it was found that pH values decreased rapidly initially in sausages. In particular, the inoculation of starter cultures results in more marked acidification than that found in the control group (no starter culture) (Dias et al., 2022). Sausages inoculated with *Lactobacillus* spp. show a strong acidifying activity during fermentation, generating a greater decrease in the pH of the sausages in comparison with the sausages of the control group. In other words, the pH decreases as the BAL count

increases. In fact, the main function of LAB is to acidify the sausage, although they can also slow down proteolytic and lipolytic activity (Munekata et al., 2021). In poultry, pork, mutton, beef and camel meat, the pH of the sausages decreased as the number of LAB increased. In contrast, the pH of horse and turkey sausages did not decrease because the number of LAB did not increase.

In the meta-regression analysis, the increase in the number of days of fermentation was related to the increase in the difference in pH between the sausages of the control group and the treated group. This is due to the fact that the inoculated bacteria perform their function as starters and cause the acidification of the sausage, which becomes more pronounced as the days pass.

It is known that the moisture content and a_w of sausages, whether inoculated or non-inoculated, decrease gradually during the manufacturing process of sausages. The water has to diffuse from the inside of the sausage to the surface and then evaporate into the chamber environment. Both rates, diffusion and evaporation, must proceed similarly. Therefore, maintaining this high humidity in the fermentation chamber is necessary to avoid excessive weight loss and to provide some control of microbial spoilage (Ducic et al., 2018). In addition, this water loss is due to the decrease in pH, which causes protein denaturation and thus a decrease in the water holding capacity of myofibrillar proteins (Krvavica et al., 2012). In the meta-analysis, significant differences in a_w and moisture at the end of maturation were found when starter culture was added compared to spontaneously fermented sausages. A sub-analysis indicated that the inoculation of sausages with *Lactobacillus* spp. was decisive in significantly lowering a_w and moisture than that of the control sausages. On the other hand, inoculation with CNS did not generate significant differences concerning the control group. In addition, the relationship between a_w and moisture with the casing diameter can be explained as a function of the bacteria used in the starter culture, where inoculation with LAB generates a greater

decrease in pH, and this contributes to the drying process with a consequent reduction in a_w and moisture.

The outcomes of the meta-analysis displayed an increase in LAB counts concerning the control group at the end of fermentation, both when the sausages were inoculated with LAB, CNS or LAB/CNS as well as with mono or multi-strains. On the one hand, the increase in LAB count in inoculated sausages was probably because both mono- and multi-strain inocula contain LAB. Therefore, this means that the added LAB adapted to the meat matrix from the beginning of fermentation to the end; the growth rate is fast during fermentation, thus becoming the dominant microbe as expected. On the other hand, in the case of inoculation with CNS, an increase in LAB counts was also observed, although this increase is lower than when LAB was used. This is evidence that the addiction of starter cultures has a favorable influence on the growth of native LAB in sausages by generating synergism, where no inhibition effects occur between the added bacteria and those of the meat matrix (Casaburi et al., 2008). In this work, we found a correlation between the higher LAB count and the decrease in pH in the inoculated sausages. In fact, carbohydrate fermentation by LAB results in the production of organic acids that lower pH (Sallan et al., 2023). This acidification has several positive technological aspects such as faster drying, inhibition of pathogenic microorganisms, activation of muscle proteases, enhanced texture profile through denaturation and coagulation of proteins and reddening through the formation of nitric oxide and nitrosyl myoglobin (Bedia et al., 2011). In addition, an increase in the dose of starter culture resulted in an increase in LAB count at the end of fermentation. In contrast, with the passage of days, LAB counts were found to decrease. This shows that although at the beginning of fermentation the inoculated sausages show higher LAB counts, as the days of fermentation pass the difference in LAB counts between the control and inoculated groups becomes smaller. It should be noted that non-inoculated sausages contain native LAB from the meat matrix, and these grow over time. However,

the time it takes for indigenous LAB to grow to levels similar to inoculated LAB is crucial to guarantee the quality and safety of dry-fermented sausages.

In the present work, the inoculated sausages presented a higher TVC count compared to the sausages of the control group at the end of fermentation. The increase in TVC counts in the studies using multi-strains and LAB inoculum is related to the fact that all these experiments contain LAB in their formulation and, therefore, have a higher LAB count compared to the control group, as described above. The TVC count in technological treatments allows us to evaluate the impact of various hygienic operations on food. A high number of mesophilic aerobic bacteria should correspond to an unacceptable product from a hygienic perspective (Cenci-Goga et al., 2016). However, a high level of bacteria may be compatible with a healthy product, as in fermented products. This is why the pathogenic bacteria count is of greater relevance (Cheng et al., 2018).

This meta-analysis displayed that the use of starter culture affected Staphylococci counts in the pool estimate. Staphylococci are among one of the important microbiological parameters in sausages because, together with LAB, they are the dominant microflora in sausages at all stages of maturation. In fact, *Staphylococcus* spp. are particularly linked to the nitrate reductase activity, lipolytic and proteolytic reactions that are very important for achieving particular sensorial properties in dry-cured meat products (Li et al., 2022).

In the results of the Staphylococci counts sub-analysis, large differences were found according to the type of starter culture strains used. When using multi-strains as starter cultures in sausages, a higher Staphylococci count was noticed at the end of fermentation compared to the control. The sausages with the addition of CNS showed, at the end of fermentation, a higher Staphylococci count compared to the control group, but this increase was lower than when LAB/CNS was used. In contrast, the growth of staphylococci in samples inoculated with LAB seems to be significantly affected by the acidification of these LAB in the meat matrix, since no increase in Staphylococci counts

was observed. Therefore, this demonstrates the importance of choosing LAB and CNS with a good interaction, so that both grow during fermentation.

In addition, the following relationship was found in the meta-regression analysis: the Staphylococci count increased with the passage of fermentation days compared to the control group, showing that the inoculated CNS are not inhibited by the other bacteria present in sausages (endogenous or inoculated). In contrast, the LAB count followed an opposite trend, as this LAB count approaches the control group sausages with the passage of days. This is possibly because the growth rate of LAB is fast at the beginning of fermentation when LAB starter cultures are added but it is slow for native LAB; however, at the end of fermentation the LAB count may equalize. All experiments with the addition of starter culture displayed a significant reduction in *Enterobacteriaceae* counts in comparison with the control group, except when CNS inocula were used. As seen above, the use of the CNS inoculum generated a decrease in pH with compared to the control batch, but to a lesser extent than when the LAB inoculum was used. It is probable that the acidity generated in the sausages was not sufficient to suppress the growth of *Enterobacteriaceae*. In this case, the need to enhance hygiene protocols and use better quality raw materials is evident. Among the subgroups of starter strains used, no significant differences were found only when the *Lactobacillus* spp. strain was not used. For this reason, it is a determining factor that the starter culture contains strains with *Lactobacillus* spp., either as mono or multi-strain inocula. *Enterobacteriaceae* are considered biomarkers of potential microbiological contamination during processing, so suppressing the growth of *Enterobacteriaceae* enhances the safety of fermented sausages (Chen et al., 2021). This decrease in the *Enterobacteriaceae* count is due to the decrease in pH caused by LAB, more specifically, the *Lactobacillus* spp. strains. The meat matrix contains the nutrients necessary for the growth of LAB, which is why they have a high growth rate, thus generating an excluding competition with *Enterobacteriaceae*. Moreover,

the antimicrobial compounds excreted by LAB, “such as bacteriocins”, may be contributed to the reduction of the number of viable cells of *Enterobacteriaceae* (Gao et al., 2014)*.*The meta-regression results showed that the difference in *Enterobacteriaceae* counts between the inoculated group and the control batch increased with the passage of fermentation days. This is related to the higher growth of LAB in inoculated sausages, which causes *Enterobacteriaceae* to be reduced during the fermentation stage.

The addition of starter culture modify the molds and yeasts count in the pooled estimate. Moulds and yeasts can be used as starter cultures, and yeasts are generally applied on the surface, usually by spraying or dipping, and can improve the sensory and external properties of the sausages (Talon et al., 2007). Surface starter cultures form a protective layer, which favors color formation and hinders the occurrence of premature fat autoxidation phenomena due to catalase activity. Certain yeasts species are capable of proliferating not only on the surface but also in the interior of fermented dry-sausages (Flores et al., 2015; Jeong et al., 2023). This work evaluated the use of LAB and CNS as starter cultures, but not molds and yeasts. Among the 77 studies eligible for inclusion, four studies included the use of surface molds in sausages with the aim of preventing the growth of undesirable molds and yeasts. Although no significant differences were observed with respect to the control group in sausages with and without surface molds, in each case the point estimates were different, and the tendency is for this count to decrease when surface molds were not used in sausages (n=29, $P< 0.01$). Therefore, it is possible that when surface molds are used, some of them pass into the meat matrix. However, these findings are based on a low number of experiments with surface molds (n=3), therefore, the effect should be interpreted with extreme care.

Color is the main factor affecting consumer acceptance of fermented meat products. The formation and stability of color are important for sausages. During fermentation, changes occur in the internal color value of L* (lightness), a* (redness) and b* (yellowness) of

fermented sausages (Škaljac et al., 2018). The meta-analysis displayed that sausage inoculation with respect to the control batch did not significantly modify the b* component, however, it modified the a* and L* components.

Higher values were observed in the L* color component in the inoculated sausages in comparison with the control at the end of fermentation when LAB was used as a starter culture, which corresponds to a higher water loss and, consequently, lower a_w and moisture as discussed above (Hernández Salueña et al., 2019). By contrast, the a* component increased at the end of fermentation in the inoculated sausages.

In the subgroup analysis for the color component a*, it was observed that the mono-strain inocula showed an increase in redness. In particular, it is observed that the increase is very marked when using CNS as starter culture. An increase is also observed when using LAB and LAB/CNS, but to a lesser extent compared to using CNS. This may be related to the autochthonous Staphylococci in the meat matrix being capable of acting on the color of the meat matrix. The variation of redness during the ripening of dry-fermented sausages is related with the formation of nitrosomyoglobin pigment (pink-red) (Miller et al., 2021). During fermentation, microorganisms have the ability to modify the color of meat from brown to bright red. In general, it is reported that staphylococci and lactic acid bacteria are involved in the degradation of nitrite to nitric oxide, favoring the formation of nitrosomyoglobin and consequently intensifying the red color of sausages (Cruxen et al., 2017; Ras et al., 2018). It is emphasized in this meta-analysis that sausages with LAB as starter culture showed an increase in red color with respect to sausages with spontaneous fermentation. This coincides with several authors who also reported that LAB probably possesses heme-independent nitrate reductase and nitrite reductase activities, which are directly involved in the mechanisms of nitrosomyoglobin formation that result in the formation of the typical pink color of fermented meat products (Chen et al., 2016; Zhu et al., 2020).

Nitrate and nitrite salts are used for the curing of meat products. In most countries, the addition of both substances, is controlled since the residual amounts are regulated. The effective substance is nitrite, which acts mainly as an inhibitor of some microorganisms. Although the role of nitrite in the formation of the pink color of cured meat products is also well known (Flores et al., 2021; Sallan et al., 2023), the sub-analysis performed for the color component a* showed that redness increases with the addition or not of nitrate/ite salts in sausages. In addition, the color a* of sausages was not associated with the percentages of nitrate/ite salt added in the meta-regression. All this suggests that these salts may play a minor role in the formation of the red colour depending on the process conditions, the addition of spices, the fermentation process and other factors applied. However, this fact does not change the importance of adding these salts to ensure antimicrobial action against pathogens such as *Clostridium botulinum*.

The results related to the type of meat used showed that the effects of the starter cultures were clearly seen in pork sausages. Although the vast majority of dry-fermented sausages are made from pork, numerous studies have assessed the possibility of reformulating fermented sausages using other types of meat, such as goat, foal, sheep, lamb, camel, among others. The addition of starter cultures in sausages promotes reddening in meats that are naturally paler or less red, whereas in sausages made from naturally redder meats the starter cultures are not able to redden the sausages further. Also, the addition of starter cultures in the sausages promoted higher values of the L* colour component in the paler meats (poultry and pork), but showed no change in the red meats (beef, camel and lamb). The sausages formulated with paler meats and starters reached a pH nearer to the protein isoelectric point where the water holding capacity decreases, generating free water in the sausages, which is related to the increase of the *L colour component (Sirini et al., 2023). The potential for using non-pork meats is the contribution of higher nutritional value and lower fat content, as well as the use of meats that are more accessible in certain

regions (Stajić et al., 2013). In the future, more experiments will need to be incorporated into the new study to elucidate the effect of other types of meat than pork.

5. Conclusions

To our knowledge, this is the first meta-analysis comparing the use or not of starter culture on physicochemical and microbiological parameters in dry-fermented sausages. The results of this meta-analysis suggest that the effect of inoculants on these parameters may differ depending on the composition of the inoculant (LAB and/or CNS). The addition of LAB or LAB/CNS to sausages showed the best effect on sausage fermentation, evidencing improvements in both sausage safety and quality. On the one hand, the addition of LAB determined a decrease in sausage pH; the higher number of LAB inhibited the growth of *Enterobacteriaceae*, which is directly related to sausage safety. Thus, positive linear relationship was found in starter culture sausages in comparison with control batch between LAB count and the dose of starter culture added, and in the pH and *Enterobacteriaceae* count with the passage of fermentation days. In contrast, a negative linear relationship was found between redness and increased casing diameter of the sausages. On the other hand, the CNS were found to be determinant in giving the optimal red color to the sausages, a characteristic that is very important for consumer acceptance. However, inocula with LAB also had the ability to generate this optimum color. Therefore, the addition of starter cultures in dry-fermented sausages is a fundamental tool to standardize technological and food safety conditions in these food products.

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Figure captions

Figure 1. Frequency of (a) LAB and (B) CNS species used as starter cultures in the experiments included in the meta-analysis.

Figure 2. PRISMA flow diagram of study selection.

Figure 3. Subgroup analysis comparing the inoculation effects of sausages with LAB and/or CNS on physical-chemical parameters: (a) pH, (b) moisture, (c) a_w, (d) color component a*, and (e) color component L*. Note: *Lactobacillaeae* refers to species included in the genus formerly named *Lactobacillus* spp. or to species included in the family *Lactobacillaeae* without the genus *Pediococcus* spp.

Figure 4. Subgroup analysis comparing the inoculation effects of sausages with LAB and/or CNS on microbiological parameters: (a) TVC, (b) LAB count, (c) Staphylococci count and (d) *Enterobacteriaceae* count (e) yeasts and molds count. Note: *Lactobacillaeae* refers to species included in the genus formerly named *Lactobacillus* spp. or to species included in the family *Lactobacillaeae* without the genus *Pediococcus* spp.

Table 1.

Effect of the use of starter culture on physicochemical and microbiological parameters of sausages at the end ripening.

Parameters	n	MD	95% CI	*P*-value	I^2 (%)
pH	141	-0.364	-0.414 ; -0.319	**<0.001**	99.39
Moisture	64	-1.443	-1.931 ; -0.955	**<0.001**	99.20
a_w	85	-0.011	-0.017 ; -0.006	**<0.001**	97.68
Color a*	38	0.859	0.266 ; 1.452	**0.004**	95.69
Color b*	36	-0.450	-1.024 ; 0.123	0.124	95.02
Color L*	38	1.288	0.433 ; 2.143	**<0.001**	97.14
LAB counts	156	0.981	0.696 ; 1.267	**<0.001**	99.82
TVC	79	0.529	0.098 ; 0.959	**0.015**	99.96
***Enterobacteriaceae* counts**	78	-1.119	-1.293 ; -0.945	**<0.001**	99.64
Staphylococci counts	116	0.484	0.293 ; 0.675	**<0.001**	99.40
Yeasts and molds count	32	-0.351	-0.619 ; -0.084	**0.010**	97.60

n= number of experiments. MD= difference in mean between starter culture and control treatments. CI= confidence interval. Significant differences (P value) are highlighted in bold.

Table 2.

Summary of random weighted meta-regression analysis for independent variables (dose of the starter culture in each experiment, casing diameter of sausages and sampling day), that influenced the effects between inoculated and non-inoculated treatments for the physicochemical and microbiological parameters.

Covariates	**Dose of starter culture**			**Casing diameter of sausages**			**Sampling day**		
	Intercept	Slope	*P*-value	Intercept	Slope	*P*-value	Intercept	Slope	*P*-value
pH	-0.712	0.054	0.070	-4.49	0.002	0.598	-0.642	0.039	**0.046**
Color a*	-0.808	0.278	0.476	4.45	-0.09	**<0.001**	0.09	0.14	0.565
Color b*	1.981	-0.210	**0.016**	-2.205	0.047	0.118	-3.122	0.394	0.109
Color L*	12.59	-1.614	**<0.00**	2.05	-0.0089	0.839	10.82	-1.326	**<0.001**
Moisture	-4.59	0.476	0.081	0.782	0.046	**0.002**	-6.73	0.704	**<0.001**
a_w	-0.006	-0.0014	0.740	-0.041	0.0006	**0.004**	-0.013	-0.0002	0.917
LAB counts	-1.393	0.344	**0.01**	1.272	-0.008	0.289	2.245	-0.183	**0.017**
TVC	0.373	0.001	0.99	0.594	-0.005	0.319	1.13	-0.10	0.08
***Enterobacteriaceae* counts**	0.224	-0.222	0.07	-0.985	-0.0032	0.625	-2.86	0.252	**0.002**
Staphylococci counts	0.126	0.009	0.283	-0.046	0.013	0.11	-2.51	0.427	**<0.001**
Yeasts and molds count	-1.391	0.343	**0.01**	0.906	-0.026	0.077	-0.965	0.097	0.369

Significant differences (P value) are highlighted in bold.

Table 3. Results of publication bias detection.

Variable response	Fail-safe n[a]	Begg and Mazumdar test	Egger's regression test	
			Intercept	p
pH	0	0.419	-2.57	0.042
Color a*	0	0.864	1.04	0.424
Color b*	0	0.570	-2.59	0.065
Color L*	11	0.036	3.57	0.018
Moisture	12	0.008	2.27	0.115
a_w	5	0.638	-2.81	0.023
LAB	20	0.265	2.41	0.082
TVC	5	0.495	1.28	0.217
***Enterobacteriaceae* counts**	0	0.233	-2.30	0.300
Staphylococci counts	4	0.096	-0.58	0.722
Yeasts and molds count	8	0.089	2.65	0.187

[a]Number of experiments required to reverse the effects are calculated on the condition of P= 0.05.

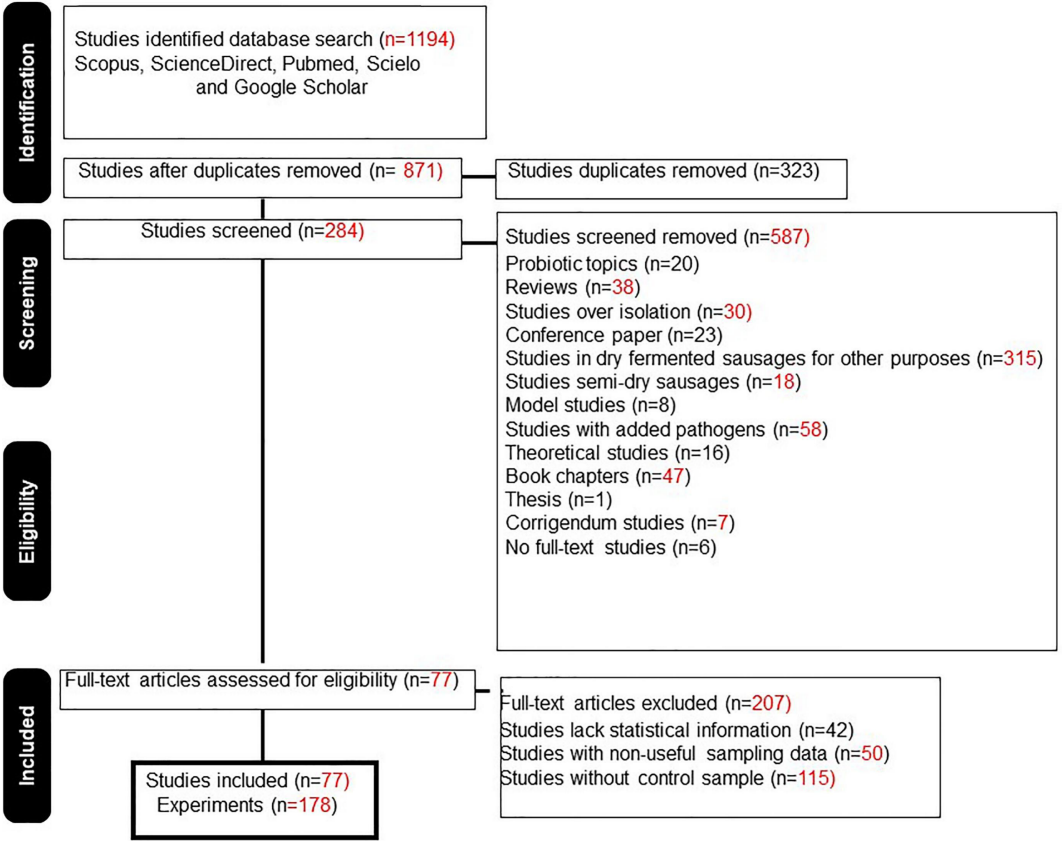
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- The meta-analysis allowed to quantify the effect of starter addition on sausages
- Sub-analysis suggests that LAB and LAB/CNS inocula has a greater effect
- Negative linear relationship was found between redness and increased casing diameters
- Positive linear relationship was found in pH and *Enterobacteriaceae* count along time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Scopus, ScienceDirect, Pubmed, Scielo
and Google Scholar
Studies after duplicates removed (n= 871)
Studies duplicates removed (n=323)
Screening
Studies screened (n=284)
Studies screened removed (n=587)
Probiotic topics (n=20)
Reviews (n=38)
Studies over isolation (n=30)
Conference paper (n=23)
Studies in dry fermented sausages for other purposes (n=315)
Studies semi-dry sausages (n=18)
Model studies (n=8)
Studies with added pathogens (n=58)
Theoretical studies (n=16)
Book chapters (n=47)
Thesis (n=1)
Corrigendum studies (n=7)
No full-text studies (n=6)
Eligibility
Included
Full-text articles assessed for eligibility (n=77)
Full-text articles excluded (n=207)
Studies lack statistical information (n=42)
Studies with non-useful sampling data (n=50)
Studies without control sample (n=115)
Studies included (n=77)
Experiments (n=178)

Figure 1

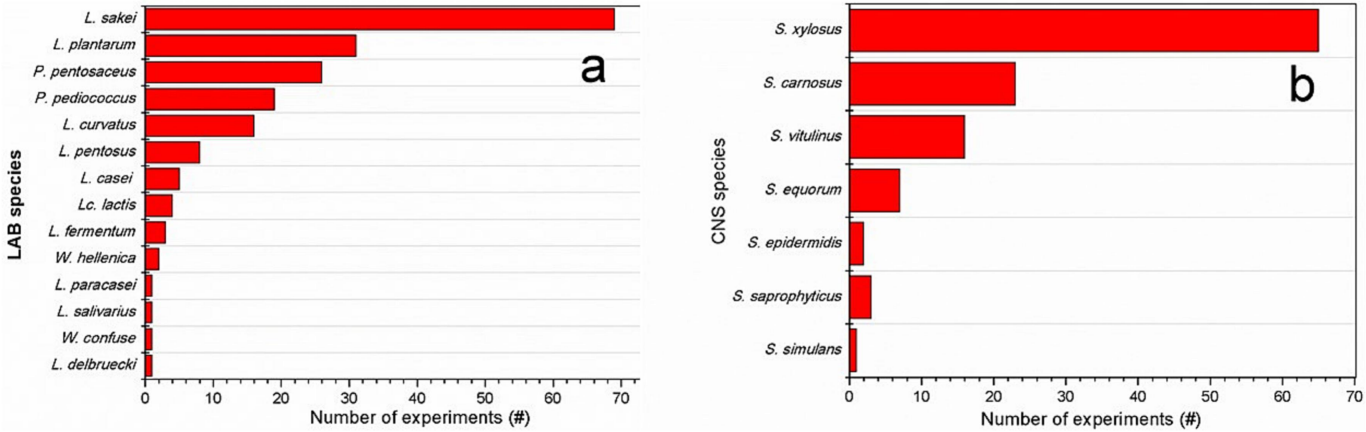

a
LAB species
L. sakei
L. plantarum
P. pentosaceus
P. pediococcus
L. curvatus
L. pentosus
L. casei
Lc. lactis
L. fermentum
W. hellenica
L. paracasei
L. salivarius
W. confuse
L. delbruecki
0
10
20
30
40
50
60
70
Number of experiments (#)
b
CNS species
S. xylosus
S. carnosus
S. vitulinus
S. equorum
S. epidermidis
S. saprophyticus
S. simulans
0
10
20
30
40
50
60
70
Number of experiments (#)

Figure 2

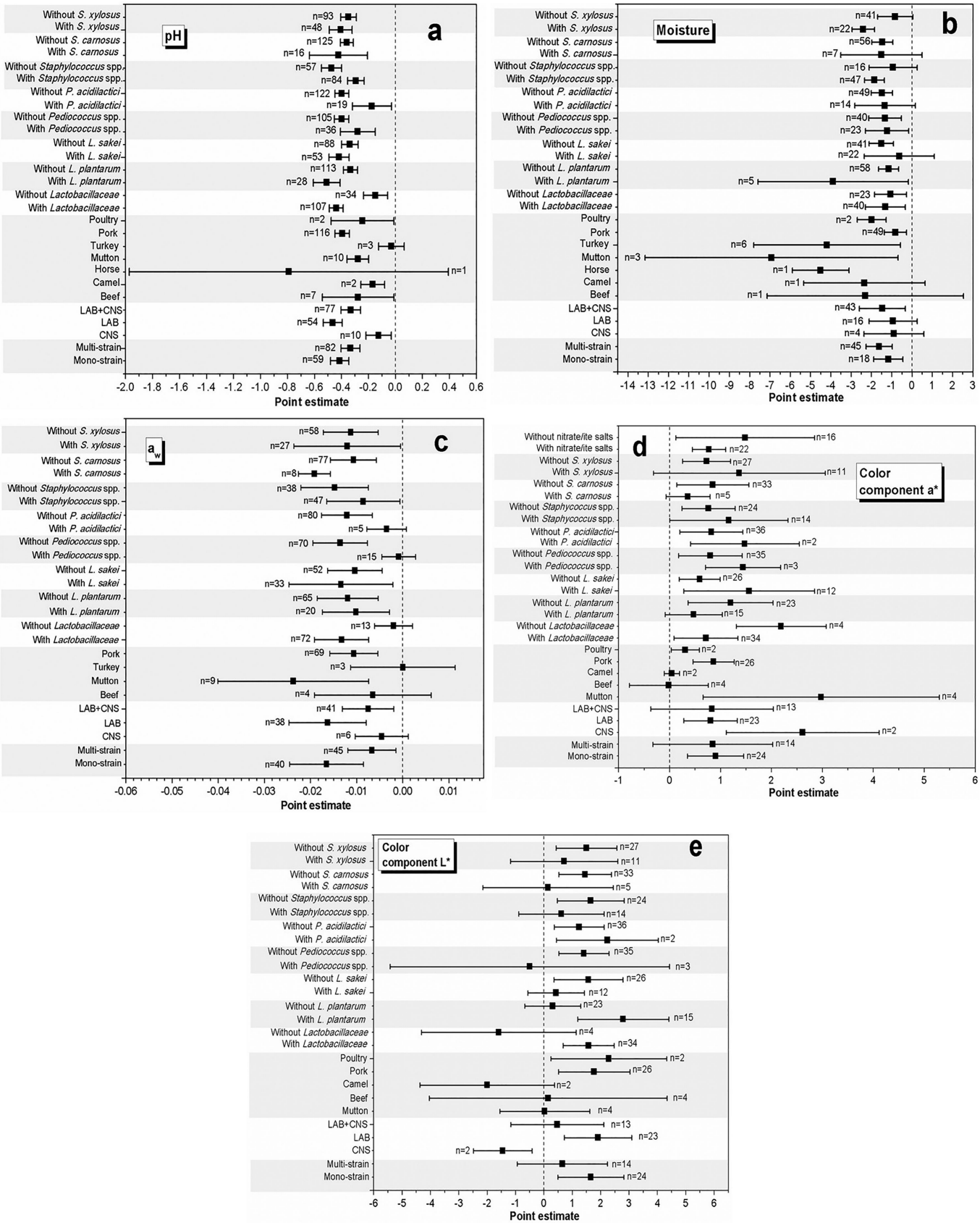
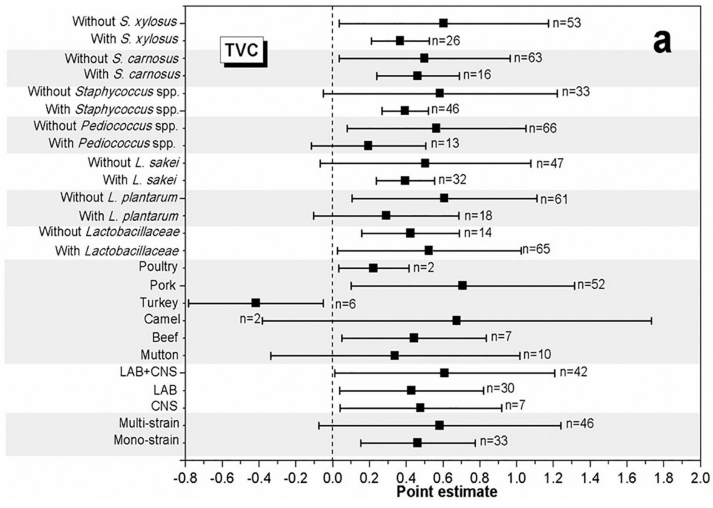
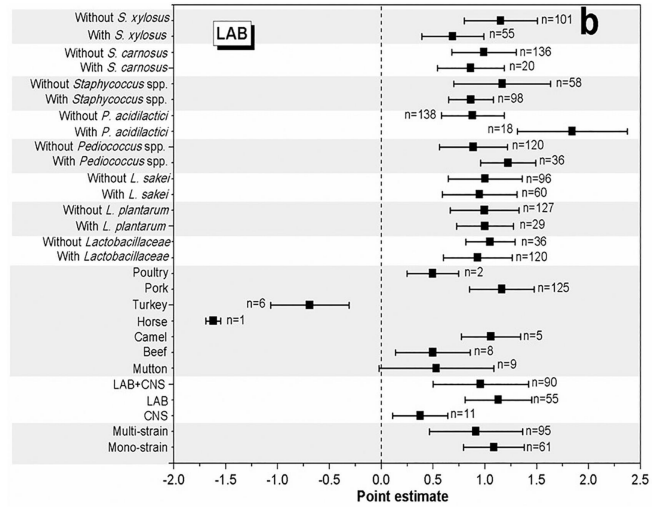
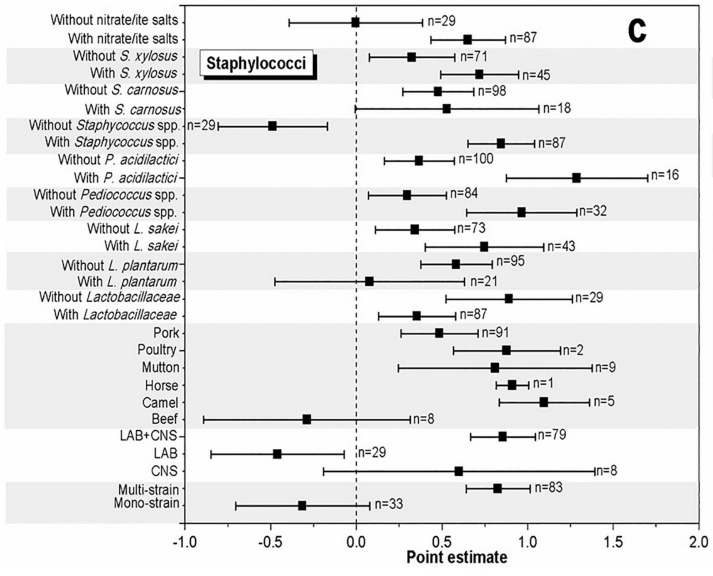

a
pH
Without S. xylosus
With S. xylosus
Without S. carnosus
With S. carnosus
Without Staphylococcus spp.
With Staphylococcus spp.
Without P. acidilactici
With P. acidilactici
Without Pediococcus spp.
With Pediococcus spp.
Without L. sakei
With L. sakei
Without L. plantarum
With L. plantarum
Without Lactobacillaceae
With Lactobacillaceae
Poultry
Pork
Turkey
Mutton
Horse
Camel
Beef
LAB+CNS
LAB
CNS
Multi-strain
Mono-strain
n=93
n=48
n=125
n=16
n=57
n=84
n=122
n=19
n=105
n=36
n=88
n=53
n=113
n=28
n=34
n=107
n=2
n=116
n=3
n=10
n=1
n=2
n=7
n=77
n=54
n=10
n=82
n=59
-2.0 -1.8 -1.6 -1.4 -1.2 -1.0 -0.8 -0.6 -0.4 -0.2 0.0 0.2 0.4 0.6
Point estimate
b
Moisture
n=41
n=22
n=56
n=7
n=16
n=47
n=49
n=14
n=40
n=23
n=41
n=22
n=58
n=5
n=23
n=40
n=2
n=49
n=6
n=3
n=1
n=1
n=1
n=43
n=16
n=4
n=45
n=18
-14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3
Point estimate
c
a_w
n=58
n=27
n=77
n=8
n=38
n=47
n=80
n=5
n=70
n=15
n=52
n=33
n=65
n=20
n=13
n=72
n=69
n=3
n=9
n=4
n=41
n=38
n=6
n=45
n=40
-0.06 -0.05 -0.04 -0.03 -0.02 -0.01 0.00 0.01
Point estimate
d
Color component a*
Without nitrate/ite salts
With nitrate/ite salts
Without S. xylosus
With S. xylosus
Without S. carnosus
With S. carnosus
Without Staphycoccus spp.
With Staphycoccus spp.
Without P. acidilactici
With P. acidilactici
Without Pediococcus spp.
With Pediococcus spp.
Without L. sakei
With L. sakei
Without L. plantarum
With L. plantarum
Without Lactobacillaceae
With Lactobacillaceae
Poultry
Pork
Camel
Beef
Mutton
LAB+CNS
LAB
CNS
Multi-strain
Mono-strain
n=16
n=22
n=27
n=11
n=33
n=5
n=24
n=14
n=36
n=2
n=35
n=3
n=26
n=12
n=23
n=15
n=4
n=34
n=2
n=26
n=2
n=4
n=4
n=13
n=23
n=2
n=14
n=24
-1 0 1 2 3 4 5 6
Point estimate
e
Color component L*
Without S. xylosus
With S. xylosus
Without S. carnosus
With S. carnosus
Without Staphylococcus spp.
With Staphylococcus spp.
Without P. acidilactici
With P. acidilactici
Without Pediococcus spp.
With Pediococcus spp.
Without L. sakei
With L. sakei
Without L. plantarum
With L. plantarum
Without Lactobacillaceae
With Lactobacillaceae
Poultry
Pork
Camel
Beef
Mutton
LAB+CNS
LAB
CNS
Multi-strain
Mono-strain
n=27
n=11
n=33
n=5
n=24
n=14
n=36
n=2
n=35
n=3
n=26
n=12
n=23
n=15
n=4
n=34
n=2
n=26
n=2
n=4
n=4
n=13
n=23
n=2
n=14
n=24
-6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6
Point estimate

Figure 3


a
TVC
Without S. xylosus
With S. xylosus
Without S. carnosus
With S. carnosus
Without Staphycoccus spp.
With Staphycoccus spp.
Without Pediococcus spp.
With Pediococcus spp.
Without L. sakei
With L. sakei
Without L. plantarum
With L. plantarum
Without Lactobacillaceae
With Lactobacillaceae
Poultry
Pork
Turkey
Camel
Beef
Mutton
LAB+CNS
LAB
CNS
Multi-strain
Mono-strain
n=53
n=26
n=63
n=16
n=33
n=46
n=66
n=13
n=47
n=32
n=61
n=18
n=14
n=65
n=2
n=52
n=6
n=2
n=7
n=10
n=42
n=30
n=7
n=46
n=33
-0.8 -0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0
Point estimate


b
LAB
Without S. xylosus
With S. xylosus
Without S. carnosus
With S. carnosus
Without Staphycoccus spp.
With Staphycoccus spp.
Without P. acidilactici
With P. acidilactici
Without Pediococcus spp.
With Pediococcus spp.
Without L. sakei
With L. sakei
Without L. plantarum
With L. plantarum
Without Lactobacillaceae
With Lactobacillaceae
Poultry
Pork
Turkey
Horse
Camel
Beef
Mutton
LAB+CNS
LAB
CNS
Multi-strain
Mono-strain
n=101
n=55
n=136
n=20
n=58
n=98
n=138
n=18
n=120
n=36
n=96
n=60
n=127
n=29
n=36
n=120
n=2
n=125
n=6
n=1
n=5
n=8
n=9
n=90
n=55
n=11
n=95
n=61
-2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0 2.5
Point estimate


c
Staphylococci
Without nitrate/ite salts
With nitrate/ite salts
Without S. xylosus
With S. xylosus
Without S. carnosus
With S. carnosus
Without Staphycoccus spp.
With Staphycoccus spp.
Without P. acidilactici
With P. acidilactici
Without Pediococcus spp.
With Pediococcus spp.
Without L. sakei
With L. sakei
Without L. plantarum
With L. plantarum
Without Lactobacillaceae
With Lactobacillaceae
Pork
Poultry
Mutton
Horse
Camel
Beef
LAB+CNS
LAB
CNS
Multi-strain
Mono-strain
n=29
n=87
n=71
n=45
n=98
n=18
n=29
n=87
n=100
n=16
n=84
n=32
n=73
n=43
n=95
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n=91
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n=9
n=1
n=5
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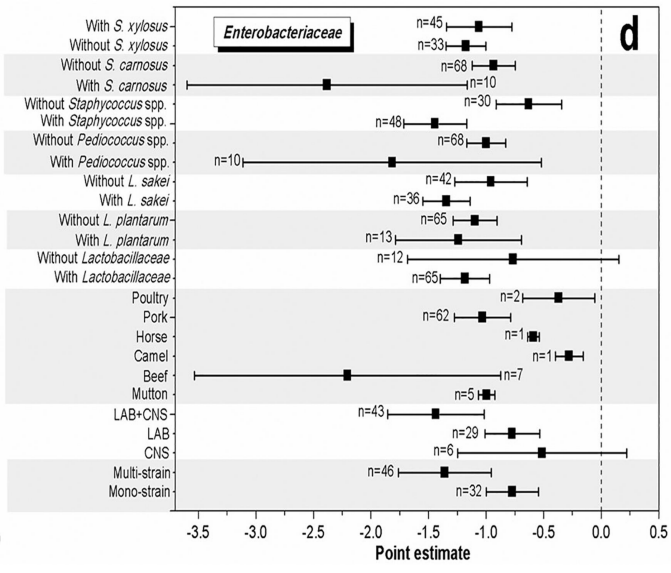
Point estimate


d
Enterobacteriaceae
With S. xylosus
Without S. xylosus
Without S. carnosus
With S. carnosus
Without Staphycoccus spp.
With Staphycoccus spp.
Without Pediococcus spp.
With Pediococcus spp.
Without L. sakei
With L. sakei
Without L. plantarum
With L. plantarum
Without Lactobacillaceae
With Lactobacillaceae
Poultry
Pork
Horse
Camel
Beef
Mutton
LAB+CNS
LAB
CNS
Multi-strain
Mono-strain
n=45
n=33
n=68
n=10
n=30
n=48
n=68
n=10
n=42
n=36
n=65
n=13
n=12
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n=2
n=62
n=1
n=1
n=7
n=5
n=43
n=29
n=6
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n=32
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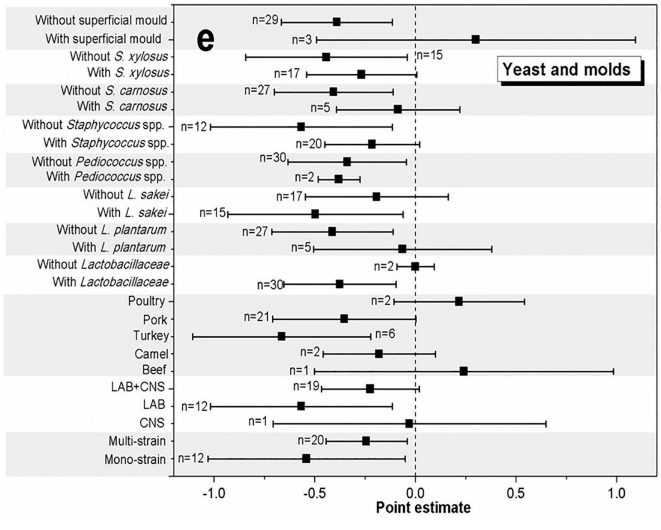
Point estimate


e
Yeast and molds
Without superficial mould
With superficial mould
Without S. xylosus
With S. xylosus
Without S. carnosus
With S. carnosus
Without Staphycoccus spp.
With Staphycoccus spp.
Without Pediococcus spp.
With Pediococcus spp.
Without L. sakei
With L. sakei
Without L. plantarum
With L. plantarum
Without Lactobacillaceae
With Lactobacillaceae
Poultry
Pork
Turkey
Camel
Beef
LAB+CNS
LAB
CNS
Multi-strain
Mono-strain
n=29
n=3
n=15
n=17
n=27
n=5
n=12
n=20
n=30
n=2
n=17
n=15
n=27
n=5
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n=30
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n=6
n=2
n=1
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n=12
n=1
n=20
n=12
-1.0 -0.5 0.0 0.5 1.0
Point estimate

Figure 4