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Review

Autochthonous cultures to improve safety and stabilize quality of traditional dry fermented meats

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Abstract: Traditional dry fermented meat products are obtained artisanally in many countries, where they represent a gastronomic heritage well distinguished from industrial counterparts. This food category is most often obtained from red meat, a food commodity that is under attack because of evidences of increased risk of cancer and degenerative diseases with high consumption. However, traditional fermented meat products are intended for moderate consumption and gastronomic experience and, as such, their production must be continued also to safeguard culture and economy of the geographical areas of origin. In this review, the main risks attributed to these products are considered and how these risks are minimized by following precise production norms, respectful of the ancient manufacturing processes is described. Moreover, results obtained in studies reporting use of autochthonous microbial cultures of lactic acid bacteria (LAB), coagulase negative staphylococci (CNS), *Debaryomyces hansenii* and *Penicillium nalgiovense* to improve safety and quality of traditional fermented meats are summarized. The role of these products as a source of microorganisms that can be beneficial to the host are also considered. From the results of the studies reviewed here it appears that the development of autochthonous cultures for these foods ensure safety and stabilize sensory characteristics with possibility to be extended to the large variety of traditional productions.

Keywords: traditional dry fermented meats; protection policy; desired microbiota; lactic acid bacteria; coagulase negative staphylococci; autochthonous cultures; safety; sensory quality

1. Introduction

Fermented meat products include dry sausages made from minced or chopped meats stuffed into natural or collagen casings, minced fermented meats with alternative surface coverings and sliced salted meats fermented without stuffing such as the South African Biltong and different types of meats sliced and dried without stuffing common in different countries. These products comprise hundreds of traditional variants whose artisanal production was transmitted between generations as a practice to preserve meat during the year for family sustenance. Families that have the possibility to rear one or a few animals in rural areas across the world also today produce sausages or other cured meat products for their own consumption according to ancient local recipes. More often, traditional fermented meat products are manufactured on artisanal scale in small manufacturing plants in the geographical areas of origin.

While nowadays there is propensity to consume meat in excess, for past generations meat was not so abundantly available and represented a precious food commodity. Indeed, it has been for long time the only source of many essential nutrients, vitamins, minerals and oligoelements in human diet [1,2], and the possibility to preserve meat by salting, fermentation and drying was very important for human nutrition in ancient times.

Based on their history and significance for the survival of mankind, traditional fermented meat products, mostly obtained from red meats, i.e. mammalian muscles [3],

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should be considered valuable foods to be consumed with moderation to not increase the risk of various cancers and chronic diseases attributed, though not with full agreement among studies, to high amounts of red meat consumption [4,5,6,7,8]. Indeed, the health problems attributed to eating red meat are probably determined by the fact that its consumption has been constantly increasing worldwide since the 1960s reaching per capita amounts that are very high in some countries [9].

Raw fermented and dry meats in European countries are represented mainly by dry fermented sausages, that, together with other traditional food productions, are the object of protection policies established to avoid the loss of a cultural heritage, of valuable gastronomic experience and economy of marginal areas as well as to respond to the citizen demand for productions with an identity linked to the geographical origin. According to the European legislation the term 'traditional' can be attributed to products with proven usage on the domestic market for at least 30 years, a period that allows transmission between generations [10]. However, most meat fermented products boast centuries [11] or millennia of history, as it can be learned by production norms publicly available, for example, from the Italian ministry of agriculture [12].

The regional dry fermented sausages represent a source of income for populations living in marginal areas, where the survival of traditional food productions means the continuation of cultural elements and the maintenance of social tissues in economically disadvantaged contexts. The protection of traditional products at European level is currently accomplished in conformity with the EU Regulation 1151/2012 [10], which sets the rules for the introduction of traditional foods in a European register of products with protected designation of origin (PDO) and protected geographical indication (PGI). Entering a product name in the register must be promoted by producer associations and approved by the European Union (EU) [13]. The European register of products with designation of origin is publicly accessible at https://ec.europa.eu/info/food-farming-fisheries/food-safety-and-quality/certification/quality-labels/geographical-indications-register/ (accessed on 13 March 2023). From this register it is possible to observe that Portugal, with 23 products, is the EU country with the highest number of dry fermented sausage types with designation of origin status, followed by Italy with 17 products. Countries with a few raw sausages in the register are Spain, France, Germany, Hungary, Poland, Romania, Croatia, Slovenia and Cyprus, while for Finland a few products from cold smoked raw reindeer meat are included. However, no literature in English can be accessed on the microbiological characteristics of many of the listed products. In addition, countries protect also the traditional products that still did not achieve the PDO or PGI status by keeping national registers. For instance, the Italian ministry of agriculture updates annually a list of national traditional products among which numerous dry fermented sausages typical of each Italian region [14]. An additional measure for the protection of Italian cured meat products, aimed at guaranteeing origin and discouraging commercial disloyal competition, is a national decree in force from year 2021 that imposes to the specify on the label the country/countries in which the animals were born, raised and slaughtered [15].

Producers are encouraged to manufacture diverse traditional foods strictly complying with operating quality schemes as long as commercial competition is fair and consumers can rely on the communication of the identity of products. This is crucial for benefiting the rural less favored areas where farming represents a main part of the economy and production costs are high [10,16]. Therefore, PDO and PGI and other protection labels, such as the Slow Food Presidium [17], are a law instruments that demonstrate to the consumer that traditional products are produced in a specific territory according to well defined production practices that determine quality, composition and identity of raw materials and the way these are obtained and transformed.

The manufacturing processes of fermented sausages are technologically simple, not requiring cooking steps or extensive changes in the structure and composition of the raw materials. What is carried out is mincing or chopping meat and fat in different ratios, mixing these in varying proportions with salt, spices and/or other natural ingredients (e.g. garlic, wine, potatoes, aromatic herbs, juniper berries, orange peel), stuffing in natural (i.e.

parts of animal intestines) or synthetic casings and letting dry the products in appropriate conditions, often still determined by local seasonal temperature and relative humidity (RH) parameters, for at least one month, depending on the diameter of the product. There exist also alternative ways to cover the surface of sausage fermenting mixture, e.g. maize flour uniform breading as in the case of the Italian Pitina sausage [12]. Some fermented meat products that can be consumed raw are not stuffed in casings. These include products manufactured in African countries, South America and Asian coutries. An example is El-Guedid, also called «el khlî», a traditional processed meat product in Algeria made from sheep, beef, goat and camel meat cut into strips, seasoned abundantly with salt and spices and exposed to the open air under the sun from one to several weeks until reaching a_w 0.625 and pH 5.2-5.5. Finally, it is stored in jars for up to one year [18]. Other not stuffed dry fermented meats are the Algerian Kaddid [19], beef jerky [20], Biltong in South Africa, that has also smoked versions [21], Kilishi, a jerky type RTE sun dried meat product popular in Northern Cameroon and Nigeria [22,23], Kitoza from Madagascar [24], Socol and Charqui in South America [25,26].

The simple manufacturing processes common to dry fermented meats do not justify equalizing these products to extensively processed meats undergoing cooking phases and/or other intense technological treatments. However, in most PDO and PGI sausages the addition of nitrate and nitrite salts and sugars, or a sugar source such as milk powder, is recommended to ensure safety by nitrite formation and acidification by lactic acid bacteria (LAB) [12,27], and this is represents a controversial safety issue for these products [28].

Sensory characteristics of traditional products must comply with the specifications described in the official norms of production that have legal value and are fixed by producer associations. Audits are carried out to establish the degree of conformity with the specifications set out in the norms and to guarantee product homogeneity among producers, during the year and in different years, as well as the distinction from similar products on the basis of multivariate chemometric approaches and sensory parameters [10,29]. These norms dictate aspects like, towns of production, animal races and farming practices, admitted feedstuffs, meat cuts to be used, product manufacturing phases and product composition also in terms of admitted additives, appearance, flavor of the end product and number of LAB at the end of ripening as a hygiene indicator. In particular, usage of microbial starter cultures is required to ensure safety for many, though not all, the PDO or PGI Italian sausages, e.g. for Salame Piemonte, Soppressa Calabrese, Ciauscolo, Salamini Cacciatore. On the other hand, for some products safety is deemed to be accomplished exclusively by modulating environmental conditions of temperature and relative humidity (RH) to ensure gradual drying and an optimal growth of the natural fermentation and ripening microbiota [12]. Indeed, inhibition of most pathogens and spoiling microbiota in these products is determined by the decrease in the a_w that can be as low as 0.843, as from the examples of Italian Ventricina del Vastese sausage, at the end of maturation [30], the decrease in pH, and the presence of NaCl and nitrites. LAB number or total mesophilic count in the product ready for consumption is taken into account by the production norms as indicators of safety, e.g. at least 100.000 CFU/g LAB for Salame di Felino, 10^7 CFU/g mesophilic microbiota, comprising LAB and coagulase negative staphylococci (CNS), for Salame Brianza [12].

Very little is specified on the origin, composition and the selection process of the microbial cultures mentioned in the production norms. An example of more detailed definition of the starter cultures was given for Salame Napoli, which has been proposed for the PGI status, for which number and type of selected cultures that is possible to add, namely lactobacilli, *Micrococcaceae*, staphylococci and pediococci, are specified, though only at microbial group or genus level [31]. It is also specified that cultures able to reduce nitrates and not too much acidifying must be used. Indeed, the final pH of sausages from Southern Europe, ranges between 5.1 and 5.79 [27,32,33]. Therefore, according to the production rules, producers are free to choose commercial starter cultures or selected autochthonous cultures. Indeed, use of a commercial starter culture including *Latilactobacillus sakei*, *Pediococcus acidilactici*, *Staphylococcus carnosus* and *S. carnosus* subsp. *utilis* was tested in a Sardinian fermented sausage and found to control *L. monocytogenes* growth Enterobacteriaceae mean levels at the end of ripening in three manufacturing plants without affecting the numbers of LAB and CNS and the product composition [34].

On the other hand, the presence on the market of producers of selected starter cultures offering customer tailored services for the development of autochthonous cultures indicates that fermented sausage producers, or producer associations, are interested to use cultures able to preserve the distinctive organoleptic characteristics of their products.

2. Technologically relevant microorganisms in dry fermented meats

The safety, sensory and nutritional quality of dry fermented meats largely rely upon the prompt development at appropriate levels of microbial groups comprising different species of LAB and CNS.

In particular, LAB contribute by lowering pH to the inhibitory activity of salt and drying on the development of pathogenic and spoiling microorganisms [35]. *L. sakei* is the LAB species most often isolated from fermented meat products and is particularly well adapted to the meat ecological niche because of the capacity to utilize substrates available in the meat matrix. One of these is arginine, an abundant amino acid in meat, that *L. sakei* metabolizes through the arginine deiminase pathway (ADI), encoded by the *arc*ABCTDR - *PTP* gene cluster, with production of ornithine, ammonia, and carbon dioxide. Concomitantly ATP is generated, thus providing a source of energy to this species. Moreover, *L. sakei* is able to ferment sugars present in meat such as ribose and the pentose moiety of nucleoside as well as *N*-acetyl-neuraminic acid. *L. sakei* is equipped with genes that favor its survival in stress conditions, in particular oxidative stress, since this species harbors a heme-dependent catalase *cat* gene and a manganese/iron superoxide dismutase gene *sod*A. Tolerance to high salt concentrations and acidic pH is strain-dependent. Genetic heterogenity was described for this species in which three intra-species clades are distinguished by multilocus sequence typing (MLST) [36]. In a study regarding the traditional product Ventricina del Vastese, 70 *L. sakei* isolates were grouped in different Rep-PCR genotypes of which only one lowered pH to 5.3 in meat extract suspension with no subsequent increase, thus indicating that only some of naturally occurring strains are able to improve product safety [37].

Coagulase-negative staphylococci (CNS) are commonly found on animal skin and mucosae from which they contaminate meats and meat manufacturing plants [38]. CNS main roles are promoting safety by reducing nitrate salts (NO_3^-), used as additives in these products, to nitrites (NO_2^-) able to inhibit *Clostridium botulinum.* With this reaction CNS also promote the development a desired red color given by the combination of myoglobin with nitric oxide NO deriving from the spontaneous reduction of nitrites to form nitrosomyoglobin ($MbFe^{II}NO$) [39]. It has been reported that inoculation with the CNS species *S. xylosus* confers a brighter red color than the autochthonous microbiota. Moreover, a lower lipid oxidation was observed in inoculated sausages, possibly due to the antioxidant capacity of nitrites [40]. In general, species belonging to the CNS group possess the nitrate reductase activity with the exception of one species associated with European fermented sausages that is *S. succinus* subsp. *succinus* [30,41,42]. However, NO can also be produced from arginine, which is abundant in meat, by the nitric oxide synthases (NOS) encoded by *nos* genes present in the genomes of all staphylococci. Therefore, CNS may contribute to the formation of nitrosomyoglobin also through the NOS pathway [39].

CNS are not in the list of microorganisms with qualified presumption of safety (QPS) status of the European Food Safety Authority (EFSA) [43] because hazardous genetic traits can be found in some strains. Safety risk characters to be examined in these bacteria are staphylococcal enterotoxin genes, panton-valentine leukocidin, toxic shock syndrome toxin-1, exfoliative toxin genes, that can be horizontally transferred from coagulase positive staphylococci, biofilm and biogenic amine (BA) formation and antibiotic resistance

(AR) genes. Other traits to be excluded are hemolytic and DNase activity [44,45]. When screening a high number of strains, PCR tests can be useful to exclude the presence of unwanted genes [44] but whole genome sequencing (WGS) is the method of choice to evaluate the most promising candidates for use as starter cultures for absence of hazardous traits [46]. In addition, AR testing at individual strain level is recommended by EFSA also for LAB strains whose QPS status is recognized at species level [46].

An example of selection scheme for CNS starter cultures was reported by Sun et al. [45]. They first selected eight CNS strains that could hydrolyze both sarcoplasmic and myofibrillar proteins, tolerated 10% of salt and 150 mg/kg nitrites, possessed good nitrate reductase activity and lipolytic activity, and did not exhibit decarboxylase activities and ARs among 143 isolates from ten geographical regions. These were identified as *S. simulans* (seven isolates) and *S. saprophyticus* (one isolate). Then they performed whole genome sequencing (WGS) to demonstrate that there were no hazardous genetic traits in the selected CNS strains.

Both LAB and CNS influence flavor and aroma by forming compounds that derive from their proteolytic and lipolytic activities which change in type and amount in a strain-specific fashion. In fermented sausages protein hydrolysis facilitates water release, favoring the drying process. Moreover, from protein hydrolysis precursors of aroma compounds that contribute to the sensory characteristics are formed. These compounds vary according to cleavage specificities of the proteolytic enzymes expressed by the bacterial strains involved in ripening [47].

In addition, some LAB strains belonging to species naturally present and adapted to fermented sausages, produce bacteriocins able to inhibit *L. monocytogenes* and *S. aureus*. In particular, *P. acidilactici* strains isolated from traditional sausages produce pediocins that inhibit the growth of pathogens such as *L. monocytogenes* and *C. perfringens* in dry-fermented sausages [48,49]. Indeed, in a number of studies, the effects of using LAB cultures producing anti-listerial bacteriocins in dry fermented sausages were examined with promising results [50].

Use of starter cultures also prevents defects caused by unwanted activities of some adventitious LAB, such as formation of excess hydrogen peroxide that may cause discoloration and lipid oxidation. Mainly CNS can neutralize pro-oxidant molecules, limiting the oxidative processes by their superoxide dismutase (SOD) and catalase (CAT) activities. The SOD enzyme lowers the amounts of free radicals present in cells by catalyzing the dismutation of the superoxide oxygen anion into oxygen and hydrogen peroxide. The latter is then converted into water and oxygen by the CAT enzyme [47].

The species *Debaryomyces hansenii*, often associated to fermented sausages, comprises strains with capacity to inhibit lipid oxidation, increase the production of volatile acid compounds [51,52] and of aroma compounds derived from amino acid degradation [53].

Finally, in dry fermented meats in which with mold development on the surface is allowed *P. nalgiovense* is the predominant species and was never reported to produce mycotoxins [54]. This mold species confers flavors from proteolysis and lipolysis and replaces naturally occurring mycotoxigenic fungi [55].

For some products a smoking procedure is carried out during drying to prevent the growth of molds on the surface [56].

3. Diversity of naturally occurring technologically relevant microorganisms in dry fermented meats

Changes in the natural microbiota composition in dry fermented meat products represents a variable in the achievement of microbiological safety and studies on the microbial ecology of traditional fermented sausages have highlighted a high variability in the microbiota composition among products and for the same product. The natural evolution during ripening of the technologically relevant microbial groups, LAB, CNS and yeasts, in products not inoculated with starter cultures differs among products but is also affected

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by batch-to-batch variation, as shown in Figure 1 for Catalão and Salsichão Portuguese sausages [27] and Ciauscolo Italian sausage [57].

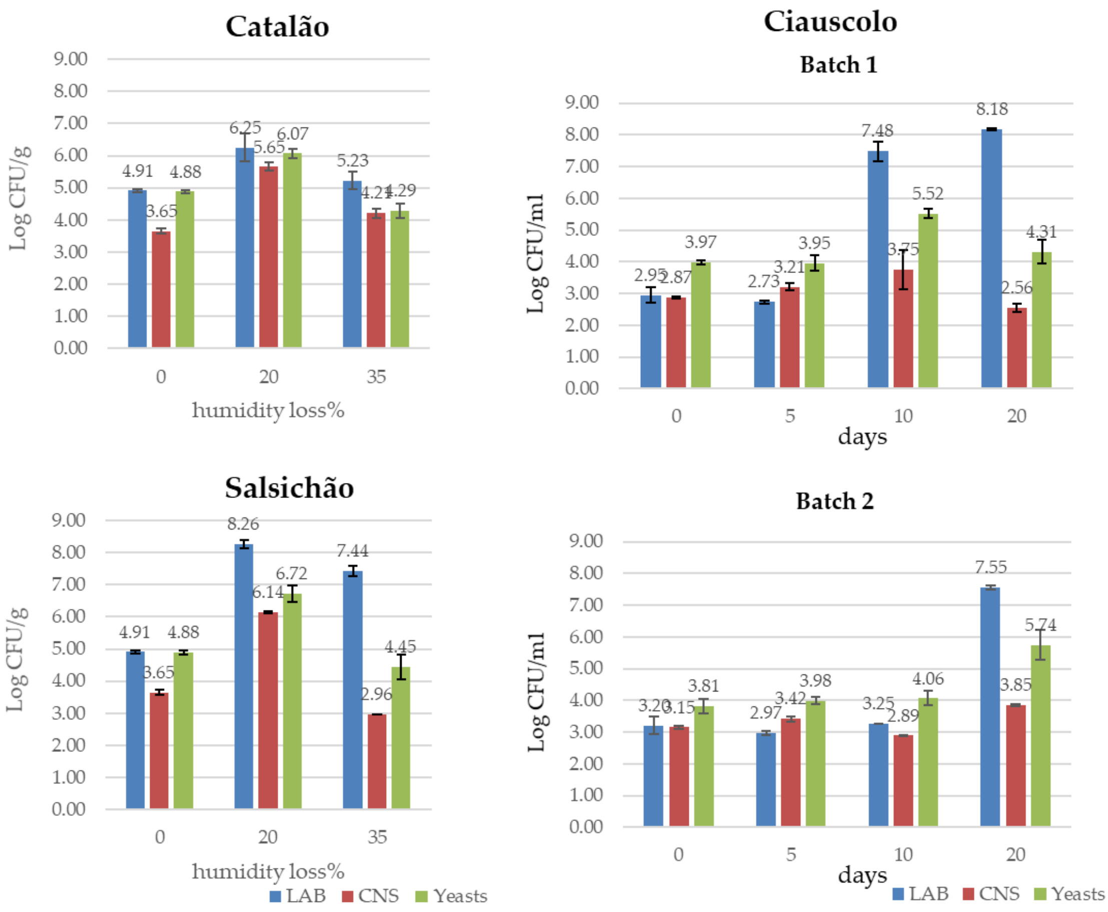

Figure 1. Natural evolution of LAB, CNS and yeasts in Catalão, Salsichão and Ciauscolo sausages as reported by Laranjo et al. [27] and Belleggia et al. [57]. A weight loss of 35%, corresponding to humidity loss, was reached in about 18 days for "Catalão" and in 35 days for "Salsichão".

These microbial groups have been found to be predominat also in not sausage products such as Kitoza, in which LAB and CNS were the two codominant populations with average counts of 6–7 Log CFU/g [24]. A high diversity of LAB and CNS species was reported for Mediterranean fermented sausages analyzed by phenotypic and molecular methods including new generation sequencing (NGS). The dominance of *L. sakei* and *S. xylosus* was observed in most products. Among LAB beyond the commonly occurring species of lactobacilli, *L. sakei*, *L. curvatus* and *L. plantarum*, other species of lactobacilli, *Enterococcus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Tetragenococcus* and *Weissella* species were identified [18,58,59,60,61]. *P. pentosaceus* was dominant in Salame Piemonte, as shown by metataxonomic analysis [61]. The CNS species more often identified in traditional fermented sausages are *S. xylosus*, *S. succinus*, *S. equorum* and *S. saprophyticus*. However, other CNS species, i.e. *S. saprophyticus*, *S. cohni*, *S. carnosus*, *S. epidermidis*, *S. pasteuri*, *S. hominis*, *S. capitis*, *S. vitulinus* and *S. warneri* were detected in some products [18,41,58,62,63,64]. In Salame Piemonte also *S. carnosus*, a species most often found in sausages from Northern Europe with pH values lower than those typical of Mediterranean sausages [62,63], was identified [61].

Yeast microbiota was mainly constituted by *D. hansenii* but also other fungal species were identified, though less frequently [61]. The mold *Penicillium nalgiovense* is the species most often present in products with a mold covered surface [55].

4. Safety concerns in traditional fermented dry meats

Microbiological hazards such as the presence of *L. monocytogenes* and other major food pathogens have been reported for dry fermented sausages. In particular, recent recalls of dry sausages due to the possible presence of *L. monocytogenes* and *Salmonella* spp. were notified [https://ilfattoalimentare.it/argomenti/richiami-e-ritiri, accessed on 25 February 2023] and according to the latest report on zoonoses in EU member states, fermented sausages one of the foods with highest occurrence of *L. monocytogenes* at distribution (3.1%). A progressive increase in number of infection outbreaks caused by *L. monocytogenes*, with 2,183 confirmed cases of invasive listeriosis in humans and the highest number of deaths in outbreaks notified to EFSA (36.4% of all deaths), was highlighted [65].

The results of a study on the survival of *L. monocytogenes* in Mediterranean-Style dry fermented sausages highlighted that, despite its constant decrease occurred during the shelf life, the pathogen was still detected in the final product, showing the capacity to overcome the hurdles of the manufacturing process [66]. Therefore, the competition by LAB and CNS microbiota and the a_w, pH values must be carefully controlled to maintain unfavorable conditions for the growth of this pathogen. Indeed, final a_w and pH values and the concentration of salts, nitrites, and spices in the fermented sausages commonly suppress or inhibit the growth of other pathogenic microorganisms, but *L. monocytogenes* can survive if the combination of pH and a_w values is permissive [67].

Use of starter cultures proved to be a valid solution to the *L. monocytogenes* threat in raw fermented meats, as demonstrated by recent investigations. For example, in Čajna sausage counts of four intentionally added *L. monocytogenes* strains belonging to serotypes 4b and 1/2a decreased significantly in all analyzed samples and were below the detection limit on day 18. Moreover, from day 7 until day 14, the decrease of *L. monocytogenes* was significantly faster in sausages in which commercial starter cultures comprising *D. hansenii*, *L. sakei*, *P. acidilactici*, *P. pentosaceus*, *S. carnosus* and *S. xylosus* were added [68].

One of the species mostly active against *L. monocytogenes* is *L. sakei*, in which different bacteriocins with anti-listerial activity were described, namely sakacin A (curvacin A), sakacin P (bavaricin); sakacin 674, sakacin K, sakacin V18, sakacin M (lactocin S), bavaricin MN, sakacin T, sakacin G, sakacin X, sakacin Q, sakacin 1 and sakacin G2, most of which are class II bacteriocins [69]. Therefore, a better exploitation of this sausage adapted LAB species by focused selection procedures should be further pursued.

A controversial safety concern attributed to dry fermented sausages regards the presence of nitrates and nitrites. These are not carcinogenic but give rise to reactive nitrogen species, including nitric oxide (NO), that combines with other compounds to form carcinogens such as nitrosoamines. In particular, N-nitrosamines are formed by combination of the nitrite reduction product NO with secondary amines [70]. Despite the fact that the main sources of dietary nitrates and nitrites are plants materials, it was hypothesized that nitrates/nitrites from animal sources determine an increase in cancer risk for the presence of amines, amides and heme iron that favor an increased production of N-nitroso carcinogens. Consequently, there is a trend to reduce or eliminate these compounds in meats [28].

Levels of added nitrites considered safe are below 150 mg/kg, the maximum amount admitted in raw cured meats by the European law [71]. However, very low levels of nitrites, such as less than 10 ppm, were detected at the end ripening in fermented sausages, since these compounds decrease after their reaction with myoglobin [72]. The most recent re-evaluation of the safety of the nitrite levels added to foods by the European Food Safety Authority was based on the estimation of the formation of nitrosoamines inside the body following their consumption. It was concluded that consumer exposure to nitrites and nitrates when used as food additives is within safe levels for all population groups. However, if all dietary sources of nitrites and nitrates are considered, the safe levels (ADIs) may be exceeded for all age groups. The EFSA experts concluded that more research is needed to address uncertainties and knowledge gaps in this area. However, when nitrites are used at approved levels, their contribution to overall exposure to nitrosoamines is of low concern for health [73]. It must be underlined that the nitrosation reaction is inhibited by substances like ascorbic acid [74] that is among the permitted additives in some Italian PDO and GPI fermented sausages [12].

There are a few available studies on the amount of nitrosoamines present in uncooked fermented sausages. One of these found low levels of N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodi-n-propylamine (NDPA), N-nitrosopyrrolidine (NPYR), N-nitrosopiperidine (NPIP) and N-nitrosodi-n-butylamine (NDBA) nitrosoamines in Sukuc Turkish fermented sausage, in other sausages, in salami and in cooked meat (Doner kebab). NDMA and NDEA are commonly found in sausages but at levels below the limit of 10 μg/kg set by some food safety agencies [74]. In a study carried out on kimchi *L. sakei* and *L. curvatus* were found to efficiently lower the amounts of NDMA and were found to directly degrade NDMA during culture in MRS broth containing NDMA [75].

Nitrite unintentionally added to meat products derives from sources such as environmental contamination and can also contribute to the formation of nitrosoamines. One study evaluated if a vegetable source of nitrates, namely chilli pepper used as ingredient in Ventricina del Vastese sausage, led to excessive levels of nitrites in the sausage and it was found that the maximum amount allowed for nitrates and nitrites was never exceeded [76].

Hazardous compounds that can be formed in fermented meats are BAs that are produced by decarboxylation of free amino acids by different bacterial groups. More often, tyramine and β-phenylethylamine are produced by enterococci since the enterococcal tyrosine decarboxylase (TDC) catalyzes also phenylalanine decarboxylation, though with lower yield compared to tyrosine [77]. Enterobacteria are the main producers of other amines, such as cadaverine and putrescine [78]. Tyramine and histamine are the most toxic BAs and the European Food Safety Authority (EFSA) has indicated a daily intake below 50 mg for histamine and below 600 mg for tyramine to be safe for healthy individuals [79]. These are formed also by individual strains of LAB belonging to different species for the presence of genetic determinants, namely amino acid decarboxylase gene clusters, which are prone to horizontal gene transfer (HGT) [80]. The absence of decarboxylase genes must be ascertained by molecular tests in bacterial strains to be used as starter cultures [41]. BA degrading bacteria may occur in fermented sausages and should be considered for use as starter cultures [81].

Another safety concern that may occur in fermented meats is the presence of mycotoxins, mainly ochratoxin A. Indeed, in some products molds are let develop on the surface to confer flavors from proteolysis and lipolysis [55]. However, the natural occurrence of also other species of molds able to produce mycotoxins in vitro was observed when their development on sausages was allowed, thus indicating the necessity to inoculate selected molds on sausage surface to outcompete the adventitous mycotoxin producers [54,82]. Regarding the prevention of mycotoxin formation, Delgado et al. [83] showed that also a strain of *P. chrysogenum* inhibited the production of the mycotoxin cyclopiazonic acid reported to be possibly present in dry fermented sausages beyond OTA when co-inoculated with the producer *P. griseofulvum* on sausages ripened at low temperature.

Development of molds on the surface in some traditional products is prevented by smoking. The smoking process for some dry fermented sausages also confers sensory connotations and is usually carried out by direct contact of the product with smoke deriving from wood combustion. The released volatile compounds (VOCs) diffuse into the product through the permeable casings, thus conferring aromas and tastes. However, among the VOCs developed during smoking the polycyclic aromatic hydrocarbons (PAH) are recognized carcinogens, so that safety limits for their amount have been fixed in terms of maximum level of the four substances benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene (PAH4), that for the smoked meat is 12 μg/kg [84]. The amounts of PAHs found in smoked meat depend on the type of wood used for smoking [56]. Strains of LAB able to subtract PAHs have been applied to lower the amount of these compounds in fermented sausages with good results also in sensory evaluation [85].

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5. Effect of autochthonous microbial cultures on safety and quality of dry fermented sausages

According to the protection rules, for traditional products recipes and manufacturing processes must be followed faithfully, and the possible interventions aimed to ensure safety must be respectful of product sensory characteristics and peculiarities. The role of the LAB and CNS microbiota on overall hygiene is crucial but safety promoting and technologically relevant characteristics, such as pH lowering capacity, proteolytic activity, and pathogen inhibiting capacity are highly variable among native bacteria [35,37]. Therefore, components of the autochthonous microbiota, adapted to the ecological niches of traditional fermented sausages, selected according to the above criteria and tested in pilot scale production, could be the best candidates as starters for the stabilization of safety in the respect of product sensory distinctness. Studies available on the application of LAB and CNS cultures isolated from traditional sausages as starters in the product of origin or similar products led often, though not always, to safety and organoleptic quality improvements. Some of those studies are reviewed below and the main findings are summarized in Table 1 for the effects observed on safety and in Table 2 for the effects observed on the sensory characteristics.

Table 1. Effect of autochthonous cultures on the safety of traditional fermented meat products.

Microbial strains	Product	Effect	Reference
L. curvatus 8427, *L. plantarum* 7423, *L. sakei* 8416, 4413 and 8426	Greek fermented sausages	inhibition of undesirable microorganisms	Baka et al. [86]
L. curvatus 54M16	fermented sausages of Campania region, Italy	lower numbers of *Enterobacteriaceae*	Casaburi et al. [87]
L. sakei CV3C2 and CECT7056, *S. equorum* S2M7, *S. xylosus* CECT7057, yeast strain 2RB4	Painho da Beira Baixa, Portugal	decrease in pH, *Enterobacteriaceae*, *L. monocytogenes* and total BAs	Dias et al. [88]
L. sakei CV3C2, *S. equorum* S2M7, yeast 2RB4	Paio do Alentejo, Portugal	decrease of *L. monocytogenes* counts and vasoactive amines tryptamine and β-phenylethylamine content	Dias et al. [89]
L. sakei CV3C2 and CECT7056, *S. equorum* S2M7, *S. xylosus* CECT7057, yeast strain 2RB4	Paio do Alentejo, Portugal	decrease of pH, *L. monocytogenes* counts and total BA content	Dias et al. [90]
L. sakei, *S. epidermidis*	Harbin sausage, China	decrease in tyramine content	Dong et al. [81]
L. curvatus 54M16	fermented sausages of Campania region, Italy	total inhibition of *L. monocytogenes* native from raw materials, inhibition of *Brochothrix*, *Psychrobacter*, *Pseudomonas* and *Enterobacteriaceae*	Giello et al. [91]
L. curvatus, *L. sakei*, *P. pentosaceus*, *S. xylosus*	Harbin dry sausage, China	decrease of a_w	Hu et al. [92]
L. curvatus SYS29, *L. lactis* HRB0, *L. plantarum* MDJ2, *L. sakei* HRB10, *W. hellenica* HRB6	traditional dry sausage, China	decrease of pH and a_w, increase of LAB counts	Hu et al. [93]

L. plantarum S50, S51, S72, S74, S85	Sucuk, Turkey	Inhibition of *L. monocytogenes*, rapid decrease of pH	Kamiloglu et al. [94]
D. hansenii	Salsiccia Sarda, Italy	Anti-mold effect	Murgia et al. [95]
S. simulans QB7	Qianwufu fermented sausage, Guizhou province, China	reduced growth of undesirable bacteria	Li et al. [44]
L. sakei 205	Salchichón, Spain	decrease of *L. monocytogenes* counts	Martín et al. [96]
D. hansenii	dry-cured meat products	decrease of aflatoxin formation by *Aspergillus parasiticus*	Peromingo et al. [97]
L. sakei, P. pentosaceus, S. carnosus, S. xylosus	Sichuan sausage, China	decreased levels of histamine, putrescine, tyramine, cadaverine and residual nitrites	Ren et al. [98]
L. sakei/ S. equorum SA25 *L. sakei* LS131/*S. saprophyticus* SB12	Galician Chorizo, Spain	pH decrease, increase of free amino acids and decrease of total BAs by approximately 20%	Rodríguez et al. [99]
L. plantarum, L. salivarius	traditional smoked horsemeat sausage, China	decrease of all indigenous microorganisms, including *Enterobacter cloacae*, *Enterococcus faecium*, *Pseudomonas* spp. and *Weissella* and of total BAs and histamine	Zhang et al. [100]

Table 2. Effect of autochthonous cultures on the sensory quality and composition of traditional fermented meat products.

Microbial strains	Product	Effect	Reference
L. curvatus 8427, *L. plantarum* 7423, *L. sakei* 8416, 4413 and 8426	Greek fermented sausages	prevention of lipid oxidation; higher scores for all sensory attributes	Baka et al. [86]
D. hansenii M4 and P2	dry-cured fermented sausages	strain P2 decreased lipid oxidation and increased acid compounds, strain M4 increased sulphur containing compounds, no differences in consumer acceptance	Cano-García et al. [51]
L. curvatus 54M16	fermented sausages of Campania region, Italy	more intense ripened flavor	Casaburi et al. [87]
L. brevis R4, *L. curvatus* R5, *L.fermentum* R6, *P. pentosaceus* R1	Harbin dry sausage	*P. pentosaceus* hydroxyl radical and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, inhibition of lipid peroxidation, high SOD and glutathione peroxidase (GSH-Px) activities	Chen et al. [101]

L. brevis R4, *L. curvatus* R5, *L. fermentum* R6, *P. pentosaceus* R1	Harbin dry sausage	*P. pentosaceus* strongest proteolysis activity, highest formation of soluble peptides and free amino acids, and VOCs from sarcoplasmic proteins	Chen et al., [102]
S. xylosus SX16, *L. plantarum* CMRC6	Chinese Dong fermented pork (Nanx Wudl)	acceleration of acidification and proteolysis, increase of total free amino acids and essential free amino acids Phe, Ile and Leu, increase of 3-methyl-1-butanol	Chen et al. [103]
L. sakei CV3C2 and CECT7056, *S. equorum* S2M7, *S. xylosus* CECT7057, yeast strain 2RB4	Painho da Beira Baixa, Portugal	higher scores in sensory attributes	Dias et al. [88]
L. sakei CV3C2 and CECT7056, *S. equorum* S2M7, *S. xylosus* CECT7057, yeast strain 2RB4	Paio do Alentejo, Portugal	negative effect on the sensory characteristics of fermented sausages	Dias et al. [89]
L. sakei, P. pentosaceus, S. xylosus	Salame Piemonte, Italy	improvement of the sensory properties	Franciosa [61]
L. curvatus, L. sakei, P. pentosaceus, S. xylosus	Harbin dry sausage, China	increase of hardness and springiness, higher percentages of aldehydes, ketones, alcohols, acids and esters	Hu et al. [92]
L. curvatus SYS29, *L. lactis* HRB0, *L. plantarum* MDJ2, *L. sakei* HRB10, *W. hellenica* HRB6	traditional dry sausage, China	increase of VOC content, decrease of total content of free amino acids, enrichment of pleasant odors for *L. sakei* and *W. hellenica*	Hu et al. [93]
L. plantarum MF1291 and MF 1298, *L. pentosus* MF1300	Traditional Norwegian salami	19 sensory parameters comparable to the commercial starter culture *L. sakei* HJ5	Klingberg et al. [62]
S. simulans QB7	Qianwufu fermented sausage, Guizhou province, China	Higher content of free amino acids, better LAB development with lower pH and a_w	Li et al. [44]
L. plantarum L125	traditional Greek dry-fermented sausage	desiderable technological characteristics	Pavli et al. [104]
L. sakei, P. pentosaceus, S. carnosus, S. xylosus	Sichuan sausage, China	lower hardness and chewiness, increased springiness; improved color and sensory attributes	Ren et al. [98]
L. sakei LS131/*S. equorum* SA25 or *L. sakei* LS131/*S. saprophyticus* SB12	Galician Chorizo, Spain	increment of the α-amino acid nitrogen, total basic volatile nitrogen and free amino acids, improvement of color	Rodríguez et al. [99]
L. fermentum BL11, *L. sakei* BL6, *P. acidilactici* BP2	Beef jerky, China	Lower pH and indicators of lipid and protein oxidation, higher VOC formation from carbohydrates for *L. sakei*; higher	Wen et al. [20]

		VOC formation from lipid β-oxidation and amino acid metabolism, esterase activity and acceptability score for *P. acidilactici*	
L. fermentum BL11, *L. sakei* BL6, *P. acidilactici* BP2	Beef jerky, China	decrease of lipid autoxidation-derived aldehydes; increment of esters for *P. acidilactici*; strain-specific flavour profiles	Wen et al. [105]

In many of the studies both safety aspects and sensory evaluation were considered. As safety aspects, the inhibition of pathogenic microorganisms, mainly *L. monocytogenes*, or proteobacteria, both pathogenic and indicators of poor hygiene, were examined in most studies. The effect of starters on BA formation was also analyzed. Sensory characteristics were defined by the analysis of proteolysis and lipolysis products, VOC formation, instrumental analysis of texture parameters and color and panel test assessments.

Baka et al. [86] evaluated the effect of inoculating five autochthonous LAB strains on the evolution of microbial groups, lipid oxidation, BA content and sensory attributes in a Greek fermented sausage. Most cultures prevented lipid oxidation, as indicated by values of malonaldehyde lower than 1 mg /kg, and treatment with *L. sakei* 4413 lowered the content of all BAs with reduction of tyramine by 13%, tryptamine by 55%, cadaverine by 60% and putrescine by 72%. Sausages produced with cultures *L. sakei* 4413 and *L. sakei* 8416 had the highest scores for all sensory attributes, namely overall flavor and color, section and external appearance and firmness.

Chen et al. [101] evaluated the role of various single starter strains or strain associations on lipolysis and lipid oxidation in Harbin dry sausages and reported that mixed cultures of *P. pentosaceus*, *L. curvatus*, *L. sakei* and *S. xylosus* modulated lipid degradation promoting lipid hydrolysis but inhibiting the formation of VOCs derived from lipid autoxidation, thus improving flavor. In another study on Harbin sausage it was found that aotochthonous *P. pentosaceus* and *L. curvatus* strains degraded sarcoplasmic proteins forming higher free amino acid amounts than in the control. *P. pentosaceus* formed the highest amount of VOCs, including aldehydes, alcohols, and acids, followed by *L. curvatus* [102].

L. curvatus 54M16 isolated from a traditional fermented sausage of Campania region, Italy, and found to carry the genes for the bacteriocins sakacin X, T and P, was able to inhibit the pathogens *L. monocytogenes* and *Bacillus cereus* and the meat spoilage bacterium *Brochotrix thermosphacta.* The strain showed a good acidifying capacity and was able to hydrolyze sarcoplasmic proteins and lipids and to reduce nitrates. Moreover, it showed high values of SOD activity, and formation of l-arginine, l-valine, l-phenylalanine and l-lysine free amino acids. In sausage fermentation it dominated the LAB population at all sampling times and lowered Enterobacteriaceae counts of about 1 Log at the end of ripening [87]. When the anti-listerial activity of this strain was analyzed in sausage production it was found to be low with pathogen levels of about 4 Log CFU/g. However, *L. monocytogenes* naturally present in the raw ingredients was totally inhibited. As demonstrated by 16S rRNA-based metagenome analysis, this strain dominated and affected the bacterial ecosystem, inhibiting spoilage bacterial genera *Brochothrix*, *Psychrobacter*, *Pseudomonas* and some Enterobacteriaceae that were still present at the end of ripening in the control sausages. The inoculated sausages were perceived with a more intense ripened flavour in sensory analysis [91].

Kamiloglu et al. [94] also observed a 2 Log CFU/g reduction of *L. monocytogenes* in Sukuk sausage inoculated with an autochthonous *L. plantarum* strain compared to the not inoculated control.

S. xylosus SX16 and *Lactiplantobacillus plantarum* CMRC6, isolated from Chinese Dong fermented pork Nanx Wudl, showed a high proteolytic activity in a preliminary screening in vitro. The combination of the two strains (starter LS) suppressed the growth of Enterobacteriaceae and accelerated acidification and proteolysis during ripening, improving the contents of total free amino acids and essential amino acids Phe, Ile and Leu. This starter increased the amount of the VOC 3-methyl-1-butanol, probably due to the inoculated *S. xylosus*, compared to the not inoculated control and the product inoculated only with *L. plantarum* CMRC6. The LS starter conferred better sensory properties to sausages, showing that the combination of lactobacilli with CNS in the added cultures enhanced sausage quality [103].

In a study aimed to evaluate the effects of different associations of autochthonous starters comprising *S. equorum* S2M7, *S. xylosus* CECT7057, *L. sakei* CV3C2, *L. sakei* CECT7056 and the yeast strain 2RB4, previously characterized [78,88,89] on the safety and quality of Paio do Alentejo, a traditional Portuguese dry-fermented sausage, the starters were inoculated at high concentrations, and an extended fermentation step of 72 h was introduced before stuffing [90]. LAB and CNS in inoculated samples reached 3 Log CFU/g and 2 Log CFU/g higher counts, respectively, compared to the control. LAB remained at almost constant numbers throughout ripening, while CNS significantly decreased at the end of ripening, as commonly observed in dry fermented sausages [41,106]. Yeast and molds maintained similar numbers throughout the ripening time with no differences among treatments, even when a yeast starter was inoculated, indicating that the autochthonous yeasts predominated. *L. monocytogenes* was present in most analyzed samples, possibly for persistence in the manufacturing plant or because originally present in the raw materials. However, in the final products its numbers were below the European legal limit of 100 CFU/g [67] for most treatments and the starter cultures significantly reduced *L. monocytogenes* counts. Moreover, the extended fermentation time allowed to reach lower pH and a_w values, thus controlling the spoiling and pathogenic microbiota with a pronounced effect on the stabilization of enterobacteria. The latter had initial numbers of 5 - 6 log CFU in the meat batter, indicating a poor hygienic quality of the raw materials [90].

The reduction of the total BA content in products inoculated with autochthonous cultures was also observed [90]. Vasoactive BAs tryptamine, β-phenylethylamine, histamine, and tyramine, showed mean values higher in control sausages (279.11 ± 131.94 mg/kg) but differences with inoculated products were not statistically significant. Use of starters showed a significant effect on the content of total BAs, with lower values in inoculated sausages (between 452.58 ± 55.11 mg/kg and 533.96 ± 63.65 mg/kg) compared to control sausages (731.95 ± 206.23 mg/kg), with lowest mean values in sausages inoculated with *S. xylosus* CECT7057 and *L. sakei* CECT7056. BA reduction compared to the not inoculated control by using autochthonous cultures was obtained also previously [88,89]. However, BA levels increased in all batches during ripening, except for β-phenylethylamine and tyramine, which was in not in line with previous findings [89]. Tyramine contents were lower than 2.20 mg/kg in all samples, while other authors observed much higher levels of this BA [107,108]. Different trends in BA formation can be explained with the predominance of bacterial groups producing different BAs among the native microbiota [90]. Regarding histamine, it was present at detectable but not at hazardous levels in end-products, similarly to what reported previously [89,107]. Tryptamine was the BA with highest concentrations in the products, as observed also in Catalão and Salsichão sausages [27] and in non-inoculated Serbian sausages [98]. This BA mainly contributed to the amount of total vasoactive amines that was higher than 200 mg/kg in end-products. However, the total content in BAs was below 1000 mg/kg, showing an improvement compared to previous reports [89,110]. The presence of the natural polyamines followed the pattern usually observed in fermented sausages, with the prevalence of spermine.

In sensory evaluation color and texture profile analysis (TPA) parameters of hardness, cohesiveness, springiness, gumminess, elasticity and chewiness were determined. Significant differences were only detected for color in sausages inoculated with *S. xylosus* CECT7057 and *L. sakei* CECT7056, that showed more reddish tones. However, control sausages showed the lowest levels of off-flavors and were the most appreciated in overall perception by the panel, although not significantly, compared to the inoculated products. Regarding sensory analysis, the association *S. equorum* S2M7/ *L. sakei* CV3C2 seemed to have a generally more positive effect than the association *S. xylosus* CECT7057/ *L. sakei* CECT7056. Moreover, the inoculated yeast did not show a significant effect in the sensory appreciation, differently than reported by Flores et al. [110] and Corral et al. [53,90].

Tyramine degrading *S. epidermidis*, *L. sakei*, and *L. curvatus* were isolated from Harbin sausage [81]. When the *S. epidermidis*/*L. sakei* association was used in Harbin sausage production it reduced tyramine content by 55% in the final product compared to the control, despite the high proteolytic activity of the *L. sakei* starter [111]. The effect of inoculation on the microbiota composition was examined by 16S rRNA gene metagenomic analysis and it was found that lactobacilli were the most abundant bacterial group in Harbin dry sausage during fermentation in all samples. Inoculation of *L. sakei* or *S. epidermidis* separately increased the abundance of lactobacilli, while, when in association, the inoculated bacteria decreased the relative abundance of *Weissella* spp. that was found to be predominant in Harbin sausage [98].

Franciosa et al. [61] showed that five selected autochthonous strains from Salame Piemonte, namely, two *P. pentosaceus*, two *L. sakei*, and one *S. xylosus* strains, already evaluated for safety, were used for pilot-scale Salame Piemonte production in seven combinations. Sausages produced with the *P. pentosaceus* strains were the most appreciated for being less bitter, less acidic, and with a more uniform aspect. However, all inoculated samples received high odor and color intensity scores and obtained higher scores than the control for the question "Would you buy it?". Samples produced with *P. pentosaceus* and *L. sakei* were evaluated as more balanced for most of the investigated attributes. Samples inoculated with *P. pentosaceus*, showed the highest scores for color intensity and general appreciation compared to samples with *L. sakei* strains, that were more acidic. In samples inoculated with *P. pentosaceus* highest concentrations of acetic acid, diacetyl and acetoin, ethanol, isopentyl alcohol, 1-hexanol, and 1-octanol were formed.

Hu et al. [92] found that inoculation with *P. pentosaceus* (Pp), *L. curvatus* (Lc), *L. sakei* (Ls) and *S.xylosus* (Sx) autochthonous strains increased the hardness and springiness of Harbin sausage and the percentages of some aldehydes, ketones, alcohols, acids and esters derived from carbohydrate catabolism, such as 3-hydroxy-2-butanone, from amino acid metabolism, such as 3-phenylpropanol, 2,3-butanediol, phenylethyl alcohol, 2-methyl-propanal, 2-methyl-butanal and 3-methyl-butanal, from β-lipid oxidation, such as 2-pentanone, 2-heptanone and 2-nonanone, and from esterification, such as ethyl esters, were significantly higher in the sausages inoculated with Pp + Sx + Lc than that in the control. Hu et al. [93] reported that *L. plantarum* MDJ2, *L. sakei* HRB10, *L. curvatus* SYS29, *W. hellenica* HRB6, and *L. lactis* HRB0 isolated from Harbin sausage conferred lower a_w and pH values to the product with an increase in LAB counts. Total free amino acids decreased in inoculated samples since possibly converted in VOCs. Electronic nose (E-nose) and sensory analysis indicated that *W. hellenica* HRB6, *L. sakei* HRB10, and *L. curvatus* SYS29 enhanced the pleasant odors with a stronger correlation with *W. hellenica* HRB6 and *L. sakei* HRB10 of most of the key VOCs determined by headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS).

Kaban et al. [112] assessed the VOC formation for autochthonous strains *L. sakei* S15, *Lactobacillus plantarum* S24, *L. plantarum* S91, *P. pentosaceus* S128b and *S. carnosus* G109 in the Turkish dry fermented sausage Sucuk and observed that most compounds were positively correlated with the products containing only *L. sakei* S15.

In the study of Li et al. [44], CNS with high protease activity were isolated from the traditional naturally fermented sausage Qianwufu. Strain *S. simulans* QB7, showing the highest proteolytic activity, tolerance to salt and nitrite concentrations and absence of virulence genes and hemolytic, decarboxylase, DNAse, and biofilm-forming activities, was used as a starter. This strain reduced growth of undesirable bacteria and determined higher contents of total free fatty acids and free amino acids, lower pH and a_w values because of enhanced LAB development.

Martín et al. [96] used *L. sakei* 205, harboring the pediocin PA gene [113] in a challenge test with *L. monocytogenes* in Salchichón traditional Spanish sausage. LAB counts in the *L. sakei* 205 inoculated samples were 7 Log CFU/g until day 30 and remained higher than 6 Log CFU/g until the end of ripening. Enterobacteriaceae were undetectable at the end of processing. Reductions in *L. monocytogenes* counts ranged from 1.6 to 2.2 Log CFU/g in all samples, showing that the processing of Salchichón did not allow the growth of this pathogen. However, the reduction in *L. monocytogenes* was significantly higher in the samples inoculated with *L. sakei* 205. The characterization of the LAB isolates by pulsed field gel electrophoresis (PFGE) showed that *L. sakei* 205 was implanted throughout the ripening time, while in the control group *L. sakei* was not detected, though similar LAB numbers were reached.

This strain was selected from the screening of 182 LAB isolates from dry-cured meat from 3 industries and 32 were able to inhibit this *L. monocytogenes* in the agar spot assay. Five of these significantly reduced of *L. monocytogenes* in a food model with *L. sakei* 205 showing the highest inhibition. In the study *L. sakei* was the species showing most frequently anti-listerial activity among the isolates. Other species active against *L. monocytogenes* isolated in one of the industries included *E. faecium*, *E. hirae*, *L. plantarum*, *Lacticaseibacillus casei* and *L. garviae*, thus showing diversity of the anti-listerial microbiota among sausage producers [113].

Strains of *L. sakei*, *P. pentosaceus*, *S. xylosus* and *S. carnosus* isolated from traditional sausages by the Meat-Processing Application Key Lab of Sichuan Province, were able to predominate during fermentation in a traditional product, while in the control *Lactobacillus* spp. and *Weissella* prevailed [98]. Undesirable microorganisms such as *Yersinia* spp., *Enterobacter* spp., *Acinetobacter* spp. and *Psychrobacter* spp. were lower in inoculated sausages where they soon decreased, while remaining in high percentage during the entire ripening time in samples with spontaneous fermentation. The levels of histamine, putrescine, tyramine and cadaverine were also significantly lower in inoculated samples being reduced by 83.09%, 69.38%, 51.87% and 57.20%, respectively, at the end of the ripening. The same starter was previously found to lower by 84.17% histamine accumulation in Cantonese sausages. The total volatile basic nitrogen (TVB-N), generally considered as an indicator of freshness and protein degradation of meat, increased substantially in both inoculated and control samples, but it was significantly lower in inoculated samples. The inoculated sausages showed less hardness and chewiness, an increased springiness and an improved color resulting in better evaluation by a sensory panel. Moreover, in inoculated sausages the concentration of residual nitrite was significantly lower with a rapid decrease from 150.21 to 4.56 mg/kg, while in the control the final nitrite concentration was 28.81 mg/kg [114].

Rodriguez et al. [99] showed that use of two autochthonous starter cultures, *L. sakei* LS131/*S. equorum* SA25 or *L. sakei* LS131/*S. saprophyticus* SB12 slightly but significantly reduced the pH values during the fermentation and increased the formation to nitrosylheme pigments with the improvement of the red coloration (a* parameter) and the yellow coloration (b* parameter) determined by colorimetric measurements. The two starters significantly decreased the Enterobacteriaceae counts in the final product, though these did not completely disappear. Both starter cultures significantly increased the α-amino acidic nitrogen (NH2-N), the total basic volatile nitrogen and the free amino acid content during manufacturing reducing the total BA content by approximately 20%. The presence of *S. saprophyticus* increased the free fatty acid content.

The effects of autochthonous LAB strains, *L. sakei* BL6, *P. acidilactici* BP2, and *L. fermentum* BL11 were lowering pH, thiobarbituric acid reactive substances (TBARS) indicators of lipid oxidation, and carbonyl indicators of protein oxidation contents. *L. sakei* BL6 produced higher carbohydrate fermentation-derived volatile compound contents, while *P. acidilactici* BP2 produced higher contents of VOCs derived from lipid β-oxidation and amino acid metabolism and showed higher esterase activity. The jerky inoculated with *P. acidilactici* BP2 had the highest acceptability score [20]. The same autochthonous LAB strains were evaluated in the ability to release VOCs in beef jerky by using electronic nose

(E-nose) and gas chromatography–ion mobility spectrometry (GC–IMS). All the three LAB strains decreased the levels of aldehydes derived from lipid autoxidation, hexanal, heptanal, octanal, and nonanal. The strains showed unique flavor profiles. In addition, inoculation of *P. acidilactici* BP2 increased the levels of esters ethyl 3-methylbutanoate, 3-methylbutyl acetate, butyl acetate, ethyl propionate, ethyl acetate, and ethyl acetate. A high correlation was found between the E-nose and GC–IMS results so that these methods were both found useful for the selection of autochthonous starter cultures for beef jerky fermentation [105].

The addition of autochthonous starter cultures able to degrade BAs through oxidative deamination catalyzed by amine oxidases can reduce the accumulation of BAs in fermented sausages. This was observed in a traditional Chinese smoked horsemeat sausage by using strains previously isolated from the product *L. plantarum*, *Ligilactobacillus salivarius* and a combination of the two. These strains also promoted the reduction in number of *Pseudomonas* spp. and Enterobacteriaceae at the end of fermentation at similar extents. Polymerase chain reaction denaturing gradient gel electrophoresis (PCR DGGE) showed that species present in starter cultures were dominant throughout fermentation, while indigenous microorganisms *Staphylococcus* spp., *S. xylosus*, *S. epidermidis*, *Enterobacter cloacae*, *L. sakei*, *Enterococcus faecium*, *P. pentosaceus*, *Pseudomonas* spp. and *Weissella* spp. showed faint bands. *Pseudomonas* spp. and *Weissella* spp. disappeared completely in all batches at day 7. In the control batch indigenous *L. sakei*, *E. faecium* and *P. pentosaceus* remained dominant throughout fermentation. Though the total BA concentration in inoculated batches was significantly lower than in the control, *L. plantarum* and *L. salivarius* did not completely abolish BA accumulation. However, the final total BA levels were low (258.87 ± 0.93 and 318.76 ± 0.34 mg/kg) and the lowest histamine concentration at the end of ripening was found in samples inoculated with *L. salivarius* (23.50 mg/kg) [100].

For autochthonous strains of the yeast species *D. hansenii* the capacity to increase safety and sensory characteristics has been described. When inoculated on the surface of Salsiccia Sarda, a selected autochthonous strain exerted an anti-mould effect without affecting sensory quality [95]. Peromingo et al. [97] found that two native *D. hansenii* strains reduced the relative expression levels of the *afl*R and *afl*S genes involved in the aflatoxin biosynthetic pathway by *Aspergillus parasiticus* in vitro and reduced the formation of these compounds on fermented sausages. Cebrián et al. [115] reported that an autochthonous strain of *D. hansenii*, alone or in combination with *S. xylosus*, prevented OTA formation by *P. nordicum*, the main OTA producer, in dry-cured sausage Sauchisson, being therefore proposed as bioprotective culture.

Since dry fermented sausages are part of the larger group of fermented foods for which it has been claimed that regular consumption improves health due to the presence of living, potentially probiotic microorganisms [116,117], they have been analyzed for the occurrence of potential probiotic bacteria also evaluating the suitability of the latter for use as starters in traditional sausages. Klingberg et al. [62] selected three potential probiotic strains, *L. plantarum* MF1291 and MF 1298 and *L. pentosus* MF1300, suitable as starter cultures for the Scandinavian-type fermented sausages that were identified among 22 dominant non-starter lactic acid bacteria (NSLAB) well-adapted to Norwegian and Swedish fermented meats. These were able to lower pH below 5.1 in a meat model, survive at pH 2.5 and in presence of 0.3% oxgall, adhered to the human colon adenocarcinoma cell line Caco-2 and expressed antimicrobial activity against potential pathogens and pathogens such as *Escherichia coli*, *B. cereus*, *Shigella flexneri*, *Y. enterocolitica*, *Salmonella* Typhimurium and *L. monocytogenes*. When applied as starter cultures for the production of a Norwegian salami according to the traditional recipe, these cultures reached high viable counts and pH values between 4.8 and 4.9 at the end of ripening. The sensory characteristics of the sausage inoculated with the autochthonous starter were comparable to those of sausages manufactured with the commercial meat starter culture *L. curvatus* HJ5, according to the evaluation of 19 parameters.

Pavli et al. [104] evaluated the performance of *L. plantarum* L125, a strain with probiotic potential isolated from a traditional Greek fermented sausage, as an adjunct culture for the production of dry-fermented pork sausages. The strain was inoculated together with a commercial starter culture and remained at high levels at the end of ripening without affecting sensory parameters. More recently Dincer et al. [118] reported the probiotic potential of *L. sakei* isolated from dry fermented Pastırma beef sausage. Namely, the two strains *L. sakei* 8.P1 and 8.P2 produced proteinaceous compounds able to inhibit *L. monocytogenes*, *S. aureus* and *P. aeruginosa* and tolerated simulated gastric juice conditions showing also good adhesion capacity. Use of potentially probiotic native LAB strains in traditional fermented sausages is still little explored and could lead to an increased benefit on health from the consumption of these products.

6. Conclusions

This analysis of studies regarding the improvement of traditional fermented meat products highlighted multiple advantages deriving from use of autochthonous cultures on both safety and sensory quality. Therefore, studies on the biodiversity of the autochthonous microorganisms should be followed by the definition of safety and health promoting attitudes and technologically relevant features of the microbial isolates to devise appropriate inoculation schemes with native cultures that can ensure safety and stabilize quality. There is still much to disclose on the numerous traditional productions not characterized microbiologically and efforts should be devoted to select native bacteria suitable for use as starter cultures. Moreover, proving that a moderate consumption of these products enriched with native probiotic microorganism can benefit health could incentivize the continuation of manufacturing traditions in marginal areas. Many of the studies summarized in this review showed that the natural microbiota includes species with a potential to prevent the development of hazardous microorganisms and substances, so their selection and application can represent an efficient tool to increase consumer's trust and appreciation for traditional fermented meat products. Guaranteeing constant quality, safety, sensory distinctness and delivery of beneficial microorganisms will motivate the protection of traditional dry fermented sausages even more than currently done.

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