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Food Research International

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Manuscript Number:	FOODRES-D-22-01442R4
Article Type:	Research Paper
Keywords:	fermented sausages, nitrate, nitrite, microbial safety, starter cultures, clean label

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Abstract:	The aim of this work was to investigate the possibility to industrially produce fermented sausages without the addition of nitrate and nitrite. Indeed, despite their antimicrobial effect and multiple technological roles, an increasing pressure for their removal has recently raised. To achieve this goal while maintaining an acceptable final product quality, we deeply modified the whole process, that was carried out at 10-15°C (i.e., temperatures lower than traditional Mediterranean products) and by using bioprotective starter cultures at high concentrations (7 log CFU/g) to lead the fermentation. Different glucose amounts (0.2 or 0.4 % w/w) were also tested to optimize the process. The results showed no significant differences between the control (with nitrate/nitrite) and the sausages without preservatives in terms of aw (value range 0.908-0.914), weight loss (about 38% in all samples), lactic acid bacteria (value range 8.1-8.3 log CFU/g) and coagulase negative cocci (value range 6.8-7.1 log CFU/g). The amount of sugar affected the final characteristics of sausages. Indeed, in the absence of curing salts, lower sugar concentration resulted in better textural features (reduced hardness and gumminess) and lower oxidation (TBARS values 0.80 vs. 1.10 mg MDA/kg of meat product in samples with 0.2% or 0.4% of glucose, respectively). Finally, challenge tests evidenced the inability of selected strains of Listeria innocua, Salmonella enterica sub. enterica and Clostridium botulinum to grow, under the adopted conditions, in fermented sausages. This research highlighted that nitrate/nitrite removal from these meat products requires accurate technological changes to guarantee the final quality.

This is the postprint of: *Safety and technological issues of dry fermented sausages produced without nitrate and nitrite* by Giulia Tabanelli, Federica Barbieri, Massimiliano Petracci, Francesca Soglia, Fausto Gardini, Rudy Magnani, Chiara Montanari, which has been published in final form in *Food Research International* 160 (2022), n. 111685. The final published version is available online at: https://doi.org/10.1016/j.foodres.2022.111685.

HIGHLIGHTS

- Nitrate/nitrite elimination requires technological changes to produce high quality salamis
- Ripening temperature was lowered for guaranteeing microbiological safety
- Higher concentrations of bioprotective starter cultures were adopted
- Low sugar concentration allowed to obtain better textural features and lower oxidation level
- Salamis were challenged with *Listeria innocua*, *Salmonella enterica* and *Clostridium botulinum*

Safety and technological issues of dry fermented sausages produced without nitrate and nitrite

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ABSTRACT

The aim of this work was to investigate the possibility to industrially produce fermented sausages without the addition of nitrate and nitrite. Indeed, despite their antimicrobial effect and multiple technological roles, an increasing pressure for their removal has recently raised. To achieve this goal while maintaining an acceptable final product quality, we deeply modified the whole process, that was carried out at 10-15°C (*i.e.,* temperatures lower than traditional Mediterranean products) and by using bioprotective starter cultures at high concentrations (7 log CFU/g) to lead the fermentation. Different glucose amounts (0.2 or 0.4 % w/w) were also tested to optimize the process. The results showed no significant differences between the control (with nitrate/nitrite) and the sausages without preservatives in terms of a_w (value range 0.908-0.914), weight loss (about 38% in all samples), lactic acid bacteria (value range 8.1-8.3 log CFU/g) and coagulase negative cocci (value range 6.8-7.1 log CFU/g). The amount of sugar affected the final characteristics of sausages. Indeed, in the absence of curing salts, lower sugar concentration resulted in better textural features (reduced hardness and gumminess) and lower oxidation (TBARS values 0.80 *vs.* 1.10 mg MDA/kg of meat product in samples with 0.2% or 0.4% of glucose, respectively). Finally, challenge tests evidenced the inability of selected strains of *Listeria innocua, Salmonella enterica* sub. *enterica* and *Clostridium botulinum* to grow, under the adopted conditions, in fermented sausages. This research highlighted that nitrate/nitrite removal from these meat products requires accurate technological changes to guarantee the final quality.

Key words: fermented sausages, nitrate, nitrite, microbial safety, starter cultures, clean label

1. INTRODUCTION

Meat curing consists in the addition of sodium chloride (NaCl) and nitrite/nitrate to meat to assure safety and prolong the shelf-life of foods. It is one of the most ancient preservation strategies and it has been adopted by humanity for long time (Toldrá & Hui, 2014). In the past centuries, nitrates and nitrites were added as impurities of the salt, which was used in considerably higher amounts than today. It is only starting from end of XIX century that the role of these salts in preserving foods has been understood. While NaCl can reduce water availability (*i.e.* a_w), nitrate and nitrite are responsible for several effects that today are well clarified and studied (Pegg & Honikel, 2014). In addition, the progressive purification of produced NaCl, makes the addition of nitrates and nitrites a specific step of cured meat manufactory process.

The addition of nitrate and nitrite in meats is explained by at least four fundamental reasons: i) antimicrobial effect exerted through the inhibition of metabolic pathways, limitation of oxygen uptake or iron sequestering; traditionally the effects of these nitrogen compounds are mainly addressed to suppress the outgrowth of clostridia spores, but its effectiveness against enterobacteria, *Staphylococcus aureus, Listeria monocytogenes* and *Bacillus cereus* is well documented (Alahakoon, Jayasena, Ramachandra, & Jo, 2015); ii) colour formation, which depends on the formation of nitric oxide able to react with many substrates, including Fe^{++} of myoglobin and Fe^{+++} of metmyoglobin, leading to the development of the typical cured meat colour (Honikel, 2004); iii) antioxidant effect, able to retard oxidative reactions and the development of rancidity due to their rapid reaction with oxygen (Ford & Lorkovic, 2002); iv) formation of cured flavour, depending on the formation of nitrite-related flavours and aromas enhanced by the suppression of rancidity (Sindelar & Milkowski, 2011).

Despite the functions exerted and their strictly controlled use in meat (Directive 2006/52/EC), nitrate and nitrite addition in food raised many concerns related to their potential of forming carcinogenic N-nitroso compounds (nitrosamine), which can be produced both in food matrices and human body (Hammes, 2012; Bernardo, Patarata, Lorenzo, & Fraqueza, 2021). This possibility was firstly suggested by Lijinsky and Epstein (1970) and confirmed by Tricker and Preusmann (1987). The reaction is potentiated by severe heat treatments and the effective health risks associated with the consumption of not thermally treated fermented sausages

has not been well elucidated (Hammes, 2012). The debate which followed these alarms showed that cured meats, and particularly fermented sausages, are nitrate sources of minor importance if compared with other potential sources, such as vegetables or water (Sindelar & Milkowski, 2011).

Nevertheless, there are increasing amounts of consumers which require fermented meats with artisanal feel (Leroy, Geyzen, Janssens, De Vuyst, & Scholliers, 2013), in which nitrate and nitrite, independently of their historical presence in sausages and their low concentration, are perceived as extraneous and, consequently, dangerous. Fermented sausage industry has the need to comply with these consumer demands requiring the elimination of nitrate and nitrite. Given the crucial role exerted by these molecules in fermented sausages, their elimination must be carefully evaluated to avoid quality decreases and maintain microbiological safety. A first strategy consists in the change of curing salts with other ingredients able to replace their activities. The use of plant extracts (such as celery, spinach, or Swiss chard) naturally rich in nitrate are a pseudo-solution (Leroy et al., 2013; Bernardo, Patarata, Lorenzo, & Fraqueza, 2021), adopted to obtain a clean label. However, European Commission recently specified that these extracts, when used with technological function, have to be considered and declared as food additives and not flavouring agents (European Commission, 2018). Other strategies are based on the use of other plant derivatives, such as essential oils or other fruit and vegetable derivatives (Ozaki et al., 2021) which, in many cases, can only partially replace nitrate and nitrite in their antimicrobial and antioxidant functions (Alahakoon et al., 2015). Also, the use of organic acids (lactate, citrate, sorbate, etc.) has been considered as an alternative to these compounds. Nevertheless, independently of their effectiveness, the use of acids, mainly due to their effect on pH, is often not compatible with the characteristics required for traditional fermented sausage, especially those produced in Mediterranean area (Van Reckem et al., 2019).

The aim of this work was to exploit the possibility to produce fermented sausages without the addition of nitrate and nitrite. The main risks of their elimination consist in the possible growth of undesired microorganisms such as *Clostridium botulinum, Salmonella* and *Listeria monocytogenes* (Bernardo et al., 2021; Patarata, Novais, Fraqueza, & Silva, 2020) and in the formation of undesirable colour of the meat, together

with a diverse sensory profile (Fraqueza, Laranjo, Elias, & Patarata, 2021). To obtains this goal, efforts was posed on the modification of process parameters adopted for the production of typical Italian salamis. In particular, the factors considered were the amounts of starter cultures and glucose added and, especially, the condition of fermentation and ripening, *i.e.* temperature and relative humidity (RH). The characteristics of the product obtained were compared with fermented sausages produced with nitrate and nitrite by analysing microbial counts, physico-chemical values, oxidation level, aroma profile, colour and texture. Finally, a challenge test was carried out to test the safety of the productive process.

2. MATERIALS AND METHODS

2.1 Sausage manufacture

The dry fermented sausages were produced in C.l.a.i. Soc. Coop. (Imola, Italy) with fresh pork meat used within 48 h from slaughtering and refrigerated at 0°C. Lean meat and fat (shoulder and neck, respectively) were minced (3.5 mm) and mixed (ratio 3:1) at approx. 0°C, added with NaCl (2.5% w/w), spices (garlic powder 0.01% and black pepper powder 0.15% w/w) and a commercial starter culture containing *Latilactobacillus sakei*, *Pediococcus acidilactici*, *Staphylococcus carnosus* and *Staphylococcus xylosus* (Chr. Hansen, Parma, Italy), at an initial concentration of approx. 7 log CFU/g for each species. Then the meat batter (300 kg) was divided in nine batches (approx. 30 kg each one): three (representing the control group, C) were added with KNO_3 (150 mg/kg) and $NaNO_2$ (50 mg/kg) and glucose 0.2% (w/w), three batches were prepared without preservatives and adding 0.2% glucose (F02) whereas the last three batches were prepared without preservatives and adding 0.4% glucose (F04). The meat mixtures were stuffed in a natural reconstituted hog casing (Varani, Castelplanio, Italy) with a diameter of 50 mm to obtain an initial weight of about 500 g. A spore suspension of *Penicillium nalgiovense* (Kerry Ingredient, Ireland) was sprayed on the casings. The fermentation and ripening process was carried out at temperature ranging from 15 to 10°C, at relative humidity (UR) from 65 to 90% for 50 days.

2.2 pH, a_w and weight loss

The analyses of pH and a_w of the fermented sausages during fermentation and ripening (0, 2, 6, 13, 24, 31 and 50 days from the production) were performed in triplicate by using a pH-meter Basic 20 (Crison Instruments, Barcelona, Spain) and an Aqualab CX3-TE (Labo-Scientifica, Parma, Italy), respectively. At each sampling time, sausages were also weighed to calculate the mean weight loss (%) with respect to the initial one. The analyses were performed in triplicate (three different sausages) and the results were expressed as mean value.

2.3 Microbial counts

The minced meat used to prepare sausages, the meat batter at time 0 and fermented sausages at the end of ripening (50 days) were analysed to determine microbial counts. After aseptic removal of the casing, a slice of approx. 10 g of sausage was transferred into a stomacher bag, mixed with 90 ml of 0.9% (w/v) NaCl sterile solution and homogenized in a Lab Blender Stomacher (Seward Medical, London, UK) for 2 min. Decimal dilutions were prepared in physiological solution and plated onto selective media to detect specific microbial groups. Lactic acid bacteria (LAB) were enumerated on de Man-Rogosa-Sharpe (MRS) agar incubated at 30°C for 48 h in anaerobic conditions. Coagulase negative cocci (CNC) were counted on Mannitol Salt Agar (MSA) incubated at 30°C for 72 h. Enterococci and *Enterobacteriaceae* were enumerated on Slanetz and Bartley medium and Violet Red Bile Glucose agar incubated for 24 h at 42°C and 37°C, respectively. Pseudomonads were counted on Pseudomonas Agar Base, supplemented with CFC Supplement and incubated for 48 h at 30°C. Sabouraud Dextrose Agar added with 0.2 g/l of chloramphenicol was used to determine yeasts by incubating plates at 28°C for 72 h. All media were provided by Oxoid (Basingstoke, UK). The analyses were performed in triplicate (three different sausages) and the results were expressed as mean value.

2.4 Colour and Texture Profile Analysis

Colour was analysed at the end of ripening (50 days). Colour [lightness (L*), redness (a*), and yellowness (b*)] was measured in five replicates on slices having a height of approximately 1 cm by a reflectance colorimeter

(Minolta Chroma Meter CR-400, Minolta Italia S.p.A., Milan, Italy), previously calibrated with a standard white ceramic tile, using illuminant source C.

Texture Profile Analysis (TPA) was assessed at 22°C using a TA-Hdi® texture analyser (StableMicro Systems, UK) equipped with a 25 kg loading cell. The test was performed on a 1 cm-high and 1.5 cm-wide cylindrical-shaped sample compressed up to 40% of its initial height by using a 5 cm-diameter aluminium probe. A time of 20 sec was set to elapse between two compression cycles. Force-time deformation curves were obtained and Hardness (kg), Springiness, Cohesiveness, Chewiness (kg), and Gumminess (kg) were calculated according to Bourne (1978). The analyses were performed in triplicate (three different sausages) and the results were expressed as mean value.

2.5 Thiobarbituric Acid Reactive Substances (TBARS)

TBARS were measured at the end of ripening according to the procedure described by Bao and Ertbjerg (2015) with slight modifications. Briefly, a 5 g sample was homogenized by Ultra-Turrax (IKA, Labortechnik, Staufen, Germany) in 15 ml trichloroacetic acid (5%, w/v) and 0.5 ml butylated hydroxytoluene (4.2% in ethanol, w/v) in ice. Then, the homogenate was filtered (Whatman 1, GE Healthcare), and the reaction prepared by boiling in water bath (100°C) for 40 min, 2 ml of filtrate mixed with 2 ml thiobarbituric acid (0.02 M). After cooling the samples, absorbance was read at 532 nm and TBARS content, expressed as mg malondialdehyde/kg of meat product, calculated from a standard curve prepared with 1,1,3,3-tetraethoxypropane. The analyses were performed in triplicate (three different sausages) and the results were expressed as mean value.

2.6 Aroma profile analysis

The volatile profile of sausages was analysed after 50 days (end of ripening). Gas-chromatography-mass spectrometry coupled with solid phase microextraction (GC-MS-SPME) technique was used to assess the aroma profile of the sausages at the end of ripening. 3 g of samples were added with known amount of 4 methyl-2-pentanol (Sigma-Aldrich, Steinheim, Germany) as internal standard and analysed according to the protocol

reported by Montanari et al. (2016). Volatile peak identification was carried out using Agilent Hewlett–Packard NIST 2011 mass spectral library (Gaithersburg, MD, United States) (NIST, 2011). The mass spectrum identification was confirmed in the same conditions by injection of the pure standards (Sigma- Aldrich, St. Louis, MO). Data are expressed as ratio between each molecule peak area and the peak area of internal standard. The analyses were performed in triplicate (three different sausages) and the results were expressed as mean value.

2.7 Challenge test

To assess the safety of fermented sausages produced without preservatives, a challenge test was performed in the sausages added with 0.2% of glucose (F02) and the control with nitrate/nitrite (C). The sausages containing 0.4% glucose were not considered in the challenge test due to the results of the previous trials, in particular higher oxidation level and hardness of the sausages. The target microorganisms were *Listeria innocua* as surrogate of *L. monocytogenes* (da Silva, de Oliveira Pena, Pflanzer, & da Silva do Nascimento, 2019), *Salmonella enterica* subspecies *enterica* and *Clostridium botulinum*. The three microbial groups considered were separately inoculated in the different batches.

For *L. innocua*, a cocktail of five strains of different origin was used (four isolated from sausage production environment and a collection strain – ATCC33090). Strains were precultured twice in Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK) at 30°C for 24 h (first step) and at 12°C for 72 h (second step), then mixed and this suspension was used to inoculate the meat batter (10 kg, 20 sausages) at a concentration of approx. 7 log CFU/g. As far as *Salmonella*, a mixture of 3 strains (*Salmonella enterica* subspecies *enterica* serovar Typhimurium ATCC14028, *Salmonella enterica* subspecies *enterica* serovar Typhimurium monophasic variant isolated from pork sausage and *S. enterica* subspecies *enterica* serovar Derby isolated from pork meat) was used, after two 24 h precultures in BHI at 30°C for 24 h. The precultures were added to reach a final concentration in the meat batter (10 kg, 20 sausages) of approx. 7 log CFU/g. Finally, for *Cl. botulinum* a cocktail of four strains was used and namely two non-proteolytic (type B and type E) and two proteolytic (type

A and type B), including a collection strain – ATCC 19397. In this case, strains were cultivated in TPGYT medium (Trypticase, Peptone, Glucose, Yeast-extract, Trypsin) at 30°C for 15 days under anaerobiosis to allow sporification. Then cell suspensions were thermally treated (80°C for 20 min), and spores were enumerated to be inoculated in the meat batter (10 kg, 20 sausages) at a concentration of approx. 3 log CFU/g.

The different meat batters were then stuffed and processed in the same conditions reported in section 1.1 and analysed during fermentation and ripening (0, 2, 6, 13, 31 and 50 days) to monitor the behaviour of these microbial species in the products. The enumeration or the detection of *Listeria* were performed according to the methods EN ISO 11290-2 and EN ISO 11290-1 (ISO, 2017a, b). *S. enterica* subsp. *enterica* was counted on Hektoen Enteric Agar (Oxoid, Basingstoke, UK) incubated at 37°C for 24 h, while the detection was performed according to ISO 6579-1 (ISO, 2017c). Finally, *Cl. botulinum* was counted following the procedure described in ISO 15213 (ISO, 2013).

2.8 Statistical analysis

Data were analysed through One-way ANOVA considering the absence of preservatives together with the eventual addition of a higher glucose content in the formulation as the main effect. Means were subsequently analysed through the parametric Tukey-HSD test.

To further investigate the effect of the absence of nitrite and nitrate on the oxidation, colour and texture profile, planned orthogonal contrasts were performed to compare the findings obtained within the control group (C) with those found in F02 and F04 samples. All statistical differences were considered significant at a level of $p \leq 0.05$. Analyses were carried out by using Statistica software (StatSoft Italy srl, Vigonza, Italy).

3. RESULTS AND DISCUSSION

3.1 Process and ripening conditions and microbial counts

Safety and quality of fermented sausages is the result of the application of several hurdles addressed to the inhibition of undesirable microorganisms. The removal of one of these hurdles (such as nitrate/nitrite) must be

220 counterbalanced by more drastic conditions for the other ones or the addition of new factors. In this trial, we
221 firstly decided to lower fermentation and ripening temperatures 10°C *vs.* 15-20°C of the traditional program
222 (Montanari et al., 2018; Tabanelli, Montanari, Grazia, Lanciotti, & Gardini, 2013) with the aim to reduce, in the
223 absence of nitrate and nitrite, the growth potential of *Enterobacteriaceae* during the first days, when a_w is still
224 high (Hospital et al., 2015). The second modification concerned the use of commercial starter cultures
225 (containing selected strains of *Lat. sakei, P. acidilactici, Staph. carnosus* and *Staph. xylosus*) added at higher
226 concentration (approx. 7 log CFU/g) with respect to the standard procedures adopted for Mediterranean
227 sausages, in which the cultures are added at about 6 log CFU/g (Montanari et al., 2018; Tabanelli et al., 2013).
228 Besides, the LAB strains used were characterized by antagonistic and bioprotective activity against *L.*
229 *monocytogenes* (Raimondi, Popovic, Amaretti, Di Gioia, & Rossi, 2014; Stahnke, 2008), which could take
230 advantage by the low temperature adopted during fermentation and ripening, especially in low contaminated
231 meats (Patarata, Novais, Fraqueza, & Silva, 2020). In fact, in order to limit the growth potential of wild
232 undesirable microbial population the sausages were manufactured using with fresh (not frozen) pork meat,
233 maintained under strictly controlled refrigeration temperature (0°C) and used within 48 h after slaughtering.
234 Three different typologies were considered in the trials: sausages without nitrate/nitrite produced with glucose
235 at 0.2% (F02), sausages without nitrate/nitrite produced with glucose at 0.4% (F04) and the control, *i.e.*
236 sausages with nitrate/nitrite produced with glucose at 0.2% (C).
237 The results of the microbial counts (Table 1) showed that LAB and CNC amounts in the meat batter
238 immediately after casing were highly dependent on the addition of the starter cultures, as expected. Indeed,
239 the counts of both these microbial groups in the minced meat before the addition of the cultures were lower
240 than 4 log CFU/g. At the end of ripening, the counts of LAB were higher than 8 log CFU/g in all the samples,
241 while CNC were approx. 7 log CFU/g and no significant difference ($p > 0.05$) in relation to the type of sausage
242 was observed. These levels of LAB and CNC are comparable with those found in similar Italian and Portuguese
243 fermented sausages at the end of ripening (Belleggia et al., 2022a, b; Tabanelli et al., 2015; Van Reckem et al.,
244 2019). In other studies, CNC were more susceptible to the presence of variable amounts of nitrate/nitrite.

245 Hospital et al. (2015) reported a double contrasting effect of nitrate/nitrite on these bacteria. On one side,
246 their presence can favour CNC growth, especially in the central part of sausages in which O_2 is scarce and
247 nitrate can act as final electron acceptor, accelerating their multiplication. On the other side, excessive
248 accumulation of nitrite can result in growth inhibition.
249 Enterococci were present at low concentration (2.5 log CFU/g) after casing and their final number never
250 exceeded 2.7 log CFU/g. Interestingly, enterococci counts in the sausages added with nitrate/nitrite were
251 significantly lower than in the sausages obtained without these preservatives.
252 A similar behaviour was observed for *Enterobacteriaceae*. Their initial count (2.6 log CFU/g) decreased in all the
253 sausages, but at a higher significant extent in the control, confirming the important role exerted by
254 nitrate/nitrite in the inhibition of these bacteria. This fact emphasizes the importance of enterobacteria control
255 in the first steps of fermentation when a_w and pH can still allow their multiplication (Christieans, Picgirard,
256 Parafita, Lebert, & Gregori, 2018; Hospital, Hierro, & Fernández, 2014; Hospital et al., 2015). Pseudomonads
257 were always below the detection limit.
258 Yeasts, initially present at 1.9 log CFU/g, grew up to more than 4 log CFU/g in all the samples without
259 significant differences. These counts are in accordance with data previously reported by other studies
260 (Belleggia et al., 2022b; Greppi et al., 2015; Montanari et al., 2018; Selgas & García, 2014).
261

262 **3.2 Chemico-physical features**

263 The results concerning pH, a_w and weight loss of the sausages during ripening are reported in Figure 1. The
264 evolution of pH in the different samples (Figure 1A) was clearly influenced by the amounts of glucose added. In
265 fact, in the sausages containing 0.4% glucose the pH decrease observed during fermentation is more relevant
266 (about 0.25 pH units) if compared with the control (C) and the sausages containing 0.2% glucose and without
267 nitrate/nitrite (F02). This difference remained constant during the ripening, and the pH increased up to 5.50 in
268 the sausages F02 and to 5.25 in the samples F04.

No significant differences were observed for a_w and weight loss during ripening (Figures 1B and 1C). The final a_w ranged between 0.908 and 0.914 and the weight losses between 37.7 and 38.7%.

3.3 Colour and oxidative stability of the lipid fraction

Overall, the addition of different glucose levels in the formulation exerted some effects on both the colour parameters and the textural traits of the dried fermented sausages (Table 2). In detail, although no significant differences were found in lightness (L*) and yellowness (b*), redness (a*) was remarkably affected by preservatives as well as by the glucose content added in the formulation. Indeed, if compared to C, a significantly lower a* value was found in F02 (13.51 *vs.* 11.58; $p < 0.01$), whereas F04 exhibited an intermediate value (12.57). This finding may be primarily ascribed to the absence of nitrate and nitrite in the formulation regardless of the glucose content added. Indeed, by means of planned orthogonal contrasts performed to assess the possible effect of the absence of preservatives (regardless of the glucose content added), a significant difference ($p < 0.05$) in a* was found between C and those experimental groups in which no preservatives were added (*i.e.*, F02 and F04) (LSM: 13.51 *vs.* 11.58 and 12.57). Within this context, it is worth mentioning that curing colour development is strongly affected by the acidification process taking place during product's fermentation (Campbell-Platt & Cook, 1995). Also, the colour parameter Chroma (C*) exhibited a similar trend: if compared to C, a significantly lower value was found in F02 (14.60 *vs.* 12.42; $p < 0.01$), whereas F04 exhibited an intermediate value (13.69). As previously observed for a*, planned orthogonal contrasts suggest that this outcome may be due to the absence of nitrate and nitrite in dry fermented sausages formulation. If compared to C, a significantly lower ($p < 0.05$) saturation index was observed, regardless of the glucose content added, in those experimental groups in which no preservatives were added (LSM:14.60 *vs.* 12.42 and 13.69). This finding may be explained by considering that the rate at which total heme pigments are converted in their nitric oxide form sharply increases as the pH decreases, especially within the pH range from 5.5 to 4.5 (Fox & Thompson, 1963).

As for the textural parameters, if compared to C, significantly higher Hardness, Gumminess and Chewness values were found in F02 and F04 by means of both analysis of variance and planned orthogonal contrasts. Regarding hardness, the values increased with the glucose content in the formulation in which no preservatives were added, with F04 exhibiting the highest value (3.74 kg). This outcome may be explained by considering that during fermentation, pH values close to the isoelectric point of the proteins are reached (Figure 1A), thus leading to a consequent increased protein aggregation that determines the development of firmer sausages (Fretheim, Egelandsdal, Harbiz, & Samejima, 1985; Gonzalez-Fernandez, Santos, Rovira, & Jaime, 2006). Indeed, a strong acidification is essential to reduce the water holding capacity of the meat proteins and thus promote water loss and evaporation (Lorenzo, Gómez, & Fonseca, 2014). In addition, under a fast acidification process, acid solubilization of collagen is also likely induced (Aktas & Kaya, 2001) and may account for the trend observed for Cohesiveness, which exhibited remarkably higher values in C samples rather than in F02 and F04 (1.83 *vs.* 1.72 and 1.73; $p < 0.001$).

Lipid oxidation level was assessed by measuring TBARS developed at the end of the ripening process. Overall, the amount of secondary products of lipid oxidation significantly differ among the experimental groups (as shown in Table 2), due to the antioxidant effect of nitrate/nitrite. In detail, as expected, the lower TBARS were observed in the sausages added with nitrate/nitrite. These findings are in agreement with the widely known antioxidant properties of nitrate/nitrite in sausages (Honikel, 2008). Indeed, nitrite can limit the development of oxidative reactions affecting the lipid fraction in several ways: i) sequestering oxygen molecules (Honikel, 2008), ii) stabilizing heme iron and sequestering free iron (Bergamaschi and Pizza, 2011), and iii) reacting with lipid radicals thus breaking the oxidative chain reaction upon solubilization of nitric oxide in fats (Skibsted, 2011). On the other hand, fermented sausages formulated with the addition of 0.2% glucose exhibited a lower oxidation level of the lipid fraction if compared to those containing the higher glucose concentration (*i.e.*, 0.4%). This outcome may be explained by considering the differences in pH directly associated to the amount of sugar added into products' formulation. Indeed, in light of the increased solubility of iron under acidic conditions (Chaijan & Panpipat, 2017; Domínguez et al., 2019) the lower pH resulting from the addition of a

higher glucose content may have promoted the development of oxidative reactions affecting the lipid fraction. In addition, it has been recently reported that reducing sugars may be involved in the oxidative deamination of the amino group residues in protein (Akagawa, Sasaki, & Suyama, 2002; Luna & Estèvez, 2018). Thus, the significantly higher lipid oxidation observed in F04 may be explained by considering the pro-oxidant potential of these compounds along with the strong interdependence existing between the oxidative modifications affecting the protein and the lipid fractions.

3.4 Aroma profile

In Table 3, the main volatile molecules detected in the sausages at the end of ripening through SPME-GC-MS analysis are reported. The molecules deriving from spices, added in the same amounts in the meat batter, are not included in the table. Nevertheless, the compounds detected were limonene, β-phellandrene, caryophyllene, α-phellandrene, *o*-cymene, copaene, terpinene-4-ol, linalool (in decreasing order, deriving from black pepper) and allyl-methyl-sulfide (from garlic). The remaining molecules were grouped according to their chemical characteristics in ketones, aldehydes, alcohols, acids, and esters.

Relevant differences related to the amount of sugar added and the presence of nitrate/nitrite were observed among ketones. These differences mainly concerned acetone, 2,3-butanedione (diacetyl) and 3-hydroxy-2-butanone (acetoin). According to Flores (2018), the origin of these compounds can be related to fermentation processes. In particular, the metabolism of pyruvate is the starting point for the production these molecules. LAB, including *Lat. sakei*, are particularly active in using pyruvate through alternative metabolic routes, especially when fermentable sugars are scarce or depleted (von Wright & Axelsson, 2011). Besides, they can also obtain pyruvate from amino acids (Barbieri, Laghi, Gardini, Montanari, & Tabanelli, 2020; Barbieri et al., 2022). This may explain their lower accumulation in the sausages produced without nitrate/nitrite containing 0.2% glucose with respect to the sausages with 0.4% of the same sugar. However, the presence of nitrate/nitrite determined the higher accumulation of diacetyl and acetoin in the control, even if at the lower sugar concentration. In effect, the reduction of nitrate/nitrite can favour the growth of coagulase positive cocci

343 (CNC) (Perea-Sanz, Montero, Belloch, & Flores, 2019) with a concomitant limitation of the metabolic potential
344 of *Lat. sakei*.
345 Hospital et al. (2015) described an opposite trend in *chorizo*, in which both diacetyl and acetoin increased with
346 the diminution of nitrate/nitrite and attributed this outcome to the higher concentration of staphylococci in
347 the sausages without nitrate/nitrite added, but in this case they used a strain of *Pediococcus pentosaceus* as
348 starter LAB. In this trial at the end of ripening, no significant difference was observed in CNC in the different
349 sausages produced (Table 1). Nevertheless, the presence of nitrate can be an alternative source for NADH
350 regeneration making pyruvate available for other metabolic pathways which can bring to the accumulation of
351 these molecules (Hammes, 2012; Sánchez-Mainar & Leroy, 2015). Perea-Sanz et al. (2019) found significantly
352 lower concentrations of diacetyl (but not of acetoin) in sausages produced with lowered nitrate addition. These
353 discrepancies may be explained also in relation to the process adopted and, in particular, to the final pH of the
354 sausages which, in the case object of this study, was rather high if compared with pH reached in the cited
355 studies. The presence of methyl ketones in sausages can be the result of microbial β-oxidation of lipids,
356 deriving from β-ketoacids produced during β-oxidation carried out in first instance by moulds and staphylococci
357 (Lorenzo, Gómez, Purriños, & Fonseca, 2016; Ordóñez, Hierro, Bruna, & de la Hoz, 1999). In particular, 2-
358 heptanone, 3-hexen-2-one and 2-pentanone concentration was higher in the samples without nitrate/nitrite,
359 indicating a possible higher activity of staphylococci.
360 Aldehydes reached their highest concentration in the sausages without nitrate/nitrite added with 0.4% glucose,
361 whilst in the control they were detected at the minimum level. A positive correlation between TBARS and linear
362 aldehydes C3-C7 detected using SPME-GC-MS has been described in literature (Olivares et al., 2011). The data
363 reported here confirmed this observation (see Table 2). In general, in fact, the quantity of aliphatic aldehydes,
364 accumulated through the action of hydroperoxydases (Ordóñez et al., 1999) was significantly lower in the
365 control. The amount of 3-methylbutanal, deriving from leucine and with a positive and important effect on
366 sausages aroma profile (Flores, 2018; Carballo, 2012) did not show differences in relation to the condition
367 considered.

The sausages produced with glucose at 0.4% were characterized by the highest alcohol accumulation. Ethanol was the major molecule of this chemical group. Ethanol can be produced during mixed acid LAB fermentation starting from pyruvate when fermentable sugars are scarce or completely depleted through mixed acid fermentation (von Wright & Axelsson, 2011) The higher content of ethanol in the sausages produced with 0.4 glucose confirm this hypothesis. Besides, other alcohols presented a similar trend, and in particular 1-octen-3-ol, 2-octen-1-ol, heptanol and hexanol.

Total acids were similar in the control (containing glucose at 0.2%) and in the samples added with 0.4% of glucose without nitrate/nitrite. These values were significantly higher if compared with the sausages produced in the absence of preservatives produced adding glucose at 0.2%. Among acids, acetic acid was the most representative. It can be produced by several pathways from LAB and staphylococci starting from pyruvate provided by sugar fermentation and other metabolisms, which can involve amino acids (Barbieri et al., 2020; Gänzle, 2015; Sánchez-Mainar & Leroy, 2015). As observed for ethanol, acetic acid is one of the final products of the metabolism of mixed acid fermentation in LAB. In particular, under defined conditions, the activity of the enzyme acetate kinase allows the accumulation of a supplementary ATP from acetyl phosphate obtained from pyruvate (von Wright & Axelsson, 2011; Zotta, Parente, & Ricciardi, 2017). This could confirm a LAB reduced activity observed determined by a higher metabolic competitiveness of staphylococci. In contrast with this data, other authors (Hospital et al., 2015; Perea-Sanz, Montero, Belloch, & Flores, 2018) found an increase of this acid in the sausages without or with lower nitrate/nitrite content. The other organic acids followed a similar behaviour, showing lower amounts in the sausages produced without nitrate/nitrite and added with glucose at 0.2%. This trend characterized also 3-methyl-butanoic (isovaleric) acid, derived from leucine metabolism. This molecule is often detected in sausages, but when its concentration is too high can be responsible for the formation of severe off-odours, due to its strong sensory impact (Montanari, Barbieri, Gardini, & Tabanelli, 2021).

3.5 Challenge test

393 Challenge tests were performed with the aim to evaluate the response of *Salmonella enterica* subsp. *enterica*,
394 *Listeria innocua* (used as surrogate of *Listeria monocytogenes*) and *Clostridium botulinum* under the process
395 conditions adopted. In this part of the work, only the sausages containing 0.2% of glucose, added or not with
396 nitrate/nitrite, have been considered due to its textural and oxidative characteristics. In fact, the oxidation level
397 increased in F02 and F04, as expects, but at a lesser extent. The lower pH of F04 caused also a relevant increase
398 in harness, if compared with the control, which was not observed in F02. A cocktail of strains belonging to the
399 three microbial groups were separately inoculated to obtain initial concentration of approx. 7 log CFU/g for *S.*
400 *enterica* subsp. *enterica* and *L. innocua* and 3 log CFU/g for *Cl. botulinum*.
401 Figure 2A represents the counts of *L. innocua* during fermentation and ripening. After 50 days of ripening, they
402 were 2 log units below the initial inoculum of the strains of *L. innocua*, regardless the presence of
403 nitrate/nitrite. However, the data of the sampling after 2 and 6 days showed significant higher counts in the
404 sausages without nitrate/nitrite. It is noteworthy the increase of the counts observed after 2 days in F02. Even
405 after 13 days, the counts are lower in the control. Only after this period *L. innocua* concentration is similar
406 (without significant differences) in both sausage type. Similar trends for *L. monocytogenes* were observed by
407 Christieans et al. (2018) using reduced levels of nitrate/nitrite. Differently from the observation of these
408 authors, the concentration of *L. innocua* at the end of ripening in the present work did not depend on the level
409 of curing salts added, probably due to the antilisterial activity of starter cultures. Nevertheless, the initial
410 increase of counts observed, although the strains of *L. innocua* were inoculated at a challenging concentration,
411 underlined the need to strict control the fermentation step.
412 Regarding the challenge test carried out with *S. enterica* subsp. *enterica* (serovars Typhimurium and Derby), the
413 results reported in Figure 2B showed no significant difference in relation to the presence of nitrate/nitrite and
414 to the sampling time. In both the trials, the ripening conditions applied allowed the decrease of the challenging
415 concentration of *S. enterica* subsp. *enterica* of about 2.5 log units after 50 days. A previous study (Hospital et
416 al., 2014) indicated that *Salmonella* Typhimurium can growth and improve its survival in the absence of
417 nitrate/nitrite. This discrepancy can be explained by the low temperature (<10°C) applied in this industrial

418 production during fermentation and ripening. In fact, even if the minimum growth temperature for *Salmonella*
419 is approx. 5°C (ANSES, 2011), the growth rate and survival of this pathogen are greatly reduced below 15°C
420 (Bell & Kyriakides, 2002). On the other hand, the effects of nitrate/nitrite on *Salmonella* spp. are rather
421 controversial, differently from other pathogens such as *Listeria* spp. and *Clostridium botulinum*, in which a
422 significant inhibition of the growth is reported (Hospital et al., 2014). Christieans et al. (2018) found that nitrite
423 is a relevant hurdle for *Salmonella* Thyphimurium in sausages. This bacterium has recently been associated
424 with several outbreaks due to fermented sausages consumption (Omer et al., 2018) and its control is becoming
425 crucial for the fermented meats. Independently on the addition of nitrate/nitrite, the results obtained in these
426 trials at the end of ripening were more relevant than those of Mataragas et al. (2015), in which both *L.*
427 *monocytogenes* and *Salmonella enterica* were reduced less than 2 log units at the end of ripening of Cacciatore
428 and Felino type sausages. In these studies, traditional temperature profiles were adopted.
429 Figure 2C represents the results of the challenge test with *Cl. botulinum*. In this case, the amount inoculated
430 was lower (about 2.7 log CFU/g). No relevant count increase was observed until the end of ripening, and, at the
431 different sampling times, no difference was observed in relation to the addition of nitrate/nitrite. Undoubtedly,
432 this toxin producer microorganism has been historically viewed as the main target of nitrate and nitrite in
433 fermented sausages. However, under industrial conditions, the number of spores present in raw materials is
434 rather low and the processes adopted are sufficient to limit it growth potential. In this case, the adoption of
435 low temperatures likely inhibits the growth of proteolytic *Cl. botulinum* while the non-proteolytic
436 psychrotrophic members of this species cannot develop when the a_w is lower than 0.97, which approximately
437 corresponded to the initial mixture a_w of the sausages. This can explain lack of registered outbreaks from
438 fermented sausages or the low risk of food poisoning from these products (Holck, Axelsson, McLeod, Rode, &
439 Heir, 2017).
440

441 **4. CONCLUSIONS**

In this research a process for producing high quality fermented sausages without the use of nitrate and nitrite or any other substitute was studied. The removal of this important hurdle to the proliferation of undesirable microorganisms was balanced by the modification of the process, that was longer and at lower temperature (10-15°C) with respect to the conditions traditionally adopted for these Mediterranean products. Moreover, high concentrations of bioprotective starter cultures were used to control the first crucial fermentation process. The absence of nitrate/nitrite did not significantly affect some chemico-physical and microbiological parameters, such as a_w, weight loss and LAB and CNC counts. On the other hand, the amount of sugar added had a relevant effect on some characteristics of the final products. Indeed, in the absence of curing salts, lower sugar concentration resulted in better textural features and lower oxidation level. These factors also affected the aroma profile, since the final product obtained without nitrate/nitrite and with 0.2% of glucose was characterized by lower amounts of ketones and acids. Among the latter, it is noteworthy the reduced accumulation of 3-methyl-butanoic acid, whose high presence in fermented sausages can be responsible for off odours. The challenge tests evidenced the inability of selected strains of *Listeria innocua, Salmonella enterica* sub. *enterica* and *Clostridium botulinum* to grow, under the adopted conditions, in fermented sausages.

FUNDING

The work was supported by Regione Emilia-Romagna in the framework of the project "Qualità e sicurezza di salami prodotti senza l'impiego di nitrati/nitriti", PSR 2014-2020 – Progetti di filiera – misura 16.2.01-progetti pilota e sviluppo dell'innovazione, CUP E23E18000080007.

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Table 1. Microbiological analyses (log CFU/g) of meat, meat batter and ripened fermented sausages produced with preservatives (C) or without nitrate and nitrite using two different glucose concentrations, (0.2% (F02) or 0.4% (F04)). Results are the mean of three independent repetitions (standard deviation is reported). For each microbial group, significant differences among samples at the end of ripening according to ANOVA are indicated by the presence of different letters.

Microbial group	Meat	Meat batter	End of ripening (50 days)		
			C	F02	F04
Lactic acid bacteria	3.2 ± 0.2	7.2 ± 0.2	8.2 ± 0.1	8.1 ± 0.2	8.3 ± 0.1
Coagulase negative cocci	3.8 ± 0.2	6.5 ± 0.2	6.8 ± 0.2	7.1 ± 0.1	7.0 ± 0.1
Enterococci	2.3 ± 0.1	2.5 ± 0.2	2.1^{a} ± 0.2	2.7^{b} ± 0.2	2.6^{b} ± 0.1
Enterobacteriaceae	2.5 ± 0.2	2.6 ± 0.2	1.5^{a} ± 0.1	2.3^{b} ± 0.1	2.3^{b} ± 0.1
Yeasts	1.8 ± 0.1	1.9 ± 0.1	4.6 ± 0.1	4.3 ± 0.1	4.4 ± 0.2

665 **Table 2.** Colour parameters, textural features and lipid oxidation level (TBARS) of ripened fermented sausages
666 produced with (C) or without the addition of preservatives (*i.e.*, nitrate and nitrite) using two different glucose
667 concentrations, namely 0.2% (F02) or 0.4% (F04). Results belong to three independent repetitions and are
668 expressed as mean values ± standard deviation.
669

Parameter	End of ripening (50 days)			p-value	
	C	F02	F04	Analysis of variance	Planned orthogonal contrasts[1]
Color					
Lightness - L*	42.27 ± 4.46	40.26 ± 3.00	40.81 ± 2.05	ns	ns
Redness - a*	13.51^{a} ± 1.22	11.58^{b} ± 1.68	12.57^{ab} ± 1.13	**	*
Yellowness - b*	5.50 ± 1.01	4.44 ± 0.50	5.38 ± 0.93	ns	ns
Chroma - C*	14.6^{a} ± 1.4	12.4^{b} ± 1.6	13.7^{ab} ± 1.2	**	*
Hue angle - h	0.39 ± 0.05	0.37 ± 0.05	0.40 ± 0.06	ns	ns
Texture Profile Analyses (TPA)					
Hardness (kg)	2.07^{c} ± 0.04	3.20^{b} ± 0.12	3.74^{a} ± 0.23	***	***
Cohesiveness	1.83^{a} ± 0.02	1.72^{b} ± 0.02	1.73^{b} ± 0.01	***	***
Gumminess (kg)	3.79^{c} ± 0.07	5.51^{b} ± 0.18	6.46^{a} ± 0.34	***	***
Springiness	1.39 ± 0.65	1.80 ± 0.03	1.75 ± 0.05	ns	ns
Chewiness (kg)	5.27^{b} ± 2.47	9.94^{a} ± 0.21	11.32^{a} ± 0.64	**	**
Oxidation of the lipid fraction					
TBARS (mg MDA/kg of meat product)	0.45^{c} ± 0.03	0.80^{b} ± 0.02	1.10^{a} ± 0.07	**	**

670
671 [1]Planned orthogonal contrasts performed to assess the eventual effect of the absence of nitrite and nitrate in
672 dry fermented sausages formulation regardless of the glucose content added (C *vs.* F02 and F04).
673 *=p <0.05, **= p < 0.01, ***= p < 0.001, ns = not significant; a,c = mean values followed by different letters,
674 significantly different among the groups (p <0.05).

675 **Table 3.** Aroma profile detected by SPME-GC-MS in the ripened sausages produced with preservatives (C) or
676 without nitrate and nitrite using two different glucose concentrations, namely 0.2% (F02) or 0.4% (F04). Data
677 are expressed as ratio between peak area of each molecule and peak area of the internal standard (4-methyl-2-
678 pentanol). Results are the mean of three independent repetitions and standard deviations are reported. For
679 each molecule significant differences between samples according to ANOVA are indicated by the presence of
680 different letters.

Volatile compound	C	F02	F04
Acetone	32.2 ± 1.0 [a]	18.6 ± 1.3 [b]	33.4 ± 1.8 [a]
2-butanone	1.06 ±0.18 [ab]	0.821 ± 0.358 [a]	1.62 ± 0.25 [b]
2-pentanone	0.643 ± 0.211 [a]	0.878 ± 0.164 [a]	2.10 ± 0.45 [b]
2,3-butanedione	4.92 ± 0.95 [a]	0.349 ± 0.098 [b]	0.714 ± 0.228 [b]
Methyl isobutyl ketone	0.449 ± 0.182	0.179 ± 0.209	0.491 ± 0.089
3-hexen-2-one	1.78 ± 0.45 [a]	5.10 ± 1.30 [b]	2.86 ± 0.57 [a]
3-hydroxy-2-butanone	11.7 ± 1.2 [a]	0.601 ± 0.411 [b]	4.26 ± 0.99 [c]
2-heptanone	1.90 ± 0.66 [a]	3.51 ± 0.91 [b]	4.07 ± 0.19 [b]
2-nonanone	0.914 ± 0.342	1.07 ± 0.14	1.41 ± 0.33
Ketones	**55.6 ± 2.8 [a]**	**31.1 ± 2.2 [b]**	**50.9 ± 3.3 [a]**
3-methylbutanal	0.175 ± 0.093	0.264 ± 0.133	0.192 ± 0.098
Hexanal	1.43 ± 0.30	1.10 ± 0.23	1.18 ± 0.15
2-heptenal	0.541 ± 0.334 [a]	2.41 ± 0.33 [b]	3.01 ± 0.53 [b]
Nonanal	1.05 ± 0.25 [a]	3.23 ± 0.51 [b]	4.94 ± 0.33 [c]
2-nonenal	1.75 ± 0.53	1.11 ± 0.65	1.83 ± 0.54
Benzaldehyde	0.591 ± 0.402	0.631 ± 0.258	1.29 ± 0.48
Decanal	0.343 ± 0.201	0.539 ± 0.249	0.202 ± 0.212
Aldehydes	**5.89 ± 0.41 [a]**	**9.28 ± 0.23 [b]**	**12.6 ± 0.4 [c]**
Ethanol	4.79 ± 0.89 [a]	5.98 ± 1.01 [a]	9.86 ± 0.75 [b]
Hexanol	0.551 ± 0.319 [a]	0.542 ± 0.291 [a]	1.15 ± 0.34 [b]
1-octen-3-ol	0.404 ± 0.264 [a]	0.810 ± 0.410 [ab]	1.318 ± 0.121 [b]
1-heptanol	0.414 ± 0.244 [a]	0.338 ± 0.173 [a]	1.04 ± 0.34 [b]
1-octanol	0.713 ± 0.331 [a]	2.94 ± 0.79 [b]	0.968 ± 0.413 [a]
2-octen-1-ol	4.15 ± 0.65 [ab]	3.35 ± 0.82 [a]	5.43 ± 0.54 [b]
Alcohols	**11.0 ±1.0 [a]**	**14.0 ±1.2 [b]**	**19.8 ± 1.0 [c]**
Acetic acid	24.0 ± 2.0 [a]	7.44 ± 1.11 [b]	19.8 ± 1.9 [c]
3-methyl butanoic acid	1.86 ± 0.15 [a]	0.724 ± 0.363 [b]	1.33 ± 0.23 [b]
Butanoic acid	1.84 ± 0.31 [a]	0.456 ± 0.329 [b]	2.09 ± 0.53 [a]
Pentanoic acid	1.12 ± 0.22 [a]	1.04 ± 0.57 [a]	2.28 ± 0.33 [b]
Hexanoic acid	1.80 ± 0.18	1.46 ± 0.31	2.05 ± 0.45
Heptanoic acid	0.692 ± 0.331	0.714 ± 0.509	1.54 ± 0.43

681

Octanoic acid	2.03 ± 0.28 [a]	2.47 ± 0.65 [ab]	3.52 ± 0.45 [b]
Nonanoic acid	2.35 ± 0.64	1.68 ± 0.83	2.66 ± 0.22
Acids	**35.7 ± 2.9 [a]**	**16.0 ± 1.4 [b]**	**35.3 ± 2.4 [a]**
Ethyl acetate	0.212 ± 0.153 [ab]	0.149 ±0.081 [a]	0.429 ± 0.222 [b]
Esters	**0.212 ± 0.153 [ab]**	**0.149 ± 0.081 [a]**	**0.429 ± 0.222 [b]**

682 **FIGURE CAPTIONS**

683 **Figure 1** pH (1A), a_w (1B) and weight loss (1C) during fermentation and ripening of fermented sausages
684 produced with preservatives (C) or without nitrate and nitrite using two different glucose concentrations,
685 namely 0.2% (F02) or 0.4% (F04). The data are the mean of three independent samples and standard error bars
686 are reported.

687

688 **Figure 2**: Microbial counts (expressed as log CFU/g) of *Listeria innocua* (2A), *Salmonella enterica* subsp. *enterica*
689 (2B) and *Clostridium botulinum* (2C) during fermentation and ripening of fermented sausages produced with
690 preservatives (C) or without nitrate and nitrite (F02). The glucose addition was 0.2 % for both samples. The
691 data are the mean of three independent samples and standard error bars are reported.

Figure 1

Click here to access/download;Figure;Figure 1.tif

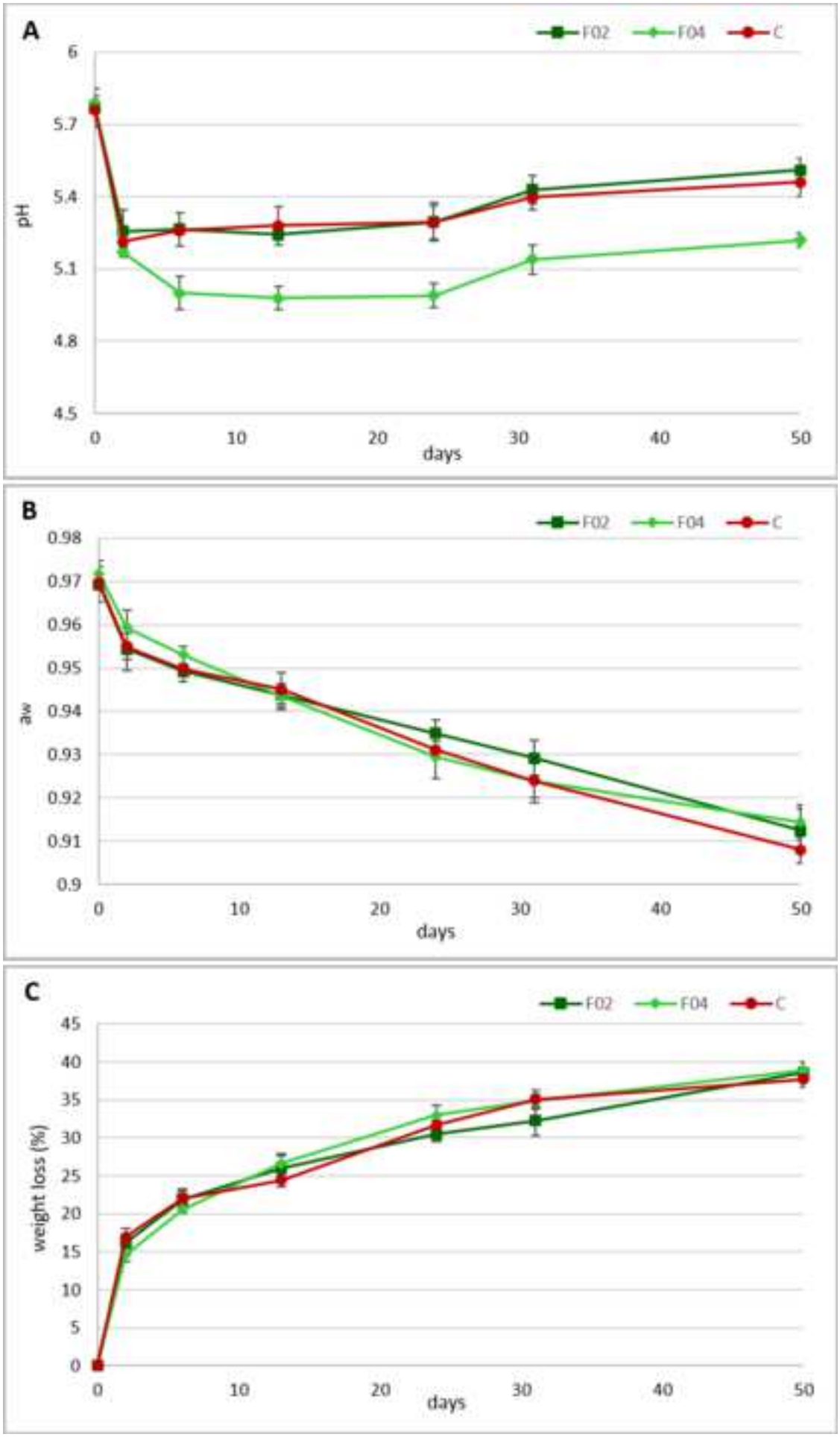

Figure 2

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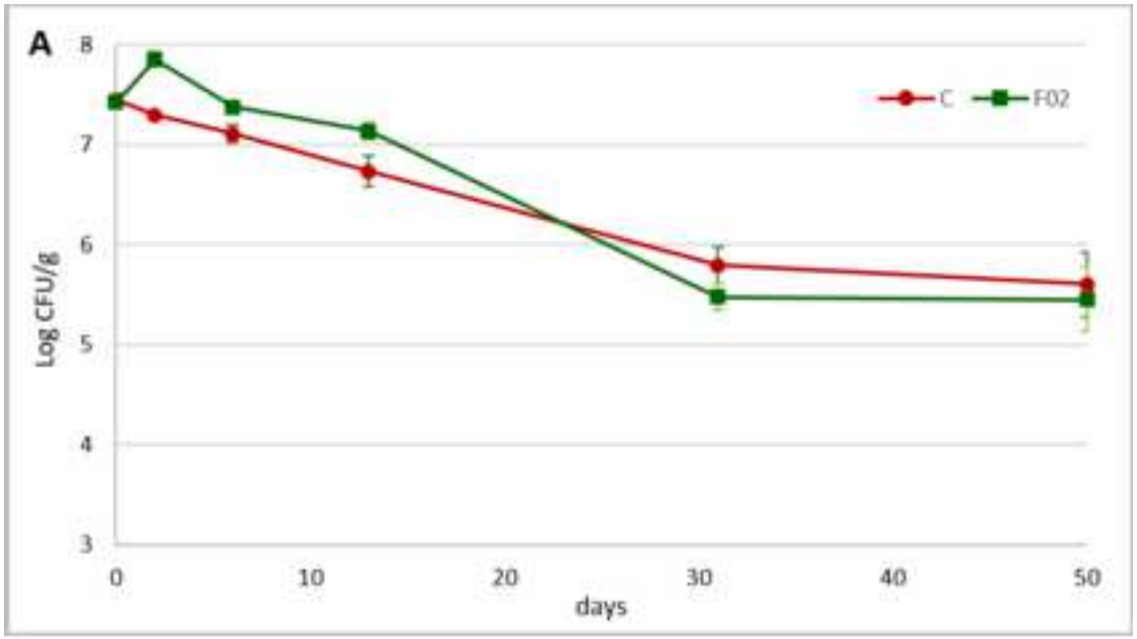
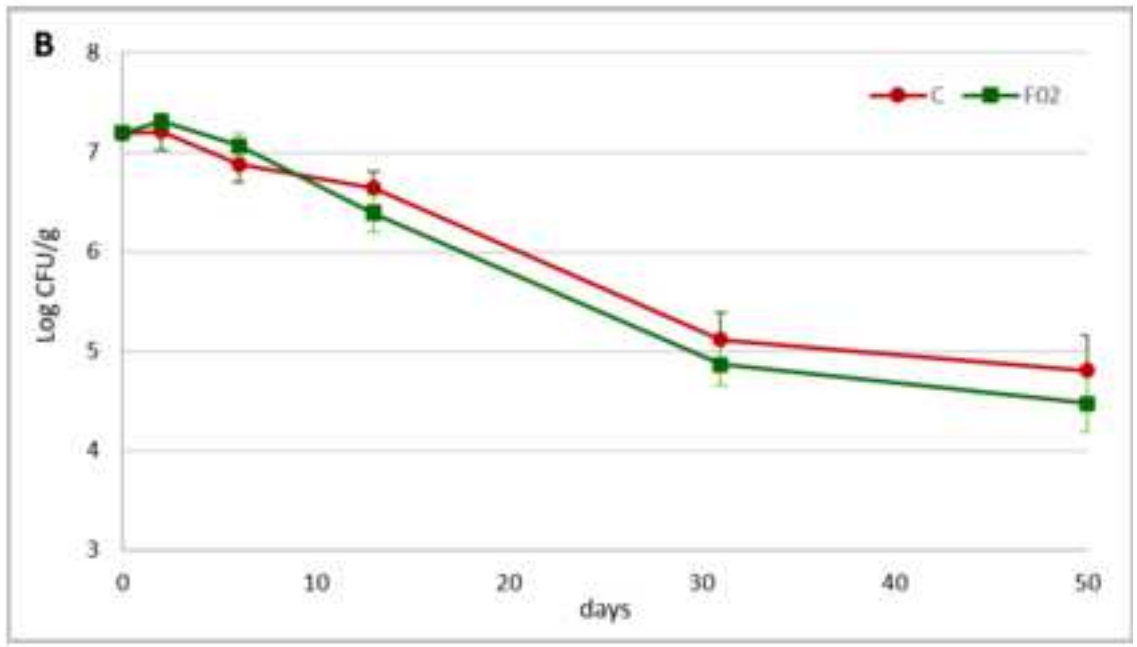
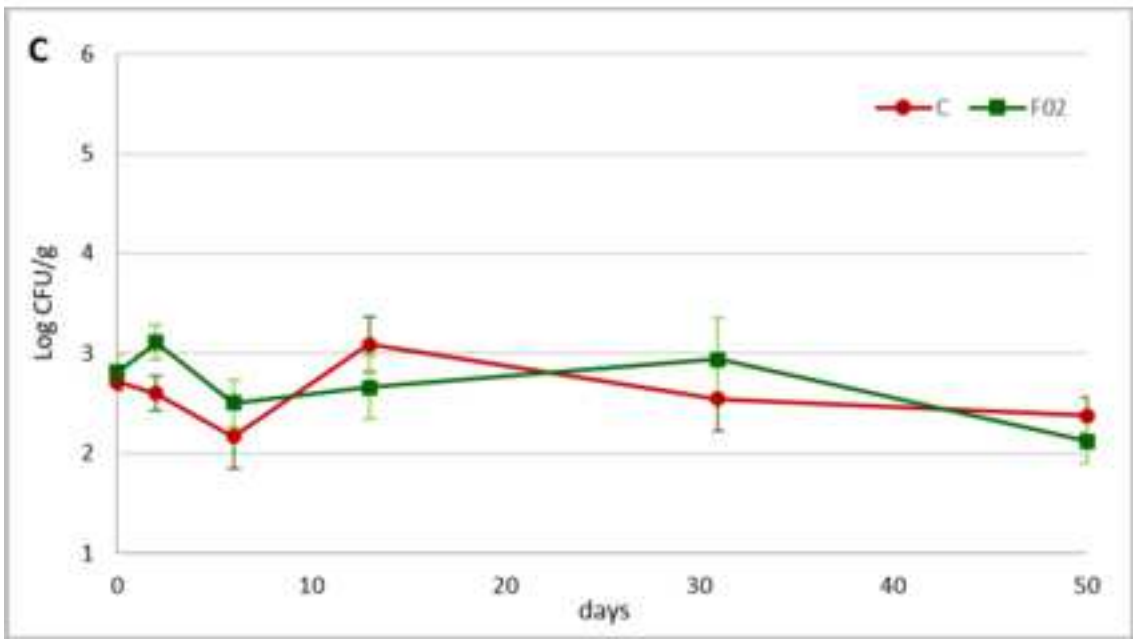






Graphical Abstract

Safety and technological issues of dry fermented sausages produced without nitrate and nitrite

Nitrate and Nitrite

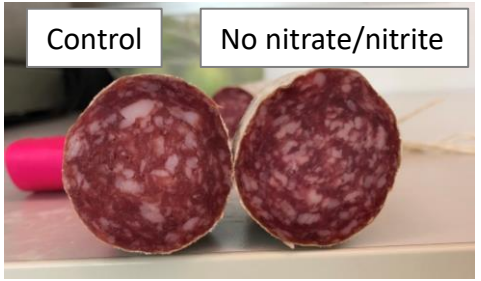
PROS
- Antimicrobial activity
- Antioxidant effect
- Color and aroma formation

CONS
- Nitrosamine accumulation
- Consumers' perception

Aim: exploit the possibility to produce fermented sausages without the addition of nitrate and nitrite

Modification of the traditional process:
- Starter cultures
- Glucose
- Fermentation and ripening conditions

M&M
- Microbiota
- pH, water activity (a_w), weight loss
- Color, texture and oxidation (TBARS)
- Aroma profile
- Challenge test (*Listeria innocua*, *Salmonella enterica* subsp. *enterica, Clostridium botulinum*)



Results
- No differences in a_w, weight loss, LAB and coagulase negative staphylococci.
- High concentration of starter cultures and long fermentation at low temperature are able to control enterobacteria.
- The lower sugar concentration tested (0.2 %) resulted in better textural features and lower oxidation level.
- No growth of the challenging strains tested.

Conclusions
- Nitrate/nitrite removal from fermented sausages requires proper technological changes to guarantee the quality of the final product.

Credit author statement

Giulia Tabanelli: conceptualization, writing-original draft preparation; **Federica Barbieri:** investigation, microbiological and chemico-physical analyses; **Francesca Soglia:** investigation, chemico-physical analyses; **Rudy Magnani:** Funding acquisition and resources; **Gabriele Gardini:** chemico-physical analyses; **Massimiliano Petracci:** writing - review & editing; **Fausto Gardini:** conceptualization writing-original draft preparation, supervision; **Chiara Montanari:** conceptualization, investigation, writing-review and editing.

Safety and technological issues of dry fermented sausages produced without nitrate and nitrite

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CONFLICT OF INTEREST

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.