

Full Length Research Paper

Effect of *Piper nigrum*, *Thymus vulgaris* and *Syzygium aromaticum* essential oils on the microbiological and sensory quality of Pork sausages during preservation

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The use of essential oils (EOs) as alternative of nitrites and nitrates in sausages will be proposed in this study. For this purpose, the minimal inhibitory concentration (MIC) of white *Piper nigrum* of *Penja*, *Thymus vulgaris* and *Syzygium aromaticum* EOs were determined *in vitro* against *Escherichia coli* ATCC25922, *Salmonella enteritidis* 155A and *Staphylococcus aureus* NCTC10652. It follows from this study that, *T. vulgaris* EO was active on *E. coli* ATCC25922, *S. aureus* NCTC10652 and *S. enteritidis* 155A with MICs of 312.5, 625 and 1250 ppm, respectively. As for *S. aromaticum* EO, it reduced the same germs with MICs of 1250, 2500 and 5000 ppm, respectively. The combination of MICs of 1250 (*T. vulgaris*) and 5000 ppm (*S. aromaticum*) was selected (CMICR) for its bactericidal effect on 03 pathogens. The CMICR totally reduced *E. coli* ATCC25922 and *S. enteritidis* 155A after 3 days of storage. The sensory analysis shows that SAUS 324 and SAUS 589 produced with white *P. nigrum* of *Penja* were appreciated by the panelists. The combination of CMICR and white *P. nigrum* of *Penja* can be considered as an alternative to chemical preservatives to limit the growth of bacteria and improve the sensory characteristics of sausages.

Key words: Sausages, *Piper nigrum*, *Penja*, *Thymus vulgaris*, *Syzygium aromaticum*, antibacterial activity.

INTRODUCTION

The transformation of meat is a process that has existed since ancient times. It is based on the use of a set of processes that lead to the modification of the texture and appearance of the meat with the main objective of increasing the shelf life of the products thus processed (Toldrá et al., 2021). The unitary operations applied to this raw material often include grinding, smoking, salting,

fermenting and cooking which lead to a wide range of products including blood sausage, andouilles, ham, pate, potted meat and sausages (Durand, 1999). Among the products mentioned earlier, fresh sausages are the most consumed and appreciated meat products in the world (Šojić et al., 2019). However, sausages are highly susceptible to microbial contamination because of their

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high protein content (Azad et al., 2022; Fursik et al., 2018). They are favorable to the development of altering and pathogenic microorganisms which are responsible on one hand for the marketable quality (taste, smell, appearance) and on the other hand for food-borne diseases such as food poisoning and intoxication (Bailly et al., 2012).

In order to solve this problem of microbial contamination, meat companies have developed several preservation methods such as the use of sulfites, nitrites and nitrates (Nair et al., 2020). However, although they improve the sanitary and organoleptic quality of meat products, these chemicals can cause health problems. High levels of nitrates and nitrites in meat products caused an estimated 600,000 deaths in Germany in the early 20th century (Honikel, 2008). Several studies have also shown that nitrites have a harmful effect on human reproduction (Manassaram et al., 2006). In addition, the International Agency for Research on Cancer (IARC) has recently concluded that the ingestion of nitrates and nitrites under certain conditions of endogenous nitrosation are probably carcinogenic (IARC, 2010).

Faced with this risk to human health, it is therefore important to reduce or eliminate the use of these preservatives in the meat industry. However, this reduction of these compounds is not without consequences. The main risks associated with the reduction or elimination of nitrite in the manufacture of charcuterie products are the reduction of product shelf life, the loss of organoleptic quality and the development of undesirable microorganisms. Aware of these risks, researchers have turned to other alternative methods such as the use of nature preservatives. This is how several works have shown the antibacterial activity of EOs extracted from cinnamon, oregano, rosemary, clove, thyme and pepper (Burt, 2004; Srinivasan, 2007; Tajkarimi et al., 2010; Evrendilek, 2015; Posgay et al., 2022). Moreover, the incorporation of natural antimicrobials in food products also allows to increase the organoleptic characteristics of the products which are appreciated by the consumers (Yu et al., 2021). In this work, the EOs of white *Piper nigrum* of *Penja*, *Thymus vulgaris* and *Syzigium aromaticum* were selected to evaluate their effect on the microbiological and sensory quality of sausages during their preservation.

MATERIALS AND METHODS

Vegetal material

The white *P. nigrum* of *Penja* used for the production of EO were purchased from small producers in the locality of *Penja* in the district of *Njombe-Penja* (Littoral, Cameroon). Indeed, the white *P. nigrum* of *Penja* is among the best peppers in the world and is the first product from sub-Saharan Africa to receive a protected geographical indication (PGI) in 2013 (Petchayo et al., 2015). *T. vulgaris* and *S. aromaticum* EOs produced by Renauld et Fils (France) have been purchased.

Microbial material

The strains of *Escherichia coli* ATCC25922, *Salmonella enteritidis* 155A and *Staphylococcus aureus* NCTC10652 were kindly donated by the laboratory of the University of Bologna in Italy.

Extraction of *Penja* EO

Five kilograms of *Penja* white *P. nigrum* were collected to extract the EO. It was carried out by the hydrodistillation method using a Clevenger type apparatus in accordance with International Standards NF ISO 212 and AFNOR (2007).

Determination of MIC of EOs

The MIC of the various EOs was determined by the Macro-dilution method. For this, 2 ml of 40 mg/ml EOs stock solutions were introduced into a sterile test tube containing 2 ml of nutritious broth with 2% of glucose. Series of dilutions were performed in order to obtain a range of EO concentrations from 20 to 0.039 mg/ml. Then, 100 μ l of each dilution was removed and replaced by 100 μ l of bacterial inoculum at a concentration of 10^8 CFU/ml to have a final bacterial concentration of 5.10^6 CFU/ml. Each tube was incubated at 37°C for 24 h and the microbial growth was evaluated using Chloride 2.3.5-triphenyltetrazolium (TTC) (Guinoiseau, 2010). The MIC was the lowest concentration of EO where no microbial growth is visible (Oulkheir et al., 2017).

Antibacterial effect of combined MICs of EOs

The combined effect of MICs of EOs was studied to optimize their effectiveness during sausage preservation. In order to evaluate this antibacterial activity, an inoculum of concentration 5.10^6 CFU/ml for each strain was mixed in a tube with the combined MICs of EOs. The different preparations were then incubated at 37°C for 24 h. After this time, 100 μ L of each combination was seeded in Petri dishes containing Chapman medium for *Staphylococcus* NCTC10652, Eosin Methylene Blue Agar (EMB) for *E. coli* ATCC25922 and *S. enteritidis* 155A. The various preparations were incubated at 37°C for 24 h. The combined MICs of EOs have inhibited the maximum of the microbial growth will be retained (CMICR).

Time to kill essay of CMICR

This inhibition test was performed following the method described by Tsuji et al. (2008). In this part of the work, the experimental setup used will be the same as the one used for the antibacterial activity of the EO combination. But in this case, only CMICR will be tested and its antibacterial activity evaluated after 1, 2, 3, 4, 8, 12 and 24 h of incubation.

Effect of CMICR on the growth of microorganisms in sausages

In order to evaluate the antimicrobial power of CMICR in sausages, 5 kg of pork meat (75 lean and 25% fat) were cleaned and then chop using a manual grinder with a 6 mm diameter mesh (Figure 1). Once chopping, 1.5% NaCl was added and the resulting product was then separated into 9 batches of 500 g each. Then, 4 batches were previously treated with CMICR. After this treatment, Batch 1, Batch 2 and Batch 3 were inoculated, respectively with *E. coli* ATCC25922, *S. enteritidis* 155A, and *S. aureus* NCTC10652, each with a concentration of 10^6 CFU/g. The Batch 4 was inoculated with

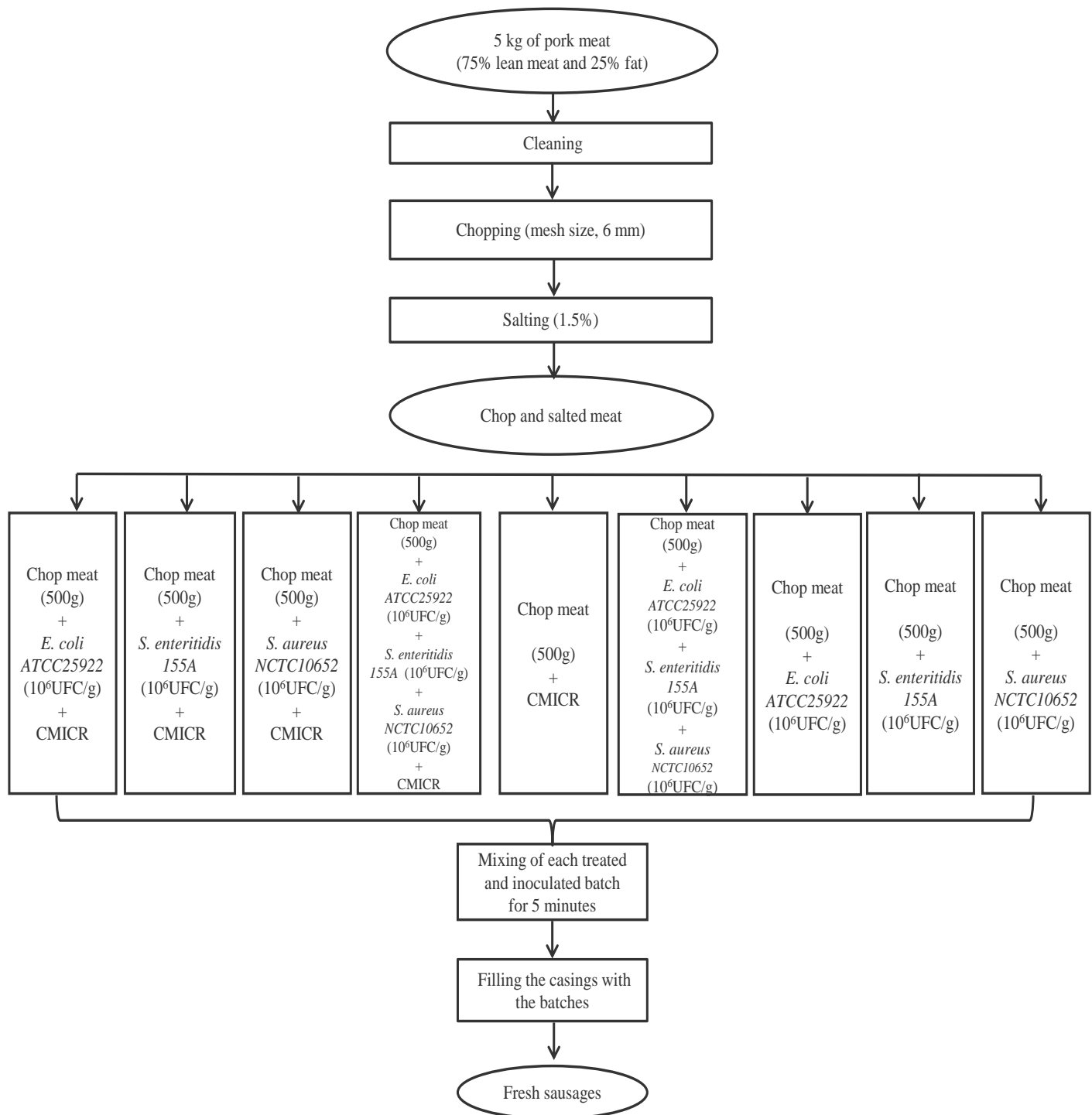


Figure 1. Production, treatment and inoculation of fresh sausages in the laboratory.
Source: Authors

les 03 pathogen bacteria in the same time. The Batch 5, Batch 6, Batch 7 and Batch 8 were inoculated, respectively with *E. coli* ATCC25922, *S. enteritidis* 155A, *S. aureus* NCTC10652, and the combination of the 03 strains served as negative control. Batch 9 was treated only with CMICR and served as a positive control. All sausages were stored at 4°C for 14 days.

Evaluation of microbial growth in the sausages

A mass of 10 g of a randomly sampled sausage was diluted in 90 ml of sterile physiological water (water + NaCl 9%). The sample prepared was homogenized for 3 to 5 min and then a decimal dilution series was carried out. Each dilution was seeded on a Petri

Table 1. Minimum inhibitory concentrations of Eos.

EOs	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella enteritidis</i>
<i>Thymus vulgaris</i>	625	312.5	1250
<i>Syzygium aromaticum</i>	2500	1250	5000
<i>Pipiper nigrum</i> of <i>Penja</i>	> 20000	> 20000	> 20000

Source: Authors

dish containing EMB media to isolate *E. coli* ATCC25922 and *S. enteritidis* 155A and Chapman for *S. aureus* NCTC10652. The Petri dish was incubated at 37°C for 24 h. The Petri dishes containing colonies between 30 and 300 were the choice for bacterial count. Microbial concentrations were obtained using the formula and expressed as CFU/g.

$$C = \frac{N \times Fd}{V}$$

Where C: Microbial concentration expressed in (CFU/g), N: Number of colonies counted on Petri dish, Fd: Dilution factor, and V: Seeded volume (mL).

Sensory analysis of sausages

For the sensory analysis test, the experiment was conducted in three rooms. A room reserved for the preparation of the samples, a room with 05 booths for the tasting and a room reserved for discussion with the panelists. During this analysis, the panelists were asked to provide in a first step their global appreciation for each sausage obtained by attributing a note on a hedonic scale going from 1 to 9 with 1 corresponding to "extremely unpleasant" and 9 to "extremely pleasant". Panelists were also asked to characterize the products by determining the intensity of attributes such as spicy taste, pink coloration, firm texture, spicy smell and flavor of the products on a scale of 1 to 3. The number 1 being "too little", 2 being "Just About Right" and 3 being "too much". To avoid errors, the order of appearance of the attributes was randomized on each sensory analysis form. This analysis also performed a penalty test that identified the various attributes that strongly reduce panelists appreciation of sausages.

Statistical analysis

The averages and standard deviations of the data obtained were calculated using the Excel spreadsheet (Office 2016) and the graphic illustrations were plotted using the Sigma plot 11.0 software. An ANOVA analysis of the sensory test data was performed using XL-Stat software version 2019-3-1 in order to highlight the differences between the panelists assessments.

RESULTS

Determination of the MICs of EOs

Table 1 gives the MICs of the different EOs tested. It can be seen that *T. vulgaris* EO had the lowest MICs compared to the other EOs. It recorded a MIC of 625,

312.5, and 1250 ppm on *S. aureus* NCTC10652, *E. coli* ATCC25922, and *S. enteritidis* 155A respectively. The lowest MIC recorded with *S. aromaticum* EO was 1250 ppm against *E. coli* ATCC25922. However, against all expectations, white *P. nigrum* of *Penja* showed no antimicrobial activity at concentrations equal to 20000 ppm. Despite the lack of activity of the high white *P. nigrum* of *Penja* EO, it will be used in sausages during sensory analysis due to its exceptional organoleptic characteristics sought after worldwide.

Effect of MIC combinations of EOs on microbial growth

Table 2 shows the antibacterial effect of the different combinations of EOs MICs on the growth of *E. coli* ATCC25922 (A), *S. aureus* NCTC10652 (B) and *S. enteritidis* 155A (C). From this analysis, it is apparent that all microorganisms were completely inactivated when tested with the 2CMI and 3CMI combinations. However, the MIC combination rather partially inactivated *S. aureus* NCTC10652 and *E. coli* ATCC25922. In contrast to these microorganisms, the bactericidal effect was observed for *S. enteritidis* 155A for MIC combinations. In view of these results, the combination of MIC of EO of concentration 1250 ppm (*T. vulgaris*) and 5000 ppm (*S. aromaticum*) were retained (CMICR) for further work because of its effectiveness.

Time to kill essay of CMICR

In order to determine the exact time required for the deactivation of microbial growth by CMICR, deactivation kinetics were performed as shown in Figure 2. It was found that after 2 h of exposure with CMICR there was a reduction of *S. aureus* NCTC10652, *E. coli* ATCC25922 and *S. enteritidis* 155A to non-detectable levels.

Antibacterial activity of CMICR in contaminated sausages

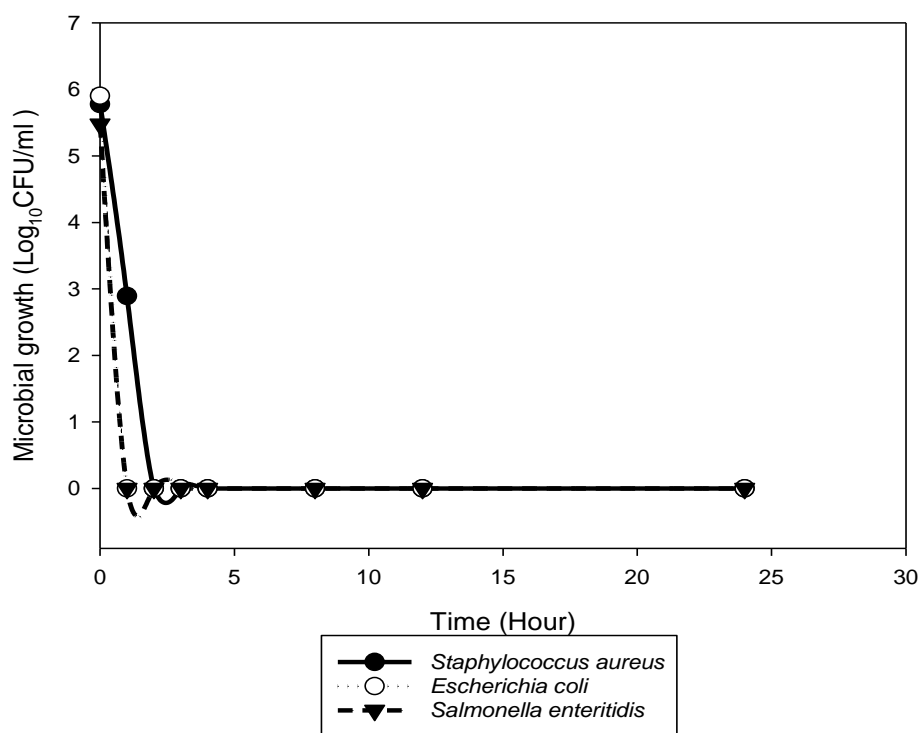
The results of the antimicrobial activity of CMICR in sausages contaminated with *E. coli* ATCC25922, *S. aureus* NCTC10652 and *S. enteritidis* 155A are as shown

Table 2. Effect of MICs combinations of EOs on microbial growth.

MIC (ppm) of EO of <i>Syzicum aromaticum</i>	MIC (ppm) of EO of <i>Thymus vulgaris</i>		
<i>Escherichia coli</i> ATCC25922 (CFU/mL)	MIC: 312.5	2MIC: 625	3MIC: 937.5
MIC: 1250	5×10	0	0
2 MIC: 2500	0	0	0
3 MIC: 3750	0	0	0
<i>Staphylococcus aureus</i> NCTC10652 (CFU/mL)	MIC: 625	2MIC: 1250	3MIC: 875
MIC: 2500	10 ²	0	0
2MIC: 5000	0	0	0
3MIC: 7500	0	0	0
<i>Salmonella enteritidis</i> 155A (CFU/mL)	MIC: 1250	2MIC: 2500	3MIC: 3750
MIC: 5000	0	0	0
2 MIC: 10000	0	0	0
3 MIC: 15000	0	0	0

2MIC: Minimum Inhibitory Concentration multiplied by 2; 3MIC: Minimum Inhibitory Concentration multiplied by 3.

Source: Authors

**Figure 2.** Kinetics of microbial deactivation by CMICR.

Source: Authors

in Figure 3. While the *in vitro* deactivation kinetics showed a total reduction of the microbial load after 2 h, the results obtained in sausages show a gradual and slow reduction of the microorganisms over an average

period of 8 days. A reduction in the order of 51, 53 and 44% was recorded for *E. coli* ATCC25922, *S. aureus* NCTC10652 and *S. enteritidis* 155A, respectively after 8, 10 and 14 days of storage before a resumption of growth.

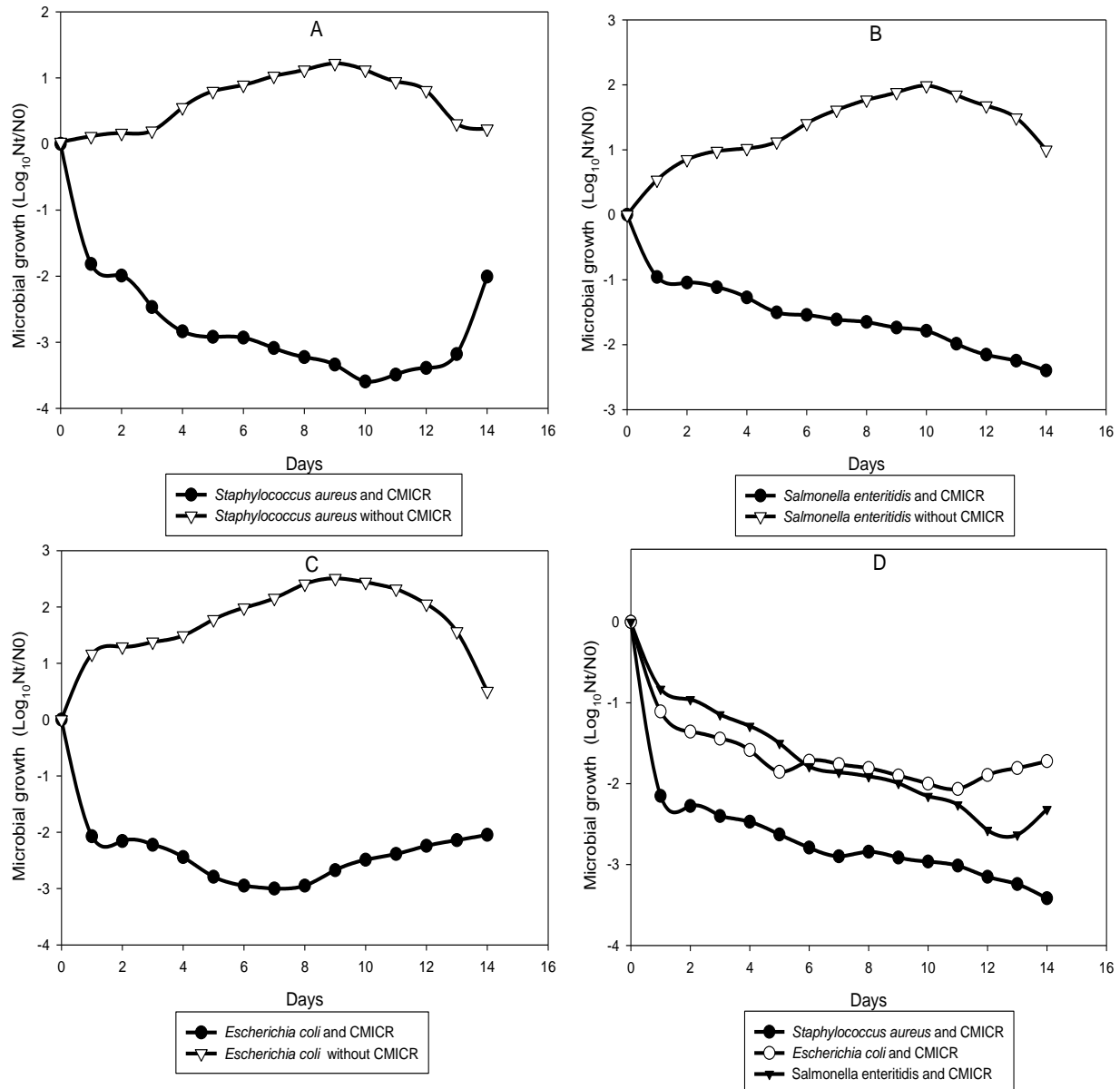


Figure 3. Reduction of *Escherichia coli* ATCC25922 (A), *Salmonella enteritidis* 155A (B), *Staphylococcus aureus* NCTC10652 (C) and combined microorganisms (D) in sausage at 4°C.

Source: Authors

Contrary to the sausages treated with CMICR where a reduction was noted during the conservation, the untreated sausages (controls) saw their average microbial load increase by 16, 33 and 45% for *S. aureus* NCTC10652, *S. enteritidis* 155A and *E. coli* ATCC25922, respectively after 9, 10 and 9 days of conservation before a reduction was observed. In this case, contrary to the sausages contaminated individually by each microorganism, a slight reduction was noted in the sausages contaminated simultaneously by these germs. A reduction of 36, 45 and 50% was recorded in *E. coli* ATCC25922, *S. enteritidis* 155A and *S. aureus*

NCTC10652, respectively after 11, 13 and 14 days of conservation before a resumption of growth was observed.

Antibacterial activity of CMICR in naturally contaminated sausages

In this work, the reduction of *S. aureus*, *E. coli* and *S. enteritidis* naturally present in fresh meat after production was evaluated (Figure 4). Compared to the results observed in the sausages contaminated in the laboratory

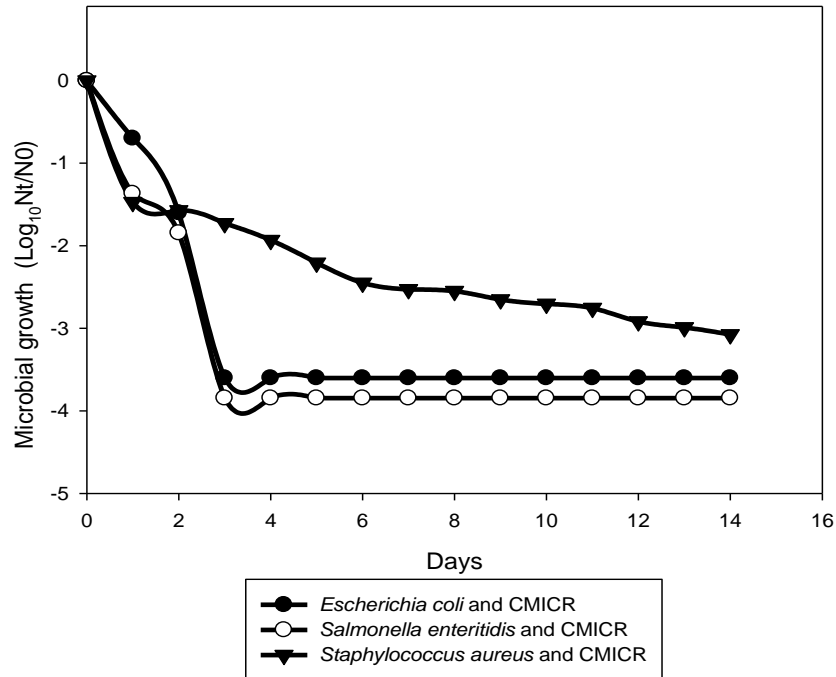


Figure 4. Reduction of microbial growth by CMICR in naturally contaminated sausages at 4°C.
Source: Authors

by microorganisms, we note a reduction of the concentration of *E. coli* and *S. enteritidis* to a non-detectable threshold in the sausages after the 3 days of conservation at 4°C. In contrast, *S. aureus* was reduced by only 48% after 14 days of storage at 4°C. This result shows that the degree of effectiveness of CMICR in deactivating *E. coli* and *S. enteritidis* at non-detectable levels is highly dependent on their concentration in the sausages.

Overall appreciation of the sausages

The sensory analysis was carried out with the sausages produced with different EOs used in this work. A total of 03 sausages were obtained. We have sausages treated with EOs of white *P. nigrum* of *Penja* (SAUS 324), sausages treated with EO of *P. nigrum* of *Penja* associated with the CMICR (SAUS 589) and sausages treated with the CMICR (SAUS 912). Not treated sausages with EOs (SAUS 102) and commercial sausages (SAUS 706) were also subjected to sensory analysis. The results show that sausage SAUS 324 and SAUS 589 were appreciated (score 6) by panelist on par with the commercial sausage (SAUS 706). The sausages SAUS 912 and SAUS 102 were less appreciated (score 5). The sausages SAUS 324 and SAUS 589 that were appreciated by the panelists are those in which white *P. nigrum* of *Penja* was part of the seasoning. Table 3

shows the overall acceptability of the different sausages.

Characteristics of the sausages

The results of the JAR test (Figure 5), which highlighted the specific characteristics of each sausage, showed that sausage SAUS 324 was judged to have a good spicy taste (67%), good spicy smell (61%), good salty flavor (72%) and good firm texture (50%). For the sausage SAUS 539, panelists characterized it as too much spicy taste (56%), too much spicy smell (50%), too much salty flavor (56%), too little firm texture (50%) and too little pink color (61%). The sausage SAUS 912 which was less appreciated compared to sausage SAUS 324 and SAUS 589 has a good spicy taste (44%), good spicy smell (50%), good salty flavor (50%), but with too little firm texture (61%) and too little pink color (89%). The sausage SAUS 102 is characterized by a good taste (56%), too little spicy smell (78%), too little salty flavor (50%), too little firm texture (61%) and too little pink color (67%).

Penalty test of the sausages

During this analysis, the attributes taken into account are those whose penalties have a threshold of 20% and which lead to an increase in the average (>1). It appears

Table 3. Overall acceptability of different sausages.

Sausages code	Composition of the sausages	Overall assessment	Significance	
			F	Pr > F
SAUS 706	Commercial sausage	6 ± 1.63		
SAUS 324	Sausages with <i>Piper nigrum</i> of Penja	6 ± 1.63		
SAUS 589	Sausages with <i>Piper nigrum</i> of Penja + CMICR	6 ± 1.54	0.88	0.48
SAUS 912	Sausages + CMICR	5 ± 1.46		
SAUS 102	Sausage without CMICR and <i>Piper nigrum</i> of Penja	5 ± 1.81		

Hedonic scale: 1= Extremely unpleasant; 2=Very unpleasant; 3= Enough unpleasant; 4=Unpleasant; 5=Not unpleasant - Not pleasant; 6= Enough pleasant; 7=Pleasant; 8=Very pleasant; 9=Extremely pleasant.

Source: Authors

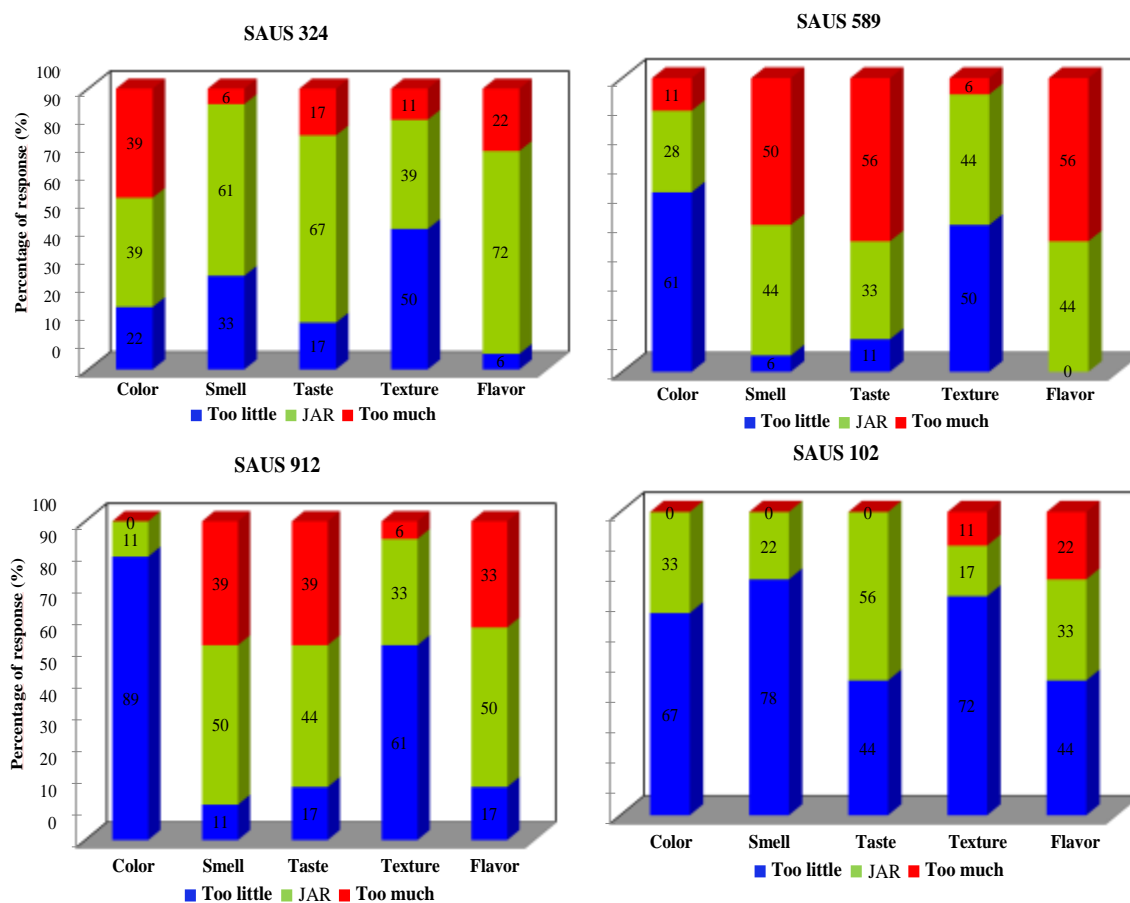


Figure 5. Characteristics of the different sausages produced.
Source: Authors

from this test that the too little spicy smell and the too little pink color of sausage SAUS 324 significantly reduce its acceptability. While too much salty flavor, too little pink color and too much spicy smell were penalizing sausage SAUS 589. Taste and too little spicy smell were the 02 characteristics that reduced the acceptability of sausage SAUS 912. The results of the penalty analysis of the

sausages are as shown in Figure 6.

DISCUSSION

In this work, the EOs of white *P. nigrum* of Penja did not show any antimicrobial activity on the main pathogens

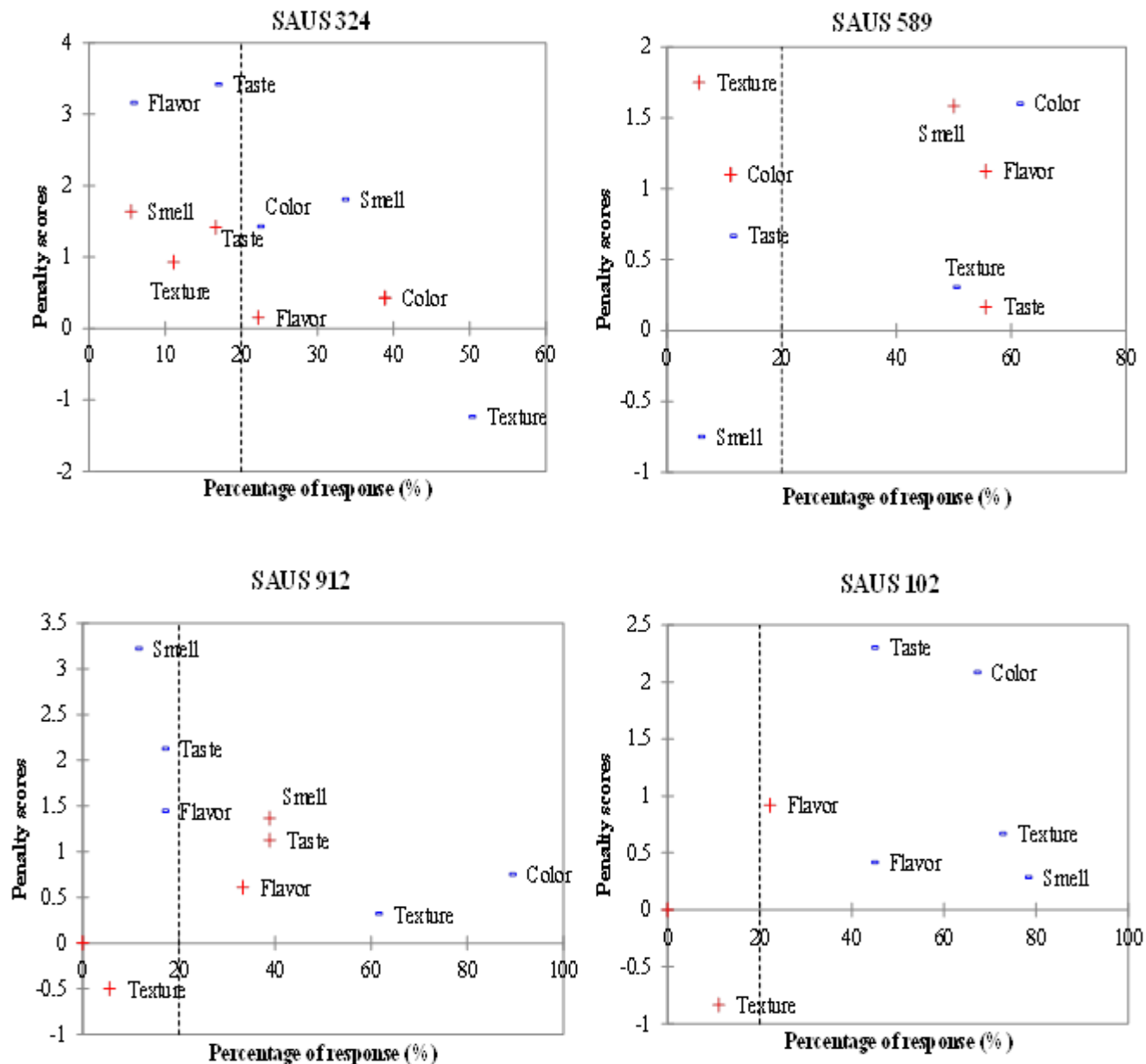


Figure 6. Results of the penalty analysis of the different sausages. Source: Authors

studied. This result is contrary to some works that showed the black *P. nigrum* would contain substances such as piperine which is responsible for its antimicrobial power. The work of Ojo et al. (2021) showed a great antibacterial power of purified piperine on *S. aureus* and *E. coli* and *S. enterica*. However, the work done by Arsana et al. (2022) showed that white *P. nigrum* would also contain Piperine. This difference observed in the antimicrobial activity could be explained by the difference in the composition of the different *P. nigrum* tested. Indeed, the antimicrobial activity of an EO is closely related to its chemical composition which itself also

depends on the species, subspecies or variety of the plant used, the geographical area and the period of harvest, the method of drying or extraction of the EO (Moraru et al., 2019). In contrast, EOs of *T. vulgaris* and *S. aromaticum* were found to be effective against *E. coli* ATCC25922, *S. enteritidis* 155A and *S. aureus* NCTC10652. This antimicrobial activity is thought to be due to the presence of numerous oxygenated terpenoid derivatives in the composition of these different EOs (Angane et al., 2022) and the higher percentage of phenolic compounds in EO higher its antimicrobial properties (Boskovic et al., 2015). The presence of

thymol and carvacrol in *T. vulgaris* EO and eugenol in *S. aromaticum* EO as the predominant compound (Ramsey et al., 2020) could also explain their high activity. These antibacterial compounds mainly act on microbial cells by destabilizing the cell architecture, leading to the increase of membrane permeability and the disruption of many cellular activities such as energy production, membrane transport and other metabolic functions (Di pasqua et al., 2006; Devi et al., 2010; Swamy et al., 2016).

Contrary to the MICs of the EOs obtained, the CMICR resulted in a significant reduction in the growth of *E. coli* ATCC25922, *S. enteritidis* 155A and *S. aureus* NCTC 10652. This significant reduction is thought to be related to the combined action of the antimicrobial components of *T. vulgaris* and *S. aromaticum* EOs acting synergistically or additively to induce a plethora of antibacterial effects (Bassolé and Juliani, 2012). Some works have shown that mixing pure compounds of Thymol and Eugenol could increase their antibacterial effect by 50% on *E. coli* and *Bacillus cereus* (Gallucci et al., 2009, Pei et al., 2009). Das et al. (2022) also stated that the EOs reduced the microbial growth and increased the shelf life of chicken meat during refrigeration at 4°C.

In the determination of the deactivation kinetics, the CMICR inhibited at a non-detectable threshold the growth of *E. coli* ATCC25922, *S. enteritidis* 155A and *S. aureus* NCTC10652 after 2 h. This rapid reduction could be explained by the contact in liquid medium of the tested germs with a diversity and high concentration of antimicrobial compounds from the combined EOs. The increase in these different parameters would have led to a series of reactions among which the increase in membrane permeability and the destruction of cell membranes (Nazzaro et al., 2013).

While *in vitro* deactivation kinetics showed deactivation at non-detectable levels of microbial growth after 2 h, in sausages contaminated with *E. coli* ATCC25922, *S. enteritidis* 155A and *S. aureus* NCTC10652 and stored at 4°C, a gradual and partial deactivation of the microbial load was observed up to the 14th day. This longer deactivation time could be explained to be the reduction of the effectiveness of CMICR due to the interaction of its compounds with the constituents of the food matrix such as lipids, proteins and ingredients present (Da Silva et al., 2021). In addition, due to their lipophilic nature, EOs are dissolved in the oil phase of foods, making them less available to act against bacteria present in the water phase (Mejlholm and Dalgaard, 2002). The work done by Tassou et al. (1995) on fish pate and egg salad showed a reduction in the antibacterial activity of mint oil on *Listeria monocytogenes* and *S. enteritidis* mainly due to the high lipid content of these foods. Similarly, Pol et al. (2001) demonstrated that the high concentrations of protein in the milk act as a limiting factor in the antibacterial activity of Carvacrol against *B. cereus*.

The growth of *E. coli* ATCC25922 and *S. aureus* NCTC10652 observed on day 8 and 10, respectively

during storage in sausages treated with CMICR is thought to be related to the instability of EOs over time due to their volatility. To this, it can be added a highly nutritious environment of the food matrix and can therefore increase the recovery rate of microorganisms that have undergone some type of stress (Gill et al., 2002).

The evaluation of the antimicrobial activity of substances in laboratory conditions is associated with specific standard microbial concentrations to define efficacy parameters (MIC and MBC). These microbial concentrations are sometimes different from those naturally encountered in food. In this work, it was noted that meat taken under real conditions of sale had a lower microbial concentration than contaminated meat in the laboratory. In the efficacy tests of CMICR on naturally contaminated meat, a total reduction of the microbial level was observed after 3 days of storage at 4°C. The antimicrobial activity of CMICR would thus depend on the microbial concentration present in the treated sausages. According to the work of Burt (2004), the effectiveness of antibacterial substances is significantly influenced by the microbial concentration of food.

While sanitary quality is an important factor in consumer choice of sausages, organoleptic characteristics also remain one of the major factors contributing to their acceptability. Among the sausages produced, only sausage SAUS 324 and SAUS 589 were appreciated in the same way as the commercial sausage (SAUS 706). We note that these two sausages have in common the presence of *P. nigrum* of *Penja* in their composition. This would explain the appreciation of these sausages by the panelists. Indeed, *P. nigrum* cultivated near the *Penja* locality in Cameroon is considered one of the best peppers in the world. *P. nigrum* of *Penja* is the first sub-Saharan African product to obtain a Protected Geographical Indication (PGI) due to its exceptional aroma and flavor (Folefack et al., 2022). Although appreciated for some of their attributes, SAUS 324 and SAUS 589 sausages have been unfortunately depreciated for their small pink color which is explained by the absence of nitrite and nitrate during their preparation. The nitrite used in the production of commercial sausages, through different chemical reactions, leads to the formation of nitric oxide (NO) which will bind to myoglobin to form nitrosomyoglobin, a stable and red compound, responsible for the typical color of sausages (Honikel, 2008).

Conclusion

At the end of this work, it was found that CMICR eliminates the growth of *E. coli* ATCC 25922 and *S. enteritidis* 155A in naturally contaminated sausages at a non-detectable level after 3 days of storage. The sensory analyses show that all the sausages treated with EO of

Penja P. nigrum in this case sausage SAUS 324 and SAUS 589 were appreciated by the consumers. But the too small pink color of these sausages must be improved in order to increase their appreciation by consumers. However, in order to ensure consumer safety and appreciation of this product, CMICR and EO *P. nigrum* of *Penja* must be mixed during sausage production.

CONFLICT OF INTERESTS

The authors have not declared any of conflict of interests.

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