

Antioxidant potential of herbs and spices in nitrite-reduced frankfurter sausages

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Nitrites are used in meat processing as a preservative, but there is a need to reduce their usage because of their potential harmful effects on consumers' health. The antioxidant potential of different herbs and spices in nitrite-reduced frankfurter type sausages and their influence on the products' sensory properties were investigated. The results show the nitrite-reduced sausages enriched with individual herbs or spices as a source of phenolic compounds had significant antioxidant potential, which was the highest in sausages with rosemary followed by sausages with thyme, black pepper, turmeric, and red paprika. Spice-enriched sausages had better sensory properties than the control sausages after chill storage.

According to the quantities produced, boiled sausages are among the most common meat products in Europe and other regions of the world, and in Serbia they account for half of the total production of all types of meat products (ŠOJČIĆ et al., 2011). The most famous products from the group of finely comminuted boiled sausages are frankfurter, hot dog, pariser, and white sausage. Frankfurter is made from meat, pork back fat, water, spices, and additives. The meat batter is prepared by comminuting and mincing the ingredients in a bowl cutter and stuffing the prepared filling into narrow diameter casings. After stuffing, the sausages are heat treated by pasteurization and during the heat treatment, dissolved myofibril proteins coagulate and give the desired consistency to the product (TEODOROVIĆ et al., 2015).

Meat products such as frankfurters are susceptible to spoilage and their shelf life is relatively short. This type of sausage is especially susceptible to oxidation because it contains a high percentage of fat, and at the same time, air is easily incorporated into the filling through damaged membranes during the intensive comminuting. By packaging frankfurters under vacuum or in a modified atmosphere, a shelf life of up to 28 days at 4 °C can be achieved (NALAN et al., 2010). Pasteurization, as a relatively mild heat treatment procedure, enables high-value nutrients such as essential amino acids, essential fatty acids, vitamins, and minerals to be well preserved (RAŠETA et al., 2018). On the other hand, bacterial spores, the most important of which are *Clostridia* spores, survive pasteurization, as do some non-sporogenic bacteria such as *Lactobacilli* and *Enterococci*. Therefore, the safety of these sausages is based not just on chill storage but also on the use of preservatives such as nitrites (VASILEV and VUKOVIĆ, 2008).

Nitrites have an indispensable role in preventing spoilage and preserving the safety of boiled cooked sausages. These compounds also have a significant antioxidant role by stabilizing the double bonds of unsaturated fatty acids in cell membranes and by chelating metal ions. The usual level of nitrites added to boiled sausages is 150 mg/kg (GOVARI and PEXARA, 2015). In addition to their preservative effects, nitrites also have a very important role in the formation of sensory properties of sausages, especially the color. The color of frankfurter type sausages is mainly based on nitroso-myoglobin, a pink pigment that is formed as a consequence of the reactions between nitrite and myoglobin, but also depends on antioxidants (reducing agents), fat content, smoking intensity, and sausage ingredients (RUIZ-CAPILLAS et al., 2014; JIN-KYU et al., 2016). However, nitrites are also a precursor for some harmful compounds, such

as N-nitrosamines, which have been shown to possess carcinogenic and mutagenic potential (GRUJIĆ et al., 2009). For this reason, some studies suggest the need to replace or at least reduce the amount of nitrite in sausages (ALIREZALU et al., 2019). Likewise, consumers are showing increasing interest in natural foods that do not contain preservatives, including nitrites (SINDELAR et al., 2007). In addition, although lipid oxidation in products can be effectively prevented by the use of antioxidant additives that are widely used in the meat industry, concerns about their potential toxicity are prompting producers to find a better solution in the form of antioxidants from natural sources, such as herbs and spices (GRUJIĆ et al., 2014).

Herbs and spices are added to meat products primarily to achieve the appropriate flavor, but research is increasingly pointing to the potential of herbs/spices as ingredients that possess preservative properties. Some common culinary herbs have a relatively high antioxidant capacity, such as rosemary and thyme (VALLVERDU-QUERALT, 2014). Other authors report that turmeric has an even stronger antioxidant capacity than ascorbic acid in burgers (MANCINI et al., 2015). According to ZHANG and HAMAUZI (2003), red paprika has higher levels of free radical scavenging and antioxidant activities (in methanol extracts) than do green and yellow paprika. GÜLCIN (2005) indicated that black pepper extract had strong antioxidant activity and radical scavenging activity in vitro, representing a good base for further investigation into application of black pepper as an easily accessible source of natural antioxidants in the food industry.

Overall, the possibility of using spices as natural preservatives and a potential substitute for nitrites in meat products could have great potential, but it has not yet been sufficiently researched at the level of a complex substrate such as the stuffing for finely comminuted boiled sausages. Therefore, the aim of this study was to examine the antioxidant potential of different herbs and spices, and their influence on sensory properties in nitrite-reduced frankfurter type sausages.

Materials and methods

Sausage production

The experimental sausages were of the following composition: **Co:** control (without added spices), 50% pork I category, 30% pork back fat, 20% ice, 1.8% table salt, sodium nitrite 50 mg/kg, 0.3% phosphates. Sausages with herbs/spices were of the same composition as the con-

KEYWORDS

- >> Antioxidant
- >> Frankfurter
- >> Nitrite reduction
- >> Sensory properties
- >> Spices

Nitrite content

Tab. 1: Chemical composition of sausages

Tab. 1: Chemische Zusammensetzung von Würstchen

Parameters	Frankfurter sausages							
	Co	Th	R	Bp	Tu	P	M	
Moisture (%)	59.41 ± 0.02 ^{ab}	60.16 ± 0.06 ^a	59.45 ± 0.18 ^{ab}	59.41 ± 0.58 ^{ab}	58.83 ± 0.09 ^{ab}	59.17 ± 0.14 ^{ab}	58.50 ± 0.24 ^b	
Fat (%)	24.12 ± 0.06 ^a	24.23 ± 0.06 ^a	23.54 ± 0.04 ^a	24.31 ± 0.52 ^a	23.82 ± 0.10 ^a	24.06 ± 0.08 ^a	23.47 ± 0.42 ^a	
Meat proteins (%)	11.82 ± 0.02 ^a	12.57 ± 0.18 ^{bc}	13.27 ± 0.13 ^c	12.42 ± 0.01 ^{ab}	12.47 ± 0.11 ^{ab}	12.42 ± 0.24 ^{ab}	12.74 ± 0.07 ^{bc}	
Ash (%)	2.56 ± 0.02 ^a	2.64 ± 0.01 ^{ab}	2.70 ± 0.01 ^{bc}	2.67 ± 0.02 ^b	2.78 ± 0.02 ^c	2.70 ± 0.01 ^{bc}	2.70 ± 0.02 ^{bc}	
NaCl (%)	1.26 ± 0.04 ^a	1.26 ± 0.01 ^a	1.28 ± 0.02 ^a	1.58 ± 0.08 ^b	1.54 ± 0.02 ^b	1.66 ± 0.04 ^b	1.68 ± 0.02 ^b	
Nitrites (mg/kg)	Storage day							
	0	34.61 ± 0.15 ^a	43.52 ± 0.09 ^b	43.50 ± 0.12 ^b	42.40 ± 0.16 ^c	43.15 ± 0.28 ^b	41.50 ± 0.25 ^d	41.87 ± 0.05 ^{dc}
	30	19.79 ± 0.18 ^a	29.07 ± 0.15 ^b	23.50 ± 0.19 ^c	23.64 ± 0.31 ^c	32.26 ± 0.30 ^d	34.43 ± 0.18 ^e	23.75 ± 0.24 ^c

*Different letters in the same row indicate a significant difference at $p < 0.05$.

Source: Micovic et al.

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trol sausages but with the individual addition of herbs or spices, as follows: **T**-sausage: 0.5% thyme; **R**-sausage: 0.5% rosemary; **Bp**-sausage: 0.5% black pepper; **Tu**-sausage: 0.5% turmeric; **P**-sausage: 0.5% sweet red ground paprika; **M**-sausage with 0.5% spice mixture comprised of 0.1% thyme, 0.1% rosemary, 0.1% black pepper, 0.1% turmeric, and 0.1% red paprika. The meat batter was prepared in a bowl cutter by first chopping the cooled meat and frozen pork back fat, then adding table salt, nitrite salt, phosphates, and half of the ice. In the case of herb/spice-containing sausages, spices were added at this time and the mass was further chopped until the mixture reached 8 °C. The other half of the ice was then added, and the batter was further comminuted and homogenized until it reached 9 °C, when it was ready for stuffing. The meat batter was stuffed into artificial casings, 22 mm diameter, and the resultant sausages were subjected to a pasteurization process at 78 °C in the chamber until 72 °C in the thermal center of the sausages was reached. After that, the sausages were cooled under cold water showers with continued air cooling until the internal product temperature was below 4 °C. The cooled sausages were vacuum-packed in groups of four in transparent foil and held in chill storage at 4 °C. The sausages were examined and tested after production and after 15 and 30 days of storage. Two replications were performed and each test was determined in triplicate.

Chemical composition

Chemical composition of the experimental sausages was analyzed according to standard methods: moisture content (ISO 1442:1997), fat content (ISO 1444:1996), protein content (ISO 937:1978), ash content (ISO 936:1998), chloride content (ISO 1841-1:1996), and nitrite content (ISO 2918:1975).

Total phenol content and antioxidant capacity

Firstly, meat extract was prepared by grinding about half a kilogram of each sample of sausages in an electric mill. Each ground sausage sample (4 g) was extracted by mixing with 40 mL of methanol and ultrasonating for 30 min. The extract was centrifuged twice for 10 min at 3500 rpm, and the supernatant was filtered through a 0.45 mm Minisart filter prior to analysis. The total phenolic content and antioxidant capacity were then determined in the filtered extracts. The total phenolic content was determined using a modified Folin-Ciocalteu colorimetric method (SINGLETON et al. 1999; LIU et al. 2002), and the results were expressed as milligrams of gallic acid equivalents/100 g. Antioxidant activity was determined using the DPPH method (BRAND-WILLIAMS et al., 1995) and the ABTS assay (RE et al., 1999). Results were expressed as μmol

trolox equivalents/100 g sausage for the DPPH method and mmol trolox equivalents/100 g sausage for the ABTS assay.

Oxidative changes

The oxidative changes in the sausages were determined by the following standard methods: acid value (ISO 660:2009), peroxide value (ISO 3960:2017), and TBARS (thiobarbituric acid reactive substances) value, which was determined according to the method of BOTSOGLOU et al. (1994).

Instrumental color

The color of the sausages was determined using a Chrome Meter CR-400 colorimeter (Minolta Co. Ltd, Tokyo, Japan) according to the CIE L*a*b* system. Measurements were performed in D-65 illumination with a standard shelter angle of 2°.

Instrumental texture

Instrumental texture profile analysis (TPA) was performed using the universal texture determination instrument TA XP (Stable Micro System, Godalming, England). For texture analysis, the casings were removed from the sausages, and cylindrical samples with a diameter of 22 mm and a height of 20 mm were prepared, which were tempered to room temperature, and then subjected to a double compression test up to 50% of the initial height. Compression was performed using an aluminum plate with a diameter of 75 mm (P/75) and a load of 5 kg. The speed of starting the contact tip before the test, during the test and after the test was 1 mm/s.

Sensory properties

The sensory properties of the sausages were examined by quantitative descriptive analysis according to the standard method ISO 6564: 1985, including the evaluation of sausage external appearance, slice surface appearance, color, odor and taste, and texture. Each property was evaluated according to a scale from 1 (unacceptable) to 5 (excellent). The panel consisted of six assessors who were selected, trained and coached in accordance with the standard method ISO 8586-2: 2008.

Statistical analysis

Statistical analysis of the obtained results was conducted by means of the software GraphPad Prism version 6.00 for Windows (GraphPad Software, USA) to determine the descriptive statistical parameters and by one-factor analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparison test. The differences in results were considered as statistically significant at the level of $P < 0.05$. Correla-

tion analyses for multiple variables included Pearson's correlation calculations in the same software.

Results and discussion

Chemical composition and nitrite content

The chemical composition and nitrite content of the experimental sausages are presented in table 1. There was no significant difference in moisture and fat content in sausages with herbs/spices compared to the control. However, a higher content of protein, ash and sodium chloride was observed in all sausages with herbs/spices than in control sausages. The greater ash content was significant ($p < 0.05$) higher for almost all sausages with herbs/spices (except thyme) than in the control, but protein levels were higher only in sausages with thyme, rosemary, and the spice mixture. Significantly higher NaCl content was measured in sausages with black pepper, turmeric, red paprika, and the spice mixture than in the other sausages.

Immediately after production (day 0), the lowest nitrite content was observed in the control sausages (34.61 mg/kg), while in sausages with herbs/spices it ranged from 41.50 to 43.52 mg/kg. The level of nitrites decreased after 30 days of storage, when the lowest nitrite content was also observed in the control (19.79 mg/kg) and the highest was in sausages with red paprika (34.43 mg/kg).

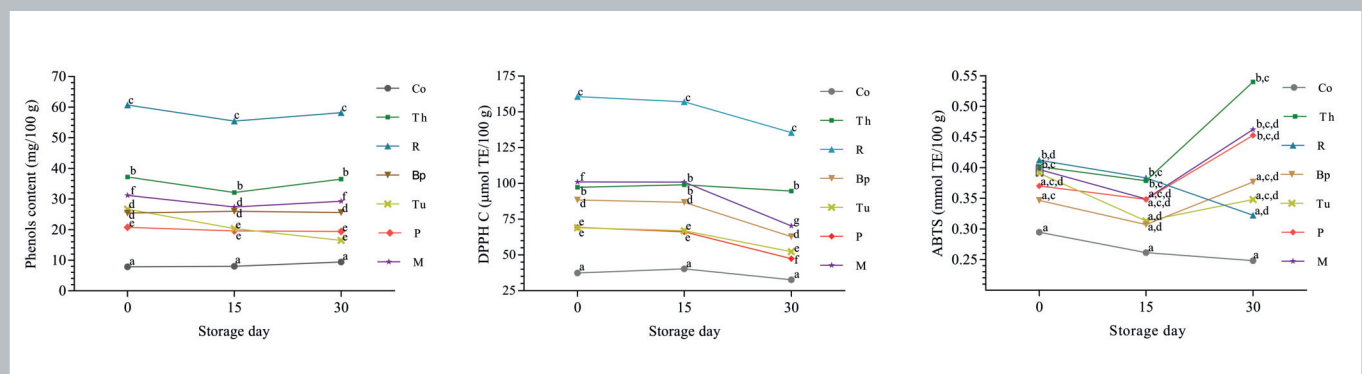
The chemical composition of experimental sausages was in accordance with the average composition of frankfurter type sausages mentioned in the literature (PEREZ-RODRIGUEZ et al., 1996; GRIZOTTO et al. 2012). The higher ash content in sausages with herbs/spices could be attributed to the addition of powdered spices as described by RUIZ-CAPILLAS et al. (2014), and higher protein content in sausages with thyme, rosemary, and the spice mixture could be a consequence of nitrogen originating from the herbs, according to KONIECZYNSKI and WESOŁOWSKI (2007). After production, the nitrite content in all experimental sausages was approximately 2.3 times lower than frankfurters of usual composition (produced with 125 mg/kg nitrite), which contain about 90 mg/kg nitrites after production (PEREZ-RODRIGUEZ et al., 1996), confirming our products were indeed nitrite-reduced. According to GOVARI and PEXARA (2015), seasonings are sources of nitrates in meat products, which could explain the higher nitrite content in sausages with herbs/spices than in the control sausages that were produced without added herbs/spices. As nitrites are highly reactive, and their content decreases during storage (PEREZ-RODRIGUEZ et al., 1996), the same pattern was also observed in our study. However, this process was the least pronounced in sausages with red paprika, which is, according to the literature data (VUKOVIĆ et al., 2011), a significant source of nitrate. This converts to nitrite in the sausage

stuffing during storage, resulting in our sausages with red paprika having the highest residual nitrite content at the end of storage. The residual nitrite plays an important role in sausage safety, which is of great importance for products with a relatively high percentage of added herbs and spices such as in this study. It is well known that herbs and spices could contain spore-forming bacteria, especially *Clostridia*, and could represent a source of sausage stuffing contamination (VASILEV and VUKOVIĆ, 2008; RAŠETA et al., 2018). Microbiological examination of the sausages from this study (the results are not presented) showed that there was no presence of *Clostridia* in the products, as well as the aerobic microbial counts in herbs/spices enriched sausages were similar to the control. Such findings confirmed that the addition of 0.5% herbs/spices did not lead to increased contamination and that the chill storage along with the residual nitrite content was sufficient to provide the safety and shelf life of the products corresponding to the literature data (NALAN et al., 2010; GOVARI and PEXARA, 2015).

Total phenolic content and antioxidant capacity

The total phenolic content and antioxidant parameters measured in experimental sausages are presented in Figure 1. Spices are recognized as a source of phenols (VALLVERDU-QUERALT et al., 2014), which have been proven to have antioxidant properties in meat products (NOWAK et al., 2016; NIKOLIĆ et al., 2020), so the phenolic content as well as their scavenging effect on DPPH and ABTS radicals were assessed in this study. Sausages with rosemary, thyme, and red paprika as well as the spiceless control sausages showed no variation in phenolic content during 30 days of storage. The levels of phenols in sausages with the spice mix and in those with turmeric slightly decreased over time. The highest phenolic content was observed in sausages with rosemary (60.7 ± 1.37 mg/100 g), with the consequent highest DPPH radical scavenging activity (160.7 ± 0.88 μ mol TE/100 g) during the whole storage period, as well as ABTS radical scavenging activity in the first 15 days of storage. Sausages with other spices had lower phenolic contents, ranging from 20.72 ± 0.06 mg/100 g in sausages with red paprika to 37.19 ± 0.34 mg/100 g in sausages with thyme, while the phenolic content in the control sausage was the lowest (7.84 ± 0.05 mg/100 g) and probably originated from the naturally occurring volatile phenols in meat fat (HA and LINDSAY, 1991). Sausages with thyme showed also strong DPPH (94.69 \pm 0.34 μ mol TE/100 g) and ABTS (0.54 \pm 0.01 mmol TE/100 g) radical scavenging activities, especially in the second phase of storage (after day 15). A high phenolic content in rosemary and thyme (VAN HECKE et al., 2017) as well as the strong antioxidant activity of these herbs was also reported by other authors (BABOVIĆ et al., 2010). Sausages containing other spices (black pepper, turmeric, red paprika, and the spice mixture), had similar DPPH

Antioxidant capacity



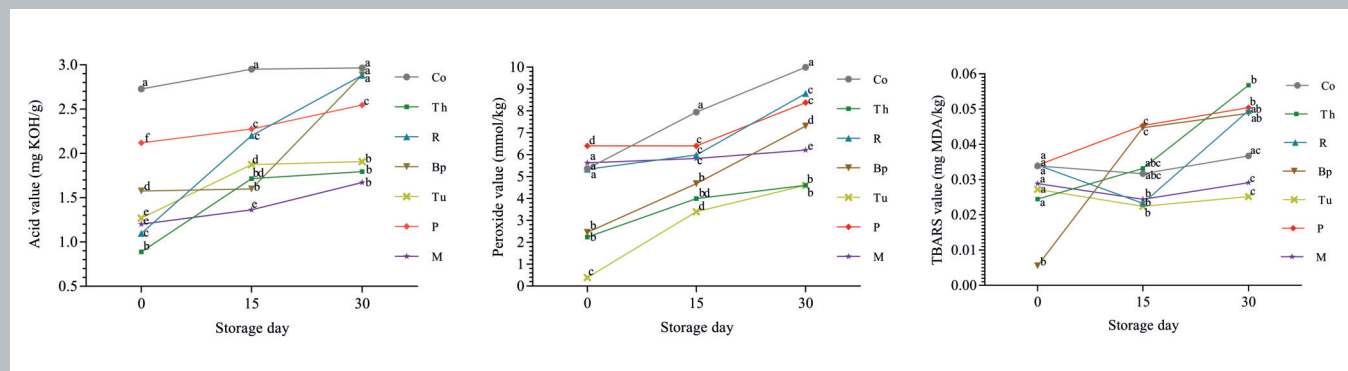
Source: Micovic et al.

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Fig. 1: Changes in antioxidant parameters of sausages during storage

Abb. 1: Veränderungen der antioxidativen Parameter von Würstchen während der Lagerung

Oxidative changes



Source: Micovic et al.

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Fig. 2: Lipolytic and oxidative changes during storage of sausages

Abb. 2: Lipolytische und oxidative Veränderungen während der Lagerung von Würstchen

and ABTS scavenging activities ($69.02 \pm 0.18 - 101.0 \pm 0.62 \mu\text{mol TE}/100 \text{ g}$ and $0.35 \pm 0.01 - 0.40 \pm 0.01 \text{ mmol TE}/100 \text{ g}$, respectively). DPPH radical scavenging activity decreased in all sausages during storage. On the contrary, ABTS radical scavenging activity increased in all sausages except in those with rosemary and the control sausages. Such an increase could be due to the ABTS decomposition rate decreasing below 5°C , at which temperature, the ABTS ion partly reverts back to ABTS, means the measured antioxidant capacity is higher (ILYASOV et al., 2020). This effect could have been augmented in our study, bearing in mind that our sausages were stored below 4°C . Based on the results in this study, the highest antioxidant activity was proven in sausages that had the highest content of polyphenolic compounds, which corresponds to the literature data (NOWAK et al., 2016; NIKOLIĆ et al., 2020). Plotting phenolic content with corresponding antioxidant capacity tests revealed a strong correlation between phenol content and DPPH activity, with coefficient of determination from 0.944 (day 0) up to 0.976 (day 30). On the other hand, phenol content and ABTS antioxidant activity results correlated poorly (coefficient of determination from 0.588 to 0.604), which leads to the conclusion that DPPH is a more suitable antioxidant test for frankfurters than is ABTS radical scavenging.

Lipolytic and oxidative changes

Lipid hydrolysis was reduced by the addition of spices, so the control sausages had the highest acid value throughout the whole storage period, being from 2.73 ± 0.06 at the beginning to $2.97 \pm 0.03 \text{ mg KOH/g}$ at the end of storage (Fig. 2). The lowest acid value during storage was observed in sausages with the spice mixture, thyme, and turmeric (ranging from 1.62 ± 0.02 to $1.91 \pm 0.03 \text{ mg KOH/g}$ after 30 days, respectively). Interestingly, although the sausages with rosemary and those with black pepper had relatively low acid values at the beginning (1.10 ± 0.01 and $1.58 \pm 0.01 \text{ mg KOH/g}$, respectively), they increased rapidly during storage, so they were similar to the acid value of control sausages after 30 days ($2.88 \pm 0.17 \text{ mg KOH/g}$ in sausages with rosemary and 2.89 ± 0.05 in sausages with black pepper). Sausages with red paprika constantly had relatively high lipolytic activity, with the acid value ranging from 2.12 ± 0.04 to $2.55 \pm 0.03 \text{ mg KOH/g}$. Lipid hydrolysis is usually attributed to microbial and tissue lipase activities (CHIZZOLINI et al., 1998), which are mostly inactivated by heat treatment during cooked sausage production. However, it is also reported that some spices, like pepper, show lipase activity (WERMAN et al., 1995) or, like turmeric, show enzyme inhibition activity (FERNANDO et al., 2019). Such data could provide an explanation for the rapid increase of the acid value in sausages with black pepper and the lowest acid value in sausages with turmeric and those with the spice mixture in our study. According to McCREA et al. (2015), paprika does not

inhibit lipases *in vitro*, which could support the high acid values in sausages with red paprika in our study. As for other spices we used in our experiment, there are no literature data about their activity on lipases. Peroxide values showed a pattern similar to acid values, being the highest in the control sausages ($9.99 \pm 0.32 \text{ mmol/kg}$) followed by sausages with black pepper, red paprika, and rosemary (7.32 ± 0.09 to $8.79 \pm 0.21 \text{ mmol/kg}$, respectively). On the contrary, the lowest peroxide value was observed in sausages with thyme and those with turmeric ($4.60 \pm 0.09 \text{ mmol/kg}$ for thyme and $4.61 \pm 0.24 \text{ mmol/kg}$ for turmeric). As lipid peroxidation relies on lipid hydrolysis (CHIZZOLINI et al., 1998), the higher peroxide values in the sausages with the higher acid values in our study were expected. The TBARS value, as a parameter of product rancidity, was far below the maximal acceptable limit of 1 mg MAL/kg (MENEZES et al., 2013) after 30 days of storage in all sausages, and ranged from $0.025 \pm 0.003 \text{ mg MDA/kg}$ in sausages with turmeric to $0.057 \pm 0.003 \text{ mg MDA/kg}$ in sausages with thyme. Although the TBARS value was surprisingly higher in some sausages with spices (thyme, rosemary, black pepper, and red paprika) than in the control sausages, the extremely low TBARS levels we determined could not be considered as a significant disadvantage compared to other TBARS values (0.49 to 2.97 mg MDA/kg) reported in frankfurters after 30 days of storage (PIL-NAM et al., 2015).

Color parameters

As all sausages in our study, including control sausages, were produced with a reduced nitrite content and without antioxidant additives but with the addition of different spices (excluding control sausages), the spiced sausages were brighter ($L^* = 75.28$ in sausages with red paprika to 80.45 in sausages with turmeric), with less redness ($a^* = -1.41$ in sausages with turmeric to 10.85 in sausages with red paprika) and more pronounced yellowness ($b^* = 9.92$ in control sausages to 34.36 in sausages with turmeric) (Tab. 2) than products with the usual nitrite content. These, according to the literature data, usually range as follows: $L^* = 68.6 - 69.9$; $a^* = 7.1 - 9.7$; $b^* = 11.4 - 12.5$ (RUIZ-CAPILLAS et al., 2014; JIN-KYU et al., 2016). The most pronounced deviations from the color parameters common for frankfurter type sausages were observed for redness and yellowness in sausages with turmeric, which is a consequence of the presence of curcumin, the main yellow pigment in turmeric (ZHANG et al., 2015), so the a^* value was actually in the green range (-1.41) at the beginning of storage and moved towards the red range after 30 days (1.29), probably as a consequence of additional nitrosomyoglobin formation in reactions of residual nitrite with myoglobin. Nevertheless, an increase in redness was observed in all experimental sausages during storage, which could be explained by that nitrosomyoglobin process. The presence of turmeric in the spice mixture did not

Color parameters

Tab. 2: Instrumental color parameters of sausages

Tab. 2: Instrumentelle Farbparameter von Würstchen

Color parameters	Storage Day	Frankfurter sausages						
		Co	Th	R	Bp	Tu	P	M
<i>L*</i>	0	80.43 ± 0.03 ^a	79.44 ± 0.03 ^b	79.83 ± 0.05 ^c	78.96 ± 0.04 ^d	80.45 ± 0.04 ^a	75.28 ± 0.05 ^e	77.09 ± 0.05 ^f
	30	79.90 ± 0.05 ^a	78.74 ± 0.04 ^b	79.31 ± 0.06 ^c	78.13 ± 0.07 ^d	79.91 ± 0.05 ^a	74.97 ± 0.06 ^e	76.64 ± 0.06 ^f
<i>a*</i>	0	5.38 ± 0.04 ^a	3.96 ± 0.04 ^b	3.66 ± 0.03 ^c	4.66 ± 0.02 ^d	-1.41 ± 0.03 ^e	10.85 ± 0.03 ^f	3.82 ± 0.03 ^g
	30	8.43 ± 0.04 ^a	5.86 ± 0.03 ^b	5.01 ± 0.03 ^c	6.73 ± 0.04 ^d	1.29 ± 0.03 ^e	12.35 ± 0.05 ^f	6.03 ± 0.05 ^g
<i>b*</i>	0	9.92 ± 0.02 ^a	11.12 ± 0.03 ^b	12.03 ± 0.04 ^c	11.67 ± 0.03 ^d	34.36 ± 0.07 ^e	26.90 ± 0.05 ^f	21.15 ± 0.04 ^g
	30	8.22 ± 0.03 ^a	10.31 ± 0.03 ^b	11.36 ± 0.04 ^c	10.31 ± 0.02 ^b	28.70 ± 0.07 ^d	25.79 ± 0.05 ^e	17.72 ± 0.06 ^f

*Different letters in the same row indicate a significant difference at $p < 0.05$.

Source: Micovic et al.

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Texture profile

Tab. 3: Texture parameters of sausages

Tab. 3: Texturparameter von Würsten

Texture Parameters	Storage day	Frankfurter sausages					
		Co	Th	R	Bp	P	M
Hardness (g)	0	3736 ± 43.58 ^a	4695 ± 48.12 ^b	4566 ± 32.80 ^b	4928 ± 65.27 ^c	4180 ± 58.27 ^d	4523 ± 56.11 ^b
	30	6212 ± 99.19 ^a	7023 ± 100.8 ^b	7492 ± 95.21 ^{cd}	7875 ± 127.7 ^d	6968 ± 126.6 ^b	7327 ± 106.0 ^{bc}
Adhesiveness (g s ⁻¹)	0	-72.88 ± 6.00 ^a	-83.46 ± 6.34 ^a	-70.9 ± 5.67 ^a	-85.7 ± 6.40 ^a	-80.62 ± 6.46 ^a	-81.84 ± 6.02 ^a
	30	-143.1 ± 14.31 ^a	-162.9 ± 13.77 ^{ab}	-147.2 ± 13.54 ^{ab}	-152.3 ± 14.96 ^{ab}	-132.6 ± 14.53 ^a	-206.1 ± 13.69 ^b
Springiness (mm)	0	0.837 ± 0.003 ^{abc}	0.827 ± 0.002 ^c	0.843 ± 0.002 ^{ab}	0.839 ± 0.002 ^{abc}	0.852 ± 0.003 ^b	0.838 ± 0.003 ^{abc}
	30	0.838 ± 0.003 ^{ab}	0.833 ± 0.003 ^a	0.845 ± 0.003 ^{ab}	0.849 ± 0.005 ^b	0.842 ± 0.004 ^{ab}	0.838 ± 0.003 ^{ab}
Cohesiveness	0	0.672 ± 0.003 ^{ab}	0.664 ± 0.002 ^a	0.692 ± 0.001 ^b	0.670 ± 0.002 ^{ac}	0.689 ± 0.012 ^{bc}	0.675 ± 0.002 ^{ab}
	30	0.688 ± 0.002 ^a	0.686 ± 0.001 ^a	0.705 ± 0.001 ^b	0.690 ± 0.002 ^a	0.702 ± 0.002 ^b	0.693 ± 0.002 ^a
Gumminess	0	2510 ± 29.48 ^a	3117 ± 26.23 ^b	3157 ± 19.73 ^{bc}	3299 ± 38.79 ^c	2875 ± 58.44 ^d	3052 ± 32.46 ^b
	30	4273 ± 63.56 ^a	4819 ± 65.55 ^b	5279 ± 63.68 ^{cd}	5437 ± 90.25 ^c	4891 ± 79.48 ^b	5072 ± 64.93 ^{bd}
Chewiness (gxmm)	0	2102 ± 29.53 ^a	2579 ± 23.39 ^{bc}	2663 ± 20.13 ^{cd}	2768 ± 34.84 ^d	2463 ± 73.99 ^b	2560 ± 30.75 ^{bc}
	30	3583 ± 58.01 ^a	4015 ± 58.36 ^b	4461 ± 64.17 ^{cd}	4619 ± 87.13 ^d	4122 ± 77.25 ^{be}	4249 ± 55.74 ^{bc}
Resilience	0	0.357 ± 0.002 ^{af}	0.353 ± 0.001 ^a	0.379 ± 0.001 ^{bd}	0.363 ± 0.001 ^{ef}	0.367 ± 0.003 ^{se}	0.370 ± 0.001 ^{ce}
	30	0.368 ± 0.001 ^a	0.365 ± 0.001 ^a	0.389 ± 0.001 ^b	0.380 ± 0.002 ^c	0.387 ± 0.001 ^b	0.381 ± 0.002 ^c

*Different letters in the same row indicate a significant difference at $p < 0.05$.

Source: Micovic et al.

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affect the color of sausages with this mixture to any great extent, since values were $a^* = 6.03$ and $b^* = 17.72$ at the end of storage. A redness, which was the closest to the values of frankfurters with the usual nitrite content, was observed in sausages with red paprika (12.35 after 30 days), and which was due to the presence of red pigments such as capsanthin and capsorubin in the paprika (VRAČAR et al., 2007). On the other hand, sausages with red paprika had more pronounced yellowness (25.79 after 30 days), due to yellow pigments such as zeaxanthin, violaxanthin, and carotenes that paprika also contains (VRAČAR et al., 2007).

Sausages with thyme, rosemary, and black pepper also had less redness and more yellowness than control sausages. The redness was probably affected by green (chlorophyll) pigments, especially from leafy herbs, as the a^* -value axis in the CIELAB color space is related to the green-red opponent colors (WESTLAND, 2012).

Texture parameters

The texture profile analysis showed (Tab. 3) that at the beginning of storage, hardness (from 4180 g in sausages with red paprika to 4928 g in sausages with turmeric), gumminess (from 2875 in sausages with red paprika to 3299 in sausages with turmeric) and chewiness (from 2463 g x mm in sausages with red paprika to 2769 g x mm in sausages with turmeric) were higher in all sausages with herbs/spices than in control sausages (3736 g, 2519 g, and 2102 g x mm, respectively). Such findings could be due to fibers originating from spices, as other authors also reported a higher hardness and chewiness in fiber-enriched frankfurters (PINTADO et al., 2016). In relation to this, the highest textural values, measured in sausages with turmeric, could be explained by the fact that turmeric powder is obtained from the fiber rich rhizome (ZHANG et al., 2015). On the other hand, ground red paprika powder is obtained from the plant fruits that are poor in fiber (VRAČAR et al., 2007). There were no

Sensory results

Tab. 4: Sensory properties of sausages

Tab. 4: Sensorische Eigenschaften von Würstchen

Quality Parameters	Storage day	Frankfurter sausages						
		Co	Th	R	Bp	Tu	P	M
External appearance	0	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	4.92 ± 0.08 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a
	30	4.60 ± 0.08 ^a	4.50 ± 0.00 ^a	4.50 ± 0.00 ^a	4.50 ± 0.00 ^a	4.50 ± 0.00 ^a	4.50 ± 0.00 ^a	4.50 ± 0.00 ^a
Slice surface appearance	0	4.92 ± 0.83 ^a	4.83 ± 0.10 ^a	4.67 ± 0.17 ^a	5.00 ± 0.00 ^a	4.83 ± 0.10 ^a	5.00 ± 0.00 ^a	4.92 ± 0.82 ^a
	30	4.90 ± 0.08 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	4.90 ± 0.08 ^a
Color	0	4.92 ± 0.08 ^a	4.75 ± 0.17 ^a	4.75 ± 0.17 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a
	30	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a
Odor and taste	0	4.75 ± 0.17 ^a	4.83 ± 0.01 ^a	4.50 ± 0.00 ^a	4.92 ± 0.08 ^a	4.75 ± 0.17 ^a	4.92 ± 0.08 ^a	4.50 ± 0.13 ^a
	30	2.10 ± 0.08 ^a	4.00 ± 0.00 ^b	4.00 ± 0.00 ^b	4.60 ± 0.15 ^c	4.80 ± 0.10 ^c	4.60 ± 0.08 ^c	4.10 ± 0.08 ^b
Texture	0	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	4.83 ± 0.16 ^a	5.00 ± 0.00 ^a	4.58 ± 0.20 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a
	30	4.90 ± 0.08 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a
Total sensory score	0	4.90 ± 0.06 ^a	4.86 ± 0.06 ^a	4.78 ± 0.04 ^a	4.98 ± 0.02 ^a	4.80 ± 0.11 ^a	4.98 ± 0.02 ^a	4.84 ± 0.04 ^a
	30	4.03 ± 0.04 ^a	4.62 ± 0.04 ^b	4.62 ± 0.01 ^b	4.80 ± 0.04 ^c	4.86 ± 0.03 ^c	4.80 ± 0.02 ^c	4.64 ± 0.03 ^b

*Different letters in the same row indicate a significant difference at $p < 0.05$.

Source: MICOVIC et al.

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significant differences ($p > 0.05$) in hardness, gumminess, or chewiness between sausages with other herbs/spices or the spice mixture, which could be explained by the similar fiber content in the leafy herbs (thyme, rosemary) and in black pepper too, even though it is obtained from the plant seeds (GÜLCIN, 2005). During storage, hardness, gumminess, and chewiness increased in all sausages, including the control, which was also reported by other authors during chill storage of frankfurters (PINTADO et al., 2016). There was no significant difference in adhesiveness between all experimental sausage groups. Concerning springiness, the highest values at the beginning of storage were measured in sausages with red paprika (0.852), but after 30 days, springiness was very similar in all sausage types and ranged from 0.833 mm in sausages with thyme to 0.849 mm in sausages with turmeric. The lowest values for cohesiveness and resilience were obtained in control sausages and sausages with thyme and those with black pepper ($p > 0.05$), while these values were the highest in sausages with rosemary and those with red paprika. Overall, the results showed that in the sausages, the turmeric powder mostly contributed to the higher firmness-associated parameters, and red paprika powder contributed to the elasticity-associated parameters.

Sensory properties

The sensory evaluation results are presented in Table 4. At the beginning of the storage, the sensory properties most affected by the addition of different herbs/spices were the slice surface appearance in sausages with rosemary (4.67), color in sausages with thyme and those with rosemary (4.75, both), odor and taste in sausages with rosemary (4.50) and texture in sausages with turmeric (4.58). Although the color of the sausages with turmeric was yellowish, it was uniform, stable and attractive to the panelists, which resulted in a very high score (5.00). On the other hand, the colors of the sausages with thyme and those with rosemary were a little greyish, which was also registered by instrumental color measurement (Tab. 2) since these products had lower a^* values compared to the control sausages. The odor and taste of sausages with rosemary was a bit "tea-like", which was considered a little repulsive. The texture of sausages with turmeric was too firm, which was in accordance with the results of texture profile analysis (Tab. 3). Total sensory scores were the highest for the sausages with black pepper and those with red paprika (4.98 for both), which are the usual spices used in frankfurter type sausages (ŠOJČIĆ et al., 2011). The sausages with black pepper and

those with red paprika were scored more favorably than the control sausage produced without spices (4.90 total score). The lowest total sensory scores were awarded to sausages with rosemary (4.78) and those with turmeric (4.80), but in general, all experimental sausages were judged as very acceptable by the panelists at the beginning of storage.

During storage, most of the sausage groups gained in scores for the cut surface appearance and color, mostly because of the increased redness that was also registered by the higher a^* -values determined by instrumental color measurement (Tab. 2). On the other hand, odor and taste became worse during storage in all sausages, except sausages with turmeric. The most pronounced odor and taste worsening was observed in the control sausages (2.10), which could be connected to the highest acid and peroxide values measured in these sausages, as described above. On the other hand, sausages with turmeric had the lowest peroxide value, which could have contributed to maintenance of their good aroma during storage. The significance of lipid oxidation for the aroma of meat products was also described by other authors (CHIZZOLINI et al., 1998).

Correlation dependence of examined parameters

The correlation dependence of some important examined parameters is presented in Table 5. As the hydrolytic and oxidative changes in lipids are considered as one of the main causes of meat product deterioration (CHIZZOLINI et al., 1998), the correlation dependence between acid value, peroxide value and TBARS value on the one hand, with antioxidant, color and texture parameters on the other hand, was analyzed. Acid and peroxide values strongly negatively correlated with the phenolic content in sausages with turmeric ($p < 0.001$), but were weakly negatively correlated to the phenolic content in all sausages with herbs/spices too. There was also a strong negative correlation ($p < 0.001$) with DPPH radical scavenging activity in almost all sausages with herbs/spices, except those with thyme. These findings confirm the antioxidative and free radical scavenging role of herbs/spices in nitrite-reduced frankfurter type sausages, as reported for some other experimental models (BABOVIĆ et al., 2010; GÜLCIN, 2005; ZHANG et al., 2015).

ABTS radical scavenging activity was both positively and negatively correlated to the acid and peroxide values, so this confirmed our previous statement that the ABTS scavenging test is not particularly suitable for this type of meat product. TBARS values were strongly negatively

Important parameters

Tab. 5: Correlation dependence (r) of some important examined parameters measured in sausages

Tab. 5: Korrelationsabhängigkeit (r) einiger wichtiger untersuchter Parameter gemessen in Würsten

		Antioxidant parameters			Color parameters			Texture parameters		
		Phenols content	DPPH	ABTS	L*	a*	b*	Hardness	Gumminess	Chewiness
Acid value	<i>Co</i>	0.307 (0.216)	-0.139 (0.582)	-0.590 * (0.009)	-0.666 * (0.003)	0.539 (0.021)	-0.514 * (0.029)	0.449 (0.062)	0.382 (0.118)	0.460 (0.055)
	<i>Th</i>	-0.330 (0.182)	-0.223 (0.373)	0.308 (0.214)	-0.705 * (0.001)	0.890* (<0.001)	-0.649 * (0.004)	0.526 * (0.025)	0.578 * (0.012)	0.623 * (0.006)
	<i>R</i>	-0.387 (0.113)	-0.814 * (<0.001)	-0.869 * (<0.001)	-0.793 * (<0.001)	0.951* (<0.001)	-0.948 * (<0.001)	0.783* (<0.001)	0.834* (<0.001)	0.844* (<0.001)
	<i>Bp</i>	-0.044 (0.862)	-0.992 * (<0.001)	0.605 * (0.008)	-0.763 * (<0.001)	0.891* (<0.001)	-0.211 (0.401)	0.410 (0.091)	0.471 * (0.048)	0.536 * (0.022)
	<i>Tu</i>	-0.805 * (0.001)	-0.805 * (<0.001)	-0.407 (0.094)	-0.430 (0.075)	0.747* (<0.001)	-0.764 * (<0.001)	0.633 * (0.005)	0.713 * (0.001)	0.802* (<0.001)
	<i>P</i>	-0.330 (0.181)	-0.815 * (<0.001)	0.472 * (0.048)	-0.602 * (0.008)	0.882* (<0.001)	-0.280 (0.260)	0.616 * (0.006)	0.630 * (0.005)	0.692 * (0.002)
	<i>M</i>	-0.235 (0.348)	-0.908 * (<0.001)	0.364 (0.138)	-0.756 * (<0.001)	0.977* (<0.001)	-0.963 * (<0.001)	0.633 * (0.005)	0.675 * (0.002)	0.719 * (0.001)
Peroxide value	<i>Co</i>	0.603 * (0.008)	-0.500 * (0.035)	-0.864 * (<0.001)	-0.822 * (<0.001)	0.644 (0.004)	-0.852 * (<0.001)	0.436 (0.070)	0.448 (0.062)	0.619 * (0.006)
	<i>Th</i>	-0.173 (0.493)	-0.296 (0.234)	0.416 (0.086)	-0.832 * (<0.001)	0.904* (<0.001)	-0.459 (0.055)	0.532 * (0.023)	0.525 * (0.025)	0.556 * (0.016)
	<i>R</i>	-0.088 (0.727)	-0.959 * (<0.001)	-0.934 * (<0.001)	-0.800 * (<0.001)	0.823* (<0.001)	-0.864 * (<0.001)	0.476 * (0.046)	0.548 * (0.019)	0.596 * (0.009)
	<i>Bp</i>	-0.042 (0.870)	-0.904 * (<0.001)	0.275 (0.269)	-0.850 * (<0.001)	0.854* (<0.001)	-0.721* (<0.001)	0.464 (0.052)	0.556 * (0.017)	0.742* (<0.001)
	<i>Tu</i>	-0.900 * (<0.001)	-0.917 * (<0.001)	-0.358 (0.145)	-0.750 * (<0.001)	0.912* (<0.001)	-0.882 * (<0.001)	0.489 * (0.039)	0.556 * (0.017)	0.634 * (0.005)
	<i>P</i>	-0.283 (0.255)	-0.688 * (0.002)	0.678 * (0.002)	-0.396 (0.103)	0.878* (<0.001)	-0.305 (0.218)	0.107 (0.674)	0.330 (0.180)	0.362 (0.139)
	<i>M</i>	-0.140 (0.581)	-0.591 * (0.001)	-0.012 (0.961)	-0.653 * (0.003)	0.742* (<0.001)	-0.725 * (<0.001)	0.163 (0.519)	0.206 (0.412)	0.274 (0.272)
TBARS value	<i>Co</i>	0.388 (0.111)	-0.280 (0.261)	-0.011 (0.966)	0.236 (0.345)	0.092 (0.715)	-0.113 (0.656)	-0.073 (0.772)	-0.049 (0.846)	-0.034 (0.893)
	<i>Th</i>	0.046 (0.857)	-0.533 * (0.023)	0.512 * (0.030)	-0.620 * (0.006)	0.702 * (0.001)	-0.331 (0.179)	0.291 (0.241)	0.318 (0.198)	0.419 (0.083)
	<i>R</i>	0.174 (0.489)	-0.798 * (<0.001)	-0.696 * (0.001)	-0.591* (0.009)	0.388 (0.112)	-0.441 (0.067)	-0.048 (0.849)	0.032 (0.898)	0.099 (0.696)
	<i>Bp</i>	0.084 (0.739)	-0.603 * (0.008)	0.003 (0.990)	-0.806 * (<0.001)	0.679 * (0.002)	-0.325 (0.188)	0.933* (<0.001)	0.951* (<0.001)	0.949* (<0.001)
	<i>Tu</i>	0.058 (0.819)	-0.025 (0.922)	0.137 (0.588)	0.072 (0.776)	-0.055 (0.829)	0.085 (0.738)	-0.107 (0.672)	-0.016 (0.948)	-0.016 (0.948)
	<i>P</i>	-0.163 (0.519)	-0.552 * (0.017)	0.177 (0.484)	-0.483 * (0.042)	0.504 * (0.033)	-0.028 (0.912)	0.672 * (0.002)	0.514 * (0.029)	0.587 * (0.010)
	<i>M</i>	0.135 (0.592)	-0.418 (0.084)	0.129 (0.610)	-0.208 (0.407)	0.109 (0.667)	-0.211 (0.400)	-0.337 (0.172)	-0.319 (0.197)	-0.228 (-0.363)

Level of significance: *

 $p < 0.05$; p -values representing differences from zero are shown in parentheses.

Source: Micovic et al.

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correlated with the DPPH scavenging activity for almost all sausages with herbs/spices (p ranged from 0.02 to <0.001) confirming it as a good test to monitor the development of product rancidity. Oxidative changes were strongly negatively correlated with L^* and b^* values on the one hand, and positively correlated with a^* values on the other hand, which was surprising, bearing in mind the fact that red pigments in meat products (myoglobin and nitroso-myoglobin) are susceptible to degradation

by peroxides (GOVARI and PEXARA, 2015). However, taking into account the strong free radical scavenging activity detected in sausages with herbs/spices in our study, it could be assumed that it was precisely this free radical scavenging activity that contributed to the preservation of meat red pigments. As for correlations between oxidative changes and texture parameters, there was a strong positive correlation for sausages with spices concerning acid and peroxide value on the one hand, and both

negative and positive correlations between TBARS value and examined texture parameters on the other hand, which was contradictory and confirmed the primary influence of some other factors on the sausage texture, such as fibers from spices, which was discussed previously.

Conclusions

The results of our study show that the frankfurter type sausages with reduced nitrite content, but enriched with individual herbs/spices as a source of phenolic compounds, had significant antioxidant potential. The highest DPPH radical scavenging activity was observed in sausages with rosemary followed by sausages with thyme, black pepper, turmeric, and red paprika. ABTS radical scavenging activity proved to be not suitable as an antioxidant capacity test for this type of sausage. The lipid hydrolysis and oxidative changes were the most intensive in the control sausages, lower in sausages with black pepper, red paprika, and rosemary, and were the lowest in sausages with turmeric and those with thyme. TBARS values were far below the rancidity level in all experimental sausages. The spice mixture produced moderate antioxidant activity in the sausages. Instrumental color measurement showed that the sausages with herbs/spices had more intensive yellowness and less redness than the control sausages, except the sausages with red paprika that had a^* values similar to sausages with usual nitrite content. Hardness, gumminess, and springiness were higher in herb/spice enriched sausages compared to control sausages. Overall, the results showed that in the sausages, the turmeric powder mostly contributed to the higher firmness-associated parameters, and red paprika powder contributed to the elasticity-associated parameters. All experimental sausages were sensorially judged as very acceptable by the panelists at the beginning of storage, but after 30 days, the control sausages received the lowest score, especially for odor and taste, while the highest scores went to sausages with turmeric, black pepper, and red paprika. The correlation testing confirmed the negative correlation between oxidative changes and DPPH radical scavenging activity in herb/spice enriched sausages.

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Literature references can be requested from the corresponding author or the editorial office, respectively.

Zusammenfassung

Antioxidatives Potenzial verschiedener Kräuter und Gewürze in nitritreduzierten Frankfurter Würstchen

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Antioxidationsmittel | Frankfurter Würstchen | Nitritreduktion | sensorische Eigenschaften | Gewürze

Nitrite werden in der Fleischverarbeitung als Konservierungsmittel verwendet, aber es besteht die Notwendigkeit, ihre Verwendung wegen ihrer möglichen schädlichen Auswirkungen auf die Gesundheit der Verbraucher zu reduzieren. Das antioxidative Potenzial verschiedener Kräuter und Gewürze in nitritreduzierten Frankfurter Würstchen und ihr Einfluss auf die sensorischen Eigenschaften der Produkte wurden untersucht. Die Ergebnisse zeigten, dass die nitritreduzierten Würste, die mit einzelnen Kräutern oder Gewürzen als Quelle für phenolische Verbindungen angereichert waren, ein signifikantes antioxidatives Potenzial hatten, das bei Würsten mit Rosmarin am höchsten war, gefolgt von Würsten mit Thymian, schwarzem Pfeffer, Kurkuma und rotem Paprika. Nach Kühlung hatten die mit Kräutern und Gewürzen angereicherten Würste bessere sensorische Eigenschaften als die Kontrollwürste.

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