Technological characterization and consumer perception of dry fermented game sausages with bay leaf (*Laurus nobilis* L.) essential oil

Tehnološka karakterizacija i percepcija potrošača trajnih kobasica od mesa divljači uz dodatak eteričnog ulja lovora (*Laurus nobilis* L.)

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Abstract

The aim of this paper was to determine basic technological traits and sensory acceptance of dry game sausages with addition of bay leaf (*Laurus nobilis* L.) essential oil. For that purpose, three treatments were established: controlled C, L1 with addition of 0.005% of bay leaf essential oil, and L2 with addition of 0.01% of bay leaf essential oil. Measurement of pH, water activity and thiobarbituric acid reactive substances (TBARS) values were performed at the end of production, while after storage for 2 months in vacuum and cold sensory hedonic test on 106 subjects was done. It was found that sausages with added bay leaf essential oil had significantly lower pH values (L1 and L2 treatment), water activity (L1 treatment) and TBARS values (L1 and L2 treatment) compared to control treatment C. Statistical analysis of sensory data revealed that sausages from C treatment were rated with significantly higher acceptability and willingness to buy than L1 and L2 treatments. It was established that sensory traits of sausages from treatment C were liked in 98.61% of consumers, from treatment L1 in 90.93%, while sausages from treatment L2 were liked in 85.71% of consumers. The highest proportion of consumers willing to buy sausages were in treatment C (91.11%), followed by sausages in treatment L1 (84.31%), and the lowest proportion were in treatment L2 (66.67%). Although beneficial impact of addition of bay leaf essential oil on technological traits was found, there was an unfavorable effect on sensory traits and willingness to buy sausages possibly due to the consumers' existing expectation of sensory characteristics of dry game sausages.

Keywords: bay leaf, dry game sausages, essential oil, sensory analysis, technological traits

Sažetak

Cilj rada bio je utvrditi osnovna tehnološka svojstva i senzornu prihvatljivost trajnih kobasica od mesa divljači s dodatkom eteričnog ulja lovora (*Laurus nobilis* L.). U tu svrhu napravljena su tri tretmana: kontrolni C, L1 tretman s dodatkom 0,005% i L2 tretman s dodatkom 0,01% eteričnog ulja lovora. Mjerenja vrijednosti pH, aktiviteta vode i reaktivnih spojeva tiobarbiturne kiseline (TBARS) izvršena su na kraju proizvodnje, dok je nakon 2 mjeseca čuvanja u vakuumu i na hladnom proveden senzorni hedonistički test na 106 potrošača. Utvrđeno je da su kobasice s dodatkom eteričnog ulja lovora imale značajno niže vrijednosti pH (L1 i L2 tretman), aktiviteta vode (L1 tretman) i TBARS (L1 i L2 tretman) u odnosu na kontrolni tretman. Statističkom analizom podataka senzorne analize utvrđeno je da su kobasice C tretmana bile ocijenjene značajno većom prihvatljivosti i spremnosti na kupnju u odnosu na L1 i L2 tretmane. Ustanovljeno je da su senzorna svojstva kobasica C tretmana bila prihvatljiva kod 98,61% ispitanika, kobasica L1 tretmana kod 90,93%, a kobasica L2 tretmana kod 85,71% ispitanika. Najveći udio ispitanika spremnih na kupnju kobasice je bio kod kobasica kontrolnog tretmana (91,11%), zatim kod tretmana L1 (84,31%), dok je najmanji udio bio kod L2 tretmana (66,67%). Iako je utvrđen koristan učinak dodatka eteričnog ulja lovora na tehnološka svojstva, pojavio se nepovoljan učinak na senzorna svojstva i spremnost na kupnju vjerojatno zbog postojanja prethodnog očekivanja senzornih svojstava trajnih kobasica od mesa divljači.

Ključne riječi: eterično ulje, lovor, senzorna analiza, tehnološka svojstva, trajne kobasice

Introduction

Oxidation is a well-known non-microbial cause of quality deterioration in meat. Oxidative deterioration in any type of meat manifests in form of discoloration, development of off flavor, formation of toxic compounds, poor shelf life, nutrient and drip losses (Falowo et al., 2014). To control the oxidation for longer time during production and storage of food, antioxidants must be used. Although there are many compounds that have been proposed to possess antioxidant properties to inhibit oxidative deterioration, only a few can be used in food products. In response to recent claims that synthetic antioxidants have the potential to cause toxicological effects and consumers' increased interest in purchasing natural products, the meat industry has been seeking sources of natural antioxidants (Karre et al., 2013). Previous reports stated that essential oils (EOs) have multiple beneficial functions which could be used in food industry. Because of the mode of extraction, mostly by distillation from aromatic plants, EOs contain a variety of volatile molecules such as terpenes and terpenoids, phenol-derived aromatic components and aliphatic components, some of them having a pronounced antioxidative impact (Sacchetti et al., 2005; Bakkali et al., 2008; Karre et al., 2013; Falowo et al., 2014).

Utilization of EOs in the manufacture of dry wild boar meat sausage could have a potential benefit due to the higher level of fat unsaturation of wild pig meat compared to domestic pig (Sales and Kotrba, 2013) and therefore higher risk for lipid oxidation and sausage deterioration during storage. Also, such sausages often have stronger and specific aroma resulting from the use of wild pig meat (Paulsen et al., 2011; Kos et al., 2015) and are often smoked so the incorporation of EOs could be more easily conducted respecting consumers' opinions.

Bay leaf (*Laurus nobilis* L.) belongs to Laureacea family, being a native plant from the warm Mediterranean region of Croatia, but also including countries like Italy, France, Spain and Portugal. It is widely used as a spice in cuisine, and in cosmetic, pharmaceutical and food industry (Dias et al., 2014). Many reports have shown the antimicrobial and antioxidative activities of bay leaf extracts and its EO in vitro and in vivo (Kaurinovic et al., 2010; Basak and Candan, 2013; Dias et al., 2014). Addition of bay leaf EO to fresh and dry sausages showed good potential for application by the industry to improve safety and shelf life. However, the results of sensory tests in sausage manufacture were questionable, and leading to sensory limitations, that does not allow its use in concentrations needed for sufficient antimicrobial and antioxidative impact (Silveira et al., 2014; García-Díez et al., 2016). Therefore, the aim of this paper was to determine the effect of the addition of bay leaf EO on basic technological traits of dry game sausages and sensory perception of randomly selected consumers and their opinion on willingness to buy such sausages.

Materials and methods

For this research, dry fermented sausages were made from meat (50% of the total) and fatty tissue (10% of the total) of female domestic pig (Large White breed) and meat from female wild pig (40% of the total). Meat and fatty tissue were grinded on 8 mm granulation, and 2.1% of salt (NaCl), 0.3% of grounded black pepper (*Piper nigrum* L.), 0.2% of grounded dry garlic (*Allium sativum* L.), 0.2% of table sugar, 0.2% of glucose, 0.1% of grounded red hot paprika (*Capsicum annuum* L.) and 2% of white wine were added. Three batches of meat batter were prepared: C - without addition of bay leaf EO, L1 - with addition of 0.005% of bay leaf EO (50 ppm), and L2 - with addition of 0.01% of bay leaf EO (100 ppm). Bay leaf EO was of chemotype 1,8 cineole and was produced by steam distillation (Pranarom, Belgium). After mixing, meat batter was stuffed into collagen casings (diameter of 38 mm) and sausages were subjected to smoking and drying in traditional conditions during winter period. Sausages were cold smoked 6 times, and the temperature conditions during production were 5-20 °C and relative humidity 60-95%. Total duration of production process was 49 days till weight loss of 45%. At the end of production, sausages for sensory analysis were vacuum packed and stored at temperatures below 8 °C for 2 months.

Technological measurement of pH, water activity and thiobarbituric acid reactive substances (TBARS) values were performed in triplicate on 10 ripened sausages at the end of the production. pH values were measured using portable pH-meter IQ 150

(IQ Scientific Instruments, USA) equipped with spear type glass electrode BlueLine 21 pH (Schott AG, Germany). Water activity values were determined using portable analyzer HygroPalm HP23-AW-A equipped with HC2-AW probe (Rotronic AG, Switzerland). The TBARS method was performed according to a modified version of the method described by Botsoglou et al. (1994). A 2 g of homogenized sample was transferred into a 50 mL centrifuge polypropylene tube, and 10 mL of 5% trichloroacetic acid (TCA) and 5 mL of 0.8% butylated hydroxytoluene in hexane were added. The content of the tube was shredded for 30 s at high speed (Ika T10 basic, UltraTurrax, Germany), homogenized for 10 s (Ika Vortex 3, Germany) and centrifuged for 5 min at 4,000 rpm (Centric 322A, Tehtnica, Slovenia). The top hexane layer was discarded, and the bottom aqueous layer was filtered thru quantitative filter paper (Munktell grade 391, Germany), and a 2 mL aliquot was pipetted into a screw-capped tube, after which 1.5 mL of 0.6% aqueous 2-thiobarbituric acid (TBA) was added. Following incubation for 30 min at 90 °C, the tube was cooled under tap water for 20 min. After cooling, the absorbance values were measured at 532 nm on spectrophotometer (Helios y, Thermo Electron Corporation, UK) and the blank values were subtracted from the sample made of 2 mL TCA and 1.5 mL of 0.6% TBA. TBARS results were expressed as $mg*kg^{-1}$ malondialdehyde (MDA), which were calculated from standard curve using 1,1,3,3 tetramethoxypropane.

Sensory analysis was performed by hedonic test using 106 untrained consumers among students and faculty staff and the basic socio-demographic characteristics of the set are presented in Table 1. Samples were scored on 10 point structured scale where 0 meant “extremely disliked” and 9 meant “extremely liked”. Four sensory traits were evaluated: cross section, odor, flavor and overall. Subjects were also asked to evaluate the willingness to buy sausages within the same scale. Samples were cut by knife on 2 mm thickness under angle 90 °, and presentation order was defined as completely balanced block design. Subjects were placed in separate booths and were instructed to use tap water and unsalted bread as palate cleansers before every sample.

The data obtained were analyzed by SAS Studio University Edition 3.4 (SAS Institute, 2015) using GLM procedure with least significant difference (LSD) test for treatment comparison for parametric analysis of pH, water activity and TBARS values. NPAR1WAY procedure was used for nonparametric analysis of sensory data after correction for outliers with Kruskal-Wallis test and DSCF (Dwas, Steel, Critchlow-Flinger) method for treatment comparison at level P=0.05. Results of parametric and nonparametric analysis are presented as mean ± standard deviation.

Table 1. Socio-demographic characteristics of the respondents in sensory analysis

Characteristic	Percentage (%)	Characteristic	Percentage (%)
Gender		Income (per month)	
Female	56.7	Low	33
Male	44.3	Middle	23.6
Age (years)		Upper Middle	33
<20	8.5	High	10.4
21-30	25.5	Education level	
31-40	30.2	Elementary school	6.6
41-50	18.9	High school	23.6
51-60	12.3	University degree	69.8
>61	4.7		

Results and discussion

Table 2 shows physico-chemical characterization of dry game sausages with different levels of added bay leaf EO. pH values found in this experiment were lower than in game sausages of comparable meat composition as it was reported by Soriano et al. (2006) and Paulsen et al. (2011), but similar to dry sausages produced from pork (Hoz et al., 2004; Tabanelli et al., 2013). Water activity and TBARS values obtained were comparable to previous research found by many authors (Nassu et al., 2003; Hoz et al., 2004; Soriano et al., 2006; Bozkurt, 2007; Abu Salem and Ibrahim, 2010; Tabanelli et al., 2013; Silveira et al., 2014; García-Díez et al., 2016).

Statistical analysis revealed that pH, water activity and TBARS values were significantly lower in treatments with bay leaf EO when compared to control group (Table 2). Silveira et al. (2014) established similar effect of added bay leaf EO to fresh Tuscan sausages that had lower pH values during storage but not at the end of manufacturing process, while effect on TBARS values was not found at all. Research on other EOs revealed different results. Abu Salem and Ibrahim (2010) added sage EO and Bozkurt (2007) added *Thymbra spicata* and sesame EO to dry sausages and they determined significantly lower TBARS values in treatments with EO, without recorded significant effect on pH value. Nassu et al. (2003) added rosemary EO to fermented goat meat sausages and found no difference in pH value nor in TBARS values, but they observed significantly lower water activity values in treatments with EO.

Table 2. Technological characterization of dry game sausages with different shares of bay leaf essential oil (mean ± standard deviation)

Trait	Treatment[1]		
	C	L1	L2
pH value	5.06 ± 0.02[a]	5.01 ± 0.006[b]	5.01 ± 0.01[b]
Water activity	0.883 ± 0.003[a]	0.868 ± 0.003[b]	0.874 ± 0.009[ab]
TBARS[2] value	0.609 ± 0.01[a]	0.478 ± 0.005[b]	0.478 ± 0.009[b]

[1]C - control treatment; L1 - sausages with addition of 0.005% of bay leaf essential oil; L2 - sausages with addition of 0.01% of bay leaf essential oil. [2]TBARS - thiobarbituric acid reactive substances expressed as mg*kg^{-1} malondialdehyde. Means within a row with different letters are significantly different (P<0.05).

The effect of addition of bay leaf EO on TBARS values could be ascribed to the known antioxidative effect of bay leaf EO as stated by many authors (Kaurinovic et al., 2010; Basak and Candan, 2013; Dias et al., 2014). Likewise, antimicrobial effect of bay leaf EO in sausages was also stated (Silveira et al., 2014; García-Díez et al., 2016). It is possible that bay leaf EO had impact on microbial growth and favoring conditions to lactic acid bacteria. This subsequently led to lower pH and to enhanced water loss, which is found to be beneficial for dry sausage production. It should be noticed that neither starter cultures nor nitrites were given to meat batter, so this effect could be useful for safe sausage production.

Wu et al. (1991) report that in case of TBARS values higher than 1 mg*kg^{-1} MDA considerable development of unwanted aroma (rancidity) could happen. Stricter criteria were proposed by Sheard et al. (2000) who found that consumers were able to notice oxidative processes at the level of 0.5 mg*kg^{-1} MDA. In this research, all sausages had TBARS values lower than 1 mg*kg^{-1} MDA, with L1 and L2 sausages having TBARS values lower than 0.5 mg*kg^{-1} MDA, suggesting that oxidized and rancid aromas should not be noticeable. Although there were statistical differences between treatments, this cannot be unambiguously marked as treatments' influence. Differences between treatments could be due to the antioxidative effect of bay leaf EO as stated by Dias et al. (2014), but also due to pepper and hot pepper (Martínez et al., 2006) or garlic (Park et al., 2008) which were all added to meat batter and have antioxidative effect. It is necessary to comprehend the effect of every spice or EO, and to explore synergistic effect with meat components as concluded by Gutierrez et al. (2008).

Visual representation of trait distribution of dry fermented game sausages with bay leaf EO is shown in Figure 1. In Figure 1, the box represents the interquartile range, a solid line within the box represents the median, squares represent the arithmetic averages, the whiskers represent the calculated minimum and maximum while the dots represent outliers of the distribution.

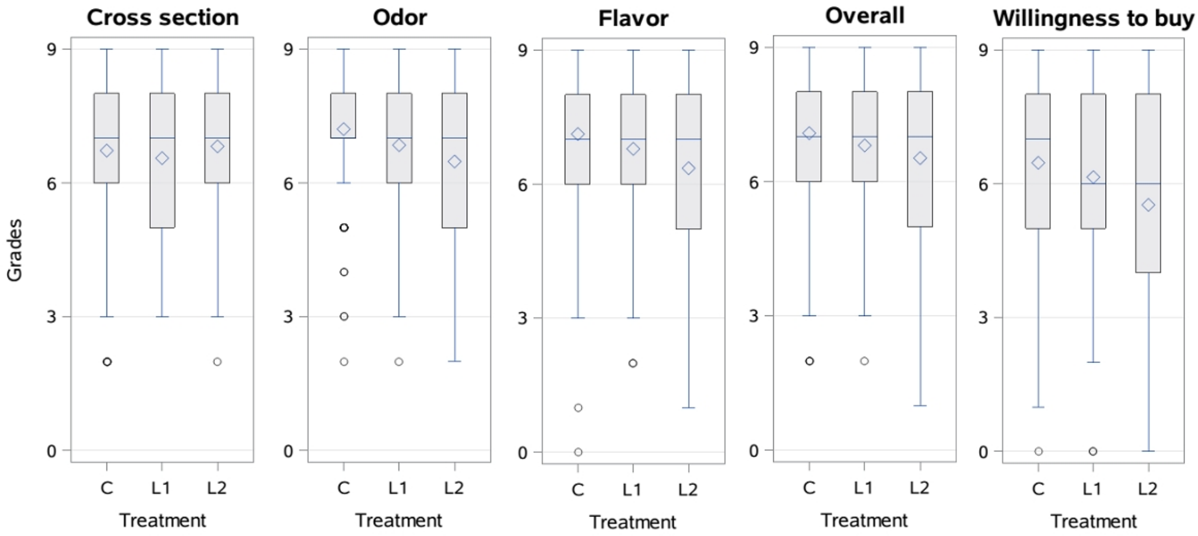

Figure 1. Visual representation of trait distribution of dry game sausages without (C) and with addition of 0.005% (L1) and 0.01% of bay leaf essential oil (L2)

Median scores of almost all traits and treatments were 7, with exception for trait willingness to buy for treatments L1 and L2 when median scores were 6. Upper quartiles of all traits and treatments were 8, while lower quartiles were more variable leading to interquartile ranges from the lowest 1 (trait odor in treatment C) to the highest 4 (trait willingness to buy in treatment L2). In some traits and treatments there were outliers (dots on the box plots) that were lower than the calculated whiskers. This was expected since untrained consumers were used in this research. According to these results, it can be concluded that the lowest variability was found in traits of treatment C, followed by traits in treatment L1, while the highest variability was established in traits of treatment L2 where the highest amount of EO was added. The reason for this could be the intensive aromatic impact of EOs on which often consumers are not accustomed to and this could be a strong disadvantage for inclusion of higher levels of EOs in meat products as it was concluded by Silveira et al. (2014) and García-Díez et al. (2016).

Figure 2 shows cumulative shares of scores 5 and higher per treatment that could be assigned to the share of consumers who liked sensory traits (averaged value) or were willing to buy sausage. It was established that sensory traits of sausages from treatment C were liked in 98.61% of tested consumers, followed by sausages from treatment L1, which were liked in 90.93% of consumers, while sausages from treatment L2 were liked in 85.71% of consumers.

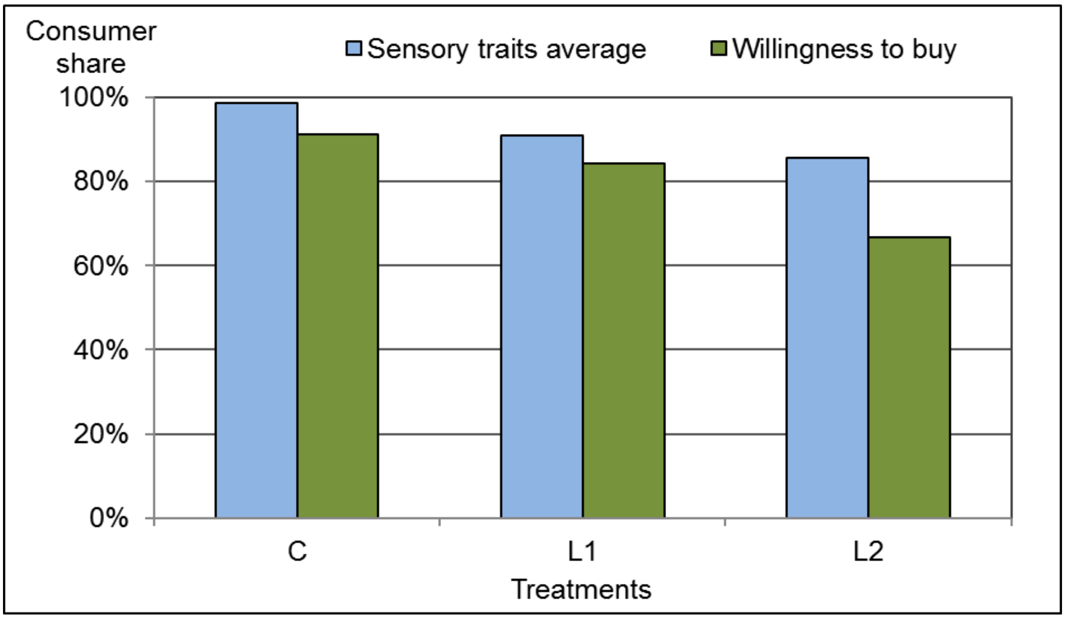

Figure 2. Cumulative shares of consumers who liked sensory traits or were willing to buy dry game sausages without (C) and with addition of 0.005% (L1) and 0.01% of bay leaf essential oil (L2)

The highest proportion of consumers willing to buy sausages were in treatment C (91.11%), followed by sausages in treatment L1 (84.31%), and the lowest proportion of consumers willing to buy sausages were in treatment L2 (66.67%). It was determined that likeability of sensory traits and willingness to buy sausages were lower with higher proportion of bay leaf EO in sausages. Similar results were found by García-Díez et al. (2016) who established that only 84% of consumers would like to consume dry sausages of control group, and around 70% of consumers showed intention to consume dry sausages with 0.005% of bay leaf EO. The same authors also established that only less than 20% of consumers would like to consume dry sausages with 0.05% of bay leaf EO, so they conclude that this proportion of EO is not applicable in practice, due to sensory reasons. In addition, with higher level of EO there was higher difference between sensory traits and willingness to buy, respectively 7.5, 6.62 and 19.05% for treatments C, L1 and L2. This could lead to conclusion that willingness to buy does not follow sensory expression linearly, and it is falling at higher rate when sensory traits are more pronounced.

Table 3 shows means and standard deviations of sensory traits and willingness to buy dry sausages with different shares of bay leaf EO. It was found that sausages from control treatment were scored significantly different when compared to sausages with addition of bay leaf EO. Namely, consumers scored sausages from treatment C significantly higher than L1 sausages in cross section, and significantly higher than L1 and L2 sausages in odor, flavor and overall. However, willingness to buy was scored significantly higher in treatment C when compared to treatment L2, but not when compared to treatment L1.

Table 3. Sensory traits and willingness to buy dry game sausages with different shares of bay leaf essential oil (mean ± standard deviation)

Trait	Treatment[1]		
	C	L1	L2
Cross section	7.15 ± 1.36[a]	6.58 ± 1.59[b]	6.87 ± 1.64[ab]
Odor	7.6 ± 1.03[a]	6.9 ± 1.54[b]	6.51 ± 1.72[b]
Flavor	7.46 ± 1.26[a]	6.92 ± 1.49[b]	6.4 ± 1.95[b]
Overall	7.44 ± 1.22[a]	6.93 ± 1.42[b]	6.54 ± 1.82[b]
Willingness to buy	6.87 ± 1.70[a]	6.34 ± 1.84[ab]	5.53 ± 2.42[b]

[1]C - control treatment; L1 - sausages with addition of 0.005% of bay leaf essential oil; L2 - sausages with addition of 0.01% of bay leaf essential oil. Means within a row with different letters are significantly different ($P<0.05$).

There were no statistically significant differences in sensory traits and willingness to buy between sausages in treatments L1 and L2, although all traits, except cross section, were scored higher in treatment L1. Based on these results, it can be concluded that addition of bay leaf EO in studied levels lead to worsening of sensory traits and to lowering of customers' willingness to buy those sausages. Possible explanation to this could be an existing expectation of consumers about sensory characteristics of dry game sausage. Similar results were reported by Silveira et al. (2014) who established that all sensory attributes of fresh sausages with addition of 0.05% and 0.1% of bay leaf EO were scored significantly lower when compared to control group. Those authors propose the adjustments in the formulation to reduce the concentration of other spices that may provide a more balanced formulation and result in a more palatable sausage.

Despite questionable reasons of addition of bay leaf EO because of its unfavorable impact on sensory traits as it was determined in previous studies (Silveira et al., 2014; García-Díez et al., 2016) and in this research, many other studies showed that different EOs could enhance sensory traits of sausages. Bozkurt (2007) found that dry sausages with 0.03% of sesame and *Thymbra spicata* EO had higher overall acceptability compared to control group. Similarly, Abu Salem and Ibrahim (2010) established that addition of 0.025% and 0.05% of sage EO resulted in higher overall acceptability of dry sausages. Nassu et al. (2003) found that dry sausages with 0.05% of rosemary EO had positive impact on overall acceptability and red color of the slice, while oxidized aroma was lower. Those results are especially important in situations when EOs are being added in amounts that have primal antimicrobial, antioxidant or medicinal impact. When adding very high proportions of EOs in meat products, superior functional effect (mostly antibacterial and antioxidative) appear but there is often noticeable reduction in sensory acceptability of the product (Busatta et al., 2008; Silveira et al., 2014; García-Díez et al., 2016). This could be even more pronounced in meat products that already have known sensory profile, which is often the case with traditional dry game sausages. Therefore, it is recommended that selection and amount of EOs added to meat products should be based on plant species that are expected in final product. In those circumstances, EOs could reveal their additional beneficial function without interfering with sensory acceptability of the final product.

Conclusions

Application of EOs in meat products has many effects including organoleptic, antimicrobial, antioxidative and medicinal as the main ones. Although being added in small amounts they are able to express noticeable impact that could sometimes impart sensory traits. Results of this research show that there is a small but noticeable impact of bay leaf EO on basic technological traits of dry game sausages leading to lower pH, water activity and TBARS values which is found to be beneficial. However, the addition of bay leaf EO in dry game sausages in amounts 0.005% and 0.01% was considered unfavorable by consumers regarding sensory traits and willingness to buy sausages. Possible explanation to this could be an existing expectation of consumers on sensory characteristics of traditional dry game sausage that already has known sensory profile. Therefore, selection and amount of EOs added to meat products should be based on plant species that are expected in final product, and added EOs could reveal their additional beneficial function without interfering with sensory acceptability of the final product.

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