J. Meat Sci. 2023, 18(2): 31-36

Research Article

ACS Publisher
www.acspublisher.com

Journal of Meat Science

Year 2023 (December), Volume-18, Issue-2

ISSN 0975-5209 **(Print)**
ISSN 2581-6616 **(Online)**

Impact of Different Concentrations of Cinnamon Essential Oil on The Physico-Chemical, Microbiological, and Sensory Characteristics of Refrigerated Pork Sausages

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ARTICLE INFO

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Received 09-11-2023; Accepted 14-01-2024

DOI: 10.48165/jms.2023.18.02.05

ABSTRACT

The investigation examined the impact of varying concentrations of Cinnamon Essential Oil (CEO) on the preservation of pork sausages. The sausages were divided into different groups: a control group,0.2 µl/g CEO (T1) 0.4 µl/g CEO (T2) and 0.6% µl/g (T3). All these samples were stored at 4°C for 15 days. Notably, the sausages treated with 0.4 µl/g CEO exhibited superior preservation qualities, as evidenced by lower levels of TBARS, free fatty acids, pH, water activity, microbial count (Total Plate Count, coliform count and staphylococcal count) and sensory properties (colour, flavour and overall acceptability) compared to all other treatments ($p<0.05$). So it can be concluded that the addition of 0.4 µl/g CEO had the most effective preservative impact on pork sausages during the refrigerated storage for a period of 15 days.

Key words: *Cinnamon essential oil, Pork sausages, Physico-chemical properties, Microbial counts, Sensorial properties*

INTRODUCTION

Meat and meat products are a valuable source of essential nutrients, yet their shelf life can be compromised by factors such as microbial spoilage, enzymatic changes, and the oxidation of lipids and proteins. Among various meat types, pork products are more susceptible to oxidation when compared to beef or lamb due to their relatively higher levels of unsaturated fatty acids (Muthukumar *et al.* 2014). To extend the shelf life of these products, antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG) can be added. However, it's worth mentioning that these synthetic antioxidants are considered potential contributors to carcinogens, raising concerns about their impact on consumer health. Instead of incorporating synthetic antioxidants, it is possible to utilize natural preservatives like essential oils, including those derived from cinnamon, thyme, rosemary, ginger, clove, and other similar options that are generally recognized as safe (GRAS) in the food industry. Essential oils are characterized by their high volatility, and when applied in vapour form, they require a smaller quantity. Moreover, this vapour application minimizes residual oil on the product, reducing concerns related to flavour

tainting. For postharvest applications, it might be more suitable to employ essential oils in their vapour phase (He et al. 2018).

The Cinnamon essential oil (CEO), derived from bark, is a common spice used to enhance the flavour and aroma of food. It is known for its high cinnamaldehyde content and a smaller concentration of eugenol, alongside various other aromatic compounds (Wang 2018). Numerous investigations have demonstrated the potent antibacterial properties of essential oils (EOs) against pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus* and many others (Yang et al. 2021). The antimicrobial effects of EOs have been attributed to their volatile components, such as terpenoids, aldehydes, and phenolic compounds. Studies suggest that EOs exert their antimicrobial action by penetrating microbial membranes, leading to the leakage of ions and cytoplasmic contents and disrupting the cell's normal functioning, as explained by (Burt 2004).

MATERIALS AND METHODS

Pigs that were raised under hygienic conditions were procured from local vendors in Gannavaram and subsequently slaughtered, dressed, and deboned in a semi-automatic pork processing facility operated by the Department of Livestock Products Technology in Gannavaram. Cinnamon essential oil is obtained from Falcon oils located in Bangalore . The media used for various bacterial analysis obtained from Himedia, Mumbai. A meat emulsion was prepared by finely chopping the deboned meat along with fat, ice flakes, a mixture of condiments, and a blend of spices, ensuring thorough mixing. Various levels of cinnamon essential oil (0.2 µl/g, 0.4 µl/g , 0.6 µl/g) were then introduced into the meat emulsion to determine the optimal amount for enhancing the quality of chicken meat sausages. A measured quantity of this emulsion was filled into sausage casings using a sausage stuffer, and the sausages were linked together. These linked sausages were then steam-cooked for 25 minutes at 90 ± 1°C. After cooking, the casings were removed, and the quality of the sausages was assessed based on various parameters.

Physico-Chemical Properties

pH

The mean pH of the preparation was assessed using the method described by Trout et al. (1992) and a deluxe digital pH meter (Systronics µ pH System, Type 371) was used for this measurement.

2-TBARS

The TBARS (Thiobarbituric Acid Reactive Substances), primarily malondialdehyde (MDA), were quantified by establishing a standard curve using 1,1,3,3-tetraethoxypropane, and the extent of lipid oxidation was expressed in milligrams of malondialdehyde per kilogram of meat. The 2-TBARS values were determined using the method outlined by Tarladgis et al. (1960).

Free fatty acids (%)

The free fatty acid content was computed and presented as a percentage relative to oleic acid. The percentage of free fatty acids was determined following the procedure described by Koniecko (1979).

Water activity (a_w)

Measuring water activity is crucial for evaluating the moisture content and stability of food products. The a_w c an be determined using the method established by Viuda-Martos et al . (2010).

Microbial analysis

The microbial quality, as indicated by the Total Plate Count (TPC) and Staphylococcal Count (SC), was determined following the procedure outlined by the International Commission of Microbiological Specifications for Foods (ICMSF), 1983. To determine microbial counts, a 10-gram meat sample was blended with 90 milliliters of 0.1% sterile peptone water. Serial 10-fold dilutions were prepared by mixing 1 milliliter of the homogenate with 9 milliliters of 0.1% peptone water. The appropriate serial dilutions were then duplicated and plated using the pour plate method. Plate count agar was used for Total Plate Count (TPC), mannitol salt agar was used for the Staphylococcal Count (SC) and Violet Red Bile Agar used for coliform count (CC). The plates were incubated at 37°C for 48 h for TPC and at 7°C for 10 days for SC.

Sensory analysis

The pork sausages were presented to a panel of six trained evaluators who assessed them for colour, flavour, and overall acceptability. A 9-point hedonic scale was employed for this evaluation, where a score of 9 indicated the highest desirability, and a score of 1 indicated the lowest desirability. This method of sensory evaluation is in accordance

with the description provided by Keeton (1983). The pork sausages were shallow-fried in a pan for 25 sec just before the sensory assessment. The sensory evaluation took place between 3:00 PM 4:30 PM, and panelists were provided with filtered tap water for rinsing their mouths in between the evaluation of different sausage samples.

Statistical analysis

The data collected from different trials in each experiment underwent statistical analysis, which included Analysis of Variance (ANOVA) to assess variations and Duncan's multiple range test (DMRT) for comparing means. This analysis was carried out using the SPSS 20 software package.

RESULTS AND DISCUSSION

Physico-Chemical Properties

pH

The mean pH values of pork sausages were significantly ($p<0.05$) affected by different concentrations of CEO (Table 1). The pH of 0.4 µl/g CEO incorporated pork sausages is less compared to other treatments during storage. The results were in accordance to Hussain et al. (2021) who added CEO to ground lamb and the increase in pH during storage due to generation of amines and ammonia by meat spoilage bacteria.

2-TBARS

The increase in TBARS during storage is often indicative of lipid oxidation is presented in Table 1. In our current study, it was observed that the samples treated with 0.4% and 0.6% CEO exhibited a significant reduction in lipid oxidation during storage when compared to both the control group and the 0.2 µl/g CEO-treated samples. This decrease in TBARS levels can largely be attributed to the presence of cinnamaldehyde and other bioactive compounds found in CEO, which have antioxidant properties and can effectively counteract lipid oxidation. These results are similar with the findings of Dashti et al. (2015), where the addition of thyme essential oil to chicken meat nuggets resulted in decreased TBARS values in the treatment groups as compared to the control group.

Free Fatty Acids

The determination of Free Fatty Acids (FFA) provides valuable information about the stability of fat during storage, as discussed by Das et al. (2008). In this study, the mean FFA values in pork sausages were significantly influenced by different concentrations of CEO, as shown in Table 1. Notably, a lower level of FFA was observed in pork sausages with 0.4 µl/g added CEO compared to both the control group and the other treatment groups. This reduction in FFA levels may be attributed to the antioxidant properties of cinnamaldehyde, a component found in CEO. These results are consistent with the findings of Para et al. (2017), who added clove oil to chicken nuggets and observed lower FFA levels compared to the control group. Similarly, Bharti et al. (2020) added 0.5 % anise essential oil and 1% caraway essential oil to chicken nuggets, leading to decreased FFA levels when compared to the control group.

Water activity (a_w)

The mean a_w values were found to be lower for the 0.4 µl/g CEO treatment group when compared to the control group, as well as the 0.2 µl/g CEO and 0.6 µl/g CEO treatment groups is seen in Table 1. This decrease in a_w can be attributed to the presence of compounds such as cinnamaldehyde, eugenol, and other volatile substances in the essential oil. These substances are released slowly from the product, reducing the availability of water to microbes and contributing to the stability of the product during storage. It's worth noting that these results differ from the findings of Arya et al. (2019), where no significant ($p>0.05$) effect of essential oil was observed in chicken meat spread. However, the results align with those reported by Aminzare et al. (2015) when adding Cinnamomum zeylanicum essential oil to Lyoner-type sausages, which also showed a reduction in a_w and improved product stability.

Microbial evaluation

The antimicrobial effects of CEO on spoilage microbial populations are summarized in Table 2. Our research findings indicated that when 0.4 µl/g CEO was used, it led to lower TPC, coliform counts, and SC compared to the control group, as well as treatment groups T1 and T2. These results align with the findings of Zhang et al. (2019), who demonstrated that the addition of 0.5% CEO to sausages resulted in a reduction in both TPC and CC. This antimicrobial action of CEO was attributed to the electronegative nature of trans-cinnamaldehyde, a compound abundantly found in essential oil. Furthermore, Karim et al. (2021) found that adding cinnamaldehyde to sausages led to a decrease in SC compared to the control group. The observed reductions in microbial counts were attributed to factors such as a pH level lower than 6, the addition of CEO, heat treatment during processing, and appropriate storage temperatures, all of which contributed to the stability of the product, as previously demonstrated by Aminzare et al. (2018).

Table 1 Effect of adding various concentrations of CEO on physico-chemical properties of pork sausages at refrigerated storage (4±1°C) (Mean ± S.E)

Parameters	Treatments	Storage days			
		0	5	10	15
pH	Control	5.61± 0.02^{c}	5.79± 0.03^{b}	5.84 ± 0.01^{c}	6.55± 0.02^{d}
	T1	5.68± 0.0^{1b}c	5.81 ± 0.04^{c}	5.79± 0.02^{b}	5.95± 0.03^{c}
	T2	5.64± 0.03^{a}	5.68 ± 0.01^{a}	5.71±0.03^{a}	5.74± 0.02^{a}
	T3	5.72 ± 0.01^{b}	5.76± 0.02^{b}	5.78 ± 0.02^{b}	5.83 ±0.01^{ab}
2-TBARS (mg malonaldehyde/kg)	Control	0.49±0.03^{ab}	0.94±0.02^{c}	1.98±0.02^{c}	2.31±0.01^{d}
	T1	0.45±001^{ab}	0.82±0.03^{b}	1.92±0.01^{c}	2.08±0.02^{c}
	T2	0.46±0.02^{a}	0.69±0.01^{a}	0.75±0.01^{a}	0.94±0.01^{a}
	T3	0.42±0.01^{a}	0.79±0.01^{b}	0.89±0.02^{b}	1.58±0.01^{b}
FFA (% of oleic acid)	Control	0.11±0.01^{a}	0.26 ±0.01^{b}	0.37±0.02^{ab}	0.92±0.01 ^{c}
	T1	0.11±0.01^{a}	0.27±0.01^{b}	0.38±0.01^{ab}	0.83± 0.02 ^{c}
	T2	0.11±0.01^{a}	0.14±0.01^{a}	0.17±0.01^{a}	0.44±0.01 ^{a}
	T3	0.10 ±0.01^{a}	0.17±0.02^{a}	0.54±0.01^{b}	0.79 ±0.03 ^{b}
a_w	Control	0.932±0.005^{c}	0.924±0.04^{ab}	0.892±0.007^{a}	0.890±0.002 ^{a}
	T1	0.922±0.003^{c}	0.919±0.002^{c}	0.887±0.003^{ab}	0.862±0.04 ^{a}
	T2	0.886±0.008^{a}	0.882±0.006^{a}	0.854±0.009^{b}	0.831±0.004^{c}
	T3	0.917±0.005^{a}	0.891±0.005^{a}	0.863±0.003^{b}	0.839±0.007^{c}

Means bearing different superscripts in the column and row differ significantly ($p<0.05$)

Table 2: Effect of adding various concentrations of CEO on microbial growth (log cfu/g) of pork sausages at refrigerated storage (4±1°C)

Parameters	Treatments	Storage days			
		0	5	10	15
TPC	Control	3.89±0.04^{a}	4.76±0.02^{b}	5.98±0.06^{c}	6.87±0.05^{d}
	T1	3.91±0.06^{a}	4.25±0.05^{b}	5.51±0.04^{c}	6.11±0.03^{cd}
	T2	3.72±0.07^{a}	4.12±0.04^{ab}	4.93±0.08^{b}	5.21±0.09^{c}
	T3	3.86±0.09^{a}	4.23±0.06^{b}	5.41±0.12^{c}	5.93±0.05^{c}
SC	Control	2.97±0.09^{d}	3.49±0.06^{c}	4.97±0.14^{b}	5.64±0.08^{a}
	T1	2.93±0.05^{dc}	3.56±0.05^{c}	4.86±0.09^{b}	5.59±0.07^{ba}
	T2	2.89±0.08^{d}	3.23±0.04^{c}	4.11±0.02^{ba}	4.96±0.03^{a}
	T3	2.91±0.05^{d}	3.29±0.06^{d}	4.53±0.11^{b}	5.21±0.09^{a}
CC	Control	1.92±0.02^{a}	2.94±0.02^{b}	3.27±0.14^{bc}	4.89±0.04^{d}
	T1	1.89±0.03^{a}	2.75±0.05^{b}	3.07±0.08^{c}	4.78±0.03^{d}
	T2	1.91±0.04^{ab}	2.19±0.07^{b}	2.98±0.02^{c}	3.15±0.04^{cd}
	T3	1.94±0.03^{a}	2.48±0.04^{ab}	3.16±0.12^{b}	4.29±0.03^{c}

Means bearing different superscripts in the column and row differ significantly ($p<0.05$)

Table 3: Effect of adding various concentrations of CEO on sensory properties of pork sausages at refrigerated storage (4±1°C) (Mean ±S.E.)

Parameters	Treatments	Storage days			
		0	5	10	15
Colour	Control	7.29±0.13^{a}	6.64±0.11^{b}	6.02±0.14^{b}	5.47±0.12^{c}
	T1	7.38±0.14^{a}	7.01±0.13^{ab}	6.89±0.17^{b}	5.86±0.11^{bc}
	T2	7.42±0.11^{a}	7.34±0.12^{a}	7.14±0.12^{ab}	6.92±0.12^{b}
	T3	6.85±0.13^{ab}	6.15±0.14^{b}	5.97±0.16^{c}	5.03±0.13^{d}
Flavour	Control	7.35±0.09^{d}	6.87±0.1^{c}	5.83±0.13^{b}	5.09±0.14^{a}
	T1	7.41±0.14^{d}	6.97±0.19^{dc}	5.96±0.17^{c}	5.75±0.12^{b}
	T2	7.44±0.18^{dc}	7.19±0.17^{c}	6.86±0.18^{b}	6.47±0.15^{b}
	T3	7.29±0.17^{d}	6.91±0.15^{d}	5.63±0.14^{b}	5.13±0.11^{a}
Overall acceptability	Control	7.54±0.08^{a}	6.78±0.13^{b}	6.12±0.12^{c}	5.34±0.09^{d}
	T1	7.51±0.14^{a}	6.95±0.15^{b}	6.32±0.17^{bc}	5.94±0.09^{c}
	T2	7.59±0.16^{ab}	7.13±0.12^{b}	6.97±0.15^{c}	6.58±0.17^{d}
	T3	7.39±0.17^{a}	6.79±0.15^{bc}	6.01±0.11^{c}	5.16±0.12^{d}

Means bearing different superscripts in the column and row differ significantly ($p<0.05$)`

Sensory Evaluation

The study investigated the impact of different concentrations of CEO on the sensory attributes of pork sausages during refrigerated storage at 4±1°C, focusing on aspects like colour, flavour, and overall acceptability, as outlined in Table 3. Notably, a significant decline in colour, flavour, and overall acceptability ratings was observed for pork sausages with varying levels of CEO incorporation. The most favourable scores were achieved with the 0.4 µl/g CEO inclusion, surpassing both the control and treatments T1 and T3. Colour scores of the pork sausages decreased significantly ($p<0.05$) from day 0 to day 15 of the storage period for the control and other formulations, mainly attributed to pigment and lipid oxidation. Notably, the 0.4 µl/g CEO formulation exhibited superior colour retention, consistent with findings from Hussain et al. (2021), where the addition of 0.05% cinnamon bark oil to refrigerated ground lamb meat similarly improved colour. Flavour experienced a reduction as the storage period increased in both the control and all treatments. T2, however, displayed a higher flavour score, likely due to the gradual release of volatile components from CEO. Higher concentrations of CEO led to lower acceptability among the panellists. These results align with Sandra et al. (2021) study, where the addition of cinnamon bark flour to nuggets yielded similar outcomes. The overall acceptability of the pork sausages exhibited a significant decrease, primarily driven by lower scores in colour, texture, and flavour qualities resulting from the addition of CEO at various concentrations. Notably, the 0.4 µl/g CEO formulation yielded the highest overall acceptability, consistent with findings from Hussain et al. (2021).

CONCLUSION

The inclusion of 0.4 µl/g in the pork sausages resulted in superior physico-chemical attributes, such as pH, 2-TBARS, FFA, and a_w, when compared to the control and other treatment groups. Additionally, the microbial counts, including TPC, CC, and SC, were lower in the sausages with 0.4% CEO. Furthermore, these sausages received higher sensory scores for colour, flavour, and overall acceptability. Consequently, it can be recommended that the use of 0.4 µl/g CEO is a viable option to maintain the quality of meat products.

ACKNOWLEDGEMENTS

The authors express their deep gratitude to Sri Venkateswara Veterinary University in Andhra Pradesh, India, for furnishing the essential resources and financial support required to conduct this original research project.

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