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Full Length Research Paper

Effects of various additives on antioxidant and antimicrobial effectiveness in emulsion-type sausages

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We investigated the effects of rosemary extract (RE), α-tocopherol (AT) and chitosan (CH) added individually or in combination as compared with butylated hydroxyanisole (BHA) on microbiological parameters [total viable count (TVC), lactic acid bacteria (LAB), enterobacteria (ENB), pseudomonas bacteria (PSY)], pH and lipid oxidation of emulsion-type sausages stored for 28 days at 4°C. TVC, LAB, ENB, and PSY counts were significantly increased (*P<0.05*) in all treatments throughout the refrigerated storage. CH and its combination with either RE or AT, or BHA alone, had the minor antimicrobial effectiveness compared to individual use of RE or AT (*P<0.05*). However, there were no differences (*P>0.05*) in all microbial counts between AT and control groups during the whole storage period. Overall storage had a significant effect on lowering pH, but no influence of additives on pH values was detected, except for 2and 28 days of storage. During refrigerated storage, CH and its combination, or BHA in emulsion-type sausages was more effective in delaying lipid oxidation compared to RE and AT (*P<0.05*). In conclusion, this study showed the minimal antioxidant and antimicrobial effects of using CH and its combination or BHA alone in emulsion-type sausages rather than single antioxidant.

Key words: Rosemary extract, α-tocopherol, chitosan, butylated hydroxyanisole (BHA), antioxidative effect, antimicrobial effect.

INTRODUCTION

A number of consumers and meat industries have shown growing interest in the development of the concept of a functional food or additive as a food or food ingredient with positive effects on public health. Much research has indicated that the application and development of various ingredients, such as rosemary extract (RE), α-tocopherol (AT), chitosan (CH), and butylated hydroxyanisole (BHA), may be useful to prolong meat shelf life delaying lipid oxidation and discoloration and inhibiting microbial growth (Fernández-López et al., 2005). For many years, BHA and synthetic ingredients has been commonly used to reduce lipid oxidation and food spoilage. However, the use of synthetic ingredients in meat and meat products can display health hazards, resulting in strict regulation over their use in meat and meat products (Kahl and Kappus, 1993). Consequently, questions regarding the safety of synthetic ingredients have led to increased demand for natural ingredients, which have been considered as a functional food or can be used as methods of controlling bacterial growth.

Rosemary (*Rosmarinus officinalis L.*) extracts are known to possess a potent antioxidant activity containing rosmanol, rosmariquinone, rosmaridiphenol, carnosic acid, and carnosol (Houlihan et al., 1984). In addition, several authors reported that phenolic diterpenoids, which are the main compounds of the RE, could have antimicrobial properties (Del Campo et al., 2000).

AT is regarded as an effective antioxidant for reducing color changes and oxidative deterioration against damage

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from oxygen free radicals and reactive products of lipid peroxidation (Sodhi et al., 2008). Positive antioxidative effects of AT in meat were generally acknowledged (Faustman et al., 1998).

CH is an important source of the naturally abundant biopolymer components with a broad range of food applications (Rudrapatnam and Farooqahmed, 2003). It shows antioxidative and preservative effects in muscle foods and antimicrobial activity against a range of food-borne microorganisms (Kanatt et al., 2008).

To maintain nutritional quality and improve economic profits, the use of these natural ingredients in combination has notably increased in recent. Several studies have stated that antioxidant blends that together can act synergistically may have superior effects compared to single antioxidant (Soultos et al., 2008). However, studies focusing on emulsion-type sausages with either single or antioxidant blends during refrigerated storage were less clear. The objective of the current study was therefore to investigate relative antioxidant and antimicrobial effectiveness of RE, AT, and CH both individually and in combination in emulsion-type sausages during re-frigerated storage as compare with BHA alone.

MATERIALS AND METHODS

The Animal Ethics Committee approval was not obtained for all experimental procedures used in this study because all samples were collected from a commercial market or source.

Natural ingredients and chemicals

The RE (Stabiloton, OS) containing 30% phenolic diterpenes (carnosic acid, carnosol, rosmanol and rosmarinic acid) was obtained from RAPS GmbH & Co. (Kulmach, Germany) and recommended at a concentration of 260 mg/kg for sausage by the manufacturer (Georgantelis et al., 2007).

AT (*all-rac*-α-tocpheryl acetate) was purchased from Sigma (Sigma Aldrich Inc., St. Louis, MO, USA) and the select level for AT used was 110 mg/kg on the basis of results from Georgantelis et al. (2007). CH, in powder form (MW: 4.9×10^5, degree of deacetylation: 85%, viscosity: 75 cps), was procured from Kumhohwasung Co., Ltd. (Uljin, Kyungpook, South Korea) and added at the level of 10 g/kg as recommended by Darmadji and Izumimoto (1994). BHA was purchased from Wako (Wako Chemicals, Osaka, Japan) and used at a concentration of 0.1 g/kg as a reference antioxidant as described by the USDA (1999).

Sausage preparation

Sausages were manufactured according to the methods generally used in emulsion-type sausages: ground pork meat (60%), pork fat (20%), cornstarch (6%), sausage seasoning (3%, contained 0.4% nitrite), salt (1.5%), polyphosphate (0.25%), and ice water (10%). Fresh boneless pork, purchased from a local meat market, was used as the raw material for emulsion-type sausage.

Pork meat was trimmed of visible fat and connective tissue and was ground together through a 5 mm grinder plate before sausage manufacture. Ground pork was mixed with other ingredients in cutting chopper. Throughout the procedure, the treatments used in this study were:

(1) no added antioxidants (C),
(2) 260 mg of RE/kg of sausage (RE),
(3) 110 mg of AT/kg of sausage (AT),
(4) 10 g of CH /kg of sausage (CH),
(5) 260 mg of RE/kg of sausage + 110 mg of AT/kg of sausage (RE + AT),
(6) 260 mg of RE/kg of sausage + 10 g of CH /kg of sausage (RE + CH),
(7) 110 mg of AT/kg of sausage + 10 g of CH/kg of sausage (AT + CH) and
(8) 0.1 g of BHA/kg of sausage (BHA).

Ice water is added to absorb the generated heat and ensure that the emulsion holds while the emulsification is processing, and when emulsions are sufficiently formed by solublizing the meat protein, fat was added. The meat is cut to a very fine particle size which encourages protein extraction while chopping. Then, the batter was blended in an emulsifier (Model FP800, Kenwood Ltd., New Hampshire, UK) for 5 min and the sausage mixture was stuffed into polyvinyliden choride casings 50 mm in diameter (Viskase Corporation, Chicago, IL, USA), which were substantially uniform in density and divided into food-casing lengths of 12 cm per unit. The casing was not stripped off for storage. The sausage unit was heated in cooking chamber for 70 min until internal temperature reached 75°C. Before storage, sausage were left to cool into ice water, and then stored at 4°C for 0, 7, 14, 21, and 28 days. All experiments were carried out in triplicate according to the entire protocol.

Measurements

Microbiological analyses

A 20 g sausage samples from each treatment was transferred to a stomacher (Lab blender 400, London, UK). The sample was homogenized in a stomacher with 180 ml of sterile peptone (BBL, Sigma-Aldrich, Inc.) water (1 g/L) for 2 min at room temperature. Serial decimal dilutions were prepared in 9 ml of peptone water and duplicate 1 ml sample of dilutions were poured-plated to give different media for the following groups of microorganism: (1) Plate count agar (PCA; Oxoid, Basingstoke, UK) for total viable count (TVC); (2) De Man Rogosa Sharpe agar (MRS; Oxoid) for lactic acid bacteria (LAB); (3) Violet red bile glucose agar (VRBG; Oxoid) for *Enterobacteriaceae* (ENB) counts; (4) *Pseudomonas* (PSY) agar base (Oxoid) for PSY counts. Plates with PCA were incubated at 32°C for 3 days (ISO, 2003) and MRS at 30°C for 3 days (De Man et al., 1960). For VRBG and PSY, plates were incubated at 37°C for 1 day (ISO, 1979) and at 25°C for 2 days, respectively. All results were counted as average colony forming units $\log_{10}$ CFU/g of sausage sample.

pH

pH measurements were determined according to AOAC (1990). A 10 g sausage sample was cut into small pieces and homogenized with 90 ml of distilled water in a blender. The pH was recorded using a pH meter (Model 520A, Orion, CO, USA). Before pH measurements, the pH meter was calibrated with standard buffers of pH 4.0 and 7.0 at 25°C.

Thiobarbituric-acid reactive substances (TBARS)

Lipid oxidation was evaluated on the basis of the concentration of malondialdehyde (MDA) in the samples (mg MDA/kg sausage) according to the method of Witte et al. (1970). Briefly, a 20 g

Table 1. Changes in total viable counts and lactic acid bacteria (log_{10} CFU/g) counts of emulsion-type sausages with RE, AT, CH and BHA during storage at 4°C for 28 days.

Item[1]	Storage days					SEM[5]
	0	7	14	21	28	
TVC[2] control	3.36^{e}	4.01^{dA}	5.14^{cA}	6.72^{bA}	7.26^{aA}	0.235
RE	3.37^{e}	3.80^{dB}	4.60^{cB}	6.03^{bB}	6.92^{aC}	0.058
AT	3.36^{e}	3.98^{dA}	5.10^{cA}	6.69^{bA}	7.15^{aB}	0.045
CH	3.36^{e}	3.57^{dC}	4.48^{cB}	5.90^{bBC}	6.79^{aD}	0.055
RE + AT	3.37^{e}	3.78^{dB}	4.63^{cB}	5.98^{bB}	6.91^{aC}	0.051
RE + CH	3.37^{e}	3.59^{dC}	4.28^{cB}	5.81^{bC}	6.73^{aD}	0.057
AT + CH	3.37^{d}	3.55^{dC}	4.59^{cB}	5.93^{bC}	6.87^{aCD}	0.269
BHA	3.36^{e}	3.80^{dB}	4.57^{cB}	5.99^{bB}	6.86^{aCD}	0.047
SEM[4]	0.009	0.196	0.215	0.050	0.055	
LAB[3] control	1.90^{e}	3.66^{dA}	4.87^{cA}	5.56^{bA}	5.99^{aA}	0.036
RE	1.87^{e}	3.50^{dB}	4.64^{cB}	5.14^{bB}	5.87^{aA}	0.036
AT	1.86^{e}	3.69^{dA}	4.88^{cA}	5.57^{bA}	5.86^{aA}	0.067
CH	1.90^{e}	3.28^{dC}	4.37^{cCD}	5.11^{bB}	5.36^{aB}	0.044
RE + AT	1.86^{e}	3.52^{dB}	4.63^{cB}	5.14^{bB}	5.64^{aA}	0.037
RE + CH	1.87^{e}	3.17^{dD}	4.30^{cD}	5.06^{bB}	5.27^{aB}	0.055
AT + CH	1.89^{e}	3.32^{dC}	4.39^{cCD}	5.15^{bB}	5.38^{aB}	0.034
BHA	1.84^{e}	3.53^{dB}	4.45^{cC}	5.14^{bB}	5.67^{aA}	0.043
SEM[4]	0.045	0.038	0.054	0.049	0.191	

$^{a-e}$, Means within same row(different storage day) with different superscript are significantly different (P<0.05); $^{A-D}$, Means within same column (different batches) with different superscript are significantly different (P<0.05); [1]RE, 260 mg of RE/kg of sausage; AT, 110 mg of AT/kg of sausage; CH, 10 g of CH/kg of sausage; RE+ AT, 260 mg of RE/kg of sausage + 110 mg of AT/kg of sausage; RE+CH, 260 mg of RE/kg of sausage + 10 g of CH/kg of sausage; AT + CH, 110 mg of AT/kg of sausage + 10 g of CH/kg of sausage; BHA, 0.1 g of BHA/kg of sausage. [2]TVC, total viable counts. [3]LAB, lactic acid bacteria. [4]Standard error of the mean within the same antioxidant group. [5]Standard error of the mean within the same storage day.

sausage sample, added to 50 ml of 20% trichloroacetic acid solution (in 2 M phosphate solution), was homogenized in a blender and mixed well in a 50 ml of distilled water. The sample was filtered through No. 1 filter paper (Whatman Inc., Clifton, NJ, USA). After filtration, 5 ml of the filtered solution was mixed with 5 ml TBA solution (0.005 M in water) in a test tube. The test tubes were placed at room temperature in the dark for 15 h and absorbance was read by a ultra-violet/visible (UV/VIS) spectrophotometer (UV-24D1(PC) 5, Shimadzu, Tokyo, Japan) at 532 nm.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) with treatments and time of storage using the general linear model (GLM) procedure of SAS (2002). Difference among treatment means were detected at the 5% level of Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Microbiological counts of emulsion-type sausages during storage

The effects of the microbiological analyses of the emulsion-type sausages with tested ingredients during the 28 days storage period are shown in Table 1 and 2. The counts of all microbiological indicators were significantly (*P<0.05*) influenced by the addition of the three natural antioxidants (RE, AT, CH) and their different combinations (RE + CH, AT + CH and RE + AT) and BHA throughout the storage period. However, after 0 day of storage, there were no differences (*P>0.05*) among all samples for TVC (from 3.36 to 3.37 log_{10} CFU/g), LAB (from 1.84 to 1.90 log_{10} CFU/g), ENB (from 1.69 to 1.74 log_{10} CFU/g), and PSY counts (from 2.80 to 2.88 log_{10} CFU/g). Overall, all microbial groups increased gradually in all samples during storage for up to 28 days. As shown in Table 1 and 2, samples with AT had similar (*P>0.05*) all microbial groups in comparison with control during 28 days of storage. These results suggest that the use of AT with emulsion-type sausages had no beneficial effects on antimicrobial activity. The noteworthy observation of the current study was that the TVC, LAB, ENB and PSY counts for the samples of CH, AT + CH, RE + CH, and BHA, which were under 6.87, 5.67, 5.62, and 6.31 log_{10} CFU/g, respectively, were lower than those for the

Table 2. Changes in ENB and PSY (log_{10} CFU/g) counts of emulsion-type sausages with RE, AT, CH and BHA during storage at 4°C for 28 days.

Item[1]	Storage days					SEM[5]
	0	7	14	21	28	
ENB[2] control	1.74^{e}	2.99^{dA}	3.99^{cA}	4.93^{bA}	5.82^{aA}	0.263
RE	1.70^{e}	2.83^{dB}	3.79^{cB}	4.85^{bAB}	5.64^{aB}	0.035
AT	1.74^{e}	2.91^{dA}	3.97^{cA}	4.98^{bA}	5.80^{aA}	0.083
CH	1.70^{e}	2.72^{dC}	3.67^{cCD}	4.63^{bBC}	5.47^{aC}	0.041
RE + AT	1.73^{e}	2.85^{dB}	3.83^{cB}	4.86^{bAB}	5.63^{aB}	0.045
RE + CH	1.72^{e}	2.68^{dC}	3.53^{cE}	4.44^{bC}	5.36^{aC}	0.039
AT + CH	1.73^{e}	2.70^{dC}	3.62^{cD}	4.55^{bBC}	5.57^{aBC}	0.039
BHA	1.69^{e}	2.79^{dB}	3.68^{cC}	4.66^{bBC}	5.62^{aB}	0.029
SEM[4]	0.048	0.035	0.031	0.029	0.070	
PSY[3] control	2.87^{e}	3.98^{dA}	5.07^{cA}	6.38^{bA}	7.45^{aA}	0.565
RE	2.85^{e}	3.81^{dB}	4.81^{cB}	5.82^{bB}	6.48^{aAB}	0.046
AT	2.80^{e}	4.00^{dA}	5.09^{cA}	6.36^{bA}	7.28^{aA}	0.084
CH	2.81^{e}	3.74^{dBC}	4.63^{cC}	5.68^{bC}	6.31^{aAB}	0.063
RE+AT	2.83^{e}	3.82^{dB}	4.79^{cB}	5.87^{bB}	6.45^{aA}	0.107
RE+CH	2.81^{e}	3.50^{dC}	4.57^{cC}	5.58^{bC}	6.08^{aB}	0.068
AT+CH	2.86^{e}	3.68^{dBC}	4.66^{cC}	5.66^{bC}	6.28^{aAB}	0.089
BHA	2.88^{e}	3.77^{dBC}	4.62^{cC}	5.87^{bB}	6.27^{aAB}	0.793
SEM[4]	0.076	0.057	0.057	0.062	0.062	

$^{a-e}$, Means within same row(different storage day) with different superscript are significantly different ($p<0.05$). $^{A-C}$, means within same column (different batches) with different superscript are significantly different ($p<0.05$). [1]Treatments are the same as in Table 1; [2]ENB, Enterobacteriaceae; [3]PSY, *Pseudomonas*; [4]standard error of the mean within the same antioxidant group. [5]Standard error of the mean within the same storage day.

remaining sample RE and RE + AT, until the end of storage period. This observation supported the findings of Georgantelis et al. (2007), who demonstrated the effectiveness of CH, added individually or in combination with rosemary on microbial growth inhibition. This implies that CH and their blends have more antimicrobial effects than other treatments. For BHA as synthetic food additive, it is interesting to note that BHA, which acts as anti-microbial effects, was equally effective as CH and their blends in emulsion-type sausages. However, Sallam et al. (2004) reported that addition of BHA in chicken sausage did not result in statistically difference in aerobic plate count (APC) when compared with the control.

In general, the most apolar phenolic compounds from RE are presumably responsible of their antimicrobial activity (Del Campo et al., 2000). In the current study, there did not show any antimicrobial properties for RE. At present, the exact mechanism of these differences is still uncertain.

pH and TBARS values

The pH and TBARS changes in the emulsion-type sausages during the 28 days storage period are shown in Table 3 and 4. There were statistically differences in pH among all samples on 0 (from 6.42 to 6.33) and 28 (from 6.27 to 6.21) days of storage (*P<0.05*). However, after 7, 14 and 21 days of storage no differences were found in pH values among samples containing RE, AT, and CH, individually or in combination (RE + AT, RE + CH, and AT + CH), BHA and control, which were ranged from 6.38 to 6.26. In all treatments, storage had a significant (*P<0.05*) effect on the pH value, which tended to decrease with storage days. This might be explained by the fact that the effectiveness of antioxidative and antimicrobial agents is relying on pH (Xiong et al., 1993; Varum et al., 1994). These results disagree with results reported by Georgantelis et al. (2007) who found that there was a gradual increase of pH in fresh pork sausages with RE, AT, and CH, or both. Work done by Soultos et al. (2008) reported that pH values increased gradually in all Greek style fresh pork sausages with CH or nitrites, or both and control. For example, an important mechanism for antimicrobial activity reduction in CH is its positive charge in acidic solution (Rhoades and Rastall, 2000). This is due to the presence of primary amines on the molecule that bind protons, as follows;

$$Chi\text{-}NH_2 + H_3O^+ \Leftrightarrow Chit\text{-}NH_3^+ + H_2O$$

Table 3. Changes in pH values of emulsion-type sausages with RE, AT, CH, and BHA during storage at 4°C for 28 days.

Item[1]	Storage days					SEM[3]
	0	7	14	21	28	
Control	6.42^{aA}	6.38^{b}	6.32^{b}	6.26^{c}	6.27^{cA}	0.027
RE	6.42^{aA}	6.38^{b}	6.34^{c}	6.29^{d}	6.21^{eB}	0.019
AT	6.44^{aA}	6.37^{a}	6.30^{b}	6.27^{b}	6.21^{cB}	0.034
CH	6.33^{aB}	6.28^{b}	6.26^{bc}	6.27^{bc}	6.23^{cAB}	0.022
RE + AT	6.43^{aA}	6.37^{b}	6.34^{b}	6.29^{c}	6.22^{dAB}	0.017
RE + CH	6.36^{aB}	6.35^{a}	6.32^{ab}	6.28^{b}	6.21^{cAB}	0.027
AT + CH	6.34^{aB}	6.30^{b}	6.30^{c}	6.26^{d}	6.21^{eB}	0.016
BHA	6.34^{aB}	6.35^{a}	6.32^{a}	6.29^{ab}	6.25^{bAB}	0.034
SEM[2]	0.032	0. 030	0.020	0.017	0.026	

$^{a-e}$, Means within same row (different storage day) with different superscript are significantly different (p<0.05). $^{A-B}$, Means within same column (different batches) with different superscript are significantly different (p<0.05). [1]Treatments are the same as in Table 1; [2]Standard error of the mean within the same antioxidant groups; [3]Standard error of the mean within the same storage days.

Table 4. Changes in TBARS (mg MDA/kg) values of emulsion-type sausages with RE, AT, CH, and BHA during storage at 4°C for 28 days.

Item[1]	Storage days					SEM[3]
	0	7	14	21	28	
Control	0.396^{eA}	0.436^{dA}	0.469^{cA}	0.485^{bA}	0.505^{aA}	0.005
RE	0.387^{dB}	0.423^{cB}	0.453^{bB}	0.476^{aB}	0.486^{aB}	0.006
AT	0.387^{dB}	0.426^{cB}	0.456^{bB}	0.476^{aB}	0.487^{aB}	0.005
CH	0.387^{dB}	0.420^{cB}	0.444^{bBC}	0.471^{aB}	0.480^{aB}	0.005
RE + AT	0.383^{eB}	0.414^{dC}	0.445^{cBC}	0.471^{bB}	0.486^{aB}	0.005
RE + CH	0.374^{eC}	0.399^{dD}	0.437^{cC}	0.465^{bB}	0.480^{aB}	0.005
AT + CH	0.374^{eC}	0.404^{dD}	0.439^{cBC}	0.466^{bB}	0.480^{aB}	0.005
BHA	0.375^{eC}	0.404^{dD}	0.437^{cC}	0.465^{bB}	0.479^{aB}	0.005
SEM[2]	0.005	0.005	0.004	0.005	0.005	

$^{a-e}$, Means within same row(different storage day) with different superscript are significantly different (P<0.05); $^{A-D}$, means within same column (different batches) with different superscript are significantly different (P<0.05); [1]Treatments are the same as in Table 1; [2]Standard error of the mean within the same antioxidant group; [3]Standard error of the mean within the same storage day.

The antimicrobial effect of CH is more pronounced in pH 6.3 for this equation (Helander et al., 2001). The solubility in most CH preparations decreases abruptly as the solution pH rises above 6.0 to 6.5 (Varum et al., 1994). Significant differences in TBARS were found between all samples and storage (*P<0.05*) TBARS values with RE, AT, and CH, or both, and BHA alone gradually increased from 0 to 28 days for emulsion-type sausages. Samples containing combination of antioxidants (RE + CH and AT + CH) or CH and BHA ranged from 0.374 to 0.480 mg MDA/kg showed a small reduction in TBARS values until the end of their storage period in comparison with those containing the individual antioxidants (RE, AT and CH), or the combination of RE + AT ranged from 0.383 to 0.487 mg MDA/kg. In addition, TBARS values showed that the highest values for control samples ranged from 0.396 to 0.505 mg MDA/kg. Although there are no reports regarding the antioxidant effect of blends of RE, AT, and CH, the combination of antioxidants (RE + CH, AT + CH, RE + AT) had lower TBARS values than the individual antioxidants (RE, AT and CH). Similar effects were observed by the application of antioxidants, either individually or both, to fresh pork sausages during 20 days of storage, among which the combined use of CH with RE or AT was the best results (Georgantelis et al., 2007). This could be attributed to their ability to act as antioxidant to break the free radical chain by donating a hydrogen atom (Pin-Der-Duh, 1998). Our observation was that BHA also had antioxidant properties, which showed a TBARS, lower than that of the control or individual antioxidants and was

equal to the combination of antioxidants. Sallam et al. (2004) reported that, in chicken sausages, the TBA values in BHA-formulated samples (0.1 g/kg) were not significantly different from any of the various garlic formulations. Previous research has shown that adding 1000 mg/kg of RE to precooked-frozen sausage was equally effective as BHA in maintaining low TBARS values (Sebranek et al., 2005). In a study conducted with ground beef patties, the antioxidative effect of RE was greater than that of AT (St. Angelo et al., 1990). According to Darmadji and Izumimoto (1994), addition of CH at the levels of 0.2, 0.5 and 1% resulted in a decrease in the TBA values from minced beef by as much as 10, 25 and 40% on the first day, respectively, and after 3 days of storage at 4°C TBA values has been reduced by 70% for CH at 1%. Moreover, during the first 28 days of storage, the TBARS values (expressed as mg MDA/kg) in control samples exceeds 0.5 mg MDA/kg which is the permissible concentration for MDA suggested by Sheard et al. (2000). It should be noted that MDA concentrations higher than 0.5 mg/kg as threshold values are considered as rancidity perception by consumers.

Conclusions

This study showed that CH (10 g/kg) added individual or in combination with RE and AT used revealed only minimal achievements in antioxidant and antimicrobial effectiveness of emulsion-type sausage. In addition, the use of BHA appears to have an important effect on the antioxidant and antimicrobial efficacy. However, because we could not report the organoleptic properties of natural ingredients alone or in combination used in the present study, further research are needed to investigate acceptance sensory evaluation and consumability of product.

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