Effect of α-tocopherol, rosemary extract and their combination on lipid and protein oxidation in beef sausages

B Nacak1, H S Kavuşan2, and M Serdaroğlu2

1Uşak University, Engineering Faculty, Food Engineering Department, 64000, Merkez, Uşak, Turkey
2Ege University, Engineering Faculty, Food Engineering Department, 35100 Bornova, İzmir, Turkey

E-mail: berker.nacak@usak.edu.tr

Abstract. This study focuses on the oxidative changes in lipids and proteins of beef sausages with incorporated α-tocopherol, rosemary extract or their combination during 3 months’ storage at 4°C. For this purpose, sausages were formulated with no antioxidant (Control, C), 200 ppm α-tocopherol (T), 200 ppm rosemary extract (R), and 100 ppm α-tocopherol + 100 ppm rosemary extract (TR). To observe oxidative changes in lipids; peroxide value, thiobarbituric acid reactive substances (TBARS), and total oxidation value (TOTOX), in proteins; sulfhydryl and carbonyl contents were measured. Use of antioxidants and storage time significantly affected oxidative stability of sausages (P<0.05). Antioxidants, individually or in combination, retarded lipid peroxidation and improve the oxidative stability of sausage during storage. The antioxidant combination showed synergistic effect on protein oxidation, as the lowest carbonyl contents were found in TR samples. As a result, a combination of antioxidants with different effect mechanisms could be the better option to prevent oxidative changes in meat products.

1. Introduction

Oxidation is the main non-microbial phenomenon in meat and meat products which creates undesirable changes in product quality, limits the shelf life and produces adverse health effects due to the development of toxic secondary oxidation products. Lipid and protein oxidations occur through free radical reactions, and due to their high amount of lipids and high concentration of prooxidants, meat products are highly susceptible to oxidative changes [1-3].

Use of antioxidants is one of the main strategies for preventing oxidation in meat products. Apart from synthetic antioxidants, natural compounds with antioxidative effect are proposed since they are safe, cheaper and consumers prefer natural sources to synthetic compounds [4]. Antioxidative effects can be achieved by different mechanisms, such as chelation of metals, scavenging free radicals, breaking chain reactions, and inhibition of lipid peroxidation [5].

α-tocopherol, an oil-soluble natural antioxidant, is widely used in the meat industry due to this compound acting as an electron donor and breaking the chain reactions. Previous reports showed the high antioxidative effect of α-tocopherol in meat products [6-8]. Rosemary (Rosmarinus officinalis L.) extract is an effective natural antioxidant for meat products due to its high amount of phenolic diterpenes and phenolic acids [9,10], and due to phenolic hydroxyl groups, rosemary extract can scavenge free
radicals and prevent lipid oxidation [11]. Previous studies have demonstrated the effects of using antioxidants individually in meat product formulations. However, the use of a combination of antioxidants with different action mechanisms could be a better way prevent lipid and protein oxidation.

To the best of our knowledge the use of α-tocopherol and rosemary extract in combination in beef sausages has not been studied yet. Therefore, the aim of this study was to investigate the effects of individually or combinative use of α-tocopherol and rosemary extract on lipid and protein oxidation in beef sausages.

2. Material and methods

Four different beef sausage formulations were prepared (Table 1). Minced beef and beef fat were purchased from a local butcher, α-tocopherol, and rosemary extract were obtained from Kimbiotek (İstanbul, Turkey). Minced beef, curing ingredients, and half of the ice were homogenized and ground for 1 min in a cutter (Alpina, Switzerland). Beef fat, α-tocopherol, and/or rosemary extract (depending on formulation), other ingredients, and the remaining of the ice were added to the meat mixture, and batters were homogenized for 3 minutes to obtained sausage emulsion. Emulsions were stuffed into casings and smoked at 40°C for 2 hours (Afos, England) then heat treated in a boiling vessel until the core temperature reached 70°C. Once cooking was completed, sausages were cooled, vacuum packaged, and stored at 4°C for 3 months. Oxidative changes of lipids were determined in terms of peroxide values [12], thiobarbituric acid reactive substances (TBARS) [13], and total oxidation value (TOTOX) [14]. Protein oxidation was investigated by the determination of sulfhydryl [15] and carbonyl groups [16]. The effects of antioxidants and storage period were investigated by using two-way ANOVA analysis. Means were compared by using Duncan’s Post-Hoc tests in the SPSS 23 software.

Table 1. Formulation of sausage samples

| Sample | Beef (g) | Beef fat (g) | Ice (g) | α-Tocopherol | Rosemary extract |
|--------|---------|-------------|--------|--------------|-----------------|
| C      | 3000    | 1000        | 1000   | -            | -               |
| T      | 3000    | 1000        | 1000   | 200 ppm      | -               |
| R      | 3000    | 1000        | 1000   | -            | 200 ppm         |
| TR     | 3000    | 1000        | 1000   | 100 ppm      | 100 ppm         |

* Sample denomination: C (Control group, without antioxidant), T (Sample with 200 ppm α-tocopherol), R (Sample with 200 ppm rosemary extract), TR (Sample with 100 ppm α-tocopherol and 100 ppm rosemary extract).
**Additives added to product formulation as 5000 g of products: 1.5% Salt, 0.15% sugar, 0.15% sodium tripolyphosphate, 0.09% ascorbic acid, 150 ppm sodium nitrite, 3% powdered milk, %3 sodium caseinate, %4 starch, 1.5% bread crumbs, spices: 0.9%

3. Results and discussion

As seen in Table 2, antioxidant and storage period showed a significant effect on peroxide values (PV), TBARS, and TOTOX of beef sausages (P<0.05). PVs of sausages were between 4.03 – 5.21 meqO2/kg at the beginning of storage, but 8.11 – 14.11 meqO2/kg at the end of storage (Table 3). Although some fluctuations were recorded during storage, in general, the impact of the storage period on PVs was significant (P<0.05). During storage, PV of C treatment increased continuously, PV of T sausages rose until month 2, and then significant decrement was observed (P<0.05). At month 3, the highest PV was recorded in C treatment (P<0.05). Antioxidant addition, whether individual or in combination, had a significant effect on peroxide content (P<0.05). Similar results were observed by Georgantelis et al. [17] wherein both α-tocopherol and rosemary extract were used in fresh pork sausages. In all cases, PVs of sausages were lower than 25 meqO2/kg, which is described as the limit for fatty foods [18].
Table 2. Analysis of variance on the effect of antioxidants and storage time on lipid and protein oxidation of beef sausages (F-values of independent variables and interactions)

| Parameter          | Source of variances | A       | B       | A x B   |
|--------------------|---------------------|---------|---------|---------|
| Lipid oxidation    |                      |         |         |         |
| Peroxide value     | A                   | 84.333* | 4.726*  | 4.147*  |
| TBARS              | B                   | 30.339* | 13.396* | 3.541*  |
| TOTOX              | A                   | 87.182* | 5.263*  | 4.261*  |
| Protein oxidation  |                      |         |         |         |
| Sulphydryl         | A                   | 55.115* | 11.178* | 7.260*  |
| Carbonyl           | B                   | 55.989* | 505.020*| 5.152*  |

A: antioxidant, B: Storage time.
*p < 0.05

TBARS values of sausages are given in Table 3. Initial TBARS values ranged between 0.15 – 0.42 mg MA/kg; the highest TBARS values were found in control treatment and R sausages, while T and TR sausages showed similar TBARS values (P>0.05). All samples showed an increased in TBARS value until month 3. C treatment had the highest TBARS value at the end of storage time. TBARS values of R, T and TR sausages increased up to month 2, then a significant reduction was observed, probably due to the decomposition of aldehydes (P<0.05). The final values in month 3 ranged between 0.35 – 0.75 mg MA/kg, with TR sausages having the lowest oxidation rate (P<0.05). Throughout the storage, TBARS values of all sausages were lower than 2.0 mg MA/kg, which is described as the limit of TBARS values in meat and meat products [13]. Similar to our results, Azizkhani and Tooryan [19] reported that using tocopherols and rosemary extract as a combination showed the greatest antioxidative effect in beef sausage storage during 3-month storage. Georgantelis [17] reported that TBARS values of fresh pork sausages formulated with chitosan and its combinations with either α-tocopherol or rosemary also showed the most intense antioxidative effect.

TOTOX, total oxidation value, is described as 2 x peroxide value + TBAR value [14]. Initial TOTOX values of sausage were between 8.24 and 10.61, and significantly increased during storage (Table 3). TOTOX values of sausages showed a similar trend as PV. The highest TOTOX value was observed for T sausage in the month 2. However, at the end of storage, C sausage had significantly higher TOTOX values compared to sausages with antioxidants. According to Decker et al. [20], the TOTOX value for food should be lower than 26; in this case, all of our sausages except C sausage in month 3 (28.98) were within the limit.

Table 3. Lipid oxidation results of sausages

| Sample | Peroxide value (meqO2/kg) | Storage time (months) | 1st | 2nd | 3rd |
|--------|---------------------------|-----------------------|-----|-----|-----|
| C      | 4.03b±0.32                | 5.75aXZ±0.77          |     |     |     |
| T      | 5.21a±0.23                | 3.67bZ±0.38           |     |     |     |
| R      | 4.72a±0.06                | 4.09bY±1.02           |     |     |     |
| TR     | 4.04b±0.10                | 2.68bW±0.58           |     |     |     |

| TBARS (mg MA/kg) | C | T | R |
|------------------|---|---|---|
| Initial          | 0.43a±0.08 | 0.19b±0.05 | 0.32a±0.06 |
| 1st              | 0.56aXY±0.05 | 0.40bY±0.04 | 0.42aY±0.02 |
| 2nd              | 0.54aXY±0.18 | 0.63aX±0.01 | 0.70bX±0.12 |
| 3rd              | 0.75aX±0.16 | 0.54bXY±0.15 | 0.45bY±0.04 |
4. Conclusion

Use of antioxidants individually in meat products is one of the effective ways to inhibit oxidative changes. However, using antioxidant combinations which have different effect mechanisms to limit oxidation reactions could be a better option. The present study indicates that using α-tocopherol, rosemary extract or their combination prevents oxidative changes in beef sausages during 3-month cold storage.

All values are means ± SD of three replicates.
Means within the same column with different superscripts (a-d) are different
Means within the same row with different superscripts (X-Z) are different

**Table 4.** Protein oxidation results of sausages

| Sample | Initial | 1st | 2nd | 3rd |
|--------|---------|-----|-----|-----|
| **Sulfhydryl groups (nmol/mg protein)** |         |     |     |     |
| C      | 1.39±0.18 | 4.86±0.72 | 2.09±0.06 | 2.27±0.52 |
| T      | 2.02±0.34 | 4.17±0.73 | 3.47±0.57 | 2.76±0.50 |
| R      | 2.12±0.46 | 4.12±0.58 | 3.62±0.39 | 1.77±0.33 |
| TR     | 2.25±0.12 | 2.92±0.09 | 1.51±0.36 | 1.87±0.09 |
| **Carbonyl groups (nmol/mg protein)** |         |     |     |     |
| C      | 1.81±0.15 | 2.78±0.08 | 3.38±0.11 | 5.46±0.21 |
| T      | 1.51±0.26 | 2.08±0.02 | 3.12±0.03 | 4.89±0.27 |
| R      | 1.34±0.15 | 2.72±0.16 | 3.30±0.02 | 4.43±0.40 |
| TR     | 1.16±0.06 | 1.69±0.01 | 2.38±0.04 | 3.83±0.52 |

All values are means ± SD of three replicates.
Means within the same column with different superscripts (a-d) are different
Means within the same row with different superscripts (X-Z) are different
storage. At the end of storage, sausages with antioxidants had significantly lower TBARS and TOTOX values. TOTOX value of C sausage in month 3 was higher than the limit value. Moreover, the α-tocopherol and rosemary extract combination showed a synergistic effect on carbonyl contents of sausages. In conclusion, the use of antioxidant combinations could be a novel approach to delay oxidative changes in meat products.

Acknowledgements
The authors would like to thank to Ege University Scientific Research Projects Coordination (EGEBAP project ID 20131) for their financial support.

References
[1] Falowo A B, Fayemi P O and Muchenje V 2014 Food Res. Int. 64 171–81
[2] Lee S, Decker E A, Faustman C and Mancini R A 2005 Meat Sci. 70 (4) 683–9
[3] Santos M M F, Lima D A S, Madruga M S and Silva FAP 2020 Poult Sci. 99 (3) 1777–87
[4] Yanishlieva N V, Marinova E and Pokorný J 2006 Eur J Lipid Sci Technol. 108 (9) 776–93
[5] Francenia Santos-Sánchez N, Salas-Coronado R, Villanueva-Cañongo C and Hernández-Carlos B 2019 Antioxidant Compounds and Their Antioxidant Mechanism Antioxidants Intech Open
[6] Chaijan M and Panpipat W 2016 Mechanism of Oxidation in Foods of Animal Origin In: Natural Antioxidants CRC Press 1–37
[7] Cropotova J, Mozuraityte R, Standal I B and Rustad T 2019 Food Control 104 1–8
[8] Li Y and Liu S 2012 J Sci Food Agric. 92 (4) 719–29
[9] Govaris A, Florou-Paneri P, Botsoglou E, Giannenas I, Amvrosiadis I and Botsoglou N 2007 LWT - Food Sci Technol. 40 (2) 331–7
[10] Weiss J, Gibis M, Schuh V and Salminen H 2010 Meat Sci. 86 (1) 196–213
[11] Haraguchi H, Saito T, Okamura N and Yagi A 1995 Planta Med. 61 (4) 333–6
[12] Koniecko E S 1979 Handbook for Meat Chemists Published online 68–9
[13] Witte V C, Krause G F and Bailey M E 1970 J Food Sci. 35 (5) 582–5
[14] Schaich K M 2016 Analysis of Lipid and Protein Oxidation in Fats, Oils, and Foods Hu M, Jacobsen C, eds. Oxidative Stability and Shelf Life of Foods Containing Oils and Fats Elsevier, 1–131
[15] Ellman G L 1959 Arch Biochem Biophys. 82 (1) 70–7
[16] Oliver C N, Ahn B W, Moerman E J, Goldstein S and Stadtman E R 1987 J Biol Chem. 262 (12) 5488–91
[17] Georgantelis D, Ambrosiadis I, Katikou P, Blekas G and Georgakis SA 2007 Meat Sci. 76 (1) 172–81
[18] Kumolu-Johson C A, Ndimele P E, Ayorinde O A and Ojikutu TI 2013 Am. J Food Technol. 10 (2) 78–84
[19] Azizkhani M and Tooryan F 2014 J Food Saf. 35 128–36
[20] Decker E A, Elias R J and McClements D J 2010 Oxidation in Foods and Beverages and Antioxidant Applications pp1–432
[21] Lund M N, Heinonen M, Baron C P and Estévez M 2011 Mol Nutr. Food Res. 55 (1) 83–95
[22] Jongberg S, Lund M N, Waterhouse A L and Skibsted L H 2011 J. Agric. Food. Chem. 59 (18) 10329–35