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COMPARATIVE ANTIOXIDANT EFFECT OF ASCORBIC ACID AND ROSEMARY EXTRACT

Elena K. Tunieva*, Victoria V. Nasonova, Nataliya M. Revutskaya
V. M. Gorbatov Federal Research Center for Food Systems, Moscow, Russia

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Abstract

The aim of the work was to study an effect of ascorbic acid and the extract of rosemary on fat oxidation, color characteristics, pH and moisture binding capacity of minced pork during cold storage. The antioxidants were introduced into minced pork in an amount of 0.05%. After addition of the antioxidant, minced meat was packed in the modified atmosphere with the high oxygen content and stored at a temperature of 4 ± 2 °C for 15 days. The indicators of the hydrolytic (acid value) and oxidative (peroxide value and thiobarbituric acid value) spoilage, color characteristics, pH and moisture binding capacity (MBC) were determined during the whole storage period (0, 5, 8, 12, 15 days). An increase in the acid value was recorded in all minced meat samples during storage without a significant difference between the control and experimental samples. Addition of the antioxidants led to a decrease in the peroxide value after 12 days of minced meat storage. Malonaldehyde began to accumulate in the control and the sample with ascorbic acid on the 8th day of storage and in the sample with the rosemary extract on the 12th day. The results obtained point to inhibition of fat oxidation in the minced meat samples with the antioxidants. Addition of the antioxidants facilitated an increase in redness compared to the control. Contrary to the rosemary extract, addition of ascorbic acid led to a decrease in pH and MBC of minced meat. Therefore, the use of the rosemary extract exerted more effective action of minced pork stability during storage compared to the same dose of ascorbic acid.

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Introduction

Lipids play a key role in formation of food product quality. An effect of fat on the sensory properties of products is linked with two main components — triglycerides and phospholipids. Triglycerides are located in adipose tissue and intermuscular adipose cells being solvents for many aromatic substances. Phospholipids are located in the myofibril membranes [1]. In the process of meat processing, fats are subjected to two main transformations — lipolysis and oxidation. The intensity of these changes depends on many factors including a raw material type, presence of non-meat recipe ingredients, packaging method, type and duration of technological processing [2–4].

The oxidative processes have a significant effect on formation of food product safety and quality, and cause changes that influence their nutritional value, sensory characteristics and shelf life. Prevention of fat oxidation has the utmost importance for the meat industry facilitating an increase in production of high-quality foods and shelf-life extension.

The self-oxidation processes can be retarded using natural and synthesized substances — antioxidants. Antioxidants are compounds that inhibit oxidation suppressing generation of free radicals as a result of the following mechanisms: scavenging species that initiate peroxidation, chelating metal ions so that they are unable to generate reactive species or decompose lipid peroxides, quenching $\bullet O^{2-}$ preventing formation of peroxides [5].

Multiple studies show that lipid oxidation in meat products can be minimized by introducing not only food additives but also substances isolated from natural sources having the antioxidant activity [6–8]. Substances with the simultaneous antioxidant and antimicrobial activities and capable of improving sensory characteristics are of special interest. For example, the rosemary extract can be used not only to improve aroma but also as a potential antioxidant and preserving agent [9–11]. Several studies point to the antibacterial activity of rosemary against *E. coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens*, *Aeromonas hydrophila*, *Bacillus cereus* and *Salmonella choleraesuis* [12]. The antibacterial and antioxidant effects of the rosemary extract were noticed both in the experiments on raw meat [12,13] and processed meat products [12,14].

The mechanism of the action of the rosemary extract as an antioxidant is extensively discussed in several publications [15,16]. Cui et al. [17] and Kontogianni et al. [18] believe that the antioxidant activity of the rosemary extract can be explained by the presence of carnosol. According to Loliger [19], carnosic acid and carnosol have an ability to scavenge peroxyl radicals. However, rosmarinic acid and hesperidin also act as scavengers of free radicals [20,21].

Aruoma et al. [22] believe that more than 90% of the antioxidant properties of the rosemary extract are conditioned by the presence of carnosic acid and carnosol. Schwarz et al. [23] reported that carnosic acid degraded less compared to other phenolic diterpenes in the conditions of the high temperature. Houlihan et al. [24] are of the opinion that the antioxidant properties of rosemary are explained by the high content of isoprenoid quinones, which scavenge free radicals and act as chelating agents for the reactive oxygen species (ROS). According to Hwang et al. [25], the antioxidant activity of rosemary is explained by the presence of the phenolic compounds that prevent free radicals as well as the spread of the free radical reaction due to chelation of ions of transition metals such as iron. Moreno et al. [26] found that the rosemary extract was similar to butylhydroxytoluene and α-tocopherol in terms of the high radical scavenging activity.

Therefore, the majority of researchers agree that carnosic acid is the main factor responsible for the antioxidant activity of the rosemary extract. Several studies point to a positive effect of the rosemary extract on a decrease in the TBA value during storage of both raw and thermally processed meat products [27–29]. On the contrary, Jin et al. [30] reported that addition of the rosemary powder did not change the TBA value in sausages compared to the control; however, rosemary showed an ability to scavenge DPPH radicals during storage. According to several studies, besides inhibiting fat oxidation, the rosemary extract facilitated an improvement in the color of meat products during storage, which was manifested, first of all, as an increase in redness stability [31–33]. However, the available data are contradictory and several authors reasoned that rosemary does not exert a significant effect on the color of meat products [34,35]. The results of the influence of the rosemary extract on the product sensory characteristics are also ambiguous. Karpińska-Tymoszczyk [36] reported that addition of the rosemary extract allowed improving sensory quality of turkey meat balls during cold storage. Racanicci et al. [37] also indicated that introduction of rosemary reduced the TBA value and enabled longer preservation of consumer-acceptable sensory characteristics of pre-cooked meat balls made from chicken breast. On the contrary, Jin et al. [30] reported about lower scores in taste and aroma assessment of sausages with rosemary. Differences in the results can be linked with a form of used rosemary (extract, powder), different types of meat raw materials, the use of thermal treatment, presence of other recipe components — salt, phosphates, sodium nitrite and others. An important method for preservation of safety and quality of meat and meat products is the use of modified atmosphere packaging (MAP) [38–40]. However, at high concentrations of CO_2 in the gas mixture, a reduction in pH can be observed, which is accompanied by changes in the natural meat color, moisture separation and appearance of off-taste. Martínez et al. [41] found that an increase in the CO_2 concentration in the gas mixture facilitated oxidation of myoglobin and lipids. Preservation of proper quality of packed sausages was achieved when using not more than 20% CO_2. Oxygen is widely used as a component of gas mixtures for meat due to the fact that myoglobin oxidation with the oxymyoglobin formation imparts the red color to meat and makes a product more attractive for consumers. The use of modified atmosphere containing the high oxygen concentration (80%) and low CO_2 concentration for meat packaging facilitates myoglobin oxygenation maintaining the attractive red color of meat [42–44]. According to Jakobsen and Bertelsen [45] the stability of meat color characteristics was achieved when using oxygen in MAP gas mixtures in amounts of 55–80%. On the other hand, Martinez et al. [46] found that the presence of O_2 in MAP gas mixtures caused a significant increase in the oxidation rate, reduction of the shelf life due to discoloration and deterioration of aroma in packed pork sausages. An improvement of color during 8-day storage was observed at an O_2 concentration of 80%. Several other studies also suggest a negative effect of O_2 in the MAP gas mixtures on oxidation of lipids and proteins [47,48]. To preserve a meat color and inhibit oxidative changes in fat and proteins, the combined use of antioxidants and modified atmosphere packaging (MAP) containing oxygen is of interest. Thus, within the framework of the present study, we carried out the comparative assessment of the antioxidant properties of the rosemary extract compared to the most widely used antioxidant ascorbic acid for pork packed in MAP with the high oxygen concentration.

Objects and methods

Samples

The objects of research were samples of m. *Longissimus dorsi* taken from carcasses of hybrid pigs (Large White × Duroc × Landrace) with a live weight of 115 ± 15 kg three days after slaughter. Meat was ground through a grinder plate with a hole diameter of 8 mm and divided into three groups: 1) control (without antioxidants); 2) experimental sample (AS) with addition of ascorbic acid in an amount of 0.05%; 3) experimental sample (RE) with addition of the rosemary extract in an amount of 0.05%. The prepared minced meat samples with a weight of 500 ± 5 g were packed in MAP (80% O_2 + 20% CO_2) and stored for 15 days at a temperature of 4 ± 2 °C. Minced meat samples were analyzed on 0, 5, 8, 12 and 15 days of storage.

Methods

The acid value, peroxide value and thiobarbituric acid were determined according Tunieva et al. [49]. The acid value was determined by the method based on titration of free fatty acids in the ether-alcohol solution of fat with the aqueous solution of alkali; peroxide value by the method based on oxidation of iodhydric acid by peroxides contained in fat with the following titration of liberated iodine with sodium thiosulphate. Determination of the thiobarbituric acid value was carried out by the method based on

the development of stained substances as a result of interaction of fat oxidation products with 2- thiobarbituric acid and measurement of the color intensity on a spectrophotometer.

Color characteristics were measured on a spectrophotometer CM-2300d (Konica Minolta, Japan). The moisture binding capacity (WBC) was determined by the pressing method consisting of pressing a sample with a load of 1 kg and the following calculation by the difference between masses before and after pressing and the area of the wet spot measured using a planimeter. The results were expressed in percent of the total mass of moisture in a product.

The pH value was registered by a potentiometric method using Testo 205 portable pH-meter (Testo, Germany).

The experiments were performed in triplicate, representing the findings as the mean ± SD. A one-way ANOVA was performed to assess the analysis. Duncan's multiple range test was used for comparison at the 95% confidential level ($p < 0.05$)

Results and discussion

Oxidation of lipids

The analysis results for the indicators of hydrolytic and oxidative spoilage of minced pork during storage are presented in Table 1.

Table 1. Changes in the acid value, peroxide value and TBARS of minced pork during storage

Indicators	Time of storage (d)	Control	AS	RE
Acid value (mg KOH/g fat)	0	1.69 ± 0.13^{ax}	1.60 ± 0.16^{ax}	1.64 ± 0.15^{ax}
	5	2.07 ± 0.14^{ax}	1.83 ± 0.18^{ax}	1.94 ± 0.18^{ax}
	8	2.44 ± 0.15^{abx}	2.33 ± 0.16^{abx}	2.36 ± 0.16^{abx}
	12	2.84 ± 0.18^{bx}	2.61 ± 0.14^{bx}	2.41 ± 0.17^{abx}
	15	3.71 ± 0.20^{cx}	3.38 ± 0.19^{cx}	3.20 ± 0.22^{bx}
Peroxide value (mmol active O_2/kg fat)	0	2.27 ± 0.11^{ax}	1.90 ± 0.14^{ax}	2.20 ± 0.18^{ax}
	5	2.98 ± 0.17^{bx}	2.58 ± 0.18^{ax}	2.24 ± 0.20^{ax}
	8	3.89 ± 0.19^{cx}	3.73 ± 0.21^{bx}	3.36 ± 0.24^{bx}
	12	5.24 ± 0.15^{dx}	4.65 ± 0.28^{by}	4.13 ± 0.21^{by}
	15	6.96 ± 0.26^{ex}	5.82 ± 0.21^{cy}	5.11 ± 0.20^{cy}
TBARS (mg MA/kg)	0	0.000^{ax}	0.000^{ax}	0.000^{ax}
	5	0.000^{ax}	0.000^{ax}	0.000^{ax}
	8	0.078 ± 0.008^{bx}	0.039 ± 0.004^{by}	0.000^{az}
	12	0.195 ± 0.020^{cx}	0.094 ± 0.009^{cy}	0.042 ± 0.008^{bz}
	15	0.284 ± 0.025^{dx}	0.158 ± 0.014^{dy}	0.097 ± 0.012^{cz}

a-e-Values with different letters within a column are significantly different (P < 0.05); x-z-Values with different letters within a row are significantly different (P < 0.05), n=3/

According to the data obtained, an increase in the acid value was observed in all minced meat samples during storage; with that, a significant difference between the control and experimental samples was not found during 8 days of storage. On the 12th day, lower acid values were noticed in the minced meat samples contained the rosemary extract. Apparently, this was conditioned by the manifestation of the inhibitory action of this antioxidant on the hydrolytic changes in fat in the sample. Similar data were obtained by Karpińska-Tymoszczyk [50] who recorded lower acid values in the samples of turkey meat balls contained the rosemary extract compared to the control. The peroxide values in the samples of minced meat did not have significant differences from the control up to 8 days of storage. Addition of the antioxidants led to a decrease in the peroxide value after 12 days of minced meat storage. By the end of storage, the highest accumulation of peroxides was noticed in the control sample. Significant differences in the peroxide value of the sample with ascorbic acid and the rosemary extract were not observed during the whole storage period. The antioxidants exerted more significant impact on accumulation of the secondary oxidation products. For example, malonaldehyde began to accumulate in the control and the sample with ascorbic acid on the 8th day of storage and in the sample with the rosemary extract on the 12th day. The results obtained indicate inhibition of fat oxidation in the minced meat samples with the antioxidants. With that, the use of the rosemary extract had the higher effect compared to ascorbic acid at the same dose. The data obtained correspond to Al-Hijazeen & Al-Rawashdeh [51] who established that the rosemary extract in an amount of 350 ppm had the higher effect on the TBA value and the level of carbonyls in chicken meat parties compared to the same dose of ascorbic acid. Manhani et al. [52] also reported that addition of the rosemary extract led to the more effective decrease in the amount of malonaldehyde during storage of frozen beef hamburgers compared to addition of the oregano extract and sodium erythorbate. Similar data were obtained by Perlo et al. [29] who did not find a significant effect of ascorbic acid on the TBARS value in pork steaks, while spraying the rosemary extract enabled significant reduction of the TBA value. On the contrary, Lund et al. [53] found that the rosemary extract (0.05%) and the mixture of ascorbate (0.05%) and citrate (0.05%) allowed the equal reduction of TBARS in beef patties during 6-day storage. Jin et al. [30] reported that addition of rosemary in amounts of 0.1 and 0.2% did not change the TBA value in sausages compared to the control; however, rosemary showed an ability to scavenge the DPPH radicals during storage. It is worth noting that a rosemary powder and not an extract was used in this study. Moreover, other recipe components in the sausage composition (salt, nitrite, phosphates) could affect the obtained results.

Color characteristics

According to several studies the rosemary extract besides inhibition of fat oxidation also facilitated an improvement in a meat product color during storage, which was manifested, first of all, as an increase in the stability of redness. However, Resurreccion & Reynolds reported that rosemary oleoresin inhibited oxidation without improving a color of frankfurters [34]. McCarthy et al. demonstrated the antioxidant action of rosemary on pork

patties; however, an effect on the product color was insignificant [35].

The results of the instrumental assessment of the minced pork color are presented in Tables 2 and 3.

Table 2. Changes in redness of minced pork

Storage duration, days	Redness (a*)		
	Control	AS	RE
0	3.28 ± 0.34^{ax}	4.59 ± 0.37^{axy}	4.97 ± 0.31^{ay}
5	3.36 ± 0.23^{ax}	4.29 ± 0.27^{axy}	5.15 ± 0.44^{ay}
8	3.35 ± 0.31^{ax}	4.90 ± 0.37^{ay}	4.75 ± 0.21^{ay}
12	3.84 ± 0.43^{ax}	5.14 ± 0.31^{ax}	4.95 ± 0.25^{ax}
15	3.23 ± 0.27^{ax}	4.72 ± 0.38^{ay}	4.84 ± 0.32^{ay}

a-e-Values with different letters within a column are significantly different (P < 0.05); x-z-Values with different letters within a row are significantly different (P < 0.05), n = 3.

Table 3.Changes in yellowness of minced pork

Storage duration, days	Yellowness (b*)		
	Control	AS	RE
0	10.34 ± 1.09^{ax}	12.37 ± 0.73^{ax}	12.23 ± 1.19^{ax}
5	11.53 ± 1.17^{ax}	12.21 ± 0.51^{ax}	12.86 ± 0.38^{ax}
8	11.83 ± 0.81^{ax}	12.83 ± 0.88^{ax}	12.51 ± 0.90^{ax}
12	12.24 ± 1.15^{ax}	13.07 ± 1.04^{ax}	12.91 ± 0.80^{ax}
15	14.18 ± 1.08^{ax}	14.01 ± 1.22^{ax}	12.98 ± 0.78^{ax}

a-e-Values with different letters within a column are significantly different (P < 0.05); x-z-Values with different letters within a row are significantly different (P < 0.05), n = 3.

According to the data obtained during minced meat storage, there were no significant changes in redness and yellowness in the internal part of minced meat in all samples. Apparently, the absence of color changes during storage is explained by oxygen dissolution and myoglobin oxygenation, which inhibited its oxidation with metmyoglobin formation. Addition of antioxidants facilitated an increase in redness compared to the control; however, it did not have a significant effect on yellowness. With that, significant differences between color indicators in minced meat with ascorbic acid and the rosemary extract were not observed. The data obtained correspond to the study of Al-Hijazeen & Al-Rawashdeh [50], who reported that addition of the rosemary extract and ascorbic acid positively affected color characteristics of chicken meat; however, significant differences between the experimental samples with the antioxidants were not revealed. Fernández-López et al. [54] demonstrated inhibition of the metmyoglobin formation by rosemary in cooked pork; with that, an effect of rosemary addition increased with an increase in product storage duration. On the contrary, Perlo et al. [29] reported an absence of a significant effect of spraying ascorbic acid and the rosemary extract on color characteristics of vacuum packed pork steaks. Lee et al. established that ascorbic acid (0.1%) significantly inhibited the metmyoglobin formation on the surface of minced beef, but not in the depth of the product; while carnosin (1.0%) significantly inhibited the metmyoglobin formation and the brown color development throughout the whole product [55].

pH value

There are different data about pH changes during meat and meat product storage. Blixt & Borch pointed to a decrease in meat pH during storage [56]. A reduction of pH during storage is reported to a greater extent for vacuum packed products. For example, the absence of oxygen in vacuum packaging promotes the development of *Lactobacillus* [57], and accumulation of their metabolites leads to pH drop [58]. In contrast, Apple et al. [59] reported about an increase in pH during storage of vacuum packed pork after 6 weeks. Perlo et al. found that ascorbic acid and the rosemary extract did not exert a significant effect on pH of vacuum packed pork steaks [29]. However, a decrease in pH was noticed during cold storage for 45 days.

According to the results of this study presented in Table 4, addition of ascorbic acid led to a reduction of pH, while the rosemary extract did not significantly affect a change in the pH value. Teruel et al. also revealed that the rosemary extract did not influence pH of frozen chicken nuggets [60]. On the contrary, Jin et al. reported that addition of the rosemary extract in amounts of 0.1 and 0.2% led to a reduction of pH in sausages [30]. During storage for four weeks, pH of sausages reduced and then increased by the 6th week of storage.

Table 4.Changes in the pH value of minced pork

Storage duration, days	pH		
	Control	AS	RE
0	$5.52 \pm 0{,}04^{ax}$	5.25 ± 0.02^{ay}	5.51 ± 0.03^{ax}
5	5.51 ± 0.02^{ax}	5.31 ± 0.02^{aby}	5.55 ± 0.01^{ax}
8	5.48 ± 0.02^{ax}	5.32 ± 0.04^{ab}	5.56 ± 0.03^{ax}
12	5.50 ± 0.04^{ax}	5.34 ± 0.01^{by}	5.58 ± 0.02^{ax}
15	5.54 ± 0.04^{ax}	5.35 ± 0.02^{by}	5.61 ± 0.03^{ax}

a-e-Values with different letters within a column are significantly different (P < 0.05); x-z-Values with different letters within a row are significantly different (P < 0.05), n=3.

Changes in pH were not revealed during storage of the control sample and minced meat sample with the rosemary extract. In contrast, pH decreased in the sample with ascorbic acid. The data obtained correspond to Ozer & Sariçoban, who reported that addition of ascorbic acid in an amount of 300 mg/kg led to a reduction of pH in chicken patties [61]. During storage, the pH values decreased initially and then increased. The initial decrease can be linked with addition of acid and an increase can be a result of the production of microbial metabolites. Fernández-López et al. reported that pH in cooked pork increased as a result of cold storage reaching the ultimate value of 5.92, while the pH values in the samples with rosemary did not have significant changes during storage [54]. These differences are, possibly, linked with the use of different packages and analysis of different meat product types including those that contained other food ingredients and additives.

Moisture-binding capacity

It is known that pH affects moisture-binding capacity (MBC) of meat. Taking into account a decrease in pH in the presence of ascorbic acid, MBC was measured in the analyzed minced meat samples (Table 5).

Table 5.Changes in MBC of minced pork

Storage duration, days	MBC		
	Control	AS	RE
0	62.7 ± 2.0^{ax}	55.0 ± 1.1^{ay}	61.6 ± 1.6^{ax}
5	56.2 ± 1.5^{abxy}	53.2 ± 1.9^{ay}	62.8 ± 2.2^{ax}
8	54.5 ± 2.1^{bxy}	51.3 ± 1.7^{aby}	61.6 ± 1.5^{ax}
12	50.6 ± 1.3^{bcy}	49.7 ± 0.7^{by}	59.2 ± 1.4^{ax}
15	52.4 ± 1.6^{by}	47.0 ± 2.8^{by}	59.8 ± 0.8^{ax}

a-e-Values with different letters within a column are significantly different ($P < 0.05$); x-z-Values with different letters within a row are significantly different ($P < 0.05$), n=3.

Addition of ascorbic acid led to a decrease in MBC of meat by 12.2% ($p < 0.05$) at the initial stage of storage compared to the control. Apparently, lower MBC values of meat in the samples with ascorbic acid are linked with a shift in pH towards the acid side, which in turn caused a reduction of the moisture binding capacity of proteins. It was established that at the initial stage of storage up to 12 days, the experimental samples with the rosemary extract did not have significant differences in the MBC value with the control ($p > 0.05$). A significant decrease ($p > 0.05$) in the MBC value of the control was found upon an increase in storage duration, which probably can be explained by a shift in pH towards the acid side during storage due to production of microbial metabolites. The MBC value in the samples with the rosemary extract was relatively stable during the whole storage period ($p > 0.05$).

Conclusions

Addition of the antioxidants ascorbic acid and the rosemary extract inhibited hydrolysis and oxidation of fat, which was especially manifested at the late period of storage after 8 days. With that, the use of the rosemary extract exerted the higher antioxidant effect compared to ascorbic acid at the same dose. Addition of the antioxidants promoted an increase in redness; however, significant differences between color indicators in minced meat with ascorbic acid and the rosemary extract were not found. Contrary to ascorbic acid, the rosemary extract did not lead to a decrease in pH and MBC compared to the control and during storage. Therefore, the use of the rosemary extract exerted more effective impact on minced pork stability during storage compared to the same dose of ascorbic acid.

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AUTHOR INFORMATION

Elena K. Tunieva, Candidate of Technical Sciences, Chief Researcher, Department of Applied Scientific and Technological Development, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalihina, 109316, Moscow, Russia. Tel: +7–495–676–95–11(306) E-mail: e.tunieva@fncps.ru
ORCID: https://orcid.org/0000-0002-6276-1679
* corresponding author

Victoria V. Nasonova, Doctor of Technical Sciences, Head of Department of Applied Scientific and Technological Development, V. M. Gorbatov Federal Research Center for Food Systems 26, Talalikhina str., 109316, Moscow, Russia. Tel.: +7–495–676–95–11 (307), E-mail: v.nasonova@fncps.ru
ORCID: https://orcid.org/0000-0001-7625-3838

Nataliya M. Revutskaya, Candidate of Technical Sciences, Chief Researcher, Department of Applied Scientific and Technological Development, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalihina, 109316, Moscow, Russia. Tel: +7–495–676–95–11(305), E-mail: n.revutskaya@fncps.ru
ORCID: https://orcid.org/0000-0002-8898-9305

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

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