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Investigations on Antioxidant and Antibacterial Activities of Some Natural Extracts

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Abstract: The main purpose of this investigation was to study the effect of adding natural antioxidants and antimicrobial extracts (rosemary, sage and their combination) on the oxidative rancidity of lipids and microbial growth in minced meat. Minced meat was blended with 0.1% rosemary extract, 0.1% sage extract and 0.05 % extract of rosemary combined with 0.05% extract of sage to evaluate quality during storage at 4°C and -18°C, The results revealed that percentage of moisture, crude protein, ether extract and ash content of fresh minced meat were 66.31, 17.62, 15.01 and 1.10%, respectively. Lipid oxidation of minced meat started to increase after 2 day of refrigeration (4°C) and increase rapidly after 4 days to reach the maximum after 10 days under the same conditions. Extract of rosemary and sage apparently retarded oxidative rancidity of minced meat during refrigerated and frozen storage. The pH and thiobarbituric acid (TBA) values demonstrated that sage was more effective as antioxidant than that of rosemary. However, the mixture of sage/ rosemary extract was the superior used antioxidant. The oxidative rancidity of minced meat apparently accelerated after 20 days of storage at -18°C. It was clear that extracts of sage and rosemary inhibited oxidizing changes of minced meat lipid stored for 100 days at -18°C. Results of psychrophillic count observed that addition of rosemary,sage and its combination showed a higher inhibitory effect against psychrophillic count as compared to the control sample. It was concluded that the adding of natural antioxidants and antimicrobial extracts (rosemary,sage and their combination) led to retard the oxidative rancidity and microbial growth during refrigerated and frozen storage of minced meat.

Key words: Extracts of rosemary · Sage · Lipid quality · Minced meat

INTRODUCTION

Fresh meat and meat products can be easily contaminated with microorganisms and, if these are not properly handled and preserved, they support growth of spoilage and pathogenic bacteria, leading to loss of their quality and constitute potential public health problems [1].

Refrigeration storage is usually the most common preservative method of fresh meat and meat products. In order to extend refrigerated storage time, antimicrobial and antioxidant additives especially those of synthetic origin, are added to beef products. However, consumers increasingly demand use of natural products as alternative preservatives in foods, as the safety of synthetic additives has been questioned in last years.

Lipid oxidation in meat is one of the reasons for quality degradation during storage. This process is associated with the presence of free radicals that lead to the production of aldehydes responsible for the development of rancid flavours and changes in the colour of meat [2]. Oxidation of meat lipids is a complex process and its dynamics depends on numerous factors, including chemical composition of the meat, light and oxygen access, as well as storage temperature.

The rate of the oxidation process is also affected by technical procedures to which meat is subjected during its processing. Because lipid oxidation leads to the formation of numerous other compounds which have an adverse effect on the quality attributes and nutritive value of meat products [3], this process frequently limits the shelf-life of the processed meat.

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The use of natural antioxidants has the advantage of being more acceptable by the consumers as these are considered as non chemical. In addition, they do not require safety tests before being used [4].

Among the methods used to reduce lipid oxidation is the application of antioxidants. Recently, natural antioxidants have been gaining increasing popularity. These antioxidants include extracts obtained from plants belonging to the Labiatae family, especially from rosemary Due to its well-documented antioxidative properties, rosemary extracts are produced on a commercial scale and used in the production of foodstuffs [5].

Recent researches are now directed towards finding naturally occurring antioxidants of plant origin. Interest in natural antioxidants has increased dramatically in recent times due to: (1) the possible carcinogenic effects of synthetic antioxidants in foods [6], (2) the antioxidative efficacy of a variety of phytochemicals, (3) the consensus that foods rich in certain phytochemicals can affect the aetiology and pathology of chronic diseases and the aging process [7].

Many natural plant extracts contain primarily phenolic compounds, which are potent antioxidants [8]. Some phenolic compounds such as sage, rosemary, thyme, hops, coriander, tea, cloves and basil are known to possess antimicrobial effects against foodborne pathogens [9,10]. Phenols are one of most important groups of natural antioxidants. They occur only in material of plant origin and they are known to easily protect oxidizable constituents of food from oxidation. Especially worthy of notice are spices and herbs which for many years have been used as additives to enhance the sensory features of food [11].

Rosemary and sage extracts apparently retarded oxidative rancidity of meat and chicken [12] and contained phenolic primary antioxidants (rosmarinic acid, carnosic acid, rosmaridiphenol, rosmariquinone, rosmanol and carnosot), which react with lipid or hydroxy radicals and convert them into stable products. In addition rosemary extracts may chelate metal ions, such as Fe+2, resulting in a reduce rate of formation of activated oxygen [13].

Raw meat can be easily contaminated by microorganisms and support the growth of pathogens, leading to serious food-borne illnesses. [14].

Natural extracts from herbs and spices have been used long time ago in meats as flavoring agents. Plant extract also exhibit antimicrobial activity by interfering and destabilizing the operation of the phospholipids bilayer of the cell membrane, enzyme systems and genetic material constituents of essential oils exhibit significant antimicrobial properties when tested separately [15].

The objective of the present study was to determine the effect of adding natural antioxidants extracts (rosemary, sage and their combination) on the oxidative rancidity of lipids in minced meat by determination of their pH and TBA values. Also, the investigation of the antimicrobial effects of this natural extract on the growth of psychrophillic bacteria in minced meat during refrigeration and frozen storage was another aim.

MATERIALS AND METHODS

Materials: Rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*) were obtained from, Medicinal Plant and Agricultural Seeds Haraz Company. Cairo Egypt.

Methods

Preparation of Rosemary and Sage Extracts: The dried herbs were individually ground by an electrically grinder to pass through 60 mesh sieve. About 5g of ground herb was shaken for 30 min. with 50 ml of 96% ethanol at room temperature. he mixture was allowed to stand for 24 hours and then filtered to obtained alcoholic particle free herb extract [16].

Natural Antioxidant Supplementation: Minced meat was blended with (a) 0.1% rosemary extract,(b) 0.1% sage extract and (c) 0.05 % extract of rosemary combined with 0.05% extract of sage as natural antioxidants. Samples were packed in polyethylene bags and divided into two portions. The first part was stored at 4°C in a refrigerator for 10 days. Samples were subjected periodically to analysis during 0, 2, 4, 6, 8 and 10 days of storage. The second one was stored in deep freezer at -18°C for 100 days. Samples for analysis were taken periodically each ten days during storage to evaluate retardation of oxidative rancidity.

Analytical Methods: Moisture, protein, fat, ash of minced beef meat were determined according to the standard methods [17]. while pH value of minced meat samples was estimated by the method of Aitken *et al.* [18].

Lipid oxidation as TBA by chemical method of stored samples was colorimetrically measured at 538 nm [19]. The results were expressed as mg maionoldehyde / kg sample.

Psychrophillic bacteria were enumerated by the pour plate method on plate count agar (Oxoid, CM0325) at

17°C. for 17 hrs plus another 72 hrs at 7°C [20] Psychrophillic counts were converted to-10 logarithms of colony forming units per g (log CFU/g sample).

Sensory Evaluation: The investigated samples were evaluated using a panel test of a point hedonic scale [21]

Statistical Analysis: Triplicate samples were analyzed for each property. Data were assessed by analysis of variance (ANOVA) as outlined by Fey and Regenstein [22].

RESULTS

Chemical Composition of Minced Meat: Mean values of the chemical composition of minced meat samples are presented in Table 1. The moisture content of minced meat averaged 66.31%, total crude protein 17. 62%, crude fat 15.01% and ash 1.10%,

Changes in pH Values of Minced Meat Contained Natural Antioxidants During Storage at 4°C and -18°C: The pH values of refrigerated minced meat storage at 4°C for the treated and untreated (control) samples during different periods are presented in Table 2 and 3. The pH values of the reference batch as well as treated samples gradually decreased As shown in Table 3, pH values of treated and untreated minced meat decreased as the storage period increased. It was clear that, extracts of rosemary or sage and their combination had significant (P= 0.05) effect on pH values of minced meat during storage.

Changes in TBA Values of Minced Meat Contained Natural Antioxidants During Storage at 4°C and -18°C: The TBA values for the minced meat during storage at 4°C and during storage at -18°C are presented in Table 4 and 5. The analysis of variance for the TBA data indicates that the TBA values were significantly affected ($P < 0.05$) by both the storage period and the extract treatments. Initial TBA values for all extract samples were significantly lower than those for the control ($P < 0.05$).

During storage at 4°C lipid oxidation of minced meat in the reference batch started to increase from 0.1 mg (0 day) to 0.41 mg (2 days) and increased rapidly after 4 days of storage at 4°C to reach 1.0 mg malonaldhyde / kg sample. The increment was further extended to rise an (5.8 mg malonaldhyde /kg sample) after 10 days of storage. While the minced meat contained extracts of both rosemary and sage 0.1 % apparently retarded oxidative rancidity of minced meat during refrigerated storage at 4°C as compared with control. TBA values demonstrated that extract of sage 0.1 % was more effective antioxidant than that of rosemary 0.1% where in minced meat contained 0.1% sage extract had 0.9 mg malonaldhyde/ kg sample after 10 days of storage while it was 1.2 mg malonaldhyde /kg for sample held 0.1% rosemary after 10 days and stored under the same conditions. While the extract of rosemary/sage extracts

Table l: Chemical composition of minced meat as fresh weight base (Mean±SE)

Content %±SE			
Moisture	Crude protein	Crude fat	Ash
67.31±0.41	17.62±0.20	14.01±0.20	1.10±0.05

Table 2: Changes in pH value of minced meat during storage at 4°C

	PH values±SE					
Natural antioxidants						
Storage (days)	0	2	4	6	8	10
Control	6.35±0.02	6.00±0.02	5.90±0.02	5.80±0.02	5.75±0.02	5.70±0.02
Rosemery 0.1%	6.40±0.02	6.40±0.02	6.35±0.02	6.30±0.02	6.28±0.02	6.15±0.02
Sage 0.1%	6.40±0.02	6.40±0.02	6.38±0.02	6.37±0.02	6.34±0.02	6.28±0.02
Rosemary 0.05% + Sage 0.05%	6.40±0.02	6.40±0.02	639±0.02	6.38±0.02	6.36±0.02	6.30±0.02

Table 3: Changes in pH value of minced meat during storage at -18°C

	PH values±SE										
Natural antioxidants											
Storage (days)	0	10	20	30	40	50	60	70	80	90	100
Control	6.35±0.041	6.30±0.02	6.25±0.01	6.20±0.03	6.18±0.02	6.15±0.01	6.10±0.02	6.00±0.01	5.80±0.02	5.75±0.01	5.62±0.02
Rosemery 0.1%	6.40±0.04	6.40±0.03	6.40±0.02	6.35±0.01	6.35±0.02	6.35±0.02	6.33±0.01	6.32±0.02	6.25±0.02	6.17±0.02	6.09±0.02
Sage 0.1%	6.42±0.07	6.40±0.02	6.36±0.03	6.36±0.02	6.30±0.01	6.25±0.01	6.25±0.02	6.27±0.01	6.18±0.01	6.14±0.01	6.15±0.01
Rosemary 0.05% + Sage 0.05%	6.35±0.11	6.41±0.02	6.40±0.01	6.38±0.01	6.40±0.02	6.41±0.02	6.42±0.01	6.41±0.02	6.22±0.02	6.11±0.02	6.02±0.02

Table 4: Change in TBA values of minced meat during storage at 4°C

Natural antioxidants	TBA mg Malonaldehyde/Kg±SE					
Storage (days)	0	2	4	6	8	10
Control	0.10±0.02	0.41±0.05	1.00±0.02	1.25±0.02	3.16±0.02	5.80±0.01
Rosemery 0.1%	0.10±0.02	0.11±0.04	0.23±0.06	0.21±0.08	0.80±0.07	1.20±0.10
Sage 0.1%	0.10±0.02	0.10±0.05	0.20±0.05	0.20±0.09	0.62±0.06	0.90±0.11
Rosemary 0.05% + Sage 0.05%	0.10±0.02	0.10±0.04	0.18±0.05	0.19±0.07	0.46±0.07	0.60±0.10

Table 5: Change in TBA values of minced meat during storage at -18°C

Natural antioxidants	TBA mg Malonaldehyde/Kg±SE										
Storage (days)	0	10	20	30	40	50	60	70	80	90	100
Control	0.10±0.02	0.17±0.05	0.40±0.08	0.5±0.11	0.6±0.07	0.8±0.12	1.4±0.14	2.1±0.12	4.21±0.23	6.34±0.17	8.21±0.24
Rosemery 0.1%	0.10±0.02	0.012±0.04	0.13±0.07	0.1±0.10	0.1±0.08	0.2±0.11	0.4±0.09	0.5±0.10	0.72±0.21	1.11±0.15	1.46±0.22
Sage 0.1%	0.10±0.02	0.11±0.03	0.12±0.08	0.1±0.09	0.1±0.08	0.1±0.10	0.2±0.07	0.3±0.11	0.47±0.22	0.67±0.18	0.84±0.21
Rosemary 0.05% + Sage 0.05%	0.10±0.02	0.11±0.04	0.11±0.08	0.1±0.10	0.1±0.07	0.1±0.11	0.1±0.10	0.2±0.10	0.30±0.23	0.49±0.16	0.62±0.21

Table 6: Change in psychrophillic bacterial count of minced meat during storage at 4°C

Natural antioxidants	Pschrophillic bacterial count log cfu/g±SE					
Storage (days)	0	2	4	6	8	10
Control	5.02±0.29	7.81±0.27	9.94±0.15	13.46±0.22	22.50±0.17	31.64±0.20
Rosemery 0.1%	4.38±0.32	3.72±0.36	4.83±0.23	5.17±0.31	10.79±0.21	16.28±0.28
Sage 0.1%	4.63±0.23	3.96±0.25	4.89±0.27	5.39±0.38	11.14±0.33	17.95±0.23
Rosemary 0.05% + Sage 0.05%	4.11±0.46	3.29±0.34	3.91±0.39	4.15±0.36	9.85±0.24	14.12±0.21

Table 7: Change in psychrophillic bacterial count of minced meat during storage at -18°C

Natural antioxidants	Pschrophillic bacterial count log cfu/g±SE										
Storage (days)	0	10	20	30	40	50	60	70	80	90	100
Control	5.02±0.17	4.71±0.20	4.56±0.22	4.90±0.33	5.67±0.24	6.74±0.14	8.69±0.17	11.59±0.11	13.53±0.15	16.87±0.27	20.31±0.30
Rosemery 0.1%	4.38±0.21	2.86±0.17	3.24±0.41	3.69±0.13	3.92±0.27	4.12±0.06	4.61±0.21	5.34±0.21	6.83±0.26	7.15±0.21	8.25±0.18
Sage 0.1%	4.63±0.11	3.02±0.23	3.59±0.29	4.22±0.24	4.78±0.13	5.17±0.09	5.86±0.18	6.42±0.26	7.11±0.34	7.93±0.26	8.84±0.24
Rosemary 0.05% + Sage 0.05%	4.11±0.18	2.16±0.25	2.89±0.37	3.41±0.32	3.67±0.19	4.02±0.12	4.46±0.14	5.20±0.30	5.98±0.28	6.43±0.22	7.16±0.20

Table 8:Mean scores of sensory characteristics of cooked minced meat treated with different natural extract.

Characteristics	Control	0.1 Rosemary	0.1 Sage	0.05+0.05 Rosemary/sage	LSD
Flavor	7.1	9.1	8.2	8.9	0.96
Tenderness	8.3	8.2	8.1	8.2	0.26
Overall acceptability	8.1	9.00	8.6	8.7	0.41

combination was the superior effective antioxidants among them since the TBA values after storage at 0 day was 0.1 mg malonatdhyde/kg sample increased to 0.6 mg/malonaldhyde /kg sample after 10 days of refrigerated storage under the same condition.

Lipid oxidation of minced meat control started to increase after 10 days and increase rapidly as the storage period extend during storage at -18°C. The TBA value at 10 days was 0.17mg malonaldhyde /kg which advanced to 8.21 mg at the end of storage period (100 days). At the end of storage time all treatments resulted in significantly lower ($P < 0.05$)

TBA values when compared to the control. The extracts of rosemary 0.1% and sage 0.1% apparently recorded oxidative rancidity of minced meat during frozen storage at - 18°C. TBA values demonstrated that extract of sage 0.1% was more efficient antioxidant than rosemary compared with extracts of rosemary/sage combination which was the most effective antioxidant among them.

Change in psychrophillic bacterial count of minced meat during storage at 4°C for 10 days and -18°C for 100 days.

Table 6 and 7 show the main values of psychrophillic bacterial count for control, 0.1% rosmary, 0.1 sage and 0.1 from its combination after storage at 4°C for 10 days and -18°C for 100 days. The results reveald that there were significant increase($P= 0.05$) in psychrophillic bacterial count for control sample which had 5.02 log cfu/g sample at zero time and increased to 31.64 log cfu/g after 10 days.While other groups treated with 0.1% rosmary, 0.1 sage and 0.1 from its combination shows littel changes in psychrophillic bacterial count at the same storage

period under the same condition.Also data observed that, psychrophillic bacterial count for samples treated with rosmary, sage and its combination during storage at 4°C for 10 days was decreased after zero time until 2 days of storage and increasing was observed reached to 16.28, 17.95 and 14.12 log cfu/g after 10 days of storage for rosmary, sage and its combination respectively.On the other hand, rosmary/sage combination was more effective as antibacterial compared others. The same traned was observed with minced meat during storage at -18°C for 100 days.

Sensory characteristics of cooked minced meat as affected by different natural extract are presented in Table 8. All formulations had more flavor than the control. Rosemary samples showed the highest score of acceptability and strongest flavor No difference (P= 0.05) was detected in the tenderness between the different formulations and control of minced meat.Generally, minced meat treated with 0.1%rosemary was more acceptable than other.

DISCUSSION

In the present experiment, adding the natural extracts of rosemary, sage and their combination to minced meat as natural antioxidants and antimicrobial led to retard the oxidative rancidity and microbial growth during refrigerated and frozen storage of minced meat. chemical composition of minced meat the present results agreed with those reported by Solomakos *et al.* [5] who found that the chemical analysis of beef meat showed a protein content of 21.870.2%, fat 2.470.1% and moisture 72.270.4%. The pH value was significantly differed between control and treated samples. Also,there were a significant differences between minced meat contained rosemary extract 0.1 % and both minced meat involved 0.1 % sage or combination of rosemary and sage. Nevertheless, no significant difference was noticed between both minced meat held sage or its mixture with rosemary. Changes in pH values may be affected by oxidative rancidity in minced meat. Regarding to the results obtained, the extracts of rosemary and sage 0.1% and their combination (0.05% of both) apparently retarded oxidative rancidity of minced meat during refrigeration at 4°C for 10 days as well as during frozen at -18°C for 100 days. The data indicated that 0.1 % rosemary extract was the most effective natural antioxidants followed by 0.1 % sage and the lowest one was the combination of both. These results agree with previously reported findings [23,24] for other natural antioxidants applied to cooked beef.

TBA value is routinely used as an index of lipid oxidation in meat products in store and the rancid flavor is initially detected in meat products between TBA values of 0.5 and 2.0 [25],data of TBA indicated that all the tested natural extracts added to minced meat showed a good antioxidant properties and extract of rosemary/sage extracts combination was the superior effective antioxidants compared with rosemary or sage alone. In this respect, [26] reported that rosemary extract could be delayed the oxidation of deboned poultry meat (DPM), processed beef gels, pork fat and chicken sausage. However, [12] reported that the addition of rosemary in the presence of salt, a prooxidant, resulted in lower TBA numbers than were recorded for the control during refrigeration storage but not during frozen storage. From a commercial standpoint, incorporation of rosemary oleoresin in sensitive meat products can substantially suppress lipid autoxidation and increase shelf life at refrigerated temperatures. Also, [27] reported that commercial rosemary extracts contain phenolic primary antioxidants which react with lipid or hydroxy radicals and convert them into stable products. Polyphenols antioxidants significantly affect the rate of the oxidation process in foodstuffs. Moreover, [13]reported that rosemary extracts may chelate metal ions, such as Fe resulting in a reduce rate of formation of activated oxygen. The effect of addition of synthetic BHA, BHT and natural antioxidants (rosemary extracts) too α- tocopherol was studied by Formanek *et al.* [28]. They found that rosemary extracts were as effective in reducing TBA as the combination of synthytic antioxidants, BHA/BHT. The use of natural antioxidants (sage, red pepper, garlic) in material constituted minced meat balls prepared from mechanically deboned turkey meat was studied by Karpinska *et al.* [29]. They found that the addition of sage and the mixture of spices retarded the process of oxidation during storage in a refrigerator for 4 days. Sage proved to be more effective than the mixture of spices, probably as a result of the antioxidant activity of polyphenols contained in the sage. Some reports found that the most apolar phenolic compounds from rosemary extracts are presumably responsible of their antibacterial activity [30].

Results of psychrophillic count observed that addition of rosemary,sage and its combination showed a higher inhibitory effect against psychrophillic count as compared to the control sample. On the other side, addition the mixture of rosemary and sage resulted in

populations of psychrophillic count significantly lower than the other treated samples during storage at 4°C for 10 days and -18°C for 100 days respectively.These results are in agreement with those reported by Oke *et al.* [31]who stated that plant extracts and essential oils constitute a natural source of antimicrobial mixtures or pure compounds for centuries. Essential oils and purified components are used as natural prevent the growth of food borne bacteria and molds antimicrobials in food systems, as well as to resulting in extension of the shelf life of processed foods.

Sensory evaluation revealed that the organoleptic properties of minced beef meat treated with rosemary were acceptable by the panelists more than sage alone or the mixture of them. These results agree with that reported by Sallam *et al.* [32].

Conclusively it could be recommended the addition of natural Antioxidant extracts of rosemary, sage and combination of them to retard the oxidative rancidity and microbial growth during refrigerated and frozen storage of minced meat and used as flavoring compounds to prepare meat products such as frankfurters, luncheon, various loaf products, sausage, fermented sausage, patties, spreads and other compounds of meat products.

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