

Antioxidant Action of Rosemary and Oregano Extract in Pre-Cooked Meat Hamburger

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Abstract

The main goal is to compare the antioxidant potential of rosemary and oregano natural extracts in precooked beef burger by assessing the lipid oxidation extent and sensory analysis. Five formulations (F) of hamburger were prepared from beef and mechanically separated as follows: meat containing sodium erythorbate (F1); deodorized rosemary extract (F2); oregano extract (F3); rosemary plus oregano extracts (F4) and without antioxidant addition, denominated control formulation (CF). The samples were frozen at -18°C for 24 hours, then submitted to heat treatment in an electric oven with internal controlled temperature of 75°C , and again frozen for a period of 30 days. The lipid oxidation extent (determined by thiobarbituric acid reactive substances—TBRS) was evaluated at 0, 15 during 30 days. After heating in plate to temperature of 75°C , the samples were submitted to color, taste and odor evaluation by 40 untrained tasters. The formulations F1, F2 and F4 presented lower concentrations of TBRS, whereas CF at day zero already showed very high values, indicating oxidation of the product. The samples showed good acceptance in the sensorial analysis.

Keywords

Rosemary Extract, Oregano Extract, Food Composition, Food Preservation,

1. Introduction

One of the main factors that affect meats and meat products quality is the occurrence of lipid oxidation, compromising significantly consumer acceptance. This process leads not only to discoloration and nutritional value loss, but also to food rejection due to rancid flavors caused by the oxidized products. Food rancidity does not only affect products sensory, but is also responsible for the emergence of toxic compounds associated with health problems, such as circulatory diseases, cancer and aging [1] [2].

For this to be avoided, synthetic substances such as butyl hydroxytoluene (BHT) and butyl hydroxyanisole (BHA) are added to retard or prevent oxidation in foods containing fat, but they have commercial limitations, since they contain a certain toxicity and phenolic odor. Currently, there is a wide variety of natural antioxidants employed in various industrialized foods and much attention has been given to those derived from herbs and spices. Food industry has an increasing interest for natural substances that can replace the synthetic ones in order to produce healthier foods that meet the demands of the consuming public [3] [4].

In this sense, spices like rosemary and oregano, widely used in cooking, were promising in studies conducted with rosemary (*Rosmarinus officianalis* L.) and oregano (*Oreganum vulgare* L.). These leaves showed inhibition of oxidative degradation in fatty foods with the possibility of industrial application [5] [6].

In research carried out with vacuum-packed, roasted and vacuum-packed meat containing 0.1% water-soluble deodorized rosemary extract, the product presented low levels of lipid oxidation during sixty days of storage at -18°C [7].

The rosemary extract obtained by the supercritical extraction technique with CO_2 -SFE showed excellent performance when used in several foods, cosmetics and pharmaceuticals, in which natural aroma, color and antioxidant and/or antimicrobial activity were desired [8].

These properties are very relevant to food industry concerning the search for possible substitutes to food synthetic preservatives. However, further studies must be done to investigate the behavior of natural extracts in food, considering all quality requirements requested by the food system.

Oxidative rancidity begins as soon as the animal dies, when blood flow stops and metabolic processes are disrupted. The steps comprising such deterioration process are formed by many chemical reactions, resulting many complex products. Unsaturated lipids provide the substrate for the reaction to occur and catalysis happens through the action of oxygen, light, heavy metals and others, being one of the major causes of meat products deterioration [9].

Particularly, in the case of cooked meat, the taste deterioration is one of the

most important revealed changes. During peroxidation, polyunsaturated fatty acids are degraded into short chain volatile components, such as aldehydes, ketones, alcohols, esters and acids, compromising both, odor and taste. This phenomenon increases during subsequent cooking and storage. It is recognized as popularly called “reheated” flavor or WOF—Warmed-Over Flavor, which is a major concern for precooked meats. The development of unpleasant flavors and odors greatly reduces consumer acceptability [7].

In phytochemistry, there is a huge interest for searching new natural sources of antioxidants and antimicrobial agents, which demands debating on the safe aspects of chemical preservatives, since these are considered carcinogenic and teratogenic, in addition to originating toxic residues [10] [11].

The rosemary (*Rosmarinus officinalis* L.) is a medicinal plant widely used around the world and one of the species with the highest antioxidant activity [12]. Many compounds have been extracted from rosemary, including flavonoids, diterpenes, steroids and triterpenes. Among these compounds, rosemary antioxidant activity has been attributed to two phenolic diterpenes, mainly: carnosine acid and carnosol [13] [14].

Five antioxidative phenolic acids were isolated from the oregano leaves (*Origanum vulgare* L.). The structure of a new compound (5a) was determined to be 2-caffeoyloxy-3-[2-(4-hydroxybenzyl)-4,5-dihydroxy]phenylpropionic acid on the basis of spectroscopic evidence. Especially, phenyl glucoside (1) and 5a showed an activity comparable to BHA [6] [15].

Vekiari and co-workers [16] studied the fractionation of oregano extract with various solvents (petroleum ether, ethyl ether, ethyl acetate and butanol) and results showed that the fraction diluted in ethyl ether was the most effective in protection against animal fat lipid oxidation when stored under light and at 65°C, equivalent to the action of butyl-hydroxy toluene. This same extract had also a good performance as an antioxidant in refined vegetable oils when stored at 35°C and 65°C and then subjected to frying.

Antimicrobial activities of six essential oils commonly used as condiments in Brazil (*Ocimum basilicum* L. (basil), *Rosmarinus officinalis* L. (rosemary), *Origanum majorana* L. (marjoram), *Mentha piperita* L. var. *Piperita* (peppermint), *Thymus vulgaris* L. (thyme) and *Pimpinella anisum* L. (anise) against *Clostridium perfringens*. The results obtained in this study demonstrated that the essential oils of basil, rosemary, marjoram, peppermint, thyme and anise exhibit, in vitro, antimicrobial activities against *C. perfringens*. The use of essential oils from commonly employed spices clearly offers an alternative to the chemical preservatives in the control and inactivation of pathogens in food, but further studies are needed in order to verify direct application in commercially produced food systems. The results suggest that the oxygenated compound, especially oxygenated monoterpenes and phenylpropanoids, might be responsible for the antimicrobial activity against *C. perfringens* [17].

In study carried out by Trindade [5], the sensorial analysis showed that

among beef hamburger samples formulated with natural antioxidants, *i.e.*, 400 mg/kg oregano extract and 400 mg/kg rosemary extract, irradiated and frozen for 90 days, those containing oregano received the highest marks among the tasters.

Therefore, the study of the vegetal origin extracts incorporated as antioxidants in foods aims to expand possibilities for other viable alternatives that ensure the integrity of the industrialized products.

Considering the literature findings, the objective of this study is to evaluate rosemary and oregano antioxidant potential in precooked beef burger stored under freezing and to determine the extent of lipid oxidation through physical-chemical and sensory analysis evaluations.

2. Materials and Methods

2.1. Materials

2.1.1. Raw Material and Additives

For the hamburgers preparation were used the following raw materials and additives: Chuck Beef (Meat), Mechanically Separated Meat (MSM), Bacon, Deodorized Oregano Extract—DANISCO S.A., Deodorized Rosemary Extract—DANISCO S.A., Iodized Salt refined—Sal Cisne/Refinaria Nacional de Sal, sodium erythorbate—SUNTRAN S.A.

Both extracts were commercially acquired (DANISCO S.A.)

2.1.2. Hamburgers Formulation, Production and Storage [18]

Sample treatment: Forty samples of five hamburger formulations—100 g each (F1, F2, F3, F4 and Control Formulation—CF) were prepared, totaling two hundred samples, containing 70% chuck, 10% MSM, 10% bacon, 8% ice water, 2% iodized salt and different concentrations of antioxidants according to **Table 1** and **Table 2**.

The samples were frozen at -18°C for 24 hours, and then subjected to heat treatment in an electric oven with internal controlled temperature of 75°C . After heat treatment, the samples were packed in plastic film, and frozen at -18°C for

Table 1. Hamburger formulations.

Raw material and additives	CF	F1	F2	F3	F4
Ground chuck beef	4.25 kg	4.25 kg	4.25 kg	4.25 kg	4.25 kg
MSM	0.50 kg	0.50 kg	0.50 kg	0.50 kg	0.50 kg
Ice water	0.40 kg	0.40 kg	0.40 kg	0.40 kg	0.40 kg
Bacon	0.25 kg	0.25 kg	0.25 kg	0.25 kg	0.25 kg
Sodium chloride	0.10 kg	0.10 kg	0.10 kg	0.10 kg	0.10 kg
Sodium erythorbate	-	0.0025 kg	-	-	-
Rosemary extract	-	-	0.005 kg	-	0.005 kg
Oregano extract	-	-	-	0.005 kg	0.005 kg

Table 2. Antioxidants concentration in the different hamburger formulations.

Formulations	Rosemary extract mg/kg	Oregano extract mg/kg	Sodium erythorbate mg/kg
CF	0	0	0
F1	0	0	500*
F2	1000	0	0
F3	0	1000	0
F4	1000	1000	0

*Concentration limit recommended by legislation [19].

a period of 30 days.

2.2. Methods

2.2.1. Determination of Lipids [20]

Food lipids determination is carried out by solvent extraction and, in general, ethyl ether or petroleum ether, which are heated in an intermittent extractor (Soxhlet apparatus). The resulting ethereal extract includes, mainly, the oil/fat resultant, although it also encompasses other soluble substances.

2.2.2. Evaluation of Lipid Oxidation [21]

Samples lipid oxidation was determined to triplicate at 0, 15 in 30 days, using a methodology recommended by Tarladgis and co-workers [21], in which thiobarbituric acid reactive substances are measured. The samples were crushed, water was added, and then subjected to distillation. Distillate aliquots were added thiobarbituric acid solution, generating colored complexes, whose intensity was measured in a spectrophotometer and subjected to the absorbance reading (A) at a wavelength of 532 nanometers. Primary lipid oxidation products are mainly hydroperoxides, which are rapidly decomposed into various thiobarbituric acid (TBA) reactive substances, particularly carbonyls; malonaldehyde is considered the most important compound.

2.2.3. Physicochemical Characterization

Moisture and ashes content determinations were conducted according to the technique described in Institute Adolfo Lutz [20].

2.2.4. Sensory Analysis

“*Antioxidant action of rosemary and oregano extracts on pre-cooked beef burger*” Project was submitted to the Research Ethics Committee of the University of São Judas Tadeu (USJT) and was approved in a consubstantiated opinion number 063/2009.

1) *Tasters Selection*

Tasters were invited to attend the Gastronomy and Dietary Technique Laboratory of the University of São Judas Tadeu—USJT for conducting the sensory acceptance tests through posters posted on USJT murals and e-mail notifications to the USJT internal community. The posters and warnings specified the type of

test and the product to be evaluated.

2) Tasters inclusion and exclusion criteria

Prior to the tests, tasters were asked to complete a questionnaire and to sign the Free and Informed Consent Form. Were selected only the tasters who met the following requirements: —be over 18 years old; sign the free and informed consent form (FICF); be a meat products consumer. During taster's selection, were also considered non-smokers, with no respiratory diseases or olfactory syndromes.

3) Sensory Analysis Performance

The sensory evaluation was performed at the Laboratory of Dietary Technique and Gastronomy—USJT in individual booths. The entire experiment was conducted according to the Good Manufacturing Practices standards [18].

The different samples, after heating in plate to the internal temperature of 75°C, were cut into four pieces. Color, taste and odor were the evaluated parameters by 40 untrained tasters, previously recruited. For this purpose, it was used a structured nine-point hedonic scale, in which, the number 1 corresponded to “extremely displeased” and the number 9, “I liked it extremely”, according to the chart model presented in **Appendix** (Sensory analysis form template), provided by Anzaldúa-Morales [22]. The samples, coded with three digits, were served in plastic cups. During the tasting, tasters received cream cracker cookies and drinking water to clean the palate. The samples were presented in monadic form.

3. Results and Discussion

3.1. Results regarding the Physicochemical Characterization of the Studied Formulations

The physic-chemical characterization of the different hamburgers formulations after oven heat treatment are presented in **Table 3**.

Different samples moisture contents ranged from 51.20% to 61.57%. This maximum variation of 10% among formulations may have occurred due to differences in the raw material itself, although it was from a single supplier. The heat inhomogeneity distribution in the furnace may have also contributed to the differences in the samples moisture content.

Table 3. Moisture/ash contents mean values and lipid percentage of the different hamburger formulations after baking.

Formulations	Physicochemical composition (%)					
	Moisture content determination	Standard deviation	Ashes content determination	Standard deviation	Determination of lipids	Standard deviation
CF	51.20	0.0006	3.73	0.0314	14.79	0.4382
F1	55.58	0.0017	3.31	0.0286	12.90	0.1947
F2	58.73	0.0011	3.15	0.0212	14.13	1.1031
F3	61.57	0.0019	3.42	0.0437	11.08	0.2446
F4	57.48	0.0040	3.29	0.118	12.82	0.2405

Meat in nature presents, on average, up to 0.9% of mineral or ash [23]. Ash contents above 1% found in hamburger samples are probably due to the addition of mechanically separated meat (MSM) to all formulations. While obtaining MSM, fragments of bird bones are incorporated into the meat, raising this ingredient ash contents.

Different formulations lipid content ranged from 11.08% to 14.79%. The ingredients that contributed to these lipids amounts were bacon, mechanically separated meat and the chuck meat itself.

The mean values of thiobarbituric acid (TBA) reactive substances (TBARS), expressed as mg malonaldehyde/kg per sample, for the five baked hamburgers formulations stored at -18°C , for zero, 15 and 30 days are presented in **Table 4**.

Lipid oxidation extent in the different formulations over the storage at -18°C is shown in **Figure 1**.

Table 4. Mean values of malonaldehyde concentration/kg of sample for the different hamburger formulations.

Formulations	mg malonaldehyde/kg of sample					
	0 day	Standard deviation	15 days	Standard deviation	30 days	Standard deviation
CF	3.76	0.75	5.51	0.29	5.04	1.36
F1	1.43	0.15	1.7	0.24	1.72	0.24
F2	0.39	0.02	1.07	0.04	1.24	0.02
F3	1.57	0.05	4.69	0.06	4.48	0.01
F4	0.36	0.08	0.83	0.05	1.04	0.10

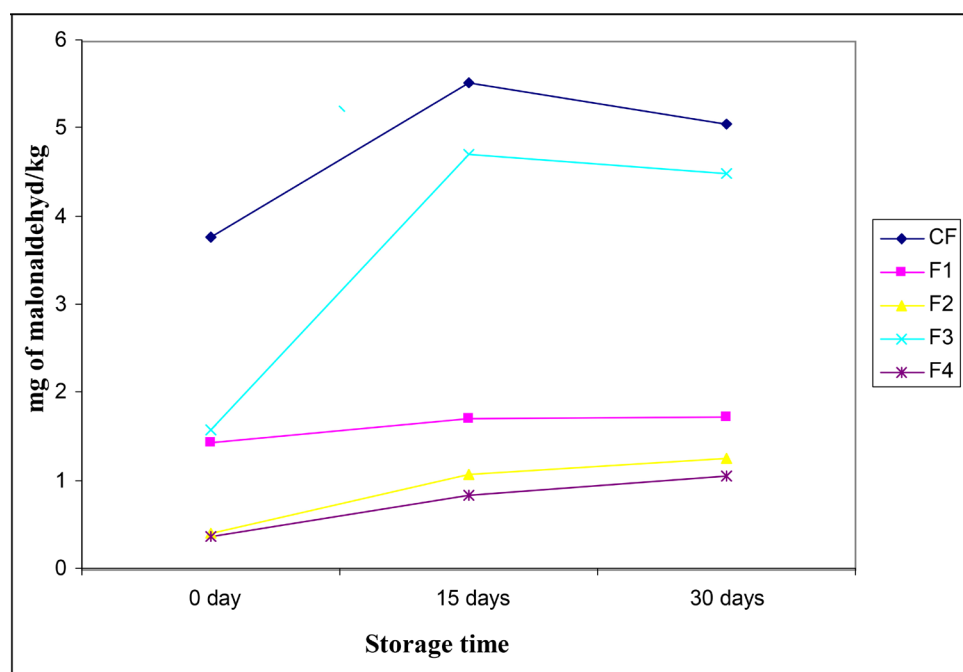


Figure 1. Extension of lipid oxidation of baked hamburger samples and stored at -18°C up to 30 days.

It is concluded from **Table 4** and **Figure 1** that the formulations F1 (sodium erythorbate), F2 (rosemary extract) and F4 (rosemary extract associated with oregano extract) presented the lowest concentrations of malonaldehyde, which means, greater stability during the storage period under freezing. The control formulation (CF) showed higher values of TBA at time zero, indicating the product oxidation due to the absence of antioxidants. This oxidation increased during storage at -18°C .

The formulation containing only oregano extract (F3) had low values of TBA at time zero, but after fifteen days of storage under freezing, it had an increase in TBA values, suggesting that oregano extract did not perform as an antioxidant in the studied concentration.

In a study conducted by Tarcitano & Mesquita [24], the Food Security of food was evaluated to guarantee the population physical and economic access to innocuous and nutritious products. This research aimed to investigate the occurrence of antimicrobial in the in natura food condiments used in the processing, preparation and/or consumption of fish, through a systematic review of the literature and covers articles published until July 2016. The authors concluded that the use of spices is a good combating the growth of bacteria, yeasts, filamentous fungi and synthesis of toxins as well as inhibiting or retarding the oxidation of fish. Similarly, oregano, rosemary and sage were the most effective antimicrobial condiments.

The results obtained in relation to the addition of rosemary extract coincide with those obtained by Pereira & Pinheiro [25] although these authors used the ethanolic extract. In the study, these authors evaluated the inhibition of the hamburgers lipid oxidation and they found that the rosemary ethanolic extract inhibited the lipid oxidation in 21 days with a low production of malonaldehyde and with values very similar to the synthetic antioxidant (BHT) and they suggested this antioxidant substitution by rosemary extract.

3.2. Results Related to Evaluated Organoleptic Characteristics: Color, Odor and Taste

The mean values of the scores attributed by the tasters to the color, odor and flavor of the different samples baked and stored at -18°C for 0, 15 and 30 days are presented in **Figures 2-4**, respectively

According to **Figure 2**, at time 0, all samples had scores between 6 (slightly liked) and 7 (moderately liked) for color, thus having a good acceptance by the tasters. On the 15th day, samples CF and F1 had scores between 5 (neither liked nor disliked) and 6 (slightly liked), while samples F2, F3 and F4 had a grade 6. At the end of storage, samples F3 and F4 received notes between 6 and 7 for this attribute.

Figure 3 shows that the control formulation (CF) received the lowest score for the odor attribute, that is, 6, but this was not very different from the average of the other samples, which had a score close to 7. After fifteen days of storage, the only sample that received mean between 6 and 7 was the F4.

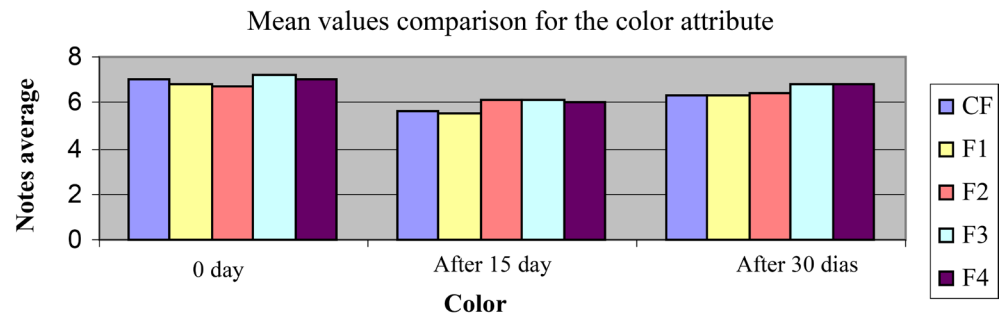


Figure 2. Mean values for the color attribute of the different hamburger samples when stored at -18°C for 0, 15 and 30 days ($n = 40$ testers).

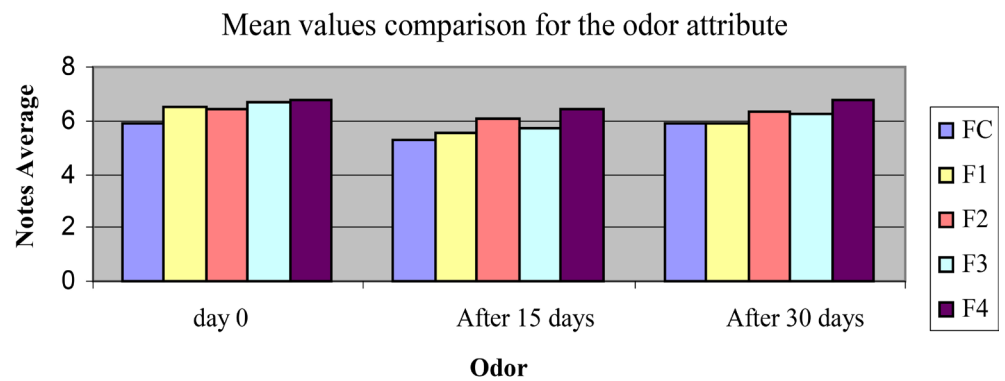


Figure 3. Mean values for the odor attribute of the different hamburger samples stored at -18°C for 0, 15 and 30 days ($n = 40$ testers).

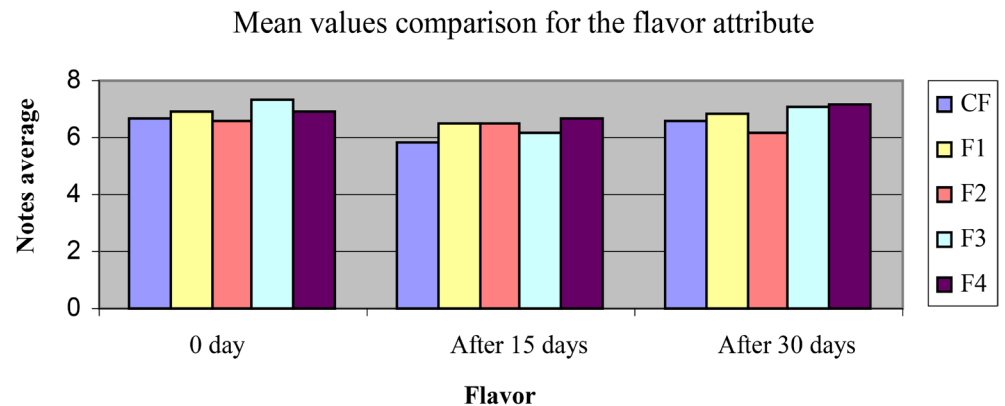


Figure 4. Mean values for flavor attribute of different hamburger samples stored at -18°C for 0, 15 and 30 days ($n = 40$ testers).

However, no sample was classified below five for the odor attribute. After 30 days, all samples had an average of 6 or slightly higher.

Regarding the flavor attribute, all the samples were well accepted by the tasters during their storage, since the averages for this attribute varied between 5.8 and 7.3. It can be inferred that there was a preference for samples F3 (with oregano extract) and F4 (with extracts of rosemary and oregano).

Although the F3 sample presented an increase in oxidation during storage, a fact proven in TBARS determination, it received satisfactory averages for the

attributes of color, odor and flavor, demonstrating that the lipid oxidation was not perceived by the evaluators during the studied period.

4. Conclusion

The study reveals that formulations F1 (with sodium erythorbate), F2 (with rosemary extract) and F4 (with rosemary and oregano extracts) showed good stability during the freezing period. The formulation containing only oregano extract did not have good stability during storage under freezing. However, reports from the sensory analysis from the tasters did not allow the establishment of a correlation between the scores attributed to color, odor and flavor and the said chemical changes.

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Appendix: Sensory Analysis form Template

SENSORY ANALYSIS OF BOVINE MEAT HAMBURGER	
Name: _____	Age: _____
E-mail: _____	
Please sample Hamburger sample to evaluate how much you liked or disliked the sample, according to the scale.	
9. I liked it very much	
8. I really liked it	
6. I liked it 7. I liked moderately	
slightly	
5. Neither liked nor disliked	
4. I slightly disagree	
3. I disliked moderately	
2. I did not like it very much.	
1. Extremely disliked	
Attributes	Note
Color	_____
Odor(smell)	_____
Flavor _____	
Comments: _____	