

# Processes in meat oxidation and usage of rosemary (*Salvia rosmarinus* (L.) Schleid.) as a natural antioxidant

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## ABSTRACT

**Objective:** Lipid oxidation of meat is one of the most important factors affecting shelf life and is one of the decisive factors in the consumer's purchase decision of the product. Therefore, information related to lipid oxidation using rosemary (*Salvia rosmarinus* (L.) Schleid.) as a natural antioxidant is described and analyzed.

**Design/methodology/approach:** Mechanisms of oxidation were explored and described, as well as the alternatives to stop this process and different methodologies to measure antioxidant activity and innovative alternatives that are currently being investigated.

**Results:** Applying antioxidants is one of the most widely used methods to counteract the oxidation process in meat. Currently, using herbs and spices has gained great acceptance, as in the case of rosemary. Its usage obtained satisfactory results for inhibiting and delaying lipid oxidation.

**Limitations of the study/implications:** Using rosemary may have some drawbacks such as incorporating a strong flavor to the meat and the effects that its active compounds may have when exposed to oxygen, heat and humidity. Therefore, it is necessary to research for alternatives that will allow better preservation and availability of its compounds.

**Findings/conclusions:** Nanoencapsulation of rosemary may be an alternative to the drawbacks of its use, working as a protective barrier for improved performance and improving food safety. However, this innovation is just being explored and is therefore not possible to have a certainty of success when using these new technological alternatives.

**Keywords:** oxidation, lipids, meat, rosemary, nanoencapsulation.

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## INTRODUCTION

Meat and meat products are rich in nutrients such as proteins, fats and minerals; they are perishable foods with a short shelf life (Heinz and Hautzinger, 2007; Rashidaie *et al.*, 2019). One of the factors that affect the most the shelf life is lipid oxidation (LO) in the unsaturated fatty acid (UFA) fraction of the membrane, forming peroxides and hydroperoxides, which are susceptible of decomposition into secondary oxidation products, such as hydrocarbons, aldehydes, ketones, alcohols, among others (Chen *et al.*, 2017; Garcia *et al.*, 2017).

These compounds produce unpleasant aromas and flavors in foods, typical of rancidity which negatively alter sensory attributes such as color, texture, odor and flavor (Gallego *et al.*, 2015). It is, therefore, necessary to use alternatives that allow meat preservation, but at the same time seek for consumer acceptance, like using natural antioxidants (Cheng *et al.*, 2017). The food industry commonly uses synthetic antioxidants, which may be unsuitable for consumers, due to their potential toxicity and carcinogenic risks (Aminzare *et al.*, 2019). Some plants are a source of bioactive substances containing phenolic compounds with antioxidant properties; among these plants is rosemary (*Salvia rosmarinus*) which contains potent antioxidants such as phenolics, diterpenes, carnosic acid, carnosol and rosmarinic acid (Chao *et al.*, 2020). However, these phenolic compounds may lose their beneficial effects when exposed to oxygen, heat, humidity and light. Therefore, it is necessary to use specific methods to protect them to achieve a higher antioxidant activity, one way is nanoencapsulation (Duarte and Larroza, 2019; Rashidaie *et al.*, 2019). The usage of rosemary oil is limited due to its hydrophobicity, as it is difficult to dissolve in the aqueous phase of food. One possible way to overcome this barrier is through nanotechnology (Boskovic *et al.*, 2019), achieving increased stability, protection, controlled release and reducing the possible adverse impact on the organoleptic properties in meat and meat products (Duarte and Larroza, 2019). On this basis, a review of the information related to lipid oxidation and the application of rosemary (*Salvia rosmarinus* (L.) Schleid.) as a natural antioxidant has been developed.

### **Shelf life of meat and meat products**

Meat and meat products are an excellent source of essential nutrients containing high-quality proteins, fats and minerals, which make them highly perishable foods, therefore their shelf life is short (Aminzare *et al.*, 2019; Tsironi *et al.*, 2019). The shelf life of meat and meat products is defined as the maximum recommended time where products can be stored, in specific temperature and humidity conditions, without losing an acceptable quality (Donohue, 2016). These products are susceptible to changes that lead to LO, whether in a fresh or cooked state. The fresh form is affected by its storage and packaging, as well as other factors related to the animal species they come from and their fat content. When cooking, oxidative stability and shelf life are again affected by the type of meat (solid, ground, or mechanically deboned) and the employed thermal processing method (boiling, frying, grilling, or curing) (Shahidi, 2016). Spoilage results in changes in the sensory characteristics of those products such as off-flavors and colorings, which make the product undesirable or unacceptable for the consumers (Donohue, 2016). Some strategies have been applied to maintain food quality and extend shelf life, these include the addition of antioxidants, which slow oxidation and extend the shelf life of packaged foods (Chao *et al.*, 2020).

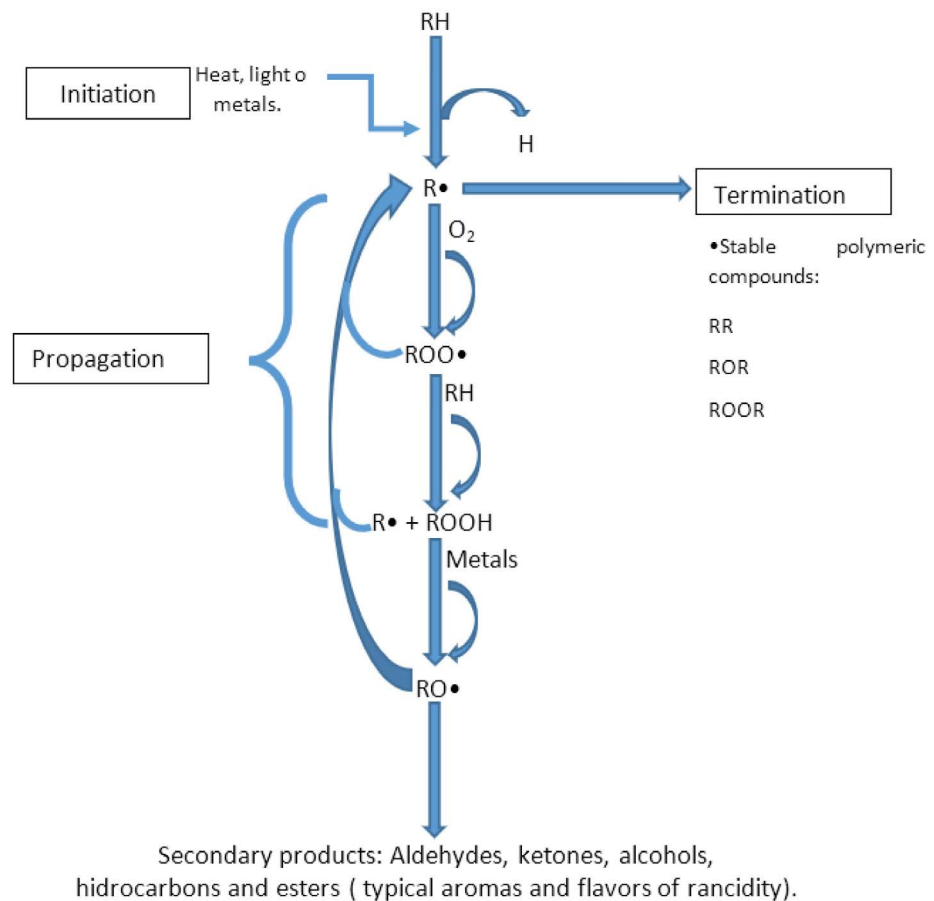
### **Meat oxidation and ways to prevent or retard it**

Oxidation is a complex and irreversible process in oils and fats. So, in meat, the membrane fraction of UFAs oxidizes resulting in the formation of hydroperoxides (Kumar *et al.*, 2015). The oxidation process comprises three stages: initiation, propagation and

termination (Figure 1). In the initiation stage, the alkyl radical is formed from UFAs. Factors such as heat, light, or metal ions generate instability in fatty acid unsaturation; this instability breaks the unsaturation, causing labile hydrogen adjacent to the double bond to be lost and forms an alkyl radical. The propagation phase is associated with increased oxygen consumption, which reacts with the alkyl radical to form peroxides and reacts with new UFAs to form hydroperoxides that are primary compounds.

These compounds are labile molecules that decompose to produce alkoxy radicals, and in turn, originate a complex mixture of secondary oxidation products, known as low molecular weight volatile and non-volatile compounds such as hydrocarbons, aldehydes, ketones, alcohols, among others. These compounds generate unpleasant aromas and flavors in foods, typical of rancidity (Wasowicz *et al.*, 2004; Venegas and Perez, 2009; Kumar *et al.*, 2015). In the termination stage, free radicals and hydroperoxides react in various combinations, forming non-radical or stable products of low molecular weight (Wasowicz *et al.*, 2004; Venegas and Pérez, 2009; Kumar *et al.*, 2015).

Lipid oxidation should be prevented and regulated to preserve food quality, given the impact that it has on the physical and nutritional characteristics of food. Lipid oxidation



**Figure 1.** Unsaturated fatty acids oxidation process. RH=unsaturated fatty acid; R• = alkyl radical; ROO• =peroxide radical; ROOH=hydroperoxide; RO• =alkoxy radical.

can negatively alter sensory attributes such as color, texture, odor and flavor (Gallego *et al.*, 2015; Cheng *et al.*, 2017). Generally, by rancid odors, discoloration, loss of nutritional values and off-flavors (Xiong *et al.*, 2020). Lipid oxidation is one of the main factors that reduce the shelf life of meat and meat products (Aminzare *et al.*, 2019; Xiong *et al.*, 2020) since these foods have high lipid content and can generate oxidized compounds such as ketones, alcohols, aldehydes, alkanes and alkenes of low molecular weight and high volatility (Rojano *et al.*, 2008; García *et al.*, 2017). Also, mincing, cooking and other processes that meat undergoes before storage and refrigeration, alter the membranes of muscle cells, thus facilitating the interaction of UFAs with prooxidant substances such as non-heme iron; LO accelerates and rapid deterioration of quality and development rancidity (Gallego *et al.*, 2015).

Heating meat decreases the UFAs content, increases hydroxyl radicals and non-heme iron and decreases the activity of endogenous antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase (Aminzare *et al.*, 2019; Xiong *et al.*, 2020). Cooking meat involves the formation of hydroperoxides that can easily decompose into various already mentioned volatile organic compounds and are responsible for reducing sensory quality due to oxidative flavors (Aminzare *et al.*, 2019).

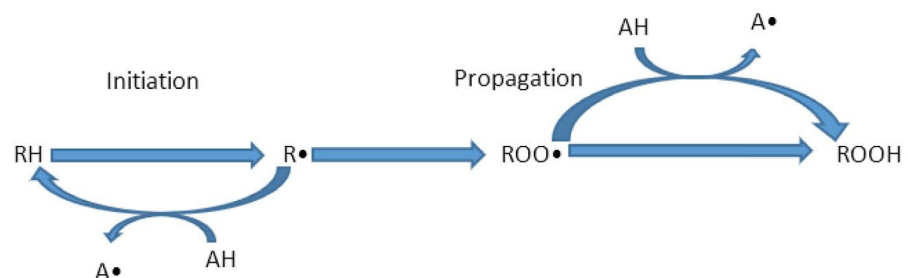
### Prevention of lipid oxidation

One of the strategies to reduce meat spoilage due to LO and improve its shelf life is applying antioxidants (Cheng *et al.*, 2017; Aminzara *et al.*, 2019). Antioxidant activity refers to the capacity of a substance to inhibit oxidative degradation caused by the reaction of free radicals (Figure 2).

Antioxidants retard the oxidation of easily oxidizable biomolecules, such as lipids in meat and meat products. These compounds can donate hydrogen radicals to free radicals, preventing oxidative damage (Londoño, 2012; Amaral *et al.*, 2018). The key mechanism in the reaction with free radicals is to form stable inactive products. The action occurs in the initiation and propagation stage when the radicals formed in these phases are removed or during the degradation of hydroperoxides (Kumar *et al.*, 2015).

### Methods to assess antioxidant activity and oxidative status in meat

Antioxidants can stabilize free radicals *via* two mechanisms, the first is called hydrogen atom transfer which comprises 2,2-diphenyl-1-picryl hydrazyl (DPPH), Oxygen Radical



**Figure 2.** Action of antioxidants on lipid oxidation. RH=unsaturated fatty acid; R•=alkyl radical; AH=antioxidant; A•=antioxidant or scavenger radical; ROO•=peroxide radical; ROOH=hydroperoxide.

Absorbance Capacity (ORAC) and N1, N1-di-methyl-1,4-phenylenediamine (DMPD). The first mechanism measures the capacity of an antioxidant by stabilizing the free radicals through transferring hydrogen atoms. The second is called electron transfer involving azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP), both of which stabilize free radicals (Londoño, 2012; Ácsová *et al.*, 2019). One of the most applied strategies to determine the antioxidant capacity of a compound, mixture, or food is to measure the activity of the antioxidant against free radical substances or solutions. Various chromogenic compounds are used to determine the capacity of phenolic compounds contained in fruits, vegetables or species extracts to trap the free radicals generated (Kuskoski *et al.*, 2005; Sotelo *et al.*, 2015). Some of them are described below.

#### **DPPH (2,2-diphenyl-1-picryl hydrazyl)**

The test is based on determining the capacity of an antioxidant to stabilize the DPPH radical, this measurement can be made by spectrophotometry with a maximum absorbance peak at 515 nm. It is one of the few stable organic radicals, it presents a strong violet coloration, whose absorbance decreases when reduced by an antioxidant. Its reduction depends on the ABTS method and the ability of the antioxidant compounds to transfer electrons or donate protons (Santacruz, 2011). It is a commercially available free radical that can be directly obtained and does not have to be generated in situ like  $ABTS^{\bullet+}$ , but it can only be dissolved in organic media. The technique has been developed to measure antioxidant capacity mainly in plants and food extracts. The method is simple and requires little instrumental material; however, on the downside, the technique makes it difficult to interpret the results when you have substances whose absorption spectrum is opposite to that of the radical (Kuskoski *et al.*, 2005; Santacruz, 2011; Londoño, 2012).

#### **ORAC (Oxygen Radical Absorbance Capacity)**

This test has advantages such as high adaptability to antioxidants, biological samples, foods and the ability to analyze the antioxidant potential of non-protein samples using a wide range of extraction agents. The reaction is conceptually simple but difficult in practice. The reactions begin with the heating of azide compounds to release nitrogen gas and generate two radicals ( $R^{\bullet}$ ). During the radical generation, it is very important to maintain the optimum heating temperature to ensure complete decomposition of the azides. If the required temperature is not maintained, unclear and incomparable results are obtained (Ácsová *et al.*, 2019).

#### **DMPD (N1, N1-Di-methyl-1,4-phenylenediamine)**

Oxidants in samples are reduced and color changes are evaluated spectrophotometrically. First, the  $DMPD^{\bullet+}$  radical is formed by mixing a solution of DMPD in an acetate buffer and ferric chloride. The prepared red-colored solution of the DMPD cation is let to rest at laboratory temperature for 12 h before being used to evaluate the antioxidant activity of the samples. The oxidative state of the  $DMPD^{\bullet+}$  substance is readable at 515 nm.

Choosing methanol as a solvent for DMPD is not suitable (Ácsová *et al.*, 2019). The results from this method are low, poorly reproducible and inconsistent (Kuskoski *et al.*, 2005).

#### **ABTS (azinobis 3-ethylbenzothiazoline-6-sulfonic acid).**

It is based on the uptake capacity of an antioxidant to stabilize the ABTS<sup>•+</sup> cation radical. It is a free radical obtained after a chemical (manganese dioxide or potassium persulfate), enzymatic (peroxidase, myoglobin), or electrochemical reaction. Azinobis 3-ethylbenzothiazoline-6-sulfonic acid can measure the activity of compounds of lipophilic and hydrophilic nature including carotenoids and flavonoids. This method is applied along with DPPH, both of which have good stability in certain conditions. It is a highly sensitive, practical and fast method. It has the advantage that its spectrum presents maximum absorbance at 414, 654, 754 and 815 nm in an alcoholic medium (Kuskoski *et al.*, 2005; Santacruz, 2011; Londoño, 2012).

#### **FRAP (Ferric Reducing Antioxidant Power)**

This method is based on the evaluation of the ability of an antioxidant to reduce ferric iron (Fe<sup>+3</sup>) present in a complex with 2,4,6-tri(2-pyridyl)-s-triazine to the ferrous form (Fe<sup>+2</sup>), causing the formation of a ferrous tripyridyltriazine complex. The reduction is indicated by an intense blue color with maximum absorption at 593 nm; however, the solution is yellow if no reduction of ferric ions occurs (Silva *et al.*, 2017; Ácsová *et al.*, 2019).

#### **TBARS (thiobarbituric acid reactive species)**

The TBARS technique assesses the oxidation state in muscle components such as meat, and is one of the most widely used techniques (Isaza *et al.*, 2013). The calculation is based on the determination of the content of the substance reactive to 2-thiobarbituric acid. A spectrophotometer at  $\lambda_{\max}=532$  nm is used to monitor the formation of a pink-colored product, resulting from the addition of 2-thiobarbituric acid with malonaldehyde (product of IFA oxidation) (Wenjiao *et al.*, 2014; Silva *et al.*, 2017). Values are expressed in mg malonaldehyde kg<sup>-1</sup>. This technique has limitations, but TBA is useful to compare the oxidation of a meat sample or byproduct at different stages. The TBARS value correlates with the results of sensory analysis in a product, as an LO indicator (Venegas and Perez, 2009).

#### **Rosemary as an antioxidant and nano encapsulation**

Synthetic and natural antioxidants have been used in the food industry to delay or prevent LO in meat (Cheng *et al.*, 2017). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ), propyl gallate (PG) and nitrite have been used in the meat industry (Gallego *et al.*, 2015; Aminzare *et al.*, 2019). However, they are unsafe for consumers, due to their toxicological and carcinogenic effects, therefore, their use has nowadays been limited.

Currently, there is a growing trend to consume natural products. Plants are a source of bioactive substances with phenolic compounds, which are the main molecules responsible for the antioxidant properties which result in an effective alternative to synthetic

antioxidants (Kumar *et al.*, 2015; Aminzare *et al.*, 2019; García *et al.*, 2020). Phenolic compounds, the main antioxidant in plants, may be classified and include phenolic diterpenes (carnosic acid and carnosol), flavonoids (quercetin and catechin), phenolic acids (gallic acid, rosmarinic acid, and caffeic acid), and volatile oils (carvacrol, eugenol, thymol, and menthol) (Aminzare *et al.*, 2019).

Among the plants that have antimicrobial and antioxidant properties is rosemary (*Salvia rosmarinus* L. (Lamiaceae), a perennial woody herb native to the Mediterranean region, worldwide cultivated as an ornamental and aromatic plant. Rosemary leaves are commonly used to flavor foods as a condiment (Rašković *et al.*, 2014; Rashidaie *et al.*, 2019). The antioxidant properties of rosemary are attributed to its major phenolic diterpenes, such as carnosic acid, carnosol and rosmarinic (caffeic acid and 3,4-dihydroxyphenylacetate ester). Rosemary has the potential effect to inhibit the LO of food, by eliminating free radicals and the chain reaction of metal ions such as Fe<sup>2+</sup> is terminated, reducing the activated oxygen molecules rate of formation (Afonso *et al.*, 2013; Rašković *et al.*, 2014; Aminzare *et al.*, 2019; Rashidaie *et al.*, 2019). Rosemary is a natural antioxidant widely used for food conservation (Feng *et al.*, 2016), due to its antioxidant activity in meat and meat products, described in Table 1.

Plant extracts such as that from rosemary have highly active compounds but can lose their beneficial effects by oxygen exposure, heat, moisture, light, or during processing. Therefore, it is necessary to use specific methods to protect them and achieve the highest antioxidant activity, one possible way is by encapsulation that reaches incorporation through nanometric delivery systems (Rashidaie *et al.*, 2019; Duarte and Larroza, 2019). Nanoencapsulation is a technology focused on coating the active agent onto another

**Table 1.** Rosemary usage (*Salvia rosmarinus*) as a natural antioxidant in meat and meat products.

Study components	Objective	Effect	Reference
Rosemary extract (RE)	Effect on the quality and stability of ground chicken meat.	The TBARS values of RE (350 ppm) were significantly (P<0.05) lower than the control at day 7 of storage time.	Hijazeen and Rawashdeh, 2019.
Lyophilized Rosemary Extract (LRE)	Evaluate the effect of the use of LRE on the oxidative stability of pork sausages stored at -12 °C.	The lipid oxidation of sausage was significantly inhibited at 49 days of frozen storage with LRE compared to the control (47.28%).	Bianchin <i>et al.</i> , 2017.
Rosemary extract	Its quality in chicken breast was evaluated during 10 days of refrigerated storage.	The lipid oxidation of chicken breast was strongly inhibited by rosemary extract. TBARS values were significantly lower than control samples from 6 to 10 days (P<0.05).	Feng <i>et al.</i> , 2016.
Rosemary essential oil (REO) and modified-atmosphere packaging (MAP)	Effect on meat quality of poultry fillets during 7 days of refrigerated storage.	The addition of REO in combination with MAP reduced the level of lipid oxidation.	Kahraman <i>et al.</i> , 2015.
Rosemary essential oil (REO)	Determine the increase in shelf life of fresh Barbarine lamb's meat.	TBARS values significantly increased for both treatments (control and REO) with storage time without significant effect.	Smeti <i>et al.</i> , 2013.
Rosemary essential oil (REO)	Improves the lipid stability and sensory characteristics of chicken meat.	Addition of essential oils of rosemary at level of 200 mg/kg (P<0.05) reduced the TBARS and (P<0.05) increased the sensory scores of beef patties during frozen storage period.	Mohamed and Mansour, 2012.

material at a nano-scale of 1 to 100 nm sizes. Nanocarriers have been made from safe materials including biodegradable polymers, lipids and polysaccharides (Mohamed *et al.*, 2020; Boskovic *et al.*, 2019). Although using rosemary essential oil is limited due to its hydrophobicity, which prevents it from dissolving in aqueous phases of food. One possible way to incorporate essential oils is by nanoencapsulation, with this technique stability, protection and a controlled release increase. The bioavailability of essential oils reduces adverse impacts on the organoleptic properties of meat and meat products by preventing undesirable interactions with food components (Aminzare *et al.*, 2019; Boskovic *et al.*, 2019; Duarte and Larroza 2019). Food acceptability mainly depends on its sensory attributes, in the quality of meat and meat products is influenced by taste perception (Cao *et al.*, 2018). Rosemary extract encapsulation or rosemary oil increases the antioxidant properties and shelf life of meat during storage. Encapsulation of rosemary extract at 1600 ppm has shown better results for antioxidant properties and increases the shelf life of beef up to day 21 of storage (Rashidaie *et al.*, 2019). The use of rosemary extract nanocapsules as a dietary supplement in broilers has also shown beneficial effects on lipid profile and antioxidant status (Mohamed *et al.*, 2020).

## CONCLUSIONS

The process of lipid oxidation (LO) in meat and meat products decreases the consumer's health. To prevent or delay this process, it is important to know how the physiological activity between the formation of oxidants and the effect of antioxidants takes place. Rosemary is one of the most widely used natural antioxidants currently under research that have satisfactory results on LO inhibition. This plant is a safe and efficient alternative for meat preservation, its incorporation can be through new technological tools such as nanoencapsulation. However, further studies on the incorporation of rosemary extract or essential oil in nanocapsules are still necessary to establish its antioxidant potential and to understand its application.

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