

# Rosemary Oil: A Natural Biopreservative for Food Preservation

Kartikeyasingh V. Chauhan\*, Samruddhi N. Thakre, Nityam P. Bais, Dr. Swati Gaikwad, Madhav Korde, Dr. Vinod Thakare

**Abstract:** Consumer safety is a top priority in the food industry, driving demand for more safer and sustainable options. Thus, there has been a growing interest in natural preservatives. Rosemary (*Rosmarinus officinalis*) has gained attention due to its bioactive compounds, including rosmarinic acid, carnosic acid, and essential oils, which exhibit strong antioxidant and antimicrobial properties. However, its poor solubility limits its full potential. To enhance the solubility of rosemary's bioactive compounds, focusing on techniques such as nanoemulsions, surfactant incorporation, and the addition of lipid-soluble antioxidants for improving solubility and stability for better application in food preservation.

The Antioxidant activity assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], and H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide) assays, confirms rosemary's ability to scavenge free radicals and reduce oxidative damage. Additionally, it demonstrates antimicrobial effects against a broad spectrum of bacterial and fungal strains, proving its potential as a natural preservative. The synergistic action of rosemary's active compounds, facilitated by beta-cyclodextrin complexation, enhances its efficacy, ensuring prolonged shelf life and improved food quality.

The combination of these properties provides a better alternative to synthetic preservatives in food and also proves a sustainable alternative to chemical preservatives. Food preservation is vital for extending shelf life and maintaining nutritional quality. Rosemary offers a natural solution that aligns with growing consumer demand for safer, sustainable ingredients, which also ensures that the product enhances quality and safety.

The use of natural preservatives, emphasizes the need to replace synthetic formulations with herb base formulations to ensure both consumer satisfaction and long-term sustainability.

## INTRODUCTION

The use of natural, plant-derived bio-preservatives has become more popular as a safer alternative to synthetic preservatives in food products due to consumer's concerns about their toxicity. Rosemary oil extract, which comes from the aromatic plant

*Rosmarinus officinalis*, which belongs to the *Lamiaceae* family, is a popular natural preservative that is rich in antioxidants like phenolic compounds, vitamin E and C, and enzymes.[1] It has been shown to have antimicrobial activity against bacteria, yeast, and moulds, effectively preventing spoilage and extending the shelf life of food products. It has been extensively researched for its hepatoprotective, antiangiogenic, and possibly therapeutic effects in Alzheimer's disease, and it also has anti-inflammatory and antioxidant qualities[1][2].

Essential oil (EOs) are natural plant-derived molecules that have antibacterial, antioxidant, and anti-inflammatory effects, making them useful food preservatives.[3] They aid to prevent microbial contamination and degradation in a variety of food goods, including vegetables, dairy, fruits, and meat, so increasing shelf life and maintaining quality. Among these, rosemary oil extract is extensively utilized because of its high antioxidant content and antibacterial action.[4] Advances in encapsulation and nano-encapsulation technology have enhanced the stability, bioavailability, and regulated release of EOs in food systems. Furthermore, smart packaging developments improve EO-based preservation, giving it a viable natural alternative to synthetic preservatives for safer and more sustainable food storage.[5]

Numerous studies have been conducted on the antibacterial and antioxidant qualities of rosemary essential oil. Minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBC), time-kill assays were used to assess its antimicrobial efficacy against Gram-positive (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative (*Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*), and fungal (*Candida albicans*, *Aspergillus niger*). The essential Oil's antibacterial and antifungal properties were more potent than those of its main constituents, 1,8-cineole and  $\alpha$ -pinene.[6][7] Additionally, atomic force microscopy

(AFM) and flow cytometry (FCM) were used to evaluate the oil's effects on *S. aureus* in order to determine morphological alterations and survival rates.[8][9] Standard assay such as the ferric reducing antioxidant power (FRAP) assay, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging were used to evaluate the antioxidant capacity of rosemary essential oil.[10][11]

## METHODS AND MATERIAL

### Extraction of Rosemary oil

The vegetable material was put in a glass column, with the lower and upper sections attached to a condenser and a water flask, respectively, in order to extract the essential oil by steam distillation. Charged with essential oil, the water vapor generated in the flask travels through the plant and is condensed in the condenser. Decantation is used to separate the oil from the water following condensation. The same circumstances as steam distillation were used for the hydro-distillation method of essential oil extraction. The sole distinction is that the vegetable matter is spread out in the water-filled flask and the unit is brought to a boil in this instance. After being created in the flask, the water-oil vapor mixtures travels to the condenser to condense. Decantation results in the recovery of the oil.[12]

### Antimicrobial activity of Rosemary oil

*Escherichia coli*, *Salmonella marcescens*, *Staphylococcus aureus*, *Aspergillus flavus*, and *Candida albicans* were among the microorganisms against which the antimicrobial activity of Rosemary oil was assessed. In order to prepare the inoculum, fungi were cultivated for seven days in Sabouraud's dextrose broth, and bacterial strains were cultivated for 48 hours in nutritional broth. For bacterial and fungal cultures, respectively, solid media were nutrient agar and Sabouraud's dextrose agar. Using a sterile cork borer, 50 µL of Rosemary oil was added to each cavity created (5 mm in diameter) in the solidified agar. The antimicrobial activity of the plates was evaluated by measuring the diameter of the inhibition zone surrounding the cavities after they were incubated at 28°C for 48 hours for bacteria and up to 7 days for fungi. Rosemary oil was diluted in DMSO to create concentrations of 12.5%, 25%, 50%, and 100% in

order to calculate the minimal inhibitory concentrations (MIC), and inhibition zones were noted. A 6 mm inhibition zone and a 25% Rosemary oil concentration were used to determine the minimum inhibitory concentrations (MIC) for *E. coli*, *S. marcescens*, and *S. aureus*. While 100% Rosemary oil generated inhibition zones ranging from 7 to 8 mm. At 0, 4, 8, 12, and 16 days, microbiological analysis was carried out using the conventional plate count method. The total bacterial count (TBC) was assessed on nutritional agar, yeast on malt extract agar, moulds on potato dextrose agar, lactic acid bacteria (LAB) on MRS agar (incubated at 37°C for 48 hours), and coliform bacteria in MacConkey broth (incubated at 32 ± 1°C). Rosemary oil had antibacterial action against the investigated strains of bacteria and fungi, and thus its efficacy rose with concentrations.[13][14]

### Antioxidant activity of Rosemary Oil

The free radical scavenging capacity (RSC) of rosemary essential oil was measured in order to evaluate its antioxidant activity.[14] Its capacity to neutralise the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and contribute to electron led to this conclusion. In this test, a DPPH solution was combined with the essential oil and an α-tocopherol methanol solution (used as a positive control). A spectrophotometer set to 515 nm was used to measure the amount of the purple-coloured DPPH radical that remained after 60 minutes. The following formula was used to determine the free radical scavenging capacity:

$$\text{RSC (\%)} = 100 - (100 \times \text{A sample} \div \text{A blank})$$

Where A sample is the absorbance following the addition of the essential oil or reference component, and

A blank is the absorbance of the DPPH solution without the sample. Every experiment was carried out three times, and the average results were taken into account. Based on the observed RSC values, a linear regression analysis was performed to calculate the IC50 value or the concentration of the essential oil needed to block 50% of the DPPH radical. Microliters of essential oil per milliliter was the unit of measurement used to express the IC50. Furthermore, the Folin-Ciocalteu method was used to ascertain the total phenolic content of Rosemary oil. The Folin-Ciocalteu reagent, a sodium carbonate solution, and the essential oil were combined in a test tube for this assay. The absorbance at 740 nm

was measured following two hours of dark incubation and vortexing. Three separate estimates of the total phenolic content were made, and the results were reported as milligrams of gallic acid equivalents (GAE) per liter.[15]

#### Solubility of Rosemary oil

Rosemary oil is an essential oil extracted from the leaves of the rosemary plant (*Rosmarinus officinalis*). Known for its fresh, herbaceous scent, it offers a range of benefits, Rosemary oil is lipophilic, meaning it is soluble in oils but not in water.

To enhance the solubility of rosemary oil, several methods can be employed, primarily focusing on the use of emulsifiers and surfactants, as well as extraction techniques. Here are some-

1. Nanoemulsion is a new method that makes preparation more stable and helps them dissolve better (increased solubility). It's a useful way to create products with essential oils, which can be used to fight fungal infections.[16][17]
2. Adding surfactants like Tween 20, PG-10-LR, PG-10-MS, and PG-10-MO to rosemary oil helps it dissolve better in water (improves solubility). These surfactants work well because they have a longer hydrophobic tail, which helps mix the oil with water more effectively.[17]
3. The rosemary lipid-soluble antioxidant, the span 20, the Tween 80 and the caprylic/capric glyceride in powder form are stirred at the constant temperature of 60 °C for 30min and cooled to obtain the rosemary lipid-soluble antioxidant.[18]

#### Synergistic Activity

Numerous studies have examined the possible synergistic effect of rosemary extract with other natural antioxidants, but the results in the scientific literature are still equivocal. When rosemary oil extract is combined with other substances, some Table-1 Comparison with Synthetic Preservatives:

Sr. No.	Aspect	Rosemary Oil	Synthetic Preservative	Reference
1.	Source	Natural (Plant derived)	Chemical (Synthetic)	[3][4][5]
2.	Antioxidant Activity	High; effective in preventing oxidation	Varies; some are effective but may pose health risks	[3][4][5]
3.	Antimicrobial Activity	Effective against various pathogens	Effective but may lead to resistance or adverse effects	[3][4][5]
4.	Consumer acceptance	High; preferred for clean labels	Lower; increasing scrutiny over safety	[3][4][5]
5.	Safety profile	Generally recognised as	Safety concerns exist regarding	[3][4][5]

research shows no increased antioxidant activity, while other studies show a beneficial effect. As an example, Resurreccion and Reynolds discovered that the antioxidant efficacy of tocopherols and rosemary extract in meat products was not enhanced when they were added together. On the other hand, found Wong et al. found that elements in rosemary oil aid in  $\alpha$ -tocopherol regeneration, indicating that rosemary may be used in place of vitamin C to preserve the stability of vitamin E. Additional research by Wada and Fang, suggested that the reason for this synergistic action is that tocopheryl radical receive hydrogen atoms from rosemary extract. The observation by Fang and Wada, that  $\alpha$ -tocopherol and rosemary extract together demonstrated noticeably more antioxidant activity in a model fish system that when used separately lends credence to this notion. They also discovered that adding rosemary extract to  $\alpha$ -tocopherol keep it stable for an additional 10 days. Likewise, Lai et al. and Stoick et al. showed that sodium tripolyphosphate (STPP) and rosemary oleoresin (OR) interacted synergistically. In avoiding warmed-over flavor (WOF) in pre-cooked beef and pork meats, the OR/STPP combination was as BHT-BHA or STPP/TBHQ.[14][19]

The combination of rosemary extract and butylated hydroxyanisole has a synergistic antibacterial effect that was substantially more effective than either chemical alone in reducing the growth of *Escherichia coli* and *Staphylococcus aureus*. This synergy resulted in a 4.4to17 fold reduction in the needed concentrations of BHA while maintaining high antibacterial activity. These findings emphasize rosemary oil potential as a natural enhancer of synthetic preservatives, decreasing the requirement for high BHA levels while boosting food safety.[19]

		safe	long-term health effects	
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#### TYPES OF FOOD ARE MOST COMMONLY PRESERVED WITH ROSEMARY EXTRACT

1. Rosemary essential oil is widely incorporated into commercial food preservation formulations, extending the shelf life of snacks, baked goods, and nut-based products by preventing rancidity and stabilizing polyunsaturated fats (PUFAs).[20]
2. Marketed formulations containing *Rosmarinus officinalis* L. oil are commonly used as natural food seasonings to enhance flavor profiles.[21]
3. Rosemary essential oil-based additives are available for dairy products, such as cream cheese, where they help maintain quality by reducing acidity.[22]
4. Natural yogurt preservatives formulated with rosemary oil effectively extend shelf life and improve stability.[3]
5. The commercially available hair oils enriched with rosemary extract are promoted for their ability to support hair growth and reduce hair loss.[23]
6. The various food and beverage industries utilize rosemary-based preservatives, leveraging their proven antimicrobial and antioxidant properties to replace synthetic additives.[20]

#### LITERATURE OF SURVEY

Kamel et al. in 2022, investigated the potential of rosemary essential oil (REO) as a natural preservative in stirred-like yogurt (SLY). The study highlighted REO's antimicrobial activity against pathogens such as *E. coli*, *S. aureus*, *Salmonella marcescens*, and fungi including *A. flavus* and *C. albicans*. Phytochemical analysis revealed active compounds like camphor and eucalyptol, which contribute to REO's antioxidant and preservative properties. The study demonstrated that the addition of REO (0.5–0.7%) to SLY enhanced lactic acid bacteria development, delayed yeast growth, and eliminated molds and coliforms during storage. The sensory evaluations showed enhanced flavor and acceptability at 0.5%, establishing REO as a viable natural alternative to synthetic preservatives.[3]

Abed & et al. in 2021, evaluated the antibacterial properties of Rosemary volatile oil and Nisin A against *Bacillus cereus* isolated from canned meat products. The results showed that Rosemary volatile oil had a more significant inhibitory effect, with a 27

mm zone at 2000 µg/ml compared to Nisin A's 19 mm at 350 µg/ml. In the In vivo study rosemary volatile oil exhibited no adverse effects on mice, while Nisin A caused changes in liver and spleen weight. The study concluded that Rosemary volatile oil is a safer and more effective alternative to Nisin A for meat preservation.[4]

A study by Kačániová et al. in 2023, explored the antimicrobial effects of *Rosmarinus officinalis* essential oil (ROEO) in the vapor phase against various bacterial and yeast strains on fruits and vegetables like apples, pears, kohlrabi, and potatoes. The results revealed that ROEO effectively inhibited bacterial growth, with the highest concentration (500 µL L-1) showing the strongest activity against pathogens such as *Enterococcus faecalis*, *Salmonella enterica*, and *Yersinia enterocolitica*. The findings suggest that ROEO has potent antimicrobial properties, which could potentially be used to extend the shelf life of fresh fruits and vegetables and as a natural alternative to synthetic preservatives.[24]

A study by Raeisi et al. in 2016, investigated the use of sodium alginate active coating solutions incorporating natural antimicrobials, including nisin, cinnamon (*Cinnamomum zeylanicum*), and rosemary (*Rosmarinus officinalis*) essential oils (EOs), to preserve the microbial quality of chicken meat fillets during storage. The findings revealed that the combination of cinnamon and rosemary EOs in the alginate coating had the most significant effect on inhibiting microbial growth, including *Listeria monocytogenes*. The study concluded that these natural preservatives could effectively extend the shelf life of chicken meat, providing an eco-friendly alternative to synthetic preservatives.[25]

The study by Fernandes, Regiane Victória de Barros, et al., in 2017 explored the effect of microencapsulated rosemary essential oil (REO) using whey protein isolate (WPI) and inulin on Minas frescal cheese shelf life was evaluated. The microencapsulated REO, added at 0.5%, showed significant antimicrobial effects, reducing aerobic mesophilic microorganisms by 1.36 log cycles after 3 days and 0.73 log cycles after 15 days compared to control cheese. The microencapsulation process did not alter the chemical composition of the essential oil and maintained the cheese's acidity control. This study suggests that microencapsulated

rosemary essential oil could be an effective natural biopreservative for enhancing the shelf life and microbial stability of cheese.[26]

The study by Hassanzadazar et al. in 2019 investigated the antimicrobial effects of rosemary essential oil nanoemulsion against key foodborne pathogens. Their study highlighted the potential of REO nanoemulsion as a powerful natural antimicrobial agent, providing an effective solution for food safety. The study demonstrated that the nanoemulsion exhibited potent antibacterial and antifungal properties, significantly inhibiting the growth of common foodborne pathogens such as *E. coli*, *Salmonella*, and *Listeria monocytogenes*. The enhanced antimicrobial effect was attributed to the increased surface area of the nanoemulsion, which allowed better penetration of the essential oil into microbial cell membranes. The study highlighted the promising use of rosemary essential oil nanoemulsions in food preservation and their potential to reduce reliance on synthetic preservatives, thus promoting safer and healthier food options.[27]

#### CONCLUSION

This study highlights rosemary as a natural preservative as it has potent antioxidant and antimicrobial properties, because of its bioactive compounds like rosmarinic acid, carnosic acid, and carnosol. However, their poor solubility in water-based systems reduce their effectiveness in food preservation. To overcome this, methods such as nanoemulsions, surfactant incorporation, and the addition of lipid-soluble antioxidants are proposed to enhance the solubility and bioavailability of these compounds, improving their stability and ensuring a more release of their active properties.

The DPPH and H<sub>2</sub>O<sub>2</sub> tests reveal that enhancing rosemary's solubility retains its strong antioxidant activity, which supports its active ability to mitigate oxidative damage and extend the shelf life of food products. The rosemary also shows broad-spectrum antimicrobial activity against many different types of microbial strains, reinforcing its potential as a natural preservative. These solubility-enhancing techniques help maintain the stability and effectiveness of rosemary's active compounds, which ensures and proves reliable preservation. The adoption of natural methods like nanoemulsions and surfactants offers a sustainable alternative to synthetic preservatives,

which aligns with the growing consumer demand for clean-label and healthier food options for longer preservation. Future research should focus on optimizing these solubility-enhancing strategies, assessing their practical applications in food products, and evaluating the sensory impacts on taste, texture, and aroma to ensure consumer acceptance.

In conclusion, improving the solubility and stability of rosemary's bioactive compounds cause its full potential as a sustainable, natural preservative, providing an effective, eco-friendly alternative to artificial preservatives.

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