

Use of *Laurus nobilis* (Noble Bay) essential oil as a preservative for a meat product, Mortadella

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Article info

Received 28/7/2025; received in revised form 7/10/2025; accepted 1/12/2025

DOI: [10.60923/issn.2281-4485/22528](https://doi.org/10.60923/issn.2281-4485/22528)

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Abstract

Our objective is to find a natural preservative for a food product as an alternative to prohibited chemical substances. The essential oils from the leaves of *Laurus nobilis* harvested in the Relizane region were characterized by physicochemical methods followed by gas chromatography coupled with a mass spectrometer detector. The effectiveness of the extracted oils was tested against microbial strains by the agar diffusion method. Indeed, two doses were incorporated into the mortadella (5 µL and 10 µL). The extraction yield was equal to $0.95 \pm 0.45\%$, with values of refractive index (1.3138 ± 0.25), acid index (0.225 ± 0.001), saponification index (31.5 ± 0.005 (mg KOH/g), ester index (31.25 ± 0.05) and relative density (0.825 ± 0.001). Chromatographic analyses showed the predominance of eucalyptol (29.79%). The antimicrobial activity test of the essential oil showed a potent activity against *Staphylococcus aureus* (80 mm) and *Pseudomonas aeruginosa* (30 mm). At T20, no growth of total mesophilic aerobic flora and pathogenic germs was observed in samples containing Essential oils.

Keywords: Preservative, extraction, essential oils, *Laurus nobilis*, antimicrobial activity

Introduction

Food safety and quality are now major issues for the food industry, health authorities and consumers. Meat products, due to their high protein, lipid and water content, provide a favourable environment for microbial growth and lipid oxidation, two main factors in their deterioration (Tajkarimi et al, 2010). To overcome these problems, industrial processes generally rely on combined methods including cooking, refrigeration, vacuum packaging, and the addition of chemical additives such as nitrites, nitrates or synthetic antioxidants. However, the use of these preservatives is increasingly controversial, particularly nitrites, due to their potential

to form carcinogenic nitroso compounds, such as nitrosamines, during digestion or under the effect of heat (Sindelar & Milkowski, 2012). This situation has led to growing interest in the development of natural preservation strategies that are more respectful of human health and the environment. In this context, essential oils from aromatic plants appear to be a promising alternative. They are recognized for their antimicrobial and antioxidant properties, widely demonstrated against a wide range of microorganisms responsible for food spoilage (Burt, 2004; Hyldgaard et al., 2012). Among them, the essential oil of noble laurel (*Laurus nobilis*) is of particular interest. Native to the

Mediterranean basin, this medicinal and aromatic plant is traditionally used for its therapeutic properties, but also as a potential natural preservative in the food industry. Noble laurel essential oil contains several major bioactive compounds, including 1,8-cineole, eugenol, linalool, and terpenyl acetate, which give it broad-spectrum antimicrobial activity, in addition to its antioxidant capabilities (Basak and Candan, 2013; Giovanelli et al., 2020). Several researches have demonstrated its effectiveness against pathogenic bacteria common in meat products such as *Listeria monocytogenes*, *Staphylococcus aureus*, or *Escherichia coli* (Bouhdid et al., 2009 Djenane et al., 2012). The main objective of this study is therefore to evaluate the effectiveness of noble laurel essential oil in the microbiological and oxidative preservation of mortadella, by determining its impact on the shelf life, chemical stability, and sensory quality of the finished product. The initial hypothesis is that the controlled incorporation of this essential oil would limit the use of synthetic additives while ensuring the safety and quality of the product. This approach has a dual objective: to meet food safety requirements and promote local natural plant resources, while contributing to the development of natural and sustainable alternatives for the agri-food industry.

Materials and Methods

Plant material

On February 23, 2025, the leaves of *Laurus nobilis* were picked in dry weather, after the dew had been cleared away by the heat. The plants should not be wet, as it could result in fermentation or the development of harmful compounds after harvesting, in the Bourmadia region (Longitude: 0.57177° or 0°34'18" East, Latitude: 35.70937° or 35°42'34" North), the plant was authenticated by a botanist from the Relizane forest conservation service. Species were identified solely by their visible macroscopic characteristics.

Microbial Strains

The microbial strains used were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. The various strains were provided by the Ecchifa analytical laboratory in Chlef city and were regularly maintained by subculture on nutrient agar, which promotes their growth, for possible future use.

Extraction of essential oils by hydrodistillation

The extraction of essential oils was carried out according to the protocol of Boukhennoufa et al., (2019).

Characterization of essential oils by CPG-MS

The chemical composition of essential oils was determined using a Hewlett Packard Agilent 6890 gas chromatograph coupled with a Hewlett Packard Agilent 5973 Quadrupole mass spectrometer detector with an analysis time of 120 min. The analyses were carried out in electron impact ionization (EI) mode with an ionization energy of 70 eV and with a HP-5 ms capillary column (Length: 30m, internal diameter: 0.25 mm, film thickness: 0.25µm). However, the stationary phase: 5% Phenyl and 95% dimethylpolysiloxane. The injections were made in split mode with 0.2 µL of essential oil volume. The chromatogram acquisition was in SCAN mode. In addition, helium was used as a carrier gas at a flow rate of 0.5 mL/min. The temperature of the ion sources and interfaces were set between 230°C and 270°C respectively. The solvent delay time was 3 min. The column temperature was programmed at 45°C for 8 min, 2°C/min up to 250°C. Mass spectra were recorded over a range of 35 to 400 m/z at a scan of 0.3 s/scan. Indeed. The compounds of the analyzed essential oils corresponding to the major peaks of each chromatogram were identified by comparing their retention times (RT) and their mass spectra with those cited in the literature and given by spectral libraries.

Physicochemical characterization

The quality and value of an essential oil were defined by accepted standards relating to physicochemical indices. The acid value was determined according to the NFV 03-906 standard (AFNOR, 1984), and the saponification value according to the NF T 60-206 standard (AFNOR, 1984). In practice, the ester value was not measured experimentally, but rather was deduced by taking the difference between the saponification value and the acid value. The refractive index (Denis et al., 1997) and the relative density at 20°C (Novidzro et al., 2019).

Testing the antimicrobial activity of essential oils using the agar diffusion method

Antimicrobial activity was assessed using the agar diffusion method in agar wells (Brahimi et al., 2020). A product was considered active if the diameter of the inhibition zone was greater than 8 mm (Ela et al., 1996).

Mortadella preparation

For the mortadella composition: Chicken = 3250 g, Rice = 250 g, Salt = 5 g, Black pepper = 7.5 g, Parsley

and garlic = 7.5 g. After mixing the ingredients, the total quantity was divided into three samples: Sample 1 = Control, Sample 2 = containing 50 μ L, Sample 3 = containing 100 μ L.

Microbiological analysis of Mortadella

Five germs must be identified : total germs (NF V 08-017), fecal coliforms (NF V08-01), *Staphylococcus aureus* (ISO 6888), *Clostridium sulfitoreductosa* (ISO 6649), and *Salmonella* (ISO 6579, 2002).

Results

Characterization of essential oils by GC-MS

The extraction yield was $0.95 \pm 0.45\%$. Chromatographic analysis of this oil showed the predominance of eucalyptol (29.97%), followed by α -terpinyl acetate (13.81%) and methyleugenol (12.49%), for a total of 92.35%. The chemical composition was grouped in Table 1.

Physicochemical parameters of essential oils

The main physicochemical parameters of the extracted essential oils are presented in Table 2.

Antimicrobial activity atudy using the agar diffusion method

This test demonstrates potent antibacterial activity of the essential oil against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with inhibition diameters of 80 mm and 30 mm, respectively. However, no antifungal activity was observed against *Candida albicans* (Fig. 1).

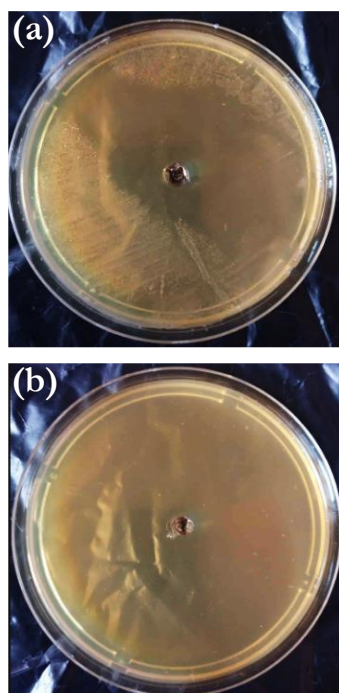


Figure 1
Antimicrobial activity
of EO against
Staphylococcus aureus (a)
and *Candida albicans* (b)

Table 1. Chemical composition of *Laurus nobilis* essential oil

N°	RT (min)	KI	Compounds	%
1	9.75	843	Ethyl 2-methylbutanoate	0.01
2	14.87	923	α -Thujene	0.50
3	15.3	928	α -pinene	2.51
4	16.25	941	Camphene	0.46
5	18.31	969	Sabinene	5.94
6	18.60	973	β -pinene	1.04
7	19.59	986	2,3-Dehydro-1,8-cineole	0.08
8	19.85	990	β -Myrcene	0.51
9	20.65	1001	α -Phellandrene	0.10
10	20.81	1003	2-Methyl-1-propyl 2-methylbutyrate	0.09
11	21.19	1008	δ -3-Carene	1.62
12	21.73	1014	α -Terpinene	0.32
13	22.34	1022	p-Cymene	0.24
14	23.31	1035	Eucalyptol*	29.97
15	24.97	1056	γ -Terpinene	0.74
16	25.59	1064	Cis-Sabinene hydrate	0.69
17	27.12	1083	α -Terpinolene	0.44
18	27.94	1094	Trans-Sabinene hydrate	0.53
19	28.4	1099	Linalool	2.34
20	29.64	1116	Cis-p-Menth-2-ene-1-ol	0.19
21	31.02	1135	Trans-p-Menth-2-en-1-ol	0.13
22	31.98	1148	Menthone	0.43
23	32.72	1158	Pinocarvone	0.04
24	33.2	1165	γ -Terpineol	0.73
25	33.84	1173	Terpinen-4-ol	3.11
26	34.93	1188	α -Terpineol	3.21
27	35.36	1194	Estragole	0.15
28	35.94	1202	3-isopropenyl-2-methylcyclohexanol	0.04
29	38.19	1234	Pulegone	1.37
30	38.39	1237	β -Citral	0.10
31	40.47	1267	Geranial	0.09
32	40.76	1271	4-Thujen-2- α -yl acetate	0.17
33	41.4	1281	Bornyl acetate	0.76
34	43.85	1313	γ -Terpineol acetate	1.20
35	46	1350	α-Terpinyl acetate	13.81
36	46.53	1358	Eugenol	2.17
37	49.65	1406	Methyleugenol	12.49
38	50.15	1414	Trans-crayophyllene	0.37
39	51.79	1441	(E)-Cinnamyl acetate	0.10
40	52.07	1447	α -Humulene	0.15
41	58.68	1557	Elemicin	1.39
42	59.67	1573	Spathulenol	1.05
43	63.98	1649	α -Eudesmol	0.97

*Main compound

Table 2. Physicochemical parameters of the extracted oils

	Value
Refraction index	1.3138 ± 0.25
Acid index	0.225 ± 0.001
Saponification index	31.5 ± 0.005 (mg KOH/g)
Ester Index	31.25 ± 0.05
Relative density	0.825 ± 0.01

Testing of microbiological application

Organoleptic characteristics. At T0, the samples exhibited a firm, uniform texture, a characteristic mortadella color, and a very pleasant odor similar to that of commercial products. After twenty days of preparation, the control sample appeared to have completely lost its

color, odor, and texture, which became somewhat rigid with the release of a foul odor. At the same time, the essential oils incorporated into the samples partially delayed the loss of color characteristic of mortadella, but much more so at the 10 μ L dose. However, no unpleasant odor was observed in these preparations.



Figure 2. Photos of mortadella prepared at T= 20. (a) Control (without essential oil), (b) sample containing 5 μ L, (c) sample containing 10 μ L.

Determination of microbiological quality

At T0 and T10, no growth of TMAF was observed in all preparations including the control. At T20, the TMAF count revealed that the samples containing the HE (5 μ L and 10 μ L) preserved their microbiological quality compared to the control (1200 CFU/g). After inoculating the samples on VF, SS and Chapman medium, no growth of germs was revealed up to T20 in the preparations incorporated by HE. On the other hand, a total absence of fecal coliforms was found at T0 and T10 in all the prepared samples. However, at T20 an alteration of the control was marked which results in a number equal to 6700 CFU/g.

Discussion

The average yield of essential oil from *Laurus nobilis* L. from Morocco extends to 1.7%. This is not the same relative to other yields found in other research conducted on species of different geography (Ouled Taarabt et al., 2017). Our refractive index was lower than the results observed oil 1.46819 (Ouled Taarabt et al., 2017). The refractive index is a measure of the essential oil's ability to deflect light and is a crucial parameter for determining the purity of the oil. A high refractive index may indicate the presence of high-quality essential oils, while a low index could suggest impurities or diluents (Wagner and Bladt, 1996). Acid index is useful for quality control in essential processing industries. Ouled Taarabt et al. (2017) found a higher acid value

compared to our results (2.5 mg KOH/g of fat). A high acid value can mean a more volatile or less stable oil, while a low value indicates a more stable oil (Cowan, 1999). The saponification value measures the amount of base required to saponify one gram of oil. This value is essential for determining the properties of the oil in soap or cosmetic manufacturing processes (Burt, 2004). A high ester value suggests a better quality oil, with greater reactive capacity in chemical processes (Rao et al., 2017). This value is important for classifying essential oils according to their chemical properties and their potential in various industrial applications. According to AFNOR (2000), the density of essential oils is generally lower than that of water. Our result is slightly close to those found by other authors: 0.9121 and 0.9043 for the same essential oil (Ouled Taarabt et al., 2017). The EO strongly inhibits the growth of *Salmonella* spp with an inhibition diameter greater than 20 mm (Ould Yerou et al., 2015). The antibacterial activity of essential oil appears to include an alteration of the bacterial membrane that occurs when this substance passes through the cell wall and cytoplasmic membrane, and disrupts the structure of their different layers of polysaccharides, fatty acids and phospholipids. In general, the antimicrobial activities have been explained mainly by C10 and C15 capable of forming covalent bonds with the active sites of the target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters contribute to the antimicrobial activity of essential oils (Belletti et al., 2004).

Conclusions

This research aimed at extracting essential oils from *Laurus nobilis* and incorporating it as a natural preservative in a meat product (Mortadella). The plant was characterized by its richness in chemical compound and their physicochemical parameters close to the standards. A powerful antibacterial activity of the essential oil was observed against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. After twenty days of preparation, it appeared that the sample containing the essential oils has preserved its color, odor, texture and total absence of the growth of pathogenic germs.

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