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Original research paper

USE OF ESSENTIAL OILS FOR FOOD SAFETY: FORMULATIONS OF EASY-TO-USE FOOD-GRADE SPRAY SANITIZERS

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Abstract: Motivated by combat against the spread of foodborne diseases, the formulations of simple and easy-to-use spray disinfectants containing *Cinnamomum zeylanicum* and *Ocimum gratissimum* essential oils were studied. Central composite experimental design was used in the development of stable products with optimal essential oil content for antimicrobial efficacy. It also allowed minimizing the alcohol content which was used to ensure miscibility between the essential oils and the aqueous phase. *Trans*-cinnamaldehyde was the major compound in the *C. zeylanicum* essential oil. Its low toxicity allowed the formulation of a risk-free product despite the great variability in the chemical composition of the essential oil. Cooked food coated with a spray containing 1% of this essential oil, in combination with *Thymus vulgaris* tincture, could be preserved for 3 days without refrigeration. Eugenol was the major component of *O. gratissimum* essential oil, followed by 1,8- cineole and methylchavicol. Sprays containing 1.5% of this essential oil were effective against *Escherichia coli* and *Staphylococcus aureus*. Xanthan gum proved to be a good essential oil-trapping agent under accelerated ageing conditions at 45 °C.

Key words: Cinnamomum zeylanicum, Ocimum gratissimum, disinfectant, food preservation, surface cleaner, hand antiseptic

INTRODUCTION

The increasing resistance of microorganisms to antimicrobial chemicals is a global concern of the third millennium. The agri-food industry is one of the sectors facing this challenge. Every year 600 million foodborne illnesses are reported worldwide. Foodborne diseases are the cause of 7.69% of the world's annual deaths (Lee & Yoon, 2021). Street food has become a growing habit for people living in big cities. Foods sold on the streets are part of a country's cuisine culture (Sezgin & Sanlier, 2016). In low- and middle-income countries, street foods

have gained socioeconomic interest in recent decades. On the one side, they contribute to attracting tourists by offering the possibility to promote and preserve cuisine culture. On the other side, they offer to local population, lowcost food with better nutritional quality in terms of protein and energy (Alfiero, Lo Giudice, & Bonadonna, 2017). The lack of good hygiene practices in street food also contributes to an outbreak of foodborne diseases (Gargiulo et al., 2022).

A multidisciplinary approach has been proposed by some scientists to combat the continuing spread and persistence of multidrug-resistant strains (Aslam et al., 2018). The use of essential oils for food safety represents a promising solution for controlling multidrug-resistant microorganisms along the food processing chain (Maurya, Prasad, Das & Dwivedy, 2021). Compared to conventional antimicrobials, essential oils are reported to be more natural, safer, environmentally friendly and better suited for use in food storage and preservation (Pandey, Kumar, Singh, Tripathi & Bajpai, 2017).

Spices and herbs improve the aroma and flavour of food while simultaneously providing some health benefits (Rafique, Baig, Mehboob, Zahid, & Irfan, 2022). *Cinnamomum zeylanicum* essential oil has demonstrated effective antimicrobial activities against strains responsible for food spoilage and infectious diseases. A recently conducted study revealed that its minimum inhibitory concentration ranged from 0.078 mg/mL to 1.25 mg/mL (Tomičić, Tomičić, Kocić-Tanackov & Raspor, 2022). *Ocimum gratissimum* essential oil has also been attributed to good activity against multidrugresistant isolates for foodborne and infectious diseases (Melo *et al.*, 2019).

Thymus vulgaris ethanol extract has demonstrated a very good antioxidant capacity and an antibacterial effect (Akin, & Saki, 2019). These two qualities are required to preserve food from rancidity and microbiological degradation. For this reason, it was associated with *C. zeylanicum* essential oil in this study.

Therefore, the present study aimed to formulate two eco-friendly disinfectants based on essential oils intended for utilisation on hand and food contact surfaces. A spray formulation was chosen due to easier utilisation, mainly for fast-food restaurants and street food vendors. The third spray product formulation was intended for direct use on cooked food to extend its shelf life.

MATERIALS AND METHODS

Materials

Thymus vulgaris (thyme) was grown in a suburb of the capital city of Antananarivo. The aerial parts were carefully washed, dried at a temperature of 50 °C for 2 h and then ground. The hydroalcoholic extract of the dried powder was prepared as described by Olah *et al.* (2017) with slight modifications. Briefly, 30 g of the powder was macerated in 300 mL of a mixture of water and ethanol in the ratio 3:7 at ambient temperature. After 15 days, the mother tincture was recuperated through filtration.

Cinnamomum zeylanicum bark (cinnamon) was harvested on the east coast of Madagascar, near Ambalatenina village. *Ocimum gratissimum* leaves (African basil) were collected in the central highlands, near the capital Antananarivo. The essential oils were distilled using a Clevenger apparatus. The extraction time was 5 h for *C. zeylanicum* bark and 3h 30 min for *O. gratissimum*.

Pharmaceutical grade ethanol was purchased from ITW Reagents, PanReacAppliChem, Milano, Italy. Citric acid, white vinegar and baking soda were food-grade of brand Leader Price, France. Xanthan gum was imported from Shaanxi Rainwood Biotech Co. Ltd, China.

Essential oil analysis

The density of essential oil at 20 °C was measured with a pycnometer. The refractive index was measured with an Abbe refractometer according to the French Standard NFT 75 – 112 (AFNOR, 1992). Rotary Index was determined as described by the French standard NFT 75 – 113 (AFNOR, 1992). The apparatus was a Jobin Yvon Laurent (France) polarimeter with a tube of 100 mm length.

The volatiles were identified using a Girdel 300 series gas chromatography equipped with a flame ionization detector. The injection temperature was set at 250 °C. The detection temperature was also 250 °C. The capillary column was a DB-WAX with 0.32 mm internal diameter and 30 m length. Temperature programming was set from 60 °C to 220 °C with a gradient of 3 °C/min. Hydrogen was used as a carrier gas in constant flow mode (60 mL/min).

The identification of the components was done by calculating the retention index (RI) using a series of C8-C22 n-alkanes in comparison with local database and with the online NIST Database (NIST WebBook SRD 69).

Experimental design

In spray product formulations, the excipient was water. Most of the ingredients were water soluble. However, the main active ingredients i.e. the essential oils were not soluble. In order to obtain a miscible mixture alcohol was added. The main difficulty in the formulation was the correct ratio between the aqueous phase, the alcohol and the essential oil, taking into account the amount of essential oil required for optimal antimicrobial effectiveness. On the other hand, the alcohol concentration should be minimized to avoid side effects on the skin or through penetration (Himabindu, Tanish, Kumari & Nayab, 2020). The experimental design enabled the correct adjustment of these parameters.

The experimental design was based on a previous formulation study, with the necessary modifications (Llinares, Santos, Trujillo-Cayado, Ramírez, & Muñoz, 2018). A central composite model with three points was chosen. Each experiment was carried out in triplicate. The variables selected were the relative proportions of alcohol, aqueous phase and essential oil. The response variable was the stability of the formulation, evaluated through the miscibility of the mixtures. The design table for the formulation of the food spray, the hand-sani tizing spray, and the surface-cleaner spray are presented in Table 1. To formulate the foodpreservative spray, the required amount of citric acid was first diluted in distilled water to prepare Mixture 1. Thyme extract was mixed with alcohol and essential oil added before stirring for 3 minutes to form Mixture 2. The two mixtures were then added and homogenized for 3 minutes. The hand-sanitizing spray was only a mixture of water, alcohol and essential oil. For the surface cleaner spray, baking soda was added to a mixture of water and vinegar and stirred for 3 minutes. Xanthan gum was then added and the mixture was heated to 55 °C for 10 minutes. After cooling, the essential oil, previously diluted in alcohol was added, and the whole was stirred for another 10 minutes.

Preference test of the food-preservative spray

The ranking test was performed according to Sharif, Butt, Sharif and Nasir (2017). The home-use method was chosen. Sixty five untrained panellists, males and females, of various ages participated in the study. Sprays containing 0.5%, 1% and 1.5% essential oil were assigned a 3 digit code.

Participants were asked to rate these samples, on a scale from 1 to 5 with 1 being very bad and 5 very good. As these were untrained panellists, no description of odour, taste or appearance was particularly required. All they had to do was express their preferences based on all these characteristics combined.

Table 1.

Experiment designs	s of spray	formulations.
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	Food-preservative spray			Hand	-sanitizing	spray	Surfa	Surface-cleaner spray		
	Aqueous	Alcohol	EO	Aqueous	Alcohol	EO	Aqueous	Alcohol	EO	
	phase	(g)	(mass %)	phase	(g)	(mass %)	phase	(g)	(mass %)	
Run	(g)			(g)			(g)			
1	80.00	1.00	0.50	80.00	1.00	2.00	90.00	1.00	1.00	
2	80.00	1.00	1.50	80.00	1.00	5.00	90.00	1.00	5.00	
3	80.00	10.00	0.50	80.00	10.00	2.00	90.00	15.00	1.00	
4	80.00	10.00	1.50	80.00	10.00	5.00	90.00	15.00	5.00	
5	90.00	1.00	0.50	90.00	1.00	2.00	100.00	1.00	1.00	
6	90.00	1.00	1.50	90.00	1.00	5.00	100.00	1.00	5.00	
7	90.00	10.00	0.50	90.00	10.00	2.00	100.00	15.00	1.00	
8	90.00	10.00	1.50	90.00	10.00	5.00	100.00	15.00	5.00	
9	76.59	5.50	1.00	76.59	5.50	3.50	86.59	8.00	3.00	
10	93.40	5.50	1.00	93.40	5.50	3.50	103.40	8.00	3.00	
11	85.00	2.06	1.00	85.00	2.06	3.50	95.00	3.77	3.00	
12	85.00	13.06	1.00	85.00	13.06	3.5	95.00	19.77	3.00	
13	85.00	5.50	0.15	85.00	5.50	0.97	95.00	8.00	0.36	
14	85.00	5.50	1.84	85.00	5.50	6.02	95.00	8.00	6.36	
15	85.00	5.50	1.00	85.00	5.50	3.50	95.00	8.00	3.00	

EO-essential oil phase

Efficacy test of the food-preservative spray

The food preservative spray was applied three times to coat the surface of previously prepared pasta dishes. Good hygiene practice was respected for their preparation (Kamboj, Gupta, Bandral, Gandotra & Anjum, 2020). Thereafter, plates were stored under two different conditions: at room temperature and in a refrigerator. Microbiological testing was carried out after 3 days (72h). From each plate, 10 g of samples were taken and diluted with buffered peptone water for microbiological testing.

The enumeration of the total aerobic mesophilic flora (TAMF) was performed according to ISO 4833-1 (2013). Plate Count Agar (Himedia, Dinhori, Nashik India) was used as a preprepared medium. The counting of the total coliforms (TC) was carried out according to ISO 4832 (2006) using Violet Red Bile Agar (Himedia, Dinhori, Nashik India). The fungal flora enumeration was carried out according to ISO 21527-1 (2008) using Sabouraud Chloramphenicol Dextrose Agar (Condalab, Madrid, Spain).

Efficacy test of the surface-cleaner and the hand sprays

Before formulating the final spray products, the antimicrobial activity of the essential oil of O. *gratissimum* was evaluated. After formulation, the sprays were divided into two samples. The first sample was kept at ambient temperature. The second sample was subjected to an accelerated ageing test at 45 °C according to the Guidelines on stability testing by CTFA & COLIPA (2004). The efficacy tests of these samples were carried out 2 and 6 weeks after formulation.

All tests in this study were performed using clinical strains of Escherichia coli and Staphylococcus aureus provided by the Microbiological Department of The National Centre for Environmental Research (CNRE) in collaboration with CHU HJRA Hospital, Antananarivo, Madagascar. The bacterial suspensions were prepared using overnight cultures and adjusted to 0.5 McFarland standard turbidity. The final values of inoculum were adjusted to 5 x 10^6 CFU/mL. The test was performed in 96 well plates using resazurin microdilution assay as described by Elshikh et al. (2016) with modifications. Resazurin (Sigma Aldrich, Saint Louis, Missouri, United States) was prepared by dissolving 0.01 g of powder in 10 ml of distilled

water. The solution was filter-sterilized through a 0.22 μ m filter and stored at 4 °C until use. A sterile 96-well plate was labelled A to H for the lines and 1 to 12 for the columns. Lines A to F were filled with 80 μ L of Mueller Hinton Broth (MHB, Himedia, Dinhori, Nashik India) and 10 μ L of resazurin. A quantity of 10 μ L of inoculum of *E. coli* was added in lines A to C and the same quantity of inoculum of *S.aureus* was added in lines D to F. 130 μ L of MHB with 10 μ L of resazurin and 10 μ L of inoculum were added to the wells of line G as a positive growth indicator. Line H was filled with a mixture of resazurin and MHB as a negative growth indicator.

The essential oil of O. gratissimum was diluted in DMSO (Merck MQ100) in the proportion of 75% essential oil in 25% solvent (v/v). Further dilutions were made by adding MHB to this stock solution to prepare a decreasing stock concentration of essential oil of 90, 82.5, 75, 67.5, 60, etc., down to 7.50 μ g/mL. From these solutions, 50 µL were added to lines A to F in triplicate. The final concentrations of essential oil ranged from 30.00 (column 1) to 2.50 (column 12) µg/mL. Plates were incubated for 18 h at 37 °C, and columns with a blue colour indicated that concentrations were above the Minimum Inhibitory value. The content of the well with the minimum concentration of essential oil in this range was subcultured in a petri dish with Plate Count Agar medium (Himedia, Dinhori, Nashik India) with further dilutions. The minimum inhibitory value was determined as the concentration that allows colony growth after incubation at 37 °C for 24 h. The minimum bactericide value referred to the concentration at which inhibition of colony growth was confirmed after incubation.

For the spray efficacy test, the same quantity of MHB, inoculum, and resazurin was filled as previously described for the essential oil. Columns 1 to 3 contained 50 μ L of the hand spray, which was stored at room temperature with 1.5%, 2.5%, and 3.5% of essential oil in triplicate. Columns 4 to 6 contained 50 μ L of the corresponding products stored at 45 °C. The same operations were performed with the surface cleaner in columns 7 to 12. After 18 h of incubation at 37 °C, subculturing in a petri dish containing a Plate Count Agar medium (Himedia, Dinhori, Nashik India) was carried out. The presence or absence of a colony was observed after incubation at 37 °C for 24 h.

Parameters	<i>Cinnamomum zeylanicum</i> bark essential oil	<i>Ocimum gratissimum</i> leaves essential oil
Appearance	Mobile liquid	Mobile liquid
Colour	Yellow to dark yellow	Pale yellow
Odour	Characteristic	Spicy, clove like
Specific gravity at 20 °C	1.035 ± 0.002	0.9862 ± 0.002
Refractive index	1.5671 ± 0.002	1.5235 ± 0.002
Rotary index	-3.25 ± 0.01	-14.6 ± 0.01

 Table 2.

 Physico-chemical characteristics of the selected essential oils

Statistical analysis

The experimental design was carried out using Chemoface 1.6, free software.

Statistical analysis for the ranking test of the sprays containing 0.5%, 1% and 1.5% essential oil was performed using JASP 0.15.0.0 software (Love et al., 2019). Descriptive analysis was conducted to compare the scores attributed to each sample by the 65 participants. Dot plots were used to analyse the variation in the results.

RESULTS AND DISCUSSION

Physico-chemical quality of essential oils

The physico-chemical characteristics of *C. zeylanicum* bark and *O. gratissimum* leaves essential oils are summarized in Table 2.

C. zeylanicum bark essential oil characteristics are consistent with commercially available oils (NHR Organic oils, 2017; Eden Botanicals, 2017). The results for *O. gratissimum* essential oil are comparable to that of Vietnamese essential oil which has a specific gravity of 0.945 and a refractive index of 1.526 (Huong *et al.*, 2020). However, for the latter, the rotary power is +15.6. For the essential oil from Tanzania, the values are very different with a specific gravity of 0.87 and an optical rotation of -110.0, only the refractive index was similar: 1.516 (Malima, Massaga, Malecela & Andrew, 2013).

Chemical composition of the essential oils

The chemical composition of *C. zeylanicum* bark essential oil and its comparison with literature data are presented in Table 3. To highlight the variability of *C. zeylanicum* essential oil composition, our samples were compared to essential oils of samples from two other locations on the Eastern coast of Madagascar, reported elsewhere. The tested sample originated from Ambalatenina. The two other locations were East Fenerive and East Ilaka (Randriamampionona, 2010). Literature data on composition of cinnamon essential oil from Iran

(Alizadeh Behbahani, Falah, Lavi Arab, Vasiee & Tabatabaee Yazdi, 2020), Turkey (Unlu, Ergene, Unlu, Zeytinoglu & Vural, 2010) and Sri Lanka (Paranagama et al., 2001) were also included in Table 3.

The essential oil studied contained 79.22% oxygenated terpenes and 13.85% terpene hydrocarbons. Trans-cinnamaldehyde is the major constituent of all the essential oils (Table 3) with relative proportions ranging from 50% to 80%. There was a significant difference in the relative proportions of the compounds of C. zeylanicum essential oils from the same Eastern Madagascan region. Eugenol content varied from 0.3% to 7.6%. Essential oil from Sri Lanka contained compounds that are non-existent in oils from other locations, like coumarin and alpha ylangene. Trans-cinnamaldehyde is endowed with antibacterial (Firmino et al., 2018), antifungal (Shreaz et al., 2016), acaricidal (Nwanade et al., 2021) and anti-inflammatory activities. WHO suggests an acceptable daily intake of 0.7 mg/kg body for cinnamaldehyde. A study reported that an intake of 400 mg daily during a clinical trial resulted in no major side effects (Gunawardena et al., 2015). The very low toxicity of this essential oil allows an easy formulation of the food spray despite the high variability of cinnamaldehyde, without a major risk of side effects.

The chemical composition of *O. gratissimum* leaves essential oil originated from Madagascar revealed that it consisted of 92.82% oxygenated terpenes and 3.34% hydrocarbon terpenes (Table 4). This sample was compared with essential oils reported from Brazil (Mohr, Lermen, Gazim, Gonçalves & Alberton 2017; Melo et al., 2019) and Kenya (Matasyoh et al., 2007). Brazilian samples presented two chemotypes: linalool / 1,8-cineole chemotype and eugenol chemotype. This latter is comparable to the Madagascar and Kenya samples. Eugenol is a broad-spectrum antimicrobial agent which is active for both gram-positive and gram-negative strains. It can be used for pharmaceuticals, food preservation and cosmetics (Marchese et al., 2017). It is well known as an anaesthetic, antioxidant and anti-inflammatory agent. In a formulation of hand-sanitizing spray, it should be noted that eugenol is a skin penetration enhancer.

Table 3.

Chemical composition of C. zeylanicum bark essential oil

	T	his stud	ły	Madagascar Fenerive Est ^c	Madagascar Ilaka Est ^c	Iran Mashhad ^d	Turkey ^e	Sri Lanka
Constituents	RI ^a	RI ^b	Area (%)	Area (%)	Area (%)	Area (%)	Area (%)	Area (%)
α pinene	1012	1015	1.30	0.56-2.51	0.67-1.57	1.30	1.64	
α thujene	1019	1022	0.22	0.00-0.06	0.00-0.35			
3 pinene	1101	1096	0.30	0.14-0.50	0.20-0.36			
penzaldehyde						0.30	9.94	0.61
α phellandrene	1160	1158	0.50	0.25-5.73	0.57-3.17			
a terpinene	1170	1172	0.67	0.20-2.62	0.43-2.14			
imonene	1195	1189	1.10	0.32-1.34	0.51-1.33	1.20	4.42	
x humulene				0.10-0.29	0.18-0.37	1.70		1.30
a copaene				0.00-0.07	0.00-0.13			
3 phellandrene	1201	1158	3.80	1.19-8.81	2.31-7.65			
l,8-cineole	1201	1211	0.15	1.17 0.01	2.51 7.05	5.40	1.55	4.60
soborneol	1205	1211	0.15			0.80	1.55	4.00
$rans-\beta$ ocimene	1233	1250	0.05	0.00-0.09	0.04-0.54	0.00		
o cymene	1233	1230	3.61	0.69-3.25	1.04-1.76	1.90		
erpinolene	1270	12/2	5.01	0.00-0.31	0.05-0.23	1.90		
cinnamyl alcohol				0.00-0.31	0.03-0.23			0.16
ohenyl ethylacohol								0.47
coumarin								0.36
x ylangene								0.70
nydrocinnamaldehyde				0 00 0 00	0.04.0.21			0.80
abinene				0.00-0.23	0.04-0.31			
5 elemene				0.00-0.07	0.00-0.04			
rans-calamenene						0.70		
3-carene				0.00-0.19	0.00-0.12			
2-phenyl ethylacetate								0.18
3-phenyl propylacetate								0.38
5 cadinene						1.40		
<i>cis</i> -β ocimene				0.00-0.14	0.03-0.10			
camphene				0.17-0.71	0.25-0.38			
3 selinene				0.00-0.20	0.00-0.52			
3 elemene				0.00-0.14	0.00-0.17			
inalool	1541	1552	3.00	1.13-4.40	1.38-3.84	7.00	1.38	
3 caryophyllene	1579	1576	2.30	0.57-1.53	1.03-2.06	6.40		8.00
erpinen-4-ol	1590	1635	0.91	0.16-1.10	0.24-0.78			
a terpineol	1650	1680	1.20	0.23-0.76	0.39-0.97			
v terpinene				0.05-0.23	0.03-0.26	0.40		
geranyle acetate	1729	1731	0.20	0.00-0.13	0.00-0.06			
nerol				0.00-0.37	0.00-0.06			
geraniol	1835	1851	0.04	0.00-0.04	0.00-0.09			
safrol	1869	1872	0.07	0.00-0.04	0.00-0.20			
aryophyllene oxide	1950	1953	0.07	0.07-0.22	0.17-0.26	0.50		
<i>rans</i> -cinnamaldehyde	2021	2025	58.40	56-71-80.97	54.63-67.04	71.50	68.95	50.50
nethyl cinnamate	2021	2023	50.40	50-71-00.97	57.05-07.04	/1.50	00.75	0.27
	2135	2141	4.65	0.28-5.42	0.62-7.65	4.60	2.77	4.15
eugenol					0.02-7.03 6.40-26.13		2.77 7.44	
cinnamyl acetate	2146	2153	8.54	1.48-13.49	0.40-20.13	0.50		8.78
cinnamic acid							1.15	0.15
nethyl isoeugenol								0.15
eugenyl acetate	0.5.40	0(0)	1.65	0.15.0.00	0.00010	0.50		0.40
penzyl benzoate Experimental retention i	2563	2636	1.65	0.15-2.99	0.62-2.16	0.50		1.10

^aExperimental retention indices ; ^bReference values according to online NIST database (NIST WebBook SRD 69); ^cRandriamampionona, 2010; ^dAlizadeh Behbahani et al., 2020; ^eUnlu, et al., 2010; ^f Paranagama et al. 2001

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		This study		Brazil (1) ^c	Brazil (2) ^d	Kenya ^e	
Constituents	RI^{a}	RI ^b	Area	Area	Area	Area	
			(%)	(%)	(%)	(%)	
α pinene	1011	1015	0.09	0.53	0.08		
α thujene	1017	1022	t				
Sabinene				0.60	0.17		
β pinene	1093	1096	0.15	1.25	0.43	1.10	
thuyadiene	1149	ND	0.12				
myrcene				0.83	0.14		
limonene	1195	1189	0.09	1.15			
α terpinene				t			
a camphene				0.46			
α phellandrene				0.50			
1,8-cineole	1208	1211	9.34	21.91	15.16		
α copaene				t			
cis-sabinene hydrate				t			
camphor				11.97		0.95	
fenchone				1.34			
3 bourbenene				t			
trans-ocimene						0.94	
germacrene D						4.25	
α farnese						0.85	
3 bisabolene						0.73	
δterpineol				t	0.12		
3 elemene				0.93			
δ guaiene				t			
a terpineol					0.31		
a bergamotene				1.07			
y cadiene				1.05			
5-cadiene				t			
cis-beta ocimene	1231	1233	0.86		0.10	7.47	
cadina-1,4-diene				0.67			
erpineol - 4				0.79	0.16		
α ĥumulene				t	0.32		
inalool	1549	1552	0.62	32.95	0.34		
3 caryophyllene	1573	1576	2.03	1.68	2.20	1.69	
a cadinol				5.18			
y muurolene					0.51		
β selinene					2.82		
a selinene					0.85		
7-epi-α selinene					0.26		
spathulenol					0.07		
nethyl chavicol	1659	1624	8.47				
epi bicyclosesquiphelandrene				t			
eugenol	2139	2141	74.39	7.42	74.83	68.81	
caryophyllene oxide						0.55	
methyl eugenol						13.21	

^a*Experimental retention indices;* ^b*Reference values according to online NIST database (NIST WebBook SRD 69);* ^c*Mohr et al., 2017;* ^d*Melo et al., 2019;* ^e*Matasyoh et al., 2007*

ND-Not documented, t-compounds present in trace quantities

Genotoxicity and immunotoxicity of eugenol have been reported, but extensive research on its chronic toxicity is very limited (Nejad, Özgüneş & Başaran, 2017).

Microbiological activity of *O. gratissimum* essential oil

Essential oil of *O. gratissimum* exhibited antimicrobial activity against both tested microorganisms. The minimum inhibitory concentration (MIC) was 11.36 µg/mL and the minimum bactericidal concentration (MBC) was 14.20 µg/ml against *E. coli* strain. In case of *S. aureus* strain, MIC/MBC was 12.12/15.15 µg/mL, respectively. *O. gratissimum* essential oil from Brazil has shown MIC and MBC of 1000µg/mL for these two strains (Melo *et al.*, 2019). Interestingly, both the Madagascan and

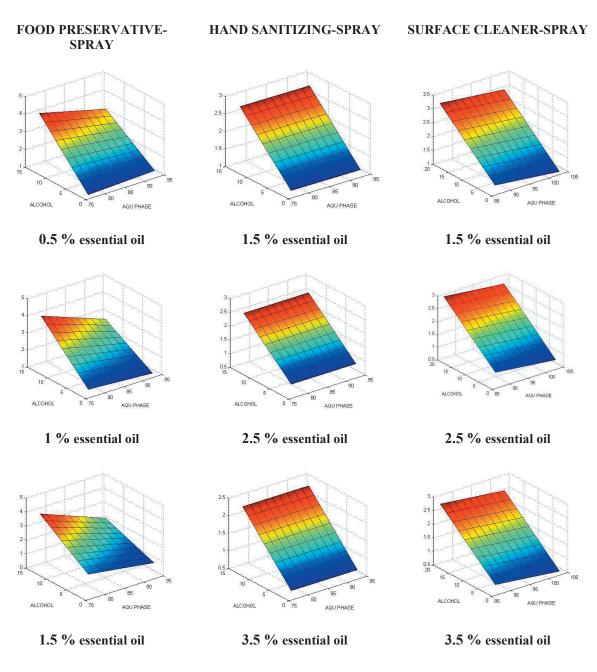


Figure 1. Relative quantities of alcohol and aqueous phase in function of essential oil mass percentage

Brazilian essential oils had around 74% eugenol as the main active principle. The main dif-ference resided in the content of 1,8-cineole which was 15.16% for the Brazilian essential oil, and 9.34% for the Madagascan essential oil contained, in addition to this, 8.47% of methylchavicol. It could be then suggested that the synergy between eugenol, 1,8-cineole and methylcha-vicol resulted in a better activity than the synergy between eugenol and 1,8-cineole alone. Methylchaviol has good antimicrobial activity and it has been studied for

integration in bio-film polymers for food preservation (Suppakul, Sonneveld, Bigger, & Miltz, 2011).

Spray formulations

Taking into account the previous results, and the toxicity of eugenol (Nejad et al., 2017) the relative percentage of *O. gratissimum* essential oil was limited between 1.5% and 3.5%. For the *C. zeylanicum* essential oil, the percentage was between 0.5 and 1.5%.

3D-surface response graphs in the function of essential oil percentages are reported in Fig. 1.

The hand-sanitizing spray presented the largest optimal area. For different concentrations of essential oil, the aqueous phase amount could be varied between 78-94 g with 12-15 g of alcohol. The food-preservative spray was the most sensitive to the variation of essential oil quantity. For a small amount of essential oil, the optimal area was quite large. As the amount of essential oil increased, the optimum area for relative quantities of alcohol and aqueous phase decreased very quickly. A formulation with 1.5% essential oil showed poor stability and 15% alcohol is needed with 83.5% of the aqueous phase. For the surface cleaner spray, the optimum area also decreased with increasing amount of essential oil but it was less sensitive than in the case of the handsanitizing spray. The hand-sanitizing spray composition allowed the most stable formulation and the food-preservative spray with the thyme extract was the most restrictive.

Characterization of the food-preservative spray

A) Ranking test

The results for the ranking test of the food preservative spray are presented in Table 5, and the corresponding distributions for the preference scores are shown in the dot plots (Fig. 2).

Table 5.

Ranking test of the tested formulations of food preservative-sprays

Sample A	Sample B	Sample C
0.5% EO	1.0% EO	1.5% EO
65	65	65
0	0	0
4.338	3.923	3.938
0.940	0.872	0.950
2	3	2
5	5	5
	0.5% EO 65 0 4.338	0.5% EO 1.0% EO 65 65 0 0 4.338 3.923



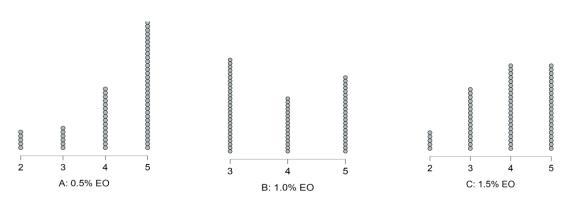


Figure 2. Dot plots showing the scores for each formulation of food preservative-spray

Table	6.
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I abic v.				
Efficacy test	for the	food-pr	eservative	sprav

2	1	1 /					
Storage		TAMF (CFU/g)		Total co (CFI	oliforms U/g)	Fungal flora count (CFU/g)	
conditions	EO content	Experimental value	Reference value ^a	Experimental value	Reference ^b	Experimental value	Reference value ^c
Control	0%	<10		0		113	
Ambient	0.5%	<10		0		28	
temperature	1%	<10	3.10^{5}	0	-	11	10^{2}
Under	0.5%	0		0		0	
refrigeration	1%	0		0		0	

TAMF - total aerobic mesophilic flora; CFU - colony forming unit

^a Le Gouvernement du Grand-Duché de Luxembourg (2018)

^b Center for Food Safety (2014)

^c Doutoum, Tidjani., Markhous, Kimassoum, & Nadlaou, 2019

Table 7.

Hand-sanitizing spray efficacy test

	Storage period						
Storage conditions	3 weeks			6 weeks			
_	1.5% EO	2.5% EO	3.5% EO	1.5% EO	2.5% EO	3.5% EO	
Ambient temperature	-	-	-	-	-	-	
Accelerated aging at 45°C	-	-	-	+	+	-	
	-: absence of colony; +: presence of colony						

Table 8.

Efficacy test for the surface cleaner spray

Storage conditions	Storage period					
	3 weeks			6 weeks		
	1.5% EO	2.5% EO	3.5% EO	1.5% EO	2.5% EO	3.5% EO
Ambient temperature	-	-	-	-	-	-
Accelerated aging at 45 °C	-	-	-	+	-	-

The maximum score was attributed to sample A with 0.5% of essential oil, for which the mean is 4.338. None of the scores for the 3 samples had a normal distribution. For sample A, the tendency was ascending from score 2 to score 5. It means that the spray was appreciated by the majority of the panellists who gave good scores of 4 and 5.

The dominant score for the spray with 1% of essential oil was 3 which means that most of the panellists find it neither good nor bad. There was no significant difference between the average scores for the sample B and C. However the high standard deviation for sample C showed large spread range of data indicating that the opinions are divergent for the spray with 1.5% essential oil.

Considering the above results on the consumer preference for low essential oil content and the stability of the formulation described previously, only sprays with 0.5% and 1% essential oil were chosen for efficacy testing. Given the fact that thyme mother tincture already contained alcohol, using an additional 15% to stabilize the formula at 1.5% essential oil would result in high alcohol content.

B) Efficacy test

Results of the microbiological testing on the third day of storage period are displayed in Table 6.

Sprays with 0.5% and with 1% of essential oil allowed the microbiological preservation of the food until day 3. The spray had a marked antifungal action and 0.5% essential oil was sufficient for preserving the food even without refrigeration.

Characterization of the hand-sanitizing spray: efficacy test

Efficacy test results for the hand sanitizing spray are presented in Table 7. The results showed inhibitory effect of the hand spray towards tested strains of *E.coli* and *S. aureus* at concentration 1.5% essential oil, stored at room temperature. In the case of storage under accelerated aging conditions for 6 weeks, only the spray with 3.5% essential oil shows good efficacy. It can be explained by the volatility of the essential oil. Utilisation of a trapping agent could help prevent this problem.

This hand sanitizing spray offered the advantage of containing a minimum quantity of alcohol while using natural active principle. Recent research results have shown a tendency to the minimization of alcohol adverse effect to the skin. WHO recommends the use of humectants in the formulation of hand sanitizing sprays. Alternative products to alcohol are also proposed, but they use non-natural chemicals as active principles (Jing, 2020). Some other researchers suggest the use of essential oils although 70% alcohol has still been the main ingredient (Wijana, Pratama, Rahmah & Arwani, 2020; Javed, Bibi, Shoaib, Perveen & Ferdosi, 2023).

Characterization of the surface cleanerspray: efficacy test

Efficacy test of the surface cleaner-spray is presented in Table 8. The results indicated that even at accelerated aging conditions, the spray with 2.5% essential oil was efficient. Wandera (2022) has demonstrated that white vinegar is a good disinfectant in food preparation settings. It is active against the two strains *E.coli* and *S. aureus*. Our study demonstrated a good synergistic effect of vinegar with *O. gratissimum* essential oil. Previous research showed that xanthan gum has good aroma trapping property (Bylaite, Adler-Nissen, & Meyer, 2005). Thus, a slower evaporation of essential oil contrary to the hand sanitizing spray could be explained. Hence, the surface cleaner spray with 2.5% essential oil was efficient even after accelerated aging condition at 45 °C.

Many scientific reviews have demonstrated the effectiveness of the essential oils in fighting multiresistant strains. Essential oils are incorporated in nanofilms and nanoparticules for preservation purpose. Many researchers focus on the development of active food packagings containing essential oils (Guidotti-Takeuchi et al., 2022). These technologies are still expensive for less developed countries, and here we offer an inexpensive solution to help with food security. In addition, the presentation in the form of a spray is a very practical and easyto-use. The lack of facilities within street food vendors is among the reasons of the outbreak of foodborne diseases (Verma & Mishra, 2020). The availability of low cost and convenient disinfectant sprays may help in the application of the food safety regulation.

CONCLUSION

Results from this study indicated effectiveness of essential oils of C. zevlanicum and O. gratissimum incorporated in simple spray products against food-borne pathogens. The surface cleaner-spray supplemented with O. gratissimum essential oil (1.5%) and white vinegar showed effectiveness against E. coli and S. aureus. Moreover, the addition of xanthan gum enables trapping the essential oil within the product, which allowed retention of its effectiveness after treatment in an accelerated aging condition at 45 °C at a concentration of 2.5%. The formulation of a hand spray without xanthan gum required a higher content of essential oil (3.5%) after accelerated aging treatment. However, both sprays stored at room temperature were active at a concentration of 1.5% essential oil. The combination of C. zeylanicum essential oil with 7% T. vulgaris tincture allowed the preservation of cooked pasta for 3 days without refrigeration. These results provide scientific evidence and contribute to the development of potent, ecological, and safer food contact disinfectants as well as food preservative agents that can be good alternatives to synthetic chemicals.

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ETERIČNA ULJA ZA BEZBEDNOST HRANE: FORMULISANJE DEZINFEKCIONIH PROIZVODA U OBLIKU RASPRŠIVAČA, JEDNOSTAVNIH ZA UPOTREBU, NAMENJENIH KORIŠĆENJU U RAZNIM FAZAMA PRIPREME HRANE

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Sažetak: U radu su ispitane formulacije proizvoda (sredstva za dezinfekciju hrane, ruku i površina u kontaktu s hranom) u obliku jednostavnih raspršivača (sprejeva), lakih za upotrebu, koji sadrže esencijalna ulja cejlonskog cimeta *Cinnamomum zeylanicum* i eugenolnog bosiljka *Ocimum gratissimum* namenjenih sprečavanju transmisije bolesti koje se prenose hranom. Eksperimentalni dizajn centralnog kompozitnog modela je omogućio razvoj stabilnih proizvoda sa optimalnim sadržajem esencijalnih ulja za efikasnu antimikrobnu aktivnost. Korišćeni eksperimentalni dizajn je omogućio minimalizovanje sadržaja alkohola koji je neophodan kako bi se omogućilo mešanje esencijalnih ulja u vođenoj fazi. (Trans)-cinamalaldehid je bio glavna komponenta esencijalnog ulja cejlonskog cimeta. Njegova niska toksičnost omogućila je formulisanje proizvoda bezbednog za zdravlje, uprkos velikoj varijaciji u hemijskom sastavu eteričnog ulja. Kuvana hrana obložena raspršivanjem 1% rastvorom esencijalnog ulja u kombinaciji sa bazičnom tinkturom od majčine dušice, bila je održiva 3 dana bez rashlađivanja u hladnjaku. Glavna komponenta eugenolnog bosiljka je bio eugenol, 1,8-cineol i metilhavikol. Raspršivači sa 1.5% ovog esencijalnog ulja bili su efikasni protiv *Escherichia coli* i *Staphylococcus aureus*. Ksantan guma se pokazala kao efikasno sredstvo za vezivanje esencijalnog ulja u testu ubrzanog starenja na 45 °C.

Ključne reči: *Cinnamomum zeylanicum, Ocimum gratissimum, dezinficijens, čuvanje hrane, antiseptik za površine, antiseptik za ruke*

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