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Full Length Research Paper

Composition of four essential oils obtained from plants from Cameroon, and their bactericidal and bacteriostatic activity against *Listeria monocytogenes*, *Salmonella enteritidis* and *Staphylococcus aureus*

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The composition of four essential oils (EOs) extracted by hydrodistillation from plants of common use in Cameroon (*Curcuma longa, Xylopia aethiopica, Zanthoxylum leprieurii* L., *Zanthoxylum xanthoxyloides*) was assessed by gas chromatography-mass. Their bactericidal and bacteriostatic activity was tested *in vitro* against three food borne pathogenic bacteria: *Listeria monocytogenes*, *Salmonella enteritidis* and *Staphylococcus aureus*. The bacteriostatic activities of this EOs on food borne bacteria were assessed *in vitro* through optical density measurements. The minimal bactericidal concentrations (MBC) were determined in broth combined with a spot plating method. *Z. xanthoxyloides* and *Z . leprieurii* showed a similar composition, with a prevalence of oxygenated monoterpenes (about 58%). The EO of *Z. xanthoxyloides* was the most effective against the microorganisms tested. Its higher concentration of geraniol could be linked to this higher activity. In almost all cases, the MBC was higher than the maximum concentration tested (3000 ppm). Notwithstanding their low bactericidal effect, the EOs studied showed interesting inhibiting activities against the tested food borne pathogens. *S. enteritidis* was the most resistant to the bacteriostatic effect of the four EOs. The knowledge of the antimicrobial potential of local plant EOs used in developing countries could help in their choice and their use to improve food safety and shelf-life.

Key words: Essential oils, composition analysis, antimicrobial activity, pathogens.

INTRODUCTION

In recent years there has been a considerable pressure by consumers to reduce or eliminate chemically synthesized additives in foods. Plants and plant products are a source of natural alternatives to improve the shelf-life and the safety of food (Lanciotti et al., 2004). The antimicrobial compounds in plant materials are commonly present in the essential oil (EO) fraction of leaves, flowers and flower buds, bulbs, rhizomes, fruits or other parts of the plant (Burt, 2004). In general the most important components of EOs are phenolic compounds, terpenes, aliphatic alcohols, aldehydes, ketones, acids, and isoflavonoids (López-Malo et al., 2005). Furthermore, the antimicrobial efficacy of EOs (and their constituents) from plants, fruits and spices are well known (Burt, 2004;

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Belletti et al., 2004).

In many areas of Africa there are unexploited sources of aromatic plants and spices which are currently used at domestic level with pharmaceutical purposes and for delaying microbial food spoilage. Moreover, some of them are used as food and beverage ingredients or as flavoring agents. It is estimated that in developing countries, up to 70% of diarrhoeal diseases indirectly responsible for children illness and death (3 million deaths out of 1500 million episodes annually for children under 5 years) are from food borne origin and caused by con-taminated food and water (Noup and Motarjemi, 1997). While technological tools and culture of food safety could need more time to be introduced in some regions, a dee-per knowledge of local plant EOs may be of a great importance because their use can help delay the growth of pathogenic bacteria and maintain them under non infection level before the consumption period.

In this work the antimicrobial activities of four EOs obtained from plants from Cameroon were assessed in relation to their composition. One of them was obtained from turmeric, a species (*Curcuma longa,* Zingiberaceae) worldwide diffused, while the others (*Xylopia aethiopica, Zanthoxylum leprieurii* and *Zanthoxylum xanthoxyloides*) were obtained from species typical of this continent.

Turmeric has been used in traditional medicine and it is also used, alone or mixed with other aromatic extracts, as spice in foods, in which it has also an antimicrobial activity (Jayaprakasha et al., 2005).

X. aethiopica (known also as West African pepper tree) belongs to *Annonaceae* and is a component of herbal medicines (Karioti et al., 2004). However, its fruits and seeds are also used in cooked foods and for spicing beverages with a daily pro-capita assumption of 1-10 g within some populations (Karioti et al., 2004).

Z. leprieurii and *Z. xanthoxyloides* (Rutaceae) are commonly used in Cameroon for the traditional treatment of some infectious diseases and are known for their antifungal activities (Tatsadjieu et al., 2003).

The main objectives of this work were to determine the composition of the EOs extracted from these 4 botanical species cultivated in Cameroon and to evaluate their antimicrobial activity against three foodborne pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella enteritidis* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Essential oil extraction and composition analysis

The EOs tested were extracted by the hydrodistillation method using Clevenger's apparatus (Lamaty et al., 1987). Gas chromatographic analyses of the EOs were done using an Agilent Technology GC/Mass 7890A / HP 5975C (Palo Alto, CA, USA). The working conditions were: injector temperature 250°C, detector temperature 280°C, EI mode (70 eV), split 1:10 capillary column Zebron ZB-5 30 m length, 0.25 mm ID and 0.25 µm film thickness (Phenomenex, Torrance, CA, USA). The temperature program was: from 50 to 240°C with increment of 3°C/min, and 1 min hold at 240°C. The EOs were dissolved in diethyl ether (Merck, KGaA Darmstadt, Germany) in a ratio 1/1000 and 1 µl injected into the GC, using helium as carrier gas at a constant flow of 1 ml/min. The identification of the volatile compounds was performed using the NIST (NIST/EPA/NIH Mass spectral Library, 2005, USA) . The identification of the volatile compounds was based on the comparison of their retention indices (RI), with those of a series of n-alkane (C6-C24) on the ZB-5 capillary column, and GC MS spectra from the NIST 2005 Mass Spectral Library and, whenever possible, by co-injection with authentic compounds.

Effect of the essential oils on the detection times *L. monocytogenes, S. aureus* and *S. enteritidis*

The strains of foodborne bacteria used in this work, that is *S. enteritidis* 155A, *L. monocytogenes* Scott A and *S. aureus* SR231, belong to the collection of the Department of Food Science of the University of Bologna. The microorganisms, stored at -80°C, were sub- cultured twice at 37°C for 24 h in BHI (Oxoid Ltd., Basingstoke, UK) before analysis. Then, each culture was appropriately diluted and 1.9 ml were added in the 8 ml medium of a MicroFoss vial (TVC medium test vials P/N 1024210, Foss Analytical A/S Slangerupgade, DK) to obtain the final cell load to be tested (2, 4 and 6 log CFU/ml). Immediately after the inoculation, 0.1 ml of EOs solutions in ethanol 97% (Sigma- Aldrich, St. Louis, Mo., U.S.A.) were added in the MicroFoss vial to obtain the desired final concentrations (300 and 600 ppm). The final ethanol concentration was 1% and in the controls only the ethanol was added in the absence of EOs. The medium contained a dye indicator (bromocresol purple) which changes its color as a consequence of the acidification, with respect to the initial pH of 6.7, induced by the microorganism growth. The vials were inoculated with three different concentrations of *L. monocytogenes*, *S. aureus* and *S. enteritidis* (2, 4 and 6 log CFU/ml) in the presence of different EOs concentrations (0, 300 and 600 ppm). Their growth ability at 37°C was assessed by measuring the colour variation of the vials through optical density (OD) measurements, using MicroFoss-32 (Foss Analytical A/S Slangerupgade, DK). Three repetitions were performed for each condition. The OD was recorded every 6 min and as soon as a colour change, determined by microbial metabolism, induced an OD change equal to a pre-set threshold (producer patent, not available for users); the system records the time of such event and calls it detection time (DT).

The OD measurements were successively modelled also with the Baranyi and Roberts (1994) model in order to estimate the parameters and compare them with the instrumental DT.

MBC analysis

For minimal bactericidal concentration analysis, 0.5 ml of broth at three different cell loads (2, 4 and 6 log CFU/ml) introduced in 1.5 ml eppendorfs and in each one, 5 µl of EOs 100 times more concentrated than the level to be tested were added. The EOs were diluted in ethanol 97% (Sigma-Aldrich, St. Louis, Mo., U.S.A.). The maximum testing concentration was 3000 ppm. After 24 h incubation, 10 µl of each eppendorf showing no visible growth were plated as spot on BHI agar plates. These latter plates were then incubated at 37°C for 24 h for the evaluation presence. After defining the first bactericidal concentration, another repetition was done reducing the intervals between two successive concentrations to 50 ppm. The MBC was defined as the lowest EO concentration that caused the death of all the inoculated cells and hence no growth after plating the 10 µl spot.

Table 1. Essential oils composition (percentage values are referred to the total peak area).

		C. longa	*X. aethiopica*	*Z. xanthoxyloides*	*Z. leprieurii*
Name	**RI**	**Area%**	**Area%**	**Area%**	**Area%**
α–thujene	856		0.30		
1Σ–α–pinene	860		18.44	1.08	
β-thujene	882		4.38	1.06	
β-pinene	884		37.80		0.21
α-phellandrene	1000	3.95	0.46		
δ-3-carene	1006				0.64
p-cymene	1009	0.58	1.00		0.40
D-limonene	1011		8.62	4.82	3.94
(E)-β-beta ocimene	1016		0.52	1.92	2.51
γ-terpinene	1031				0.93
terpinolene	1040	0.43			
2,6-dimethyl-2,6-octadiene	1274			9.33	11.38
santolina triene	1471				0.23
Monoterpenes		4.96	71.53	18.21	20.24
1,8-cineole	1013	2.79	5.38		
p-menth-8-en-1-ol	1030		0.76		
linalool	1046		0.80	1.31	0.64
(Z)-rose oxide	1052			0.73	
pinocarveol	1065		0.86	0.69	
isopulegol	1069			5.36	2.23
β-citronellal	1073			4.73	6.51
L-4-terpineol	1085		1.10	1.35	3.57
p-menth-1-en-8-ol	1092		1.18		
α–thujenal	1095		1.06		
(Z)-carveol	1206				0.36
(R)-(+)-beta citronellol	1212			18.11	17.37
(E)-citral	1218				0.10
geraniol	1225			16.17	5.91
terpinol hydrate	1264			1.55	1.07
citronellic acid	1260				15.99
geranic acid	1277				1.12
geranyl acetate	1289			5.89	2.77
citronellyl acetate	1854				0.66
Oxygenated monoterpenes		2.79	11.14	55.89	58.32
γ-elemene	1266		0.96		
α-cubebene	1285		0.77		0.29
β–elemene	1299				0.14
(Z)-βfarnesene	1419	0.50			
β-cubebene	1437	1.96	7.74	0.82	4.03
α-zingiberene	1447	3.46	0.53		0.69
β-bisabolene	1451	0.34			
γ–cadinene	1459	2.32	0.39		1.12
γ-gurjunene	1605				0.20
γ-himachalene	1610		1.20		
kaurene	2008		0.70		
Sesquiterpenes		8.58	12.29	0.82	6.47
Nerolidol	1479				0.59

Table 1 contd

(-)-spathulenol	1485		0.91	0.67	
τ-muurolol	1617				0.73
γ–eudesmol	1621				0.30
τ-cadinol	1626			1.10	0.51
ar-tumerone	1631	17.63			
tumerone	1631	43.09			
curlone	1647	17.47			
caryophyllene oxide	1486	0.48			
manoyl oxide	2012			5.53	
farnesol	2034			0.93	0.27
Oxygenated sesquiterpenes		78.67	0.91	8.23	2.40
decanal	1200				0.74
2,4-dimethyl-1,3-cyclopentanedione	1256			2.56	
eugenol	1282				0.24
cyclooctane	1402				0.32
p-tert-octylphenol	1497	0.74			
α-ionene	1635			1.32	1.98
sotolon	1674	0.43			
Others		1.17	0.00	3.88	3.28
Not identified		3.83	4.13	12.97	9.11

Statistical analysis

Microsoft Office Excel 2003 was used for the statistical analyses while Baranyi and Roberts model was fitted using the DMFit Excel macro freely available from the Institute of Food Research (IFR) Norwich, UK.

RESULTS

Essential oil composition

The composition of the four EOs was evaluated through a GC -Mass technique. Table 1 reports the percentage (on the basis of the relative peak area) of each compound present, as well as the cumulative percentages of each class of compounds (monoterpenes, sesquiterpenes, oxygenated monoterpenes, oxygenated sesquiterpenes, others and not identified).

The EO from fruits of *X. aethiopica* was characterized by a high concentration of monoterpenes (more than 70%) among which -pinene, 1S- -pinene and limonene were the most important.

The compositions of the oils of *Z. xantoxyloides* and *Z. leprieurii* were similar: oxygenated monoterpenes were the most abundant molecules (about 58%) in both cases, with a relevant presence of (R)-(+)- -citronellol (17-18%), geraniol (especially in *Z. xantoxyloides* in which it accounted for 16.2%), isopulegol (2-5%), -citronellal (5-6%) and geranyl acetate (3-6%). Among monoterpenes 2, 6 -dimethyl- 2, 6-octadiene and limonene were the most important molecules. *Z. leprieurii* was characterized by a higher concentration of sesquiterpenes (especially -cubebene), while in *Z. xanthoxyloides* a relevant amount (5.5%) of manoyl oxide was detected.

The EO obtained from *C. longa* rhizomes was characterized by the prevalence of oxygenated sesquiterpenes; in fact, turmerone, ar-turmerone and curlone accounted for more than 78% of the oil constituents.

MBC of the essential oils

The MBC of the four essential oils was found to be higher than 3000 ppm at inoculum level of 6 log CFU/ml independently of the target microorganism or the oil. Moreover, the MBC was higher than 3000 ppm when tested on *S. enteritidis* 155A, *L. monocytogenes* Scott A at inoculum levels of 4 and 2 log CFU/ml. Unique exceptions of MBC lower than 3000 ppm to be mentioned are *X. aethipoica* and *Z. leprieurii* EOs tested against *S. aureus*. The MBC was 2800 and 2650 for *X. aethipoica* when the microorganism was inoculated at 4 and 2 log CFU/ml, respectively. Moreover, MBC of 2000 and 1800 were observed for *Z. leprieurii* when *S. aureus* was inoculated at 4 and 2 log CFU/ml, respectively.

In spite of the high MBCs observed, the further trails were carried out using lower concentrations of EOs (600 ppm) with the aim to evaluate the possible slowing effect on pathogen growth (which can be reduced in prolongation of the time needed to reach infective cell load) with amounts of oils which can be compatible with a use in real food.

Table 2. Detection times, expressed in hours, determined by the MicroFoss instrument (DT) and parameters (param.) obtained with the Baranyi and Roberts model for the different conditions evaluated. The standard deviations for three independent repetitions are also reported (± standard deviation).

Essential oil	Conc. (ppm)	Inoculum (CFU/ml)	*L.monocytogenes* Scott A		*S. aureus* SR231		*S. enteritidis* 155A	
			DT (h)	param. (h)	DT (h)	param. (h)	DT (h)	param. (h)
Control	0	10^4	7.50±0.01	7.57±0.02	6.90±0.02	5.93±0.09	7.30±0.22	6.78±0.34
Z. xanthoxyloides	300		12.45±0.75	11.44±0.34	19.50±0.25	17.49±0.77	9.75±0.05	9.30±0.09
X. aethiopica			20.70±4.41	18.89±2.32	19.30±2.71	20.99±5.65	8.40±0.10	7.49±0.10
C. longa			9.35±0.15	9.46±0.33	8.65±0.05	7.16±0.02	7.55±0.05	6.90±0.28
Z. leprieurii			12.20±0.32	11.66±0.26	13.35±0.35	11.52±1.22	7.90±0.30	7.35±0.33
Control	0	10^2	11.20±0.19	11.55±0.17	9.90±0.03	10.15±0.24	11.50±0.32	10.94±0.42
Z. xanthoxyloides	600		26.60±1.33	18.02±3.12	62.30±1.27	50.29±2.51	28.60±0.32	30.28±2.24
X. aethiopica			46.60±0.91	48.02±1.25	50.00±2.23	n.d.*	14.10±0.24	13.32±1.75
C. longa			15.00±0.21	15.27±0.75	13.90±0.16	13.87±0.12	12.40±0.36	11.83±1.15
Z. leprieurii			24.80±0.33	20.92±0.13	25.20±1.10	22.19±0.83	18.30±0.12	8.35±1.64
Control	0	10^4	7.50±0.01	7.4585±0.24	6.90±0.02	4.81±0.11	7.30±0.21	7.15±0.06
Z. xanthoxyloides	600		22.15±1.65	17.035±2.12	48.30±0.85	45.68±0.99	11.95±1.55	10.41±0.11
X. aethiopica			32.65±1.15	28.75±0.14	32.15±4.45	31.27±0.01	10.40±0.03	8.97±1.34
C. longa			10.55±0.15	10.16±1.97	9.60±0.74	7.48±0.02	8.85±0.05	8.02±0.20
Z. leprieurii			16.90±1.31	13.44±1.17	n.d.	n.d	12.30±0.12	11.01±0.01
Control	0	10^6	4.30±0.14	4.188±0.36	4.10±0.11	3.83±0.31	4.20±0.12	3.61±0.28
Z. xanthoxyloides	600		8.50±0.23	4.50±0.64	12.20±0.65	7.79±1.27	7.90±0.26	6.17±0.67
X. aethiopica			16.00±1.13	n.d.	17.40±0.24	15.03±0.25	5.40±0.11	4.46±0.23
C. longa			6.10±0.34	6.09±0.26	5.70±0.20	5.56±0.31	4.70±0.01	3.95±0.02
Z. leprieurii			8.20±0.26	4.11±0.21	8.10±0.45	5.85±0.37	7.60±0.05	5.43±0.34

The inhibiting activity of the EOs was tested by using the MicroFoss equipment. In first instance, the detection time (DT) provided by the instrument was used as indirect measure of the inhibition induced by the presence of the EOs on the growth dynamics of the pathogenic microbial species (*S. enteritidis* 155A, *L. monocytogenes* Scott A and *S. aureus* SR231) . In an indirect method (such as MicroFoss), DT can be defined as the time required to attain a measurable change of the growing medium in relation to the metabolic activity of the growing cells. In the case of Microfoss, the color change induced by pH variation is detected in a semi-fluid layer at the base of a disposable vial containing the culture medium and test sample (Odumeru and Belvedere, 2002).

In Table 2 the DT data obtained in the different conditions for the 3 microorganisms are reported. In the same table are also reported the values of the parameters as evaluated by the equation of Baranyi and Roberts (1994) used to model the OD data provided by the MicroFoss. Estimated parameters are compared with the DT data provided by MicroFoss.

The parameters estimated by the Baranyi and Roberts model were very close to the DT estimated by Microfoss. The linear regression between the DT and the parameters showed a good correlation between the data (R=0.991) and the slope of the regression line was very close to 1 (1.102) indicating a good concordance between the results obtained with the two calculation methods. In this framework, it can be assumed that

assumed that the two approaches gave the same information about the EOs ability to delay the growth of the different organisms studied and for this reason only the DT has been used for the discussion.

As expected, the DT values of the three microbial species in the absence of the EOs depended on the initial inoculum levels, increasing as the cell loads decreased. They ranged between 4.30 and 11.20 h, 4.10 and 9.90 h and 4.20 and 11.50 h for *L. monocytogenes* Scott A, *S. aureus* SR231 and *S. enteritidis* 155A, respectively. Each EO exerted different effects on DT values in relation to the target species and its inoculum level as well as to the oil concentration used.

In particular, the EO of *X. aethiopica* was the most active in inhibiting *L. monocytogenes* Scott A. At a concentration of 300 ppm it increased of about 3 times the DT of this species, while at 600 ppm there was a four-fold increase of DT, independently of the initial inoculum level. The same oil had a marked inhibiting effect also on *aureus* SR231, whose DT increased three times when its concentration was 300 ppm. The inhibition was more pronounced in the samples added with 600 ppm of EO, independently of the initial inoculum level. However, the highest inhibition was observed at the lowest inoculum, that is 2 log CFU/ml, with a 5-fold DT prolongation. *S. enteritidis* 155A showed a low sensitiveness to this EO and its DT was only slightly increased (1 h) by the presence of 300 ppm of the oil, while its addition at 600 ppm caused DT increases lower than 50%, even at the lowest inoculum. -pinene and its isomer 1S- -pinene were the major component of *X. aethiopica*.

Z. xanthoxyloides doubled the DT values of *L. monocytogenes* Scott A when added at 300 ppm or at 600 ppm in the presence of the higher inoculum level, while when the cells were inoculated at 2 and 4 log CFU/ml DTs increased 3 folds. A similar effect, even if less marked, was exerted by *Z. leprieurii*. In the presence of 300 ppm the EO of *Z. xanthoxyloides* tripled the DT values of *S. aureus* SR231. A concentration of 600 ppm of this EO increased up to 6 folds DTs with the exception of the highest inoculum level (6 CFU/ml). In this case a four time *S. aureus* SR231 DT prolongation was observed. The antimicrobial efficacy of the *Z. leprieurii* oil against this species was lower with respect to *Z. xanthoxyloides*. As observed for *X. aethiopica* EO, *S. enteritidis* 155A was again the most resistant to the inhibitory effect of these two EOs. The presence of 300 ppm of Z. *xanthoxyloides*, with an inoculum of 4 log CFU/ml, prolonged the DT only two hours with respect to the control (from 7.30 to 9.75 h), while no significant effect was observed in the presence of 300 ppm of *Z. leprieurii* EO. The increase of oil concentrations to 600 ppm caused a more pronounced inhibition of *S. enteritidis* 155A. However, the DTs were delayed of two times or less.

Curcuma longa was the EO showing the lowest antimicrobial activity among those considered. It was the less effective against *L. monocytogenes* Scott A and determined an increase of 50% of DT independently of oil concentration and initial inoculum. Moreover, it was also the EO less efficient in prolonging DT of *S. aureus* SR231. At the lowest *S. aureus* SR231 inoculum level considered, the supplementation with 600 ppm of this EO caused a 4 h DT prolongation. *C. longa* EO did not have noticeable inhibitory effect on *S. enteritidis* 155A independently of oil concentration and inoculum level.

DISCUSSION

Essential oil composition

As previously reported, the EO of *X. aethiopica* was characterized by a high concentration of monoterpenes (more than 70%) among which -pinene, 1S- -pinene and limonene were the most important. Also Karioti et al., (2004) found in *X. aethiopica* oil produced in Ghana relevant proportions of - pinene and germacrene D (absent here) but did not found relevant amounts of limonene, -thujene, and - cubebene, characterizing the Cameroon EO analysed in this study. In another work (Keita et al., 2003) the presence of sabinene was reported but this compound was not detected in the EO from Cameroon. Similarly to sabinene, also (*Z*)- -ocimene and -pinene, which were the principal components of *Z. leprieurii* and *Z. xanthoxyloides* EOs according to other studies (Tatsadjieu et al., 2003; Tchoumbougnang, 2005), were found in minor amounts in this work.

-pinene was found in significant concentrations in citrus oils (lemon, citron, sweet lime) with antimicrobial activity against *Saccharomyces cerevisiae* (Belletti et al., 2004). This terpene was one of the most important components of a sage EO, able to reduce the growth kinetics of many microorganisms (Tepe et al., 2005). The EO of *X. aethiopica* analysed was characterized also by high concentrations of (eucalyptol) and -cubebene. Although there are no specific data about the antimicrobial activity of -cubebene, it was an important constituent of an antimicrobic *Satureja cuneifolia* EO (Sko ibuši and Bezi , 2004). The antimicrobial activity of 1, 8-cineole is better documented. It was tested with positive results against fungi and bacteria (Dadalio lu and Evrendilek, 2004; Terzi et al., 2007). However, 1, 8-cineole was very less effective against *L. monocytogenes* than -pinene (Mourey and Canillac, 2002).

In the oil of *C. longa*, turmerone and ar-turmerone are commonly dominant even if different concentrations are reported. In fact, the total percentage of these two molecules ranged from about 32% of Chinese oil to more than 50% in the oil from turmeric rhizomes produced in Sri Lanka (Jayaprakasha et al., 2005). In a recent work (Leela et al., 2002) turmerone and ar-turmerone constituted alone about 80% of Indian turmeric oil. Curlone amount was about 17.5% of the EO. -zingiberene, found here at a concentration of about 3.5%, can represent even more than 30% of the oils having different geogra-

phical origin (Uehara et al., 1992).

Antimicrobial effect of essential oils

The EO of *Z. xanthoxyloides* was more effective against the microorganisms tested. A reason for this higher activity could be found in the higher concentration of geraniol. In fact, geraniol showed a great efficacy in inhibiting the growth and survival in food of spore forming bacilli (*Bacillus* sp. and *Paenibacillus* sp.) and in some cases, of *S. aureus* (Grande et al., 2007a; 2007b). The same terpene had a marked antimicrobial activity against *L. innocua* and *S. enteritidis* (Raybaudi-Massilia et al., 2006). The composition of *Z. xanthoxyloides* EO differed from that of *Z. leprieurii* also for high concentration of manoyl oxide. However, few reports are available on the antimicrobial activity of this molecule (Demetzos et al., 2001) while according to Kalpoutzakis et al. (2001), diterpenoids possessing an ent- manoyl oxide skeleton are powerful antimicrobial agents.

Previous works showed that *C. longa* inhibited the growth and activity of some bacteria and fungi (Jaya-prakasha et al., 2005). However, the antimicrobial effi-cacy resulted strictly dependent of concentration, micro-bial species, EO fraction and, especially, of modality of extraction used. The antimicrobial activity of *C. longa* ex-tract has been attributed to compounds belonging to fla-vonoids and particularly to borneol, cymene, cuparene, and carene (Thongson et al., 2005) . The absence of such compounds in the EO considered in the present study can in part explain its low antimicrobial activity. On the other hand, turmerone, ar-turmerone and curlone consti-tuted more than 78% of the oil constituents. No data are available on the antimicrobial activity of these oxygenated sesquiterpenes; however it is presumable that their high boiling points and, consequently, their low vapour pre-ssure at 25°C can reduce their possibility to approach the cell membrane that is the main or primary microbial target of antimicrobial compounds (Lanciotti et al., 2004).

The lower sensitivity of *S. enteritidis* 155 A, with respect to the *L. monocytogenes* Scott A and *S. aureus* SR231, to the EOs considered was not surprising. This is in agreement with previous literature reports (Thongson et al., 2005), that stated that Gram -positive bacteria are more sensitive to EOs of spices than Gram-negative bacteria. In fact, the inherent resistance of Gram negative bacteria to many antimicrobial agents is well known and mainly attributable to the outer membrane, which acts as an efficient permeability barrier against macromolecules and hydrophobic substances (Helander et al., 1997) as well as to the high content in cyclopropanic fatty acids of the inner membrane (Chang and Cronan, 1999).

Most of the Cameroonian EOs considered in this work showed interesting inhibiting activities against three food-borne pathogens such as *L. monocytogenes, S. enteri-tidis* and *S. aureus*. Only the EO of *C. longa* had weak inhibiting properties, especially against *S. enteritidis*. On the other hand, the latter microbial species was the most resistant also to the oils of *X. aetiopica, Z. xanthoxyloides* and *Z. leprieurii*. The bacteriostatic effect the EOs can be mainly attributed to the presence of molecules whose bioactivity against microorganisms has been already described, such as geraniol and geranyl acetate, -pinene and in *X. aetiopica*.

Although their high MBC values and the low con-centration used, all the tested essential oils significantly delayed the DT (and parameter from the Baranyi model) of the pathogenic species tested, and particularly of *S. aureus*. These results indicated such EOs as pro-mising instruments to delay the growth of foodborne pathogens usually present in food in developing coun-tries, due to low hygiene and wrong storage conditions. The 3-4 fold delay observed *in vitro* could be important in countries in which, due to lack of a reliable cool chain, food storage is very limited. It should be noted that the conditions found in real foods are usually more stringent than those adopted in this study (optimal for the target organisms considered). Moreover the antimicrobial efficacy of these EOs used at concentrations organo-leptically compatible with real foods could be enhanced throughout their combination with other available hurdles.

On the other hand, a deeper knowledge about the characteristics of these oils can contribute to their con-scious use in food preparation even at a domestic or small scale and, consequently, to improve shelf life and safety of food. Obviously, the results of this screening have to be validated by further experimentation on real food matrices in order to verify their interaction with food constituents (such as proteins, lipids, starch) and the effects deriving from their use in combination with other preservation technique.

Conclusions

This study proved that MicroFoss can represent a rapid alternative tool for the screening of the bioactivity of non conventional antimicrobials. Moreover it revealed the great applicative potential of the essential oils of *C. longa, X. aethiopica, Z. leprieurii* and *Z. xanthoxyloides* to inc-rease shelf -life and safety of foods. The growth delay of pathogenic species observed in optimal *in vitro* condition are promising, foreseeing a more interesting exploitation in real foods that are usually more stringent for the micro-bial growth. These results can represent a starting point for the set up of a multi-hurdle strategy for food manu-facture and preservation.

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