academicJournals

Vol. 8(11), pp. 1157-1169, 12 March, 2014
DOI: 10.5897/AJMR12.1021
ISSN 1996-0808 ©2014 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Phytochemical analysis and antimicrobial bioactivity of the Algerian parsley essential oil (*Petroselinum crispum*)

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Accepted 22 July, 2013

In this paper, we extracted, analyzed and studied the antimicrobial activity of Algerian parsley essential oil on several microbes that cause infectious diseases and its effects on kinetics of lactic acid production by *Lactobacillus casei subsp. rhamnosus*. The essential oil of parsley (*Petroselinum crispum Hoffm*) obtained by hydrodistillation was characterized by its physicochemical properties and by its chromatographic profiles. Myristicin and dillapiol were identified by gas chromatography-spectrometry mass (GC/MS). The essential oil showed a high antimicrobial spectrum towards *Bacillus cereus* and *Candida albicans*, average effectiveness against *Clostridium perfringens*, *Staphylococcus aureus* and *Enterococcus faecalis* and no influence on *Escherichia coli*. The key odorant effects of parsley in the growth of *Lactobacillus casei subsp. rhamnosus* was studied. The results showed that *L. rhamnosus* can produce up to 10.96 and 13.78 g.L^{-1} of lactic acid on the control fermentation and on the second fermentation, respectively, characterized by the addition of 20 µL of the essential oil in the growth exponential phase.

Key words: Parsley, essential oil, dillapiol, myristicin, antimicrobial activity, *Lactobacillus rhamnosus*.

INTRODUCTION

Essential oil in plants presents great interests in food, pharmaceutical, cosmetic and perfume industries by virtue of their aromatic properties. Parsley (*Petroselinum crispum Hoffm*) belonging to the *Apiaceae* family is considered as an aromatic and medicinal plant used often in traditional medicine for their diuretic, vermifuge, emmenagogue and purgative properties (Lopez et al., 1999; Marczal et al., 1997). Parsley is known for its antidiabetic (Manderfeld et al., 1997), antimicrobial, antihypertensive, anticoagulant, antihyperlipidaemic, anti-hepatotoxic, membrane protective (Fejes et al., 2000) and antioxidant (Nielsen et al., 1999) effects. Phyto-chemical analysis shows the presence of flavonoids, carotenoids, ascorbic acid, myristicin, apiole, terpenoids and coumarins (Anand et al., 1981; Davey et al., 1996; Pino et al., 1997; Tomas et al., 1972; Tunal et al., 1999). The active principles have not yet been fully investigated in food and biotechnology. Researches made were related to the study of stigmasterol, stigmasterol palmi-tate, coumarin, phenol acids, essential oils, carbohy-drates and hydroalcohol extracts (Beaux et al., 1997; Harbone and Saleh, 1971; Hegnauer, 1973; Ravid et al., 1983; Tanira et al., 1996; Trenkle, 1971). The natural extracts stemming from this plant contain a variety of

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phenolic derivatives and essential oils with power inhibittion effect against bacteria.

Lactic acid, a natural organic acid, and its derivatives are widely used in food, pharmaceutical, leather and textile industries (Hujanen and Linko, 1996). Furthermore, since lactic acid has an excellent reactivity derived from both carboxylic and hydroxyl groups, it can undergo a variety of chemical conversions into potentially useful chemicals such as propylene oxide, propylene glycol, acrylic acid, 2,3-pentanedione and lactate ester (Litchfield, 1996; Yun et al., 2003). Recently, there has been an increased interest in L-lactic acid production because it could be used as a raw material for the production of polylactic acid, a polymer used as special medical and environmental-friendly biodegradable plastic, and hence a substitute for synthetic plastics derived from petroleum feedstocks (Amass et al., 1998; Datta et al., 1995).

Antibiotics have been utilized in the farm environment as therapeutic agents and growth promoters for over 50 years (Joerger, 2003). But the emergence of multidrug resistant pathogens and imposed restrictions on the use of antibiotic feed additives have intensified the search for novel possible alternatives (Bedford, 2000; Wierup, 2000; Diep and Nes, 2002; Gillor et al., 2005). In this regard, much interest has been focused on usage of the active principles of plants such as the essential oil, the flavonoids and the alkaloids due to their great potential applications in medicine. In the present work, we investigated for the first time, the determination of physico-chemical properties and identification of major components of the essential oil of parsley stalks and shoots from the region of Mascara (North-west of Algeria). Then, the antimicrobial activity of this essential oil was studied against resistant microbial strains responsible for diseases infections (*Escherichia coli, Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, Clostridium perfringens* and *Candida albicans*) and the study of the effect of the parsley essential oil addition on the kinetics of the lactic acid production by *Lactobacillus casei subsp. rhamnosus*.

MATERIALS AND METHODS

Essential oil isolation

Fresh aerial part of parsley was collected during April and May 2011 from the region of Mascara, North West of Algeria. The sample (100 g of fresh parsley in 500 mL of water distilled) was submitted to hydrodistillation for 3 h. The distillate was extracted with the methane dichloro and dried over anhydrous sodium sulphate. The resulting essential oil was subsequently analysed.

Identification

The identification of the main constituents of the essential oil of parsley was realized by chromatography (TLC, GC and GC/MS).

Thin layer chromatography (TLC)

On a TLC same patch, we mixed a drop of essential oil of the parsley and a drop of the dillapiol (leading product). Elution was done with ether: petroleum ether (1:4) and visualized using UV, vanillin.

GC and GC-MS analysis

GC-FID

The analysis by GC of the essential oil of parsley was carried out on a Typify VARIAN 3900 gaz chromatograph equipped with a flame ionization detector (FID). A capillary column: [CP-Sil 5CB (30 m x 0.25 mm), DF 0.25 µm], the pulse of the vector gas (N_2), 30 mL/min. The analysis was performed using the following temperature program: 80°C/min at the rate of 20°C/min to 280°C, injector and detector temperature were held, respectively, at 220 and 300°C. The injection volume was 1 µL.

The identification of the dillapiol is made by its co-injection with the essential oil of parsley and with comparison of the retentions indices, back with those of the various peaks revealed in the chromatogram of the studied essential oil.

GC/MS

GC-MS analysis was performed on a gas chromatogram VARIAN SATURN 2100 T interfaced with a mass spectrometer with impact ionization (70eV). A capillary Column [CB 8 (30 M × 0.25 mm), DF 0.25 µm] was used. The column temperature was programmed to rise from 80°C/min to 280°C/min at the rate of 20°C/min. The carrier gas was helium with a flow rate of 1 mL/min, injector and detector temperature were held, respectively, at 280 and 300ºC, the injection volume was 1 µL.

The compound identification was based on their retention indices and mass spectra was compared with the data from the Baser Library.

Antimicrobial activity of the essential oil of parsley

On some microorganisms responsible for diseases infectious

Tested microorganisms: Antimicrobial activity of the essential oil of parsley was tested on 6 clinical strains chosen because of their nosocomial resistance which were *E. coli*, *S. aureus*, *B. cereus*, *C. perfringens*, *E. faecalis* and *C. albicans*. All bacterial strains were provided by the Laboratory of Medical Analysis located in Dr. Yessaâd Khaled Hospital (YKH) of Mascara City, situated in western Algeria. The presumptive identification of these strains was determined by morphological characters and biochemical characterization (Brennan et al., 2001).

The antibiogram: It is the analytical method that allows *in vitro* interpretation of the sensibility of bacteria to antibiotics. Antibiotics selected are: Tetracycline, gentamycine, ofloxacine, ampicillin, penicillin g, clindamycine, oxacilline, chloramphenicol and amoxicillin. We poured a suspension of microorganisms in the limp, dried them at 37°C then deposited the discs of antibiotics in the surface of the agar and incubated at 37°C/24 h. The measure of the diameter of the zones of inhibition allows classifying bacteria in three categories: sensitive, intermediate or resistant (Al-Obeïd, 1991; Joffin and Guey, 1995).

Antimicrobial activity determination: Antimicrobial activity of the parsley essential oil was determined by the agar disc diffusion method. A suspension of each tested micro organism (average concentration is 10^8 cells per mL) was mixed with Mueller Hinton Agar (MHA), then poured on Petri plates with sterilized Whatman No.3 filter paper discs (diameter 6 mm) impregnated with a tested dose of the oil (10, 20, 30 and 40 µL) and were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured (Luu, 2002; Rubio et al., 2003).

Enumeration after culture: The enumeration of the viable bacteria is a collectively used method. Culture is made on agar medium in box. After incubation in a suitable temperature, the number of appeared colonies corresponds to cells present in the volume analysed by the suspension (Meyer and Deiana, 1988).

On the kinetics of the lactic acid production by Lactobacillus casei subsp. rhamnosus

Bacterial strain and media

The *L. casei subsp. rhamnosus* (LBC80 10D) strain used in all our experiments was supplied by the Rhone Poulenc group (Nancy France). It was maintained on MRS medium in the presence of 10% glycerol and stored at -20°C. The MRS medium used in growth and inoculum preparations contained the following components (in $g.L^{-1}$): 10 Soya peptone, 10 beef extract, 5 yeast extract, 2 K_2HPO_4, 5 $NaCH_3CO_2,3H_2O$, 2 triammonium citrate, 0.2 $MgSO_4,7H_2O$, 0.05 $MnSO_4, H_2O$, 15 glucose and 1 mL Tween 80. The pH was adjusted to 6.25 by 25% (w/w) NH_4OH aqueous solution prior to sterilization at 108°C for 15 min. The reactivation phase is realized after two successive transplantations at 42°C during 2 h on liquid MRS medium (Amrane and Prigent, 1994).

Fermentation conditions and methods

All experiments were carried out in a 2 L jar fermenter (Applikon Biocontroller ADI1030) with an initial volume of 1.5 L at 42°C. The agitation speed was set at 200 rpm to insure complete mixing of the fermentation medium. The inocula were incubated at 42°C for 12 h at 200 rpm before their transfer to the fermenter in a 10%. The culture pH was maintained at 6.25 by automatic addition of 25% (w/w) NH_4OH solution using a computer coupled peristaltic pump during the 24 h of fermentation. The samples were withdrawn at desired intervals and frozen for further analysis. The fermentation batch (control) of *L. rhamnosus* for production of L-lactic acid at a glucose concentration of 15 $g.L^{-1}$ was carried out. Other culture led in the same conditions was also prepared containing 20 µL of the parsley essential oil added during the exponential growth phase (this concentration is chosen after preliminary tests). The evolution of the biomass, the residual glucose amount and the lactic acid production are followed in regular time intervals.

Microbiologic method

Gram colouring is made regularly to control at the same time any risk of contagion and the presence of spores.

Analytical methods

The biomass was determined by measurement of the optical density (OD) at 570 nm by a spectrophotometer HITACHI 4-2000. Culture samples were centrifuged (13200 g at 4°C for 5 min), diluted and filtered. Residual glucose and lactic acid concentrations were determined by Multi parameter Medical Analyzer. The enzymatic kit used for the lactic acid dosage is the PAP Ref-61 192 and for the glucose dosage it is the Elitech diagnosis ref - GPSL-0500.

Data processing treatment

The calculation of the fermentation kinetic parameters requires a preliminary data processing (smoothing) of the rough experimental data with the software KALEIDAGRAPH. This data processing is based on the technique of the averages slipping by using a second degree polynomial.

Calculation of the fermentation kinetic parameters

The various analyses carried out allow the following time evolution of the component concentrations present in the culture medium: [Biomass: X(OD) = f (t), sugars: S = f (t) and the metabolite produced (lactic acid): P = f (t)]. From these raw data, it is possible to calculate the fermentation kinetic parameters in the batch culture by the calculation of the volumetric growth rate (r'''_x in $g.L^{-1}.h^{-1}$) and the specific growth rate (µ in h^{-1}):

$$r'''_X = \frac{dX}{dt}, \quad \mu = \frac{r'''_X}{X}$$

The calculation of the volumetric sugar consumption rate ($r'''s$ in $g.L^{-1}.h^{-1}$) and specific sugar consumption rate (Qs in $g.g^{-1}.h^{-1}$) is also possible:

$$r'''_S = -\frac{dS}{dT}, \quad Qs = \frac{r'''_S}{X}$$

as well as the volumetric lactic acid production rate ($r'''p$ in $g.L^{-1}.h^{-1}$) and the specific lactic acid production rate (Ql.a in $g.g^{-1}.h^{-1}$):

$$r'''_P = \frac{dP}{dt}, \quad Ql.a = \frac{r'''_P}{X}$$

The maximal specific growth rate (µmax) was determined from the slopes of the plotted linear curve:

$\ln X/X_0 = f(t)$ (Bimbenet and Loncin, 1995)

Outputs in biomass and lactic acid (the slopes)

The biomass (Yx/s) and products (Yp/s) yields are defined as the mass ratios in biomass and metabolites formed per gram of consumed carbonaceous substrate. During this fermentation, we were interested in the yields of sugar conversion into biomass and lactic acid.

X_0, S_0, L_0, represents the concentrations in respectively biomass, substrate and metabolites (lactic acid) produced at time t_0 of the fermentation and *X, S, L* at time t of the fermentation.

The approach used to determine the outputs consists in plotting linear curves: $[(X–X_0) = f (S_0–S)]$ and $[(L.A–L.A_0) = f (S_0–S)]$. The slopes obtained from these straight lines represent respectively the outputs of sugar conversion into biomass and lactic acid (Djidel, 2007).

RESULTS AND DISCUSSION

Characterization and identification of the parsley essential oil

The essential oil of the parsley was obtained with a yield on 0.14 and 0.08%, respectively, for the dichloromethane and the pentane. This can be explained by the strong solubility of the essential oil in a polar solvent. The isolated essential oil of brown yellow colour and characteristic smell of the parsley was characterized by its physico-chemical indications (Table 1).

Table 1. Physicochemical characterization of parsley essential oil.

Physicochemical parameter	Result
Density	1.17
Rotators power	+2°
Indication of refraction	1.322
Miscibility in the ethanol	18
Indication of iodine	2.03
Indication of acid	11.22
Indication of ester	54.71
Iindication of saponification	46.56

Table 2. The time of retention indices and the percentage of every peak.

Number Pic	Retention indices (min)	Percentage (%)	Products identified
1	6.82	1.6	-
2	7.44	47.5	Dillapiol
3	7.72	12.66	-
4	12.53	38.22	-

Thin layer chromatography (TLC)

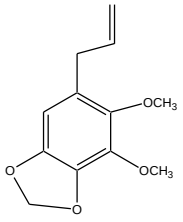
Dillapiol was revealed by TLC by comparison of its factor of keeping back (Rf = 0.57) with those of the essential oil.

Gas chromatography (GC)

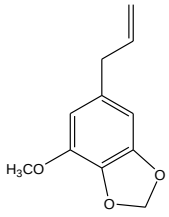
Table 2 gives the time of retention indices and the percentage of every peak. Peaks 1, 3 and 4 were not able to be identified by absence of leading products. The chromatogram (Photo 1), representing the co-injection of the dillapiol with parsley essential oil, indicates the presence of this heterocyclic. Indeed, one observes a net increase of the peak no 2. The percentage passes from 19.11 to 47.5%.

Gas chromatography/spectrometry mass (GC/MS)

With the aim of confirming the presence of the dillapiol, we appealed to the chromatography in phase vapour coupled with the mass (GC/MS). We notice that the spectre of mass of the majority peak is in accordance with the fragmentation of the dillapiol: M +: 222, 207, 191, 177, 149, 121 and 77. Besides, the presence of the myristicin was listed M+: 192, 161, 119, 91 and 65.



Dillapiol



Myristicin

Comparison of the chemical composition of parsley essential oil with other studies

By comparison between our results and those reported in the literature, we are going to quote some examples of the works, which showed the chemical composition of essential oil of parsley. Essential oil of parsley of Germany contains the apiol, the monoterpenes α- and β-pinene, of the myristicin (Spraul et al., 1992).

That of Egypt contains 23.8% of myrcene, 39.7% of myristicin, 6.94% α- pinene, 4.57% β- pinene, 1.11% α-phellandrene, 17.1% 1.3.8 p-menthatriene, 1.03% of dillapiol, 0.71% of bisabolole and 0.11% of camphor (Hashem and Sahab, 1999).

Studies showed the variety of the chemical composition of the essential oil of parsley. The major product is the 1,3,8-p-menthatriene for several foreign oils. Table 3 gives the main constituents of the essential oil of the parsley for several countries (Simon and Quinn, 1988).

Contrary to the foreign oils, the Algerian essential oil of parsley is constituted mainly of dillapiol isomer of the apiol.

Antimicrobial activity of the parsley essential oil

On tested microorganisms

Characterization and identification of the tested microorganisms: On the Mac Conkey medium, *E. coli* suspected colonies presented a diameter from 2 to 3 mm; they are red to pink, smooth, brilliant and flat with regular edges. *E. coli* are Gram negative (Photo 2). The most important biochemical characteristics are presented in Table 4.

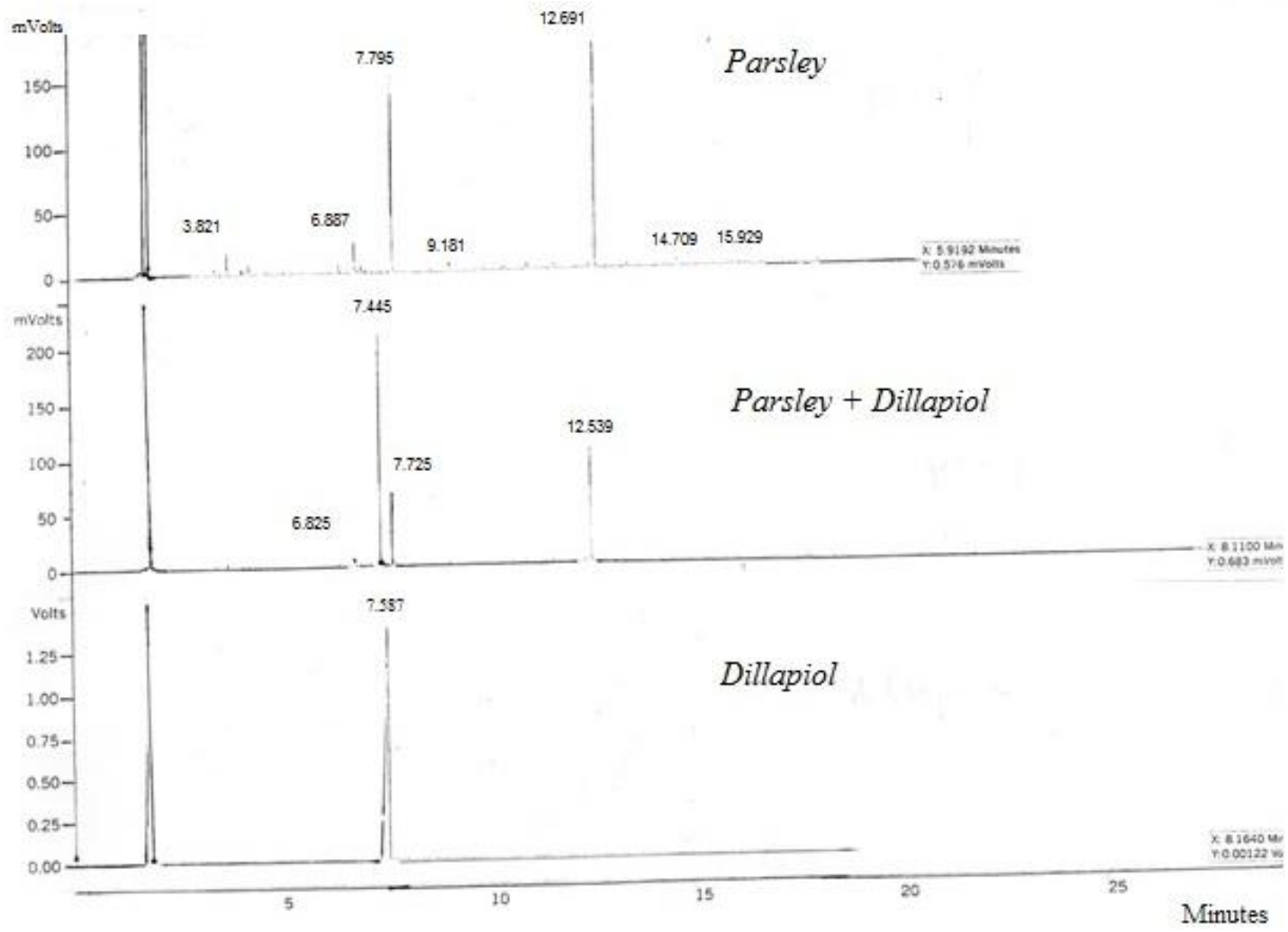


Photo 1. Chromatogram in gaz phase of parsley essential oil.

Table 3. Percentages of the main constituents of the essential oil foreign of Parsley.

Country / Product	Turkey	Arabie Saoudite	Yougoslavie	Iran
α- Pinene	2.3	1.1	0.1	0.6
β- Pinene	1.6	0.8		0.5
Myrcene	9.7	10.4	2.8	2.9
α-Phellandrene	1.1	0.8	0.3	0.9
β-phellandrene	12.1	8.0	6.2	9.7
Terpinene	0.1	0.1		0.2
Terpinolene	2.9	13.9	2.1	5.4
1,3,8-p-Menthatriene	62.8	44.0	20.1	64.7
Thymol		2.0		
Myristicin		1.5	60.5	0.2
Apiol			1.4	

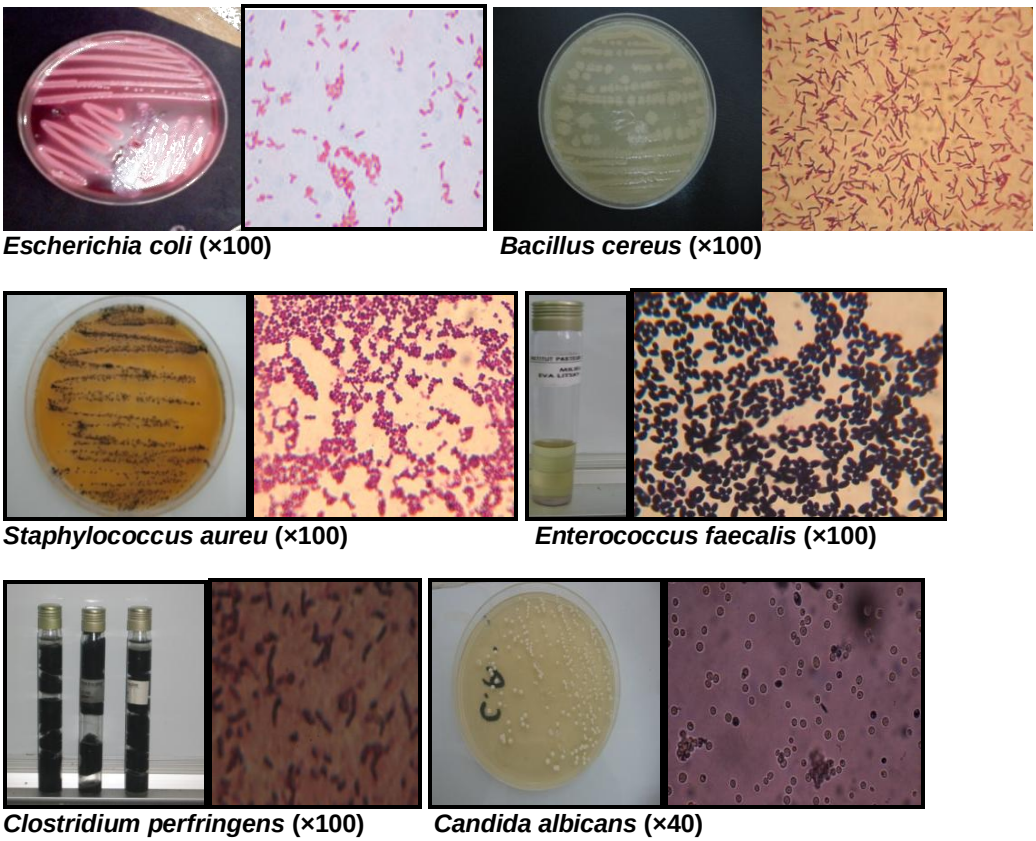


Photo 2. Macroscopic and microscopic aspects of tested microorganisms.

Table 4. Biochemical characteristics of *E. coli.*

Biochemical tests		Results
Oxidase test		-
O.N.P.G.		+
Simmons citrate		-
Reductase nitrate		+
	Lactose	+
	Saccharose	+
	Glucose	+
TSI	Gaz	+
	H_2S	-
	Urea	+
Urea-indol	Indol	+
	Mannitol	+
Mannitol-mobility	Mobility	-
	RM	+
RM/VP	VP	-
ADH/ LDC/ ODC		-/+/+

B. cereus appears under shape of big dry, extensive and irregular colonies of a whitish colour. This microorganism is Gram positive. The most important biochemical characteristics are presented in Table 5.

Characteristic colonies of *S. aureus* are black brilliant 1 to 2 mm in diameter, surrounded with zones of transparency. Microscopic observation shows that these microorganisms are Gram positive. For biochemical tests, we realized for the first time, the tests of catalase and of coagulase which are then supported by the gallery API Staph (Table 6).

In Eva Litsky liquid medium, the faecal Streptococci form a trouble and a purple pastille in the heart of tube. The isolation of *E. faecalis* was made from the faecal Streptococci. Gram colouring shows that these

Table 5. *Bacillus cereus* (characters of determination).

Biochemical test	Result
Catalase	+
Respiratory type	Facultative
Fermentation of sugars (Lactose)	+
Haemolyse	+
Degradation of the casein	+

Table 6. *S. aureus* identification (positive reactions after 24 h at 37°C).

Primordial test				
Catalase			**Coagulase**	
+			**+**	
API Staph.				
0	GLU	FRU	MNE	MAL
-	+	+	+	+
LAC	TRE	MAN	XLT	MEL
+	+	+	-	-
NIT	PAL	VP	RAF	XYL
/	/	/	-	-
SAC	MDG	NAG	ADH	URE
-	-	-	+	+

Table 7. Identification characters of *E. faecalis*.

Biochemical test		Result
Catalase		-
Mannitol-mobility	Mannitol	+
	Mobility	-

Table 8. *C. perfringens* identification.

IND	URE	GLU	MAL	LAC
+	+	+	+	+
SUC	MAN	SAL	XYL	ARA
+	+	+	+	-
GEL	ESC	GLY	CEL	MNE
+	+	-	+	+
MLZ	RAF	SOR	RHA	TRE
-	+	+	+	+

microorganisms are pods and Gram positive (Photo 2). Two biochemical tests were realized: the catalase and mannitol-mobility (Table 7).

The colony suspect of *C. perfringens* is a black colony of a diameter superior to 0.5 mm. A microscopic morphology reveals that these microorganisms are bacilli to positive Gram. Adding to the test of the catalase, *C. perfringens* was identified by using a miniaturized technique called the gallery API 20 A (Table 8).

C. albicans cultured on the selective medium Sabouraud gives white, creamy and smooth colonies. The simple colouring of blue in the lactophenol shows the presence of the egg-shaped cells (Photo 2).

The antibiotic sensitivity testing: The results of the antibiogram (Table 9 and Photo 3), showed that the *E. coli* strain was sensitive to gentamycine and to ofloxacine, it can be treated in a usual and intermediate dose in tetracycline and ampicillin. According to Bachir and Benali (2008), this strain is sensitive to chloramphenicol, resistant to ampicillin, doxycycline and pristinamycine and intermediate resistant to erythromycin and nitroxoline. According to the results obtained, we can classify *B. cereus* in two categories of sensitivity (intermediate and sensitive). *B. cereus* is sensitive to gentamycine, tetracycline and ofloxacine. It is treated in a usual and intermediate dose in penicillin G and clindamycine. It is can be treated by the increase of the dose of the penicillin G or clindamycine. *S. aureus* is intermediate in the penicillin G and sensitive to gentamycine, clindamycine, tetracycline and oxacilline. *E. faecalis* is resistant to all the chosen antibiotics. *C. perfringens* is sensitive to chloramphenicol and tetracycline. It is called intermediate in the penicillin G in amoxicillin and in ampicillin.

Antimicrobial activity of the parsley essential oil: According to the results of aromatogram obtained in Table 10 and Photo 4, we can note that the various doses applied to the *B. cereus, S. aureus* and *C. perfringens* presents an inhibitory activity increasing to 10, 20 and 40 µL. The doses of 10 and 20 µL have an identical effect on the *E. faecalis* representing a diameter of 14 mm. *C. albicans* countered sensitive and *E. coli*, showed insensitivity against the action of the various doses of the *Petroselinum crispum* essential oil.

We noticed that the *E. coli*, which is a bacterium to negative Gram, showed insensitivity to the action of the various doses of the *P. crispum* essential oil, contrary to bacteria that was Gram positive. This result is supported by the variation of the result by the different composition and the cellular structure of bacteria to Gram positive and negative bacteria, because and according to Larpent and Larpent-Gourgaud (1985), the Gram negative bacteria cells contain a wall rich in lipids and a double membrane equipped with a died plasmic space. This confers her degree of protection against the constituents of the essential oil. So, we supposed that *E. coli* inhibition requires high concentrations of essential oil. A lessening spectre of activity was observed with the studied essential oil which represents a good activity on the bacterial strain *B. cereus* and *C. albicans* with a diameter of zone of inhibition, respectively of 26 and 24 mm in means and a weak activity on *S. aureus* and *C. perfringens* which represent a diameter of zone of inhibition of 18.5 mm in mean and finally the *E. faecalis* with an average of zone of inhibition of 17 mm. This sensibility can be due to the action of one or several essential oil of parsley.

The lactic acid production by L. casei subsp. rhamnosus

The results of the two batch fermentations of *L. rhamnosus* control and with 20 µL of parsley essential oil are presented in Figures 1 to 3, respectively. We noticed the increase in the concentrations of biomass, residual

Table 9. Antibiogram results of tested microorganisms with traditional antibiotics.

Antibiotic	*E. coli*		*B. cereus*		*S. aureus*		*E. faecalis*		*C. perfringens*	
	IZD	R	IZD	R	IZD	R	IZD	R	IZD	R
Tetracycline	8	I	30	S	32	S	0	R	22	S
Gentamycine	20	S	20	S	18	S	-	-	-	-
Ofloxacine	20	S	27	S	-	-	-	-	-	-
Ampicillin	7	I	-	-	-	-	0	R	8	I
Penicillin G	-	-	9	I	10	I	0	R	8	I
Clindamycine	-	-	9	I	20	S	0	R	-	-
Oxacilline	-	-	-	-	16	S	-	-	-	-
Chloramphenicol	-	-	-	-	-	-	0	R	31	S
Amoxicillin	-	-	-	-	-	-	-	-	13	I

IZD: Inhibition zone diameter (mm); R: results.

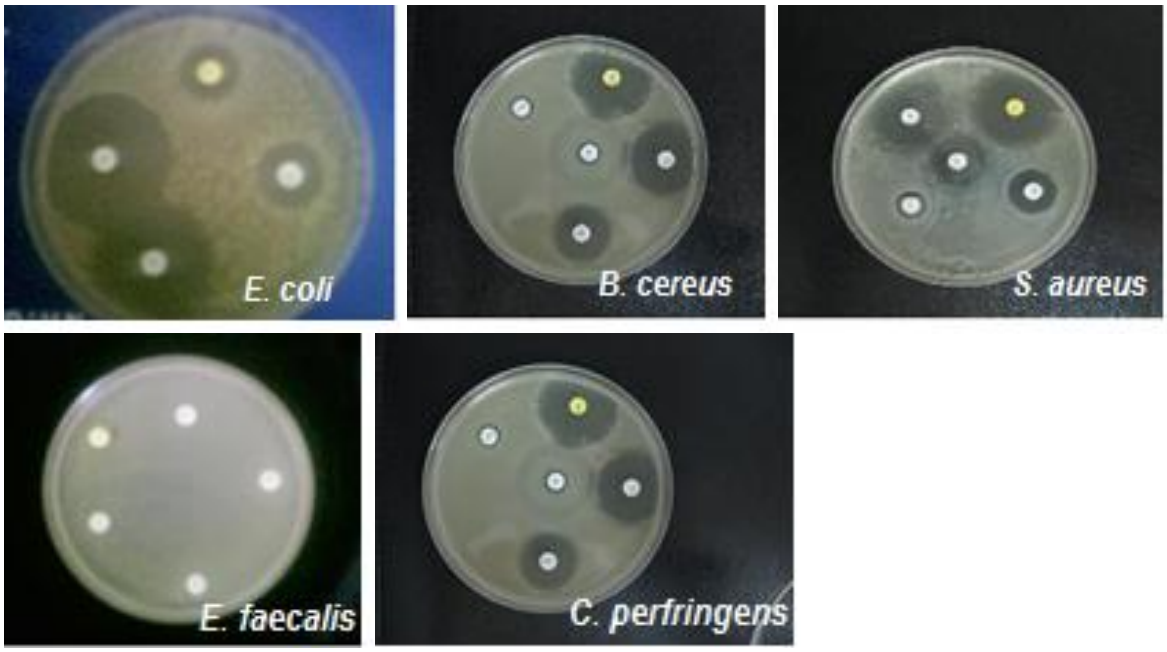


Photo 3. Antibiogram of tested microorganisms.

Table 10. Zone of inhibition (mm) of tested microorganisms in the presence of various doses of the parsley essential oil.

Doses of parsley EO / Inhibition zone diameter (mm)	10 µL	20 µL	30 µL	40 µL
Escherichia coli.	0	0	0	0
Bacillus cereus	23	27	-	29
Staphylococcus aureus	13	20	22	24
Enterococcus faecalis	14	14	16	20
Clostridium perfringens	12	18	21	25
Candida albicans	18	20	24	30

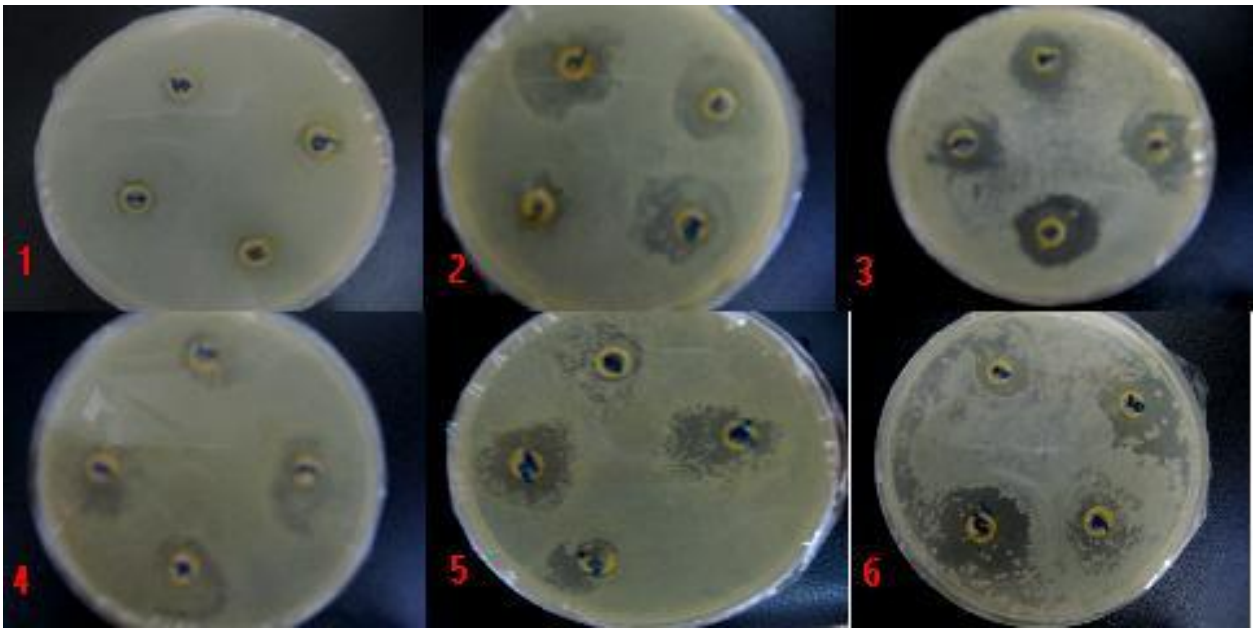


Photo 4. Aromatograms made on microorganisms tested in the presence of various doses of the essential oil of parsley. 1. *Escherichia coli*, 2. *Bacillus cereus*, 3. *Staphylococcus aureus*, 4. *Enterococcus faecalis*, 5. *Clostridium perfringens*, 6. *Candida albicans*.

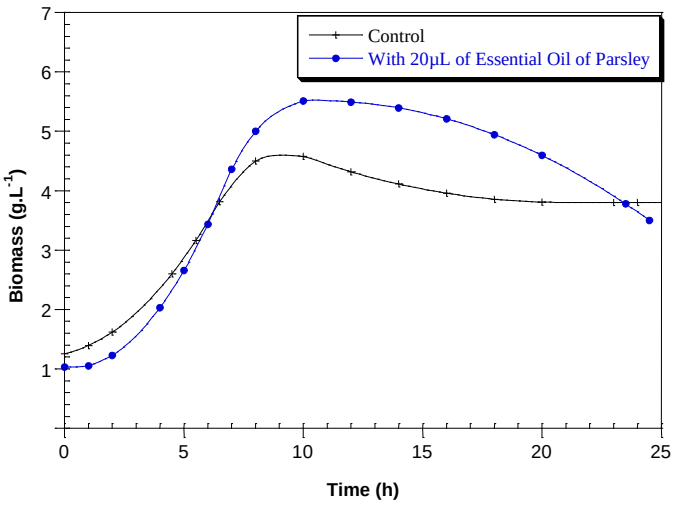


Figure 1. Biomass production during the culture of *Lactobacillus rhamnosus* in two batch fermentations (control with 20 µL of essential oil of parsley).

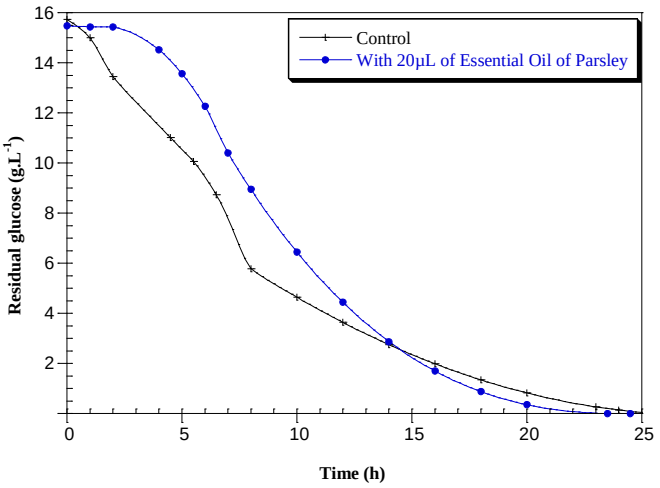


Figure 2. Residual glucose during the culture of *L. rhamnosus* in two batch fermentations (control with 20 µL of essential oil of parsley).

sugars and lactic acid. The initial concentration in glucose (15 $g.L^{-1}$) was the same for the samples. They were inoculated with the same quantity of biomass (1.2 $g.L^{-1}$). According to the obtained results, it was observed that the *L. rhamnosus* growth for the batch fermentations is characterized by a short duration of the latency phase which indicates that the inoculated cells were in full exponential phase.

For the control fermentation, a weak biomass initial concentration of 1.2 $g.L^{-1}$ was observed which increases after 6 h of fermentation until 3.5 $g.L^{-1}$, then until 4.575 $g.L^{-1}$ after 8 h of culture (at the end of the exponential phase). The growth end is due to the glucose exhaustion in the culture medium which reaches a final value of 0.008 $g.L^{-1}$ after 24 h of fermentation. During the stationary phase (after 8 h of culture), the strain consumed the glucose and used it only for the cellular maintenance. In parallel, the lactic acid production started from a value

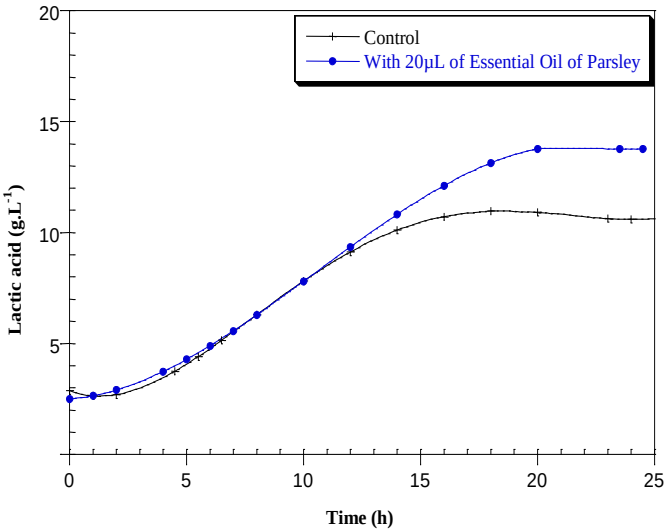


Figure 3. Lactic acid production by *L. rhamnosus* during two batch fermentations (Control with 20 µL of essential oil of parsley).

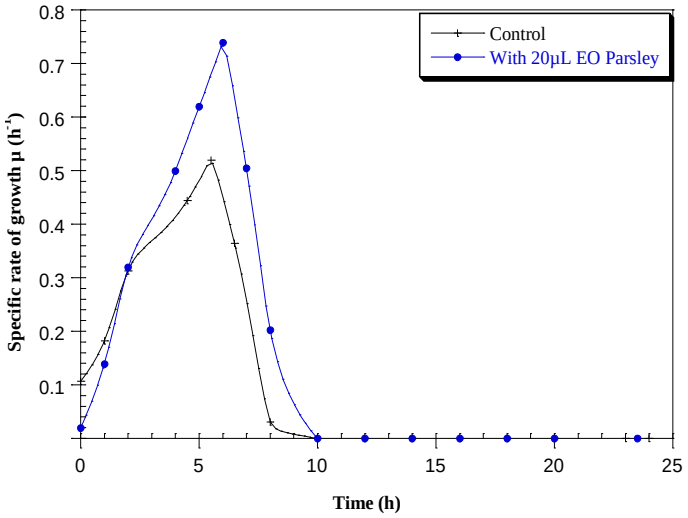


Figure 4. Specific rate of growth of *L. rhamnosus* during two batch fermentations (control with 20 µL of essential oil of parsley).

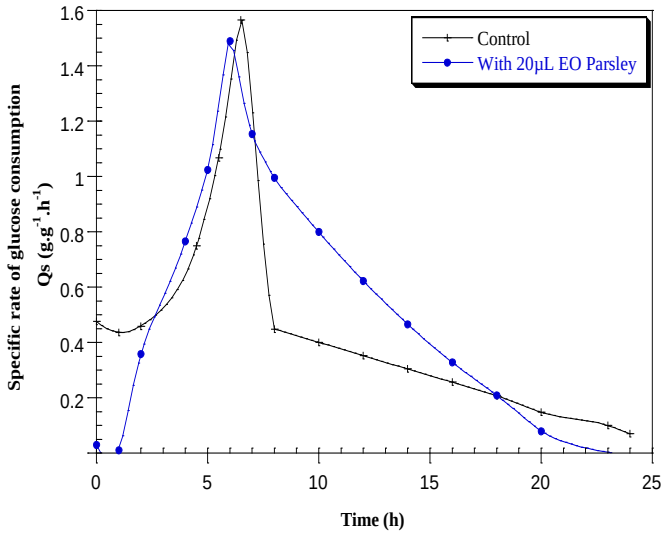


Figure 5. Specific rate of glucose consumption by *L. rhamnosus* during two batch fermentations (control with 20 µL of essential oil of parsley).

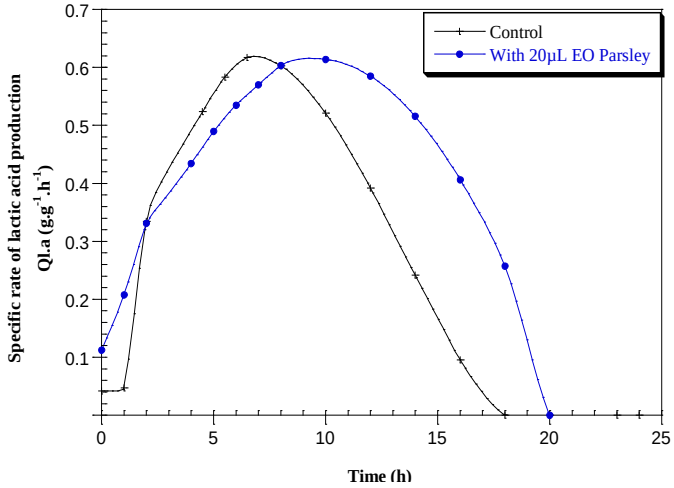


Figure 6. Specific rate of lactic acid production by *L. rhamnosus* during two batch fermentations (control with 20 µL of essential oil of parsley).

of 3 g.L^{-1} and reached 10.96 g.L^{-1} of lactate after 24 h of fermentation. During this fermentation that lasted for 24 h, the quantity of consumed sugar was 15 g.L^{-1}.

However, for the batch fermentation, the *Lb. rhamnosus* biomass started with an initial concentration of 1.2 g.L^{-1} and the parsley essential oil was added during the full exponential phase after 6 h of culture corresponding to a biomass quantity of 3.5 g.L^{-1}. After 10 h of fermentation, cellular concentration increased at a value of 5.51 g.L^{-1}. For the production of lactic acid, the *L. rhamnosus* culture reached the maximum value of 13.78 g.L^{-1} after 24 h of fermentation.

The specific rate of *L. rhamnosus* growth (µ) in the control MRS medium started at 0.11 h^{-1} and reached its maximum value of 0.5 h^{-1} after 6 h of fermentation, then it decreased to 0 h^{-1} after 10 h of culture. For the parsley fermentation, µ started with an initial value of 0.02 h^{-1} increasing to 0.74 h^{-1} after 6 h of fermentation (Figure 4).

The maximal sugar consumption specific rate (Qs max) was 1.5 g.g^{-1}.h^{-1} for the control and parsley and the maximal (Ql.a.max) lactic acid production specific rate was of 0.61 g.g^{-1}.h^{-1} for the control as well as parsley cultures (Figures 5 and 6).

The growth kinetics may be characterized by a maximal growth specific rate (µmax) which is equal to 0.17 h^{-1} in the MRS medium (control) and increases to 0.22 h^{-1} in the

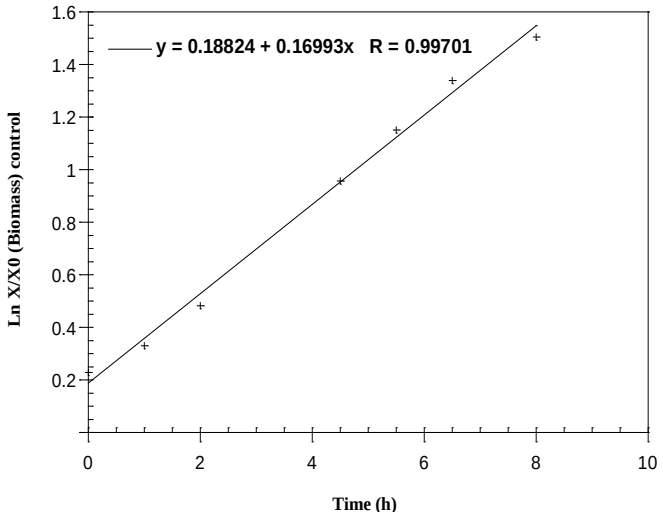


Figure 7. Maximal specific rate of growth µmax (slopes) during batch fermentation (control).

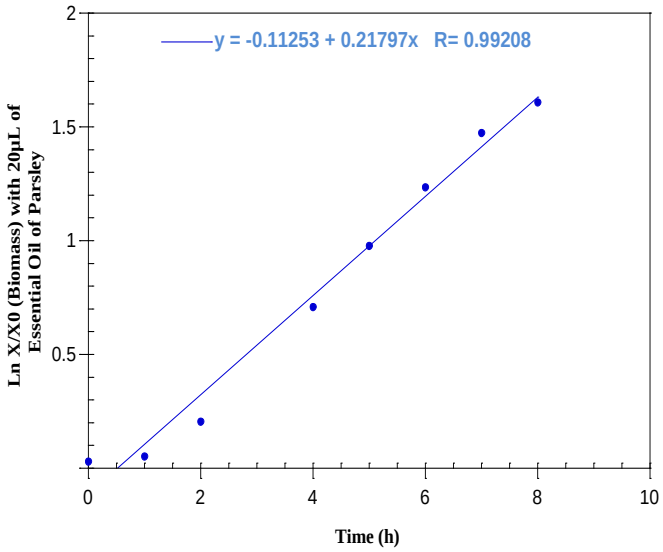


Figure 8. Maximal specific rate of growth µmax (slopes) during batch fermentation (control with 20 µL of essential oil of parsley).

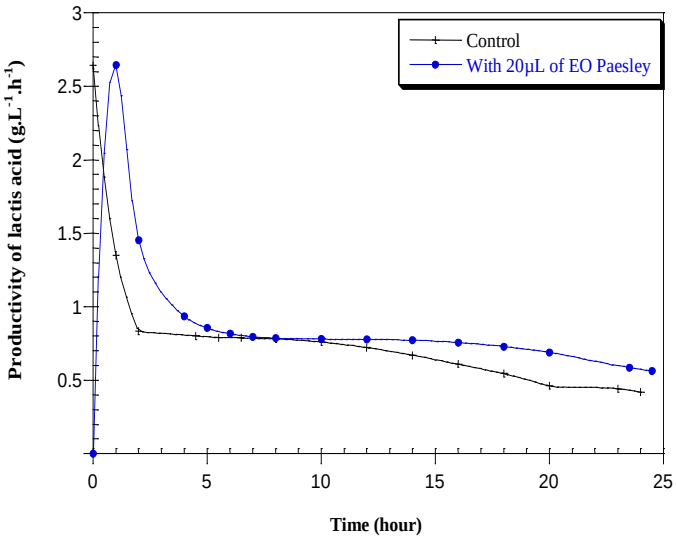


Figure 9. Productivity of lactic acid in two batch fermentations (control with 20 µL of essential oil of parsley).

the presence of the 20 µL of parsley essential oil. The lactic acid productivities are identical for both control and parsley cultures (Figures 7 to 9 and Table 11).

For the control fermentation, the yield of sugar conversion to biomass was 0.84 [1] and 0.71 $g.g^{-1}$ into lactic acid. It seems that the majority of the sugar is used for the cellular maintenance and multiplication and the remainder for the lactic acid production. The addition of the parsley essential oil in the culture stimulates the lactic acid production with a conversion yield of 0.87 $g.g^{-1}$.

According to the results in Table 11, one finds a positive correlation between the lactic acid production and the sugar consumption rate. This result shows the fate of consumed sugar: the majority of the consumed sugar is used for the lactic acid production (Table 11).

Conclusion

Medicinal plants represent a plentiful source of bioactive natural substances among which are the essential oils. This work aims to characterize and estimate antimicrobial activity of these substances on *E. coli, B. cereus, S. aureus, E. faecalis, C. perfringens and C. albicans*. The dillapiol constitutes 47.5% of the essential oil of parsley. The identification could be made by the use of chromatographic methods. Besides, the existence of the myristicin in essential oil of parsley was confirmed. It is shown that ethers-oxides are bi-functional having a function of dioxymethylene and one or two functions as methoxylic, found in our essential oil, and possess numerous therapeutic actions. They show antalgic and antispasmodic properties and anti-infectious power comparable to phenols methyl-ethers. *In vitro* analyses, allowed us to determine the antimicrobial effect of essential oil of *P. crispum* by the agar disc diffusion method which turned out more active especially on *B. cereus* and *C. albicans* (*C. perfringens*, *S. aureus* and *E. faecalis* are less sensitive), while it did not have any effect on the *E. coli*. The results of estimation of the effect of the parsley essential oil over the kinetics of lactic acid production by *L. rhamnosus* show a stimulating effect of the *P. crispum Hoffm* essential oil on the kinetics of lactic acid production and growth by *L. rhamnosus*.

ACKNOWLEDGEMENTS

We gratefully acknowledge Pr. J.M. Pons from reactivity

Table 11. Kinetics parameters of batch fermentations.

Fermentation / Parameter	Control	With 20µL of essential oil of parsley
Biomass max ($g.L^{-1}$)	4.57	5.51
Lactic acid max ($g.L^{-1}$)	10.96	13.78
Residual glucose ($g.L^{-1}$)	0.008	0.007
µmax (h^{-1})	0.17	0.22
Qsmax ($g.g^{-1}.h^{-1}$)	1.56	1.50
Q.l.a max ($g.g^{-1}.h^{-1}$)	0.61	0.61
Yx/s ($g.g^{-1}$)	0.84	0.41
Yp/s ($g.g^{-1}$)	0.71	0.87
Productivity in lactic acid ($g.L^{-1}.h^{-1}$)	2.64	2.64

µmax: Maximal specific rate of growth; Qs.max: maximal specific rate of consumption of sugars; Ql.a. max: maximal specific rate of production of lactic acid; Yx/s: yield on conversion of sugars in biomass; Yp/s: yield on conversion of sugars in lactic acid.

in organic synthesis of Paul Cezanne University Aix-Marseille and Pr. M. Fick (ENSAIA, INPL, France).

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