4

Physico-Chemical, Biochemical and Microbiological Phenomena of the Medicinal and Aromatic Plants Extract Used in the Preparation of *Tassabount* Date Juice in Morocco

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1. Introduction

In the Moroccan oases, traditional preparations of dates, the fruits of date palm (*Phoenix dactylifera*), are often associated with medicinal and aromatic plants (MAPs) which provide the properties of flavoring, preservation and medication (Harrak, 2007). For the traditional dates juice, *Tassabount*, its nutritional and organoleptic qualities and its therapeutic virtues come from both the date genotypes (cultivars and wild hybrids) and a multitude of MAPs used in its preparation (Harrak et al., 2009). Considering its promising applications, the *Tassabount* juice can get out of household manufacturing and consumption to emerge as a local product for a wider market. Such valorization requires a deep description and understanding of the different steps of the traditional juice processing.

Harrak et al. (2009) described the household process of preparing *Tassabount* which consists of two main steps. The first one is to prepare an aqueous extract of MAPs. This step is commonly called "fermentation" by households. About thirty MAPs can be used for preparing *Tassabount* (Zirari et al., 2003; Harrak, 2007). These are crop or wild plants used to make beverages, perfumes, medicinal extracts and herbal teas and tea bags (Table 1). Made empirically, these MAPs confer various therapeutic virtues and aromatic notes to *Tassabount* juice. The second step is juice processing. A viscous mixture is made with dates and a gradual incorporation of the MAPs extract. The rich foam reminiscent of soap forming, gave the name *Tassabount*. The mixture is finely sieved to remove seeds and part of the pulp retentate (Harrak et al., 2009).

Plants	Scientific names(1)
Basil	*Ocimum basilicum* L.
Bitter almond	*Prunus amygdalus* Stokes
Buttercup	*Pulicaria arabica* (L.) Cass.
Clove	*Eugenia cariophyllata* Thunb. (*Syzygium aromaticum* (L.) Merr.)
Date palm (fruits: dates)	*Phœnix dactylifera* L.
Fumitory	*Fumaria capreolata* L. / *Fumaria officinalis* L. / *Fumaria agraria* Lag. / *Fumaria parviflora* Lam. / *Euphorbia obtusifolia* Poiret / *Euphorbia helioscopa* L.
Gaillonia	*Gaillonia reboudiana* Coss. et Dur.
Haloxylon	*Haloxylon scoparium* Pomel
Harmel (roots)	*Peganum harmala* L.
Henna (leaves)	*Lawsonia inermis* L. (*Lawsonia alba* Lamk.)
Hundred petaled rose	*Rosa damascena* Mill. / *Rosa centifolia* Mill.
Iris (roots)	*Iris germanica* L. / *Iris florentina* L.
Lemon (fruits: lemons)	*Citrus limon* (L.) Burm.
Lime (fruits: limes)	*Citrus limon* (L.) Burm. / *Citrus aurantiifolia* Swingle
Mandrake	*Mandragora autumnalis* L.
Mint round	*Mentha suaveolens Ehr. (Mentha Rotundifolia* (L.) Hudson)
Myrtle	*Myrtus communis* L.
Nutmeg	*Myristica fragrans* Houtt
Oregano	*Origanum compactum* Benth. / *Origanum vulgare* L.
Ormenis	*Ormenis africana* Jord. et Fourr. / *Ormenis scariosa* (Ball.) Lit. et Maire
Pennyroyal	*Mentha pulegium* L.
Rosemary	*Rosmarinus officinalis* L.
Round shoveler	*Cyperus rotondus* L.
Sagebrush	*Artemisia herba-alba* Asso.
Sarghine (roots)	*Corrigiola telephiifolia* Pour.
Thyme	*Thymus satureioides* Coss. et Ball. / *Thymus broussonetii* Boiss. / *Thymus pallidus* Coss. / *Thymus maroccanus* Ball. / *Thymus vulgaris*
Zygophylle (roots)	*Gactulum album* / *Zygophyllum gaetulum* Emb. et Maire *Zygophyllum waterloti* Maire / *Zygophyllum fontanesi* Webb.

(1) (Sijelmassi, 1996).

Table 1. Medicinal and aromatic plants used in the preparation of *Tassabount* dates juice.

Depending on physical, chemical and microbiological parameters of MAPs extract, this step is critical to the *Tassabount* quality. The aim of this work is to provide a better understanding of the main physical, physico-chemical, biochemical and microbiological phenomena that take place during the preparation of MAPs aqueous extract. Nutritional, organoleptic and hygienic qualities of this extract are evaluated as well as its impact on the quality of *Tassabount* for a better valorization of this juice.

2. Materials and methods

2.1 Standardization of the aromatic extract

To set the conditions for preparation of standardized aqueous extract, preliminary tests for the preparation of aromatic extracts were performed. These trials included the selection of MAPs, temperature and maceration time.

2.1.1 Selection of medicinal and aromatic plants

Six plants were chosen among the most used by the oases households in the preparation of *Tassabount*. They are known for their antiseptic, antispasmodic, diuretic, carminative, antibacterial and / or antioxidant proprieties. These are oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.), hundred petaled rose (*Rosa centifolia*), pennyroyal (*Mentha pulegium* L.), sarghine (*Corrigiola telephiifolia*) and harmel (*Peganum harmala* L.) (Fig. 1).

Fig. 1. Medicinal and aromatic plants used for the preparation of the aqueous aromatic extract.

2.1.2 Medicinal and aromatic plants concentration

In general, concentrations of MAPs used in traditional food or pharmaceutical preparations are based on the know-how of the oases population who experienced from the earliest times how to control the active ingredients and to avoid harmful overdoses.

Based on the experience of oases women, the quantities of plants used for a volume of five liters of water were set as follows: 25 g of origano, 25 g of hundred petaled rose, 25 g of thyme, 25 g of pennyroyal, 12.5 g of sarghine roots and 12.5 g of harmel roots (Harrak, 2007). The amount used of harmel roots, much less rich in alkaloids than seeds, is tiny compared to the doses known to induce toxicity (Hammiche & Merad, 1997, as cited in Ben Salah et al., 1986). However, awareness and information on the toxic potential of plants, used in various traditional preparations, are an important preventive measure.

Before use, the MAPs are first sifted, sorted, washed and drained. Three dates of the *Black Bousthammi* variety (Fig. 2) chosen for the preparation of the juice were added as a source of carbohydrates and aromas in the MAPs maceration step.

Fig. 2. Dates of *Black Bousthammi* variety during ripening (on the left) and mature (on the right).

2.1.3 Temperature and duration of medicinal and aromatic plants maceration

Oases women immerse plants in water at room temperature and let marinate for two to three days. This period is deemed sufficient by the women for both an effective diffusion of plants constituents in the aqueous phase and for a good "fermentation". However, some women, yielding to the facility and depleting PAMs, use extracts leftover exceeding 5 days of maceration, accepting therefore a decline in the quality. To this end, we felt more appropriate in our work to extend the maceration beyond three days to detect signs of possible alteration of the quality of the extract.

2.2 Characterization of physical, physico-chemical and biochemical phenomena of medicinal and aromatic plants extract

2.2.1 Preparation of the medicinal and aromatic plants extract

Three jars, each containing water and aliquots of the six plants as described above, were covered with a cloth (not airtight closure) and placed at an average ambient temperature of 23 °C +/- 1 °C for monitoring the maceration.

The different physico-chemical and biochemical analyses were performed on filtered extracts collected from the three jars during five days or more.

2.2.2 Physical and physico-chemical criteria

1. Weight loss: The weight loss of the extract, providing information on gas release and / or water evaporation during the MAPs maceration, was followed by the weighing of the three jars. It is expressed in %.
2. Brix: The Brix of the extract during the MAPs maceration was determined at 20 °C using a digital refractometer (Pocket Refractometer PAL-1 (0~53%), ATAGO). It is expressed in °Bx (AOAC, 1990).
3. pH: The pH of the extract during the MAPs maceration was measured at 20 °C using a pH meter (SCHOTT) on a sample of 20 ml of filtered extract, under continuous stirring (AOAC, 1990).
4. Total titratable acidity: The total titratable acidity of the extract during the MAPs maceration was determined by titration of 20 ml of filtered extract using TitroLine Easy (SCHOTT) to pH 8.1 with a solution of sodium hydroxide 0.1 N. The acidity is expressed in meq / 100 ml (AOAC, 1990).
5. Ultra violet / visible spectrum: The evolution of the absorbance of the MAPs extract according to the wavelength from 190 nm to 900 nm (UV / Visible zone) was determined at four maceration times (20 min, 78 h, 102 h and 212 h). The measurements were made with a UV / visible spectrophotometer (UVIKON 933 Spectrophotometer Double Beam UV/VIS) using quartz cells with optical path equal to one centimeter. The color intensity of the extract during the MAPs maceration was determined as the sum of the luminous absorbance at 420, 520 and 620 nm.

2.2.3 Biochemical criteria

1. Total polyphenols: The total polyphenols contained in the aqueous extract during the MAPs maceration were extracted by an acetone / water mixture. They were determined by the Folin-Ciocalteau method (colorimetric method) revealing a blue color. Elimination of molecules with reducing properties, disturbing the determination of polyphenols by the Folin-Ciocalteau method, was performed on a cartridge with absorptive capacity of the polyphenols. Elution of the polyphenols was carried out with methanol. Quantification was performed using an external calibration of gallic acid at 760 nm and was expressed in mg GAE (Gallic Acid Equivalent) per 100 g of aqueous extract (Georgé et al., 2005). Measurements of absorbance were performed using a UV / Visible spectrophotometer (UVIKON 933 Spectrophotometer).
2. Ethanol: The dosage of ethanol of MAPs extract was achieved using an Enzytec™ fluid ethanol Kit for the photometric determination of ethanol. The samples were introduced into small cells of PS type for spectrophotometer. Measurements of absorbance at the wavelength of 340 nm were performed using a UV / Visible spectrophotometer (UVIKON 933 Spectrophotometer). The ethanol content was expressed in g / l of the aqueous extract of plants.
3. Lactic acid: The dosage of lactic acid of MAPs extract was achieved by using an Enzytec™ L-lactic Acid/D-lactic acid Kit for the photometric determination of lactic acid. The samples were introduced into small cells of PS type for spectrophotometer.

Measurements of absorbance at the wavelength of 340 nm were performed using a UV/ Visible spectrophotometer (UVIKON 933 Spectrophotometer). The lactic acid content was expressed in g / l of aqueous extract of plants.

2.2.4 Production of carbon dioxide

The release of carbon dioxide during the maceration of plants was followed in three sealing jars. The concentrations of CO_2 and O_2 in the jars, expressed in %, were measured using an O_2/CO_2 analyzer (PBI Dansesor, Checkmate 9900).

2.2.5 Evolution of the aromatic profile

The aromatic profile of the extract was followed during the MAPs maceration by implementing an experimental system based on Solid Phase Micro Extraction (SPME, SUPELCO Inc). This technique allows direct sampling and concentration, of the volatile emissions present in the headspace above the aqueous extract. The aromatic profile changes were monitored during three days by taking samples every two hours.

Trapping of the volatile aromatic fraction of the extract was performed using a manual fiber holder for 20 minutes. Volatile substances adsorbed by the fiber of polydimethylsiloxane type (PDMS) were then injected and desorbed thermally at 250 °C.

Separation and identification of volatile compounds were carried out by gas chromatography coupled with mass spectrometry (Agilent 5973N) using (J&W, DB-WAX) capillary column 0.25 mm (inner diameter) x 30 m x 0.25 µm (film thickness). The flow rate of carrier gas (helium) was 1.0 ml / min. Oven temperature programmation was from 40 °C to 220 °C for 10 min, at the rate of 3 °C / min (Fig. 3).

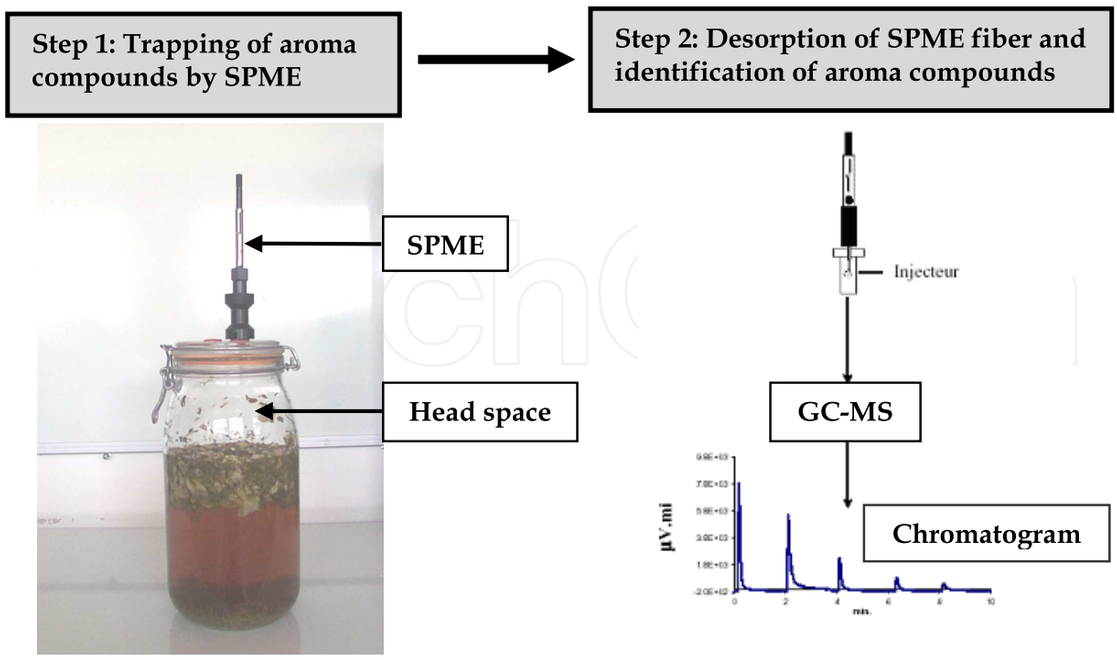

Fig. 3. Experimental system monitoring the aromatic potential of medicinal and aromatic plants extract.

Kovats retention indices were generated by using a series of *n*-alkanes from C-5 to C-22 eluted under the same chromatographic conditions as samples of aromatic extract.

The acquisition, visualization and analysis of data were made using the MSD ChemStation software (Agilent) and the NIST 2.0 a library of reference mass spectra.

2.2.6 Global olfactory fingerprint

The study of the aromatic profile during the maceration was also performed on the aqueous extract of MAPs by monitoring the global olfactory fingerprint using the electronic nose. A total of 15 samplings were carried out during the five days of maceration. At each sampling, 6 replicates of 3 ml of extract were withdrawn using a precision syringe and introduced into 10 ml chromacol headspace vial then immediately crimped.

The headspace was generated by incubation at 60 °C during 15 min with stirring (500 rpm). 2 ml of headspace were sampled and injected at a rate of 1.5 ml/s. They were injected (flow rate of purified air of 150 ml / min) in the Prometheus electronic nose (Alpha MOS) equipped with 18 MOS (metal oxide sensor). The sensor response, based on adsorption-desorption phenomenon, depends on the affinity and concentration of the molecules present in the head space. Time acquisition was 2 minutes, followed by 8 minutes of relaxation for a correct return to baseline.

So, each sample was associated to 18 different values of the sensors, considered as variables. They were analyzed by factorial discriminant analysis (FDA) using the Prometheus Alphasoft software (version 7), generating a global olfactory fingerprint which is considered as a real identity card.

2.3 Microbiological evolution of the extract

2.3.1 Aromatic extract sampling

A jar containing water and plants, prepared by the same method as the standard extract, was sealed to prevent outside contamination. An amount of 9 ml of extract was taken from the aqueous portion under stirring at four different times of plants maceration (0 h, 24 h, 48 h and 72 h) and then it was filtered. The removal and filtration of the extract were carried out in aseptic conditions (in laminary flow microbiological cabinet).

2.3.2 Enumeration of the microbial flora

Culture media: PCA (Plate Count Agar, Bio Merieux), PDA (Potato Dextrose Agar, Bio Merieux) and MRS (Man Agar, Rogosa, Sharpe) were respectively used to monitor the evolution of total mesophilic microflora, fungal microflora and lactic microflora. Serial dilutions up to 10^{-6} of the extract were performed using sterile physiological water (containing 0.85 % NaCl). For PCA and PDA media, 1 ml of extract was inoculated in the mass and incubated at 30 °C for 48 h. For the MRS medium, 0.1 ml was spread on the surface using sterile glass balls. The Petri dishes were then placed in airtight jars containing a Genbox anaer bag (generator for the cultivation in the jar of anaerobic bacteria) (Bio Merieux). Monitoring of anaerobic conditions was achieved using a moistened indicator paper. Incubation was carried out at 37 °C during 72 h. Control dishes (extract free) were

also prepared for the three culture media. The results were expressed as logarithm (Log) of colony-forming units (cfu) per liter of aqueous extract of plants.

3. Results and discussion

3.1 Evolution of physical and physico-chemical criteria of aromatic extract

3.1.1 Weight loss

Fig. 4 shows the evolution of weight losses of MAPs extract during the maceration, calculated from the initial weight. Weight losses during the first four days of maceration were respectively 0.59 %, 1.12 %, 1.52 % and 2.03 %, reaching 4.08 % after eight days of maceration. This weight loss could be mainly explained by gas release during the maceration.

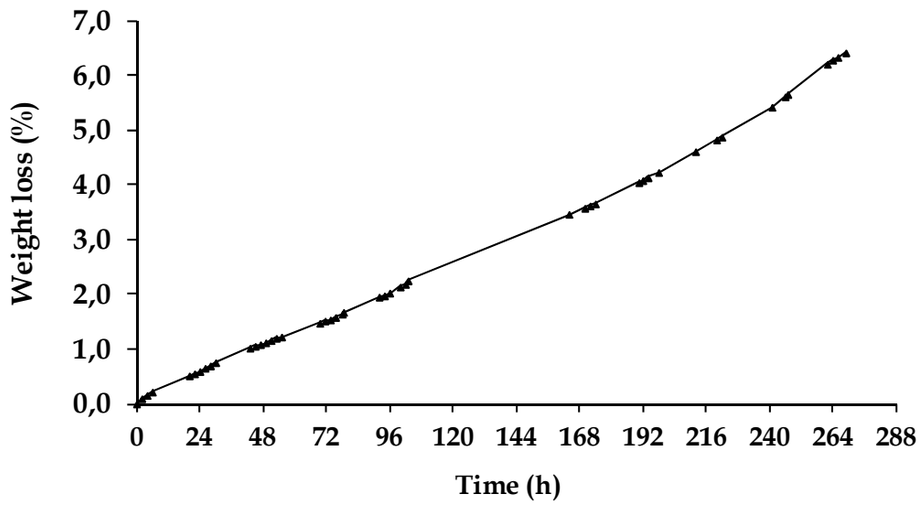

Fig. 4. Weight loss (%) of the aromatic extract during the maceration of medicinal and aromatic plants.

3.1.2 Brix

The largest change of Brix took place during the first twenty-eight hours of maceration (Fig. 5). In fact, the Brix value doubled during this time, from 0.4 °Bx to about 0.9 °Bx and this demonstrated the migration, into the aqueous phase, of soluble solids including sugars present in plants and the dates pulp added. This was followed by stabilization at this value during the second day, then a slight decrease during the third day. This decrease could be due to the use of sugars by microorganisms for their metabolism.

3.1.3 pH and total titratable acidity

The pH was about 7.3 at the beginning of the maceration and decreased steadily to the value of 5.3 after 44 h (Fig. 6). Then, the pH tended to stabilize at this value. The acidity followed the opposite trend. It recorded at the beginning a value of 0.8 meq / 100 ml, which increased with maceration time to reach 5.8 meq / 100 ml after 44 h. This increase can be explained by the production of organic acids during this MAPs maceration step. After this time, measures stabilized.

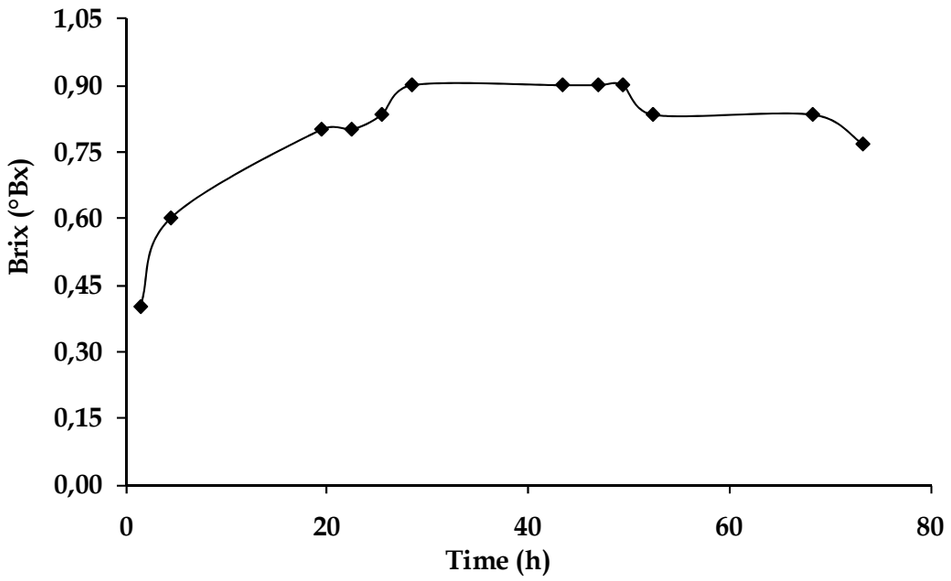

Fig. 5. Evolution of the aromatic extract Brix during the maceration.

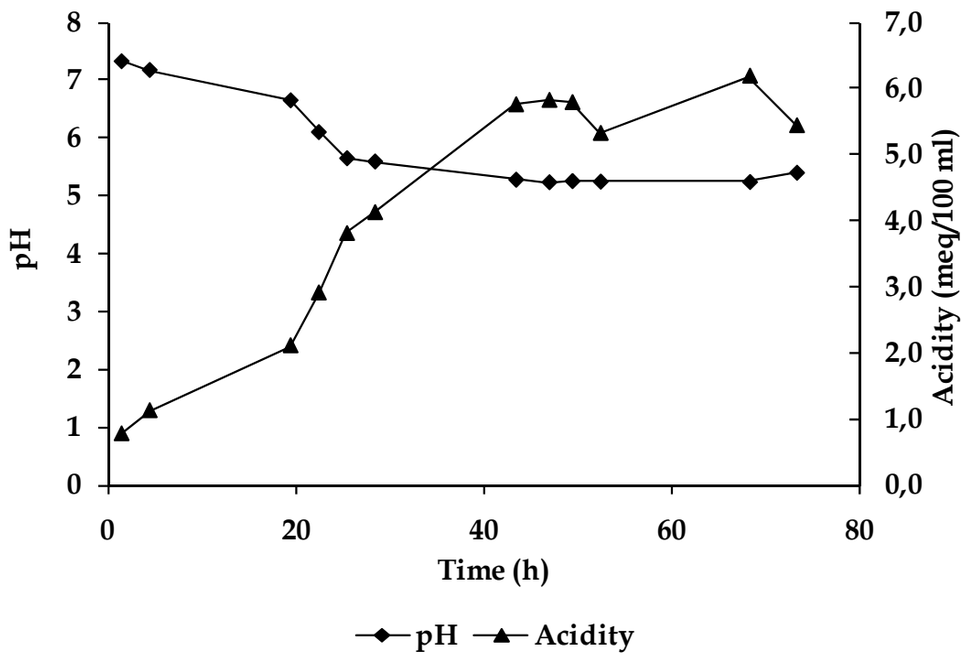

Fig. 6. Evolution of pH and total titratable acidity of the aromatic extract during the maceration.

The aromatic extract was slightly acidic (pH near 5), which could inhibit the growth of certain pathogenic bacteria. In practice, some households use, among MAPs, pieces of lemon or lime to increase the acidity of the extract.

3.1.4 UV / Visible spectrum

The evolution of extract UV / Visible spectrum during the maceration of MAPs showed the presence of peaks at some wavelengths. The presence of these peaks indicated release into water of some compounds of MAPs as phenolic compounds. Three major peaks appeared in the UV region at wavelengths: 338 nm, 281 nm and 197 nm (main peak). The latter was also observed in the extract just at the beginning of the plants maceration (after 20 min). The two spectra taken after 3 days (102 h and 212 h) coincided perfectly, indicating about a saturation of the liquid extract and / or depletion of MAPs. These two spectra were close and had the same pace as that recorded after a maceration time of 78 h. Only the peak intensities differed, heralding a lower concentration in different components of the extract at 78 h compared to those recorded after three days. The spectrum taken at the beginning of maceration had the same profile as those taken at the end of maceration, while showing very significant differences in intensity, so in concentration of absorbing compounds at these different wavelengths. This indicated a rapid diffusion of these compounds in water (Fig. 7).

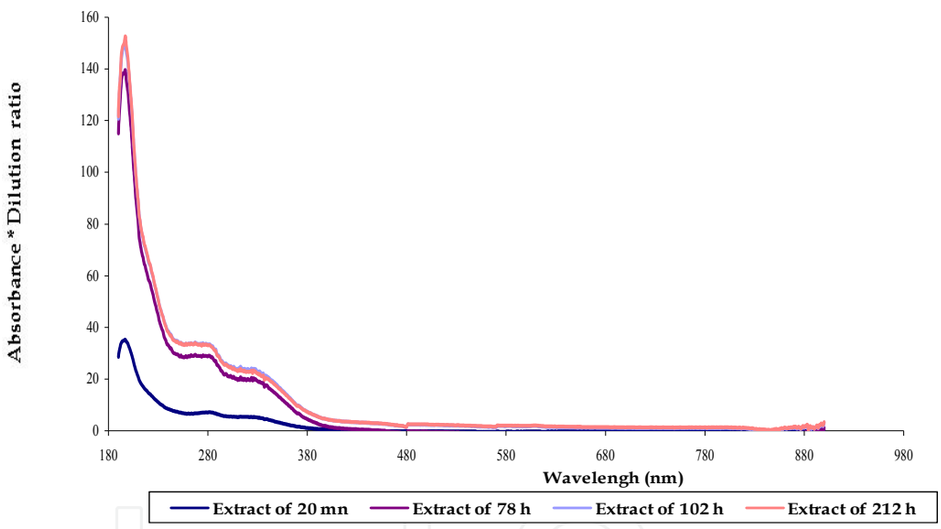

Fig. 7. Spectra of the extract in UV/Visible obtained at different times of maceration of aromatic and medicinal plants.

Moreover, a similar study on the kinetics of the absorbance at different wavelengths of green tea extracts also revealed an increase in absorbance at 280, 330 and 350 nm with time of infusion in water (Nkhili et al., 2007). The compounds corresponding to these peaks were, respectively, total phenols, hydroxycinnamic derivatives and flavonols.

Concerning the color intensity, it increased slightly during the first three days of maceration (from 0.164 recorded at 20 min to 0.797 recorded after 3 days), and recorded a strong increase (up to 7.200) in the fourth day to be stable thereafter. This increase could be due to the diffusion of colorants and to the browning of the extract due to oxidative phenomena. Its stabilization could be attributed to a depletion of plants in these compounds and eventually

to an equilibrium established between the aqueous phase and plants. This coloration of the extract, however, did not affect the brown color of the dates juice.

3.2 Evolution of biochemical criteria of aromatic extract

3.2.1 Total polyphenols

The total polyphenols content increased during the first three days of maceration of plants and tended to stabilize at a value of about 100 mg gallic acid equivalent (GAE) / 100 g of extract after the fourth day. These values were in fact 24.21, 95.00 and 99.98 mg GAE / 100 g extract, respectively, after 20 min, 78 h and 102 h of plants maceration.

3.2.2 Ethanol

The evolution of the ethanol content was fast at the beginning of maceration: the concentration had indeed increased from zero, recorded at 20 min, to 2.17 g / l, recorded after the third day. Between the third and the eighth days of maceration, we recorded a slight increase, reaching only 0.7 g / l. However, we may retain that the content of the aromatic extract ethanol was generally low, even after eight days of maceration.

3.2.3 Lactic acid

The lactic acid concentration increased with the duration of maceration. It reached 0.92 g / l after three days (78 h) and 1.30 g / l after almost nine days (212 h). The presence of lactic acid in the extract is an asset for the stabilization of the *Tassabount* juice. Indeed, lactic acid is widely used as a preservative in foods. Its combination with essential oils of MAPs could be valuable to preserve the sensory quality of juice and to prevent pathogens contamination that might require a higher acidity for their destruction or their limitation of growth (Dimitrijević et al., 2007).

3.3 Evolution of carbon dioxide

The evolution of carbon dioxide production during the maceration of plants for a period of ten days is shown in Fig. 8. It is clear that the production of carbon dioxide increased with time reaching a maximum average of 76.6 % after one week. The anaerobiosis was reached after 46 h. This change in the reverse direction of carbon dioxide (CO_2) and oxygen (O_2) indicated a fermentative process occurring during maceration of MAPs. The intense release of CO_2 during the first four days was consistent with the weight loss of the aromatic extract recorded during the maceration of MAPs.

It should be reminded that the monitoring of concentrations of both gases was performed in airtight conditions. The gas phase above the extract, estimated to be about one third of the total volume, is very important for the interparticle transfer of oxygen and carbon dioxide generated by the metabolism thus affecting the microbial metabolism (Desgranges & Durand, 1990).

Furthermore, the increase of CO_2 concentration during the maceration of MAPs generated a pressure in the gas phase. Previous studies have shown that the partial pressure of CO_2 had an effect on the physiological behavior of filamentous fungi by inhibiting their growth (Desgranges & Durand, 1990).

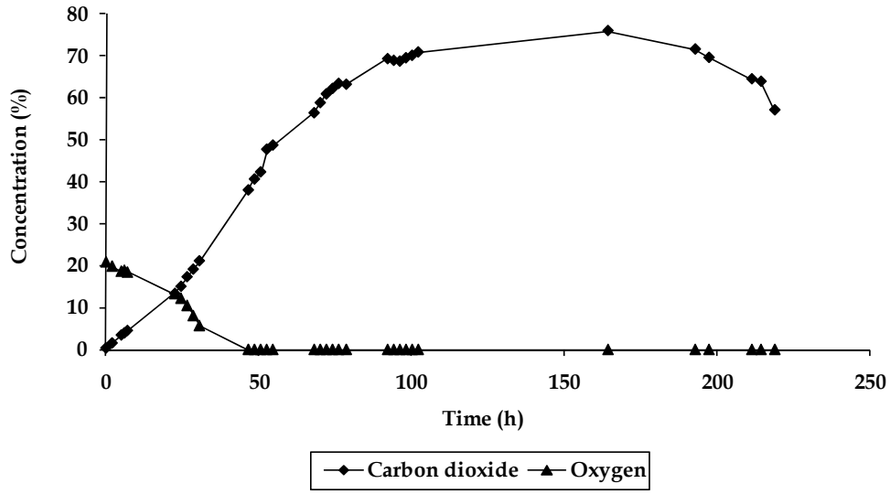

Fig. 8. Evolution of carbon dioxide and oxygen concentrations (%) during the maceration of medicinal and aromatic plants.

3.4 Evolution of the aromatic profile of plants extract

3.4.1 Quantitative and qualitative analysis of aroma compounds

A full qualitative analysis, taking into account all of the volatile compounds extracted by SPME, has identified a total of 92 volatile compounds in the aqueous extract of MAPs (Table 2). Of these, 96.7 % were identified in the extract taken after 6 h of maceration of plants, against only 54.3 % for the extract taken after 48 h. The compounds known for their functional properties such as thymol, carvacrol and α-pinene, were identified in both situations. These qualitative observations suggested that the maceration time of 6 h allowed the best expression of aromatic plants.

The 92 identified aroma compounds showed the predominance of terpenic hydrocarbons and their alcoholic and ketonic derivatives. Terpenic esters, oxygenated sesquiterpenes, aliphatic alcohols, aliphatic ketones and aldehydes were found as well.

For some aromatic compounds identified by SPME, the interpretation of results was based on a semi-quantitative approach, by determining the dominant compounds (with relatively large peaks), compounds with low peaks and compounds that were not detected in the extract at different studied maceration times (Table 3). The data in this table show mainly quantitative variations of the compounds at different times of maceration. The qualitative composition of the extract at these different times remains substantially the same with a few exceptions, where we record the absence or presence at a low concentration. The majority of compounds having low Kovats indices (ranging from 997 to 1243) were identified in the headspace, in important amounts, at the beginning of maceration (recorded after 20 min, the requisite trapping time) until the third day. Other compounds were also found at the beginning of maceration, this was the case especially of β-linalool, (-)-bornyl acetate, thymyl methyl ether, β-caryophyllene, isoborneol, thymol and carvacrol.

However, some compounds had a random detection during the maceration as cis-geranylacetone and mint furanone. This could be due to saturation of the SPME fiber or possibly they have competed with the other compounds. As for the humulene oxide, it has been appeared after the sixth hour of maceration.

N°	Aromatic compound	Kovats index	Extract of 6 h(2)	Extract of 48 h(2)
1	Tricyclene	997	+	+
2	Alpha-thujene	1010	+	+
3	Alpha-pinene	1012	+	+
4	Camphene	1045	+	+
5	Cyclobutanol(1)	1060	-	+
6	Beta-pinene	1088	+	+
7	Alpha-phellandrene(1)	1104	+	+
8	3-heptanone(1)	1126	+	+
9	Delta-3-carene(1)	1129	+	+
10	Beta-myrcene	1143	+	+
11	Pseudolimonene(1)	1148	+	+
12	Alpha-terpinene	1158	+	+
13	D-limonene	1178	+	+
14	Beta-phellandrene	1186	-	+
15	Eucalyptol	1190	+	+
16	Beta-*trans*-ocimene	1215	+	-
17	Gamma-terpinene	1225	+	+
18	3-octanone	1228	+	+
19	Beta-*cis*-ocimene	1230	+	+
20	*p*-cymene	1243	+	+
21	Terpinolene	1259	+	+
22	Delta-4-carene(1)	1264	+	+
23	3-methylcyclohexanone(1)	1286	+	-
24	2-octanol(1)	1295	+	-
25	3-octylacetate(1)	1315	+	+
26	*Cis*-Rose oxide	1328	+	-
27	3-nonanone	1331	+	-
28	*Trans*-Rose oxide	1341	+	-
29	L-fenchone(1)	1361	+	-
30	Nonanal	1366	+	-

N°	Aromatic compound	Kovats index	Extract of 6 h(2)	Extract of 48 h(2)
31	3-octanol	1369	+	+
32	Thujone(1)	1386	+	-
33	Beta-thujone(1)	1404	+	-
34	Linalool oxide(1)	1410	+	-
35	1-octen-3-ol(1)	1420	-	+
36	*Trans*-limonene oxide(1)	1423	+	-
37	Isomenthone	1429	+	+
38	*Trans*-sabinenehydrate	1436	+	+
39	Alpha-cubebene(1)	1442	+	-
40	Menthofurane(1)	1446	+	+
41	Alpha-campholenal(1)	1451	+	-
42	D-isomenthone	1455	+	+
43	Camphore	1471	+	+
44	Alpha-copaene(1)	1475	+	-
45	Alpha-bourbonene(1)	1499	+	-
46	Alpha-gurjunene(1)	1510	+	-
47	Beta-linalool	1517	+	+
48	Pinovarvone(1)	1521	+	-
49	Bergamol(1)	1527	+	-
50	*Trans*-isopulegone	1529	+	+
51	Bornyl formate	1535	+	+
52	Isopulegone	1542	+	+
53	(-)-Bornyl acetate	1548	+	+
54	Menthol acetate(1)	1550	+	-
55	Thymol methyl ether(1)	1558	+	-
56	Dihydrocarvone	1565	+	-
57	Thymyl methyl ether	1567	+	+
58	Beta-caryophyllene	1571	+	+
59	Thujanol	1575	+	+
60	Carvone(1)	1582	+	-
61	Delta-elemene(1)	1585	+	-
62	(+)-isomenthol(1)	1597	+	-
63	Pulegone	1601	+	+
64	*Trans*-pinocarveol(1)	1606	+	-

N°	Aromatic compound	Kovats index	Extract of 6 h(2)	Extract of 48 h(2)
65	Aromadendrene(1)	1610	+	-
66	Estragole(1)	1611	+	-
67	Cinerone	1614	+	+
68	*Cis*-verbenol(1)	1619	+	+
69	Alpha-caryophyllene(1)	1620	+	-
70	Isoborneol	1655	+	+
71	Alpha-terpineol	1662	+	+
72	D-carvone	1666	+	-
73	Neral	1668	+	-
74	Beta-bisabolene	1688	+	-
75	(R)-(+)-beta-citronellol	1718	+	+
76	Alpha-curcumene(1)	1728	+	-
77	Anethole	1757	+	+
78	*Cis*-carveol(1)	1770	+	-
79	*p*-cymen-8-ol(1)	1775	+	-
80	Nerol	1785	+	-
81	*Cis*-geranylacetone(1)	1795	+	-
82	Beta-phenylethanol	1821	+	+
83	Piperitone	1833	+	+
84	Caryophyllene oxide	1900	+	+
85	Cinerolone(1)	1903	+	-
86	Methyleugenol(1)	1923	+	-
87	Humulene oxide	1960	+	+
88	Spathulenol(1)	1975	+	-
89	Eugenol(1)	1994	+	-
90	Thymol	2045	+	+
91	Carvacrol	2064	+	+
92	Mint furanone(1)	2213	+	-
Total of identified compounds			89	50

(1) Identification attempt based on Kovats retention indices on polar column, the spectra reference of NIST library and the comparison with mint chromatograms.

(2) +: Presence of the aromatic compound; -: absence of the aromatic compound.

Table 2. Volatile compounds identified in the extract of medicinal and aromatic plants after 6 h and 48 h of maceration.

Aromatic compound	Kovats index	Maceration time (h)(1)															
		0	2	4	6	8	23	25	27	29	31	48	50	52	54	56	71
Tricyclene	997	*	*	*	+	*	+	*	+	+	+	+	+	+	+	+	+
α-thujene	1010	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
α-pinene	1012	*	*	*	+	+	+	+	+	+	+	+	+	+	+	+	+
Camphene	1045	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
β-pinene	1088	*	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+
β-myrcene	1143	*	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+
α-terpinene	1158	*	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+
D-limonene	1178	*	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+
β-phellandrene	1186	*	*	*	-	-	*	*	*	+	*	*	+	+	*	+	*
Eucalyptol	1190	*	*	*	+	+	+	*	*	+	*	*	*	+	*	*	*
γ-terpinene	1225	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3-octanone	1228	*	*	*	+	*	*	*	*	+	*	*	+	+	+	+	+
p-cymene	1243	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3-octanol	1369	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*
Isomenthone	1429	*	*	*	+	*	+	*	*	+	*	*	*	*	*	*	*
Trans-sabinenehydrate	1436	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*
D-isomenthone	1455	*	*	*	+	*	*	*	*	*	*	*	*	*	-	*	*
Camphor	1471	*	*	*	+	*	*	*	*	*	*	*	*	*	-	*	*
β-linalool	1517	+	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+
Trans-isopulegone	1529	*	*	*	+	*	*	*	*	*	*	*	*	*	-	*	*
Bornyl formate	1535	*	*	*	+	*	+	*	*	*	*	*	*	*	-	*	*
Isopulegone	1542	*	+	*	+	+	+	+	+	+	*	*	*	+	-	+	*
(-)-Bornyl acetate	1548	+	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+
Dihydrocarvone	1565	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*
Thymyl methyl ether	1567	+	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+
β-caryophyllene	1571	+	+	*	+	+	+	+	+	+	*	*	+	+	*	+	*
Thujanol	1575	*	*	-	+	*	*	*	*	*	*	*	+	+	*	+	*
Pulegone	1601	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cinerone	1614	*	*	-	+	*	*	*	*	*	*	*	*	*	*	*	*
Isoborneol	1655	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
α-terpineol	1662	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*
β-bisabolene	1688	*	*	*	+	-	-	*	*	*	*	*	*	*	*	*	-
(R)-(+)-β-citronellol	1718	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*

Aromatic compound	Kovats index	Maceration time (h)(1)															
		0	2	4	6	8	23	25	27	29	31	48	50	52	54	56	71
Anethole	1757	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*
Cis-geranylacetone	1795	-	*	-	+	*	*	-	-	*	-	-	*	*	*	*	-
β-phenylethanol	1821	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*
Piperitone	1833	*	*	*	+	*	*	*	*	*	*	*	*	*	*	*	*
Caryophyllene oxide	1900	*	*	-	+	*	*	*	*	*	*	*	*	*	*	*	*
Humulene oxide	1960	-	-	-	+	*	*	*	*	*	*	*	*	*	*	*	*
Thymol	2045	+	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+
Carvacrol	2064	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mint furanone	2213	-	*	-	+	*	*	-	*	*	*	-	*	*	-	*	-

(1) + Major presence; * Weak presence; - "Analytical" absence.

Table 3. Main aroma compounds identified in the aromatic extract during the maceration of the medicinal and aromatic plants.

3.4.2 Functional properties of the main aroma compounds

Carvacrol, with an important flavoring power, is approved by Food and Drug Administration (FDA - USA) for food use. It was included by the European Union in the list of category B chemical aroma that can be added to food products, for example 2 ppm in drinks and 25 ppm in candy (De Vincenzi et al., 2004). Several studies have shown that carvacrol has several biological activities. It is anthelmintic, antibacterial, antidiuretic, anti-inflammatory, antioxidant, antiseptic, antispasmodic, antitussive, expectorant, carminative, fungicidal, irritating, pesticide and worming (Akrout, 2004, as cited in Duke, 1998). Just like carvacrol, thymol has strong antioxidant activities comparable to those of known antioxidants such as α-tocopherol and butylated hydroxytoluene (BHT). Therefore, the ingestion of these aroma compounds can help prevent *in vivo* oxidation damages such as lipid peroxidation that is associated with cancer, premature aging, atherosclerosis and diabetes (Lee et al., 2005).

The α-pinene, present among the major compounds of the PAMs extract and among the aroma compounds of Moroccan dates as well (Harrak et al., 2005), has several biological activities: it is antibacterial, antiviral, anti-inflammatory, expectorant, sedative, herbicide, insect repellent and flavoring (Akrout, 2004, as cited in Duke, 1998).

In addition, numerous investigations have confirmed the antimicrobial action of essential oils in model food systems and in real foods. In this regard, many studies have shown that the essential oils of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) are among the most active ones against a number of food spoilage and pathogen microorganisms (Dimitrijević et al., 2007).

3.5 Global olfactory fingerprint evolution changes

Factorial discriminant analysis was applied to the rectangular table of data whose rows are samples of aromatic extracts divided into groups by time of maceration and whose columns

are the responses of 18 sensors of the electronic nose. For each of the available 15 periods of maceration (groups), the six extract samples gave us a table of dimension 90 x 18. Maceration times studied were: 0 h, 2 h, 5 h, 18 h, 24 h, 26 h, 29 h, 42 h, 46 h, 49 h, 52 h, 69 h, 73 h, 76 h and 139 h. The goal was to find the discriminant axes separating the best possible in projection of the 15 groups based on the responses of the electronic nose sensors. In this analysis, the first three axes explained respectively 91.4 %, 4.4 % and 3.8 % of the total variance.

The group projection in the space formed by the first three axes confirmed that the first axis had a much greater discriminatory power than the two other, because the separation of groups was in fact much clearer along this axis. It distinguished samples with a maceration time greater than or equal to 24 hours from samples with a maceration time shorter than 24 hours (Fig. 9).

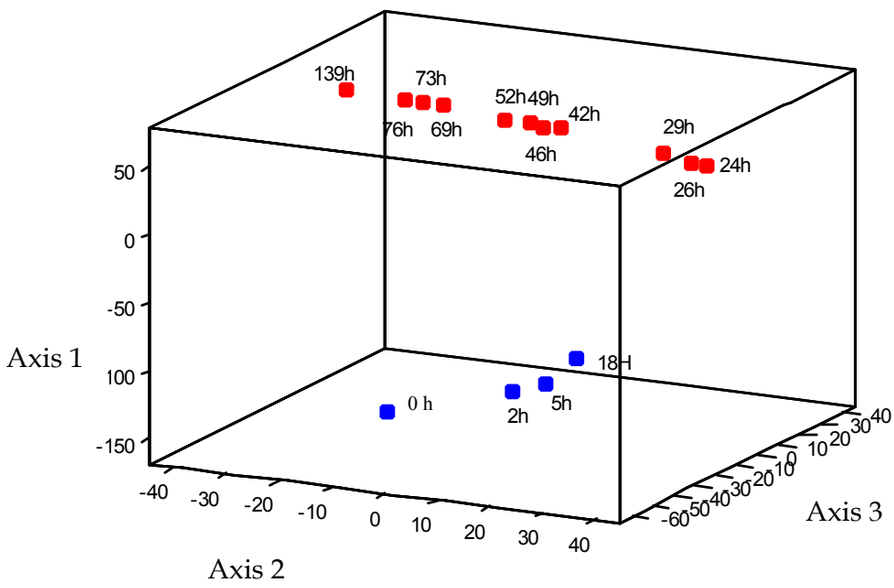

Fig. 9. Representation in the space formed by the first three discriminant axes of the factorial discriminant analysis (FDA) of the average scores of the olfactory fingerprint of the aromatic extract at 15 maceration times.

In addition, the second axis, which explained only 4.4 % of the variability, discriminated better between samples that have been macerated for at least 24 hours, and the third axis, which explained much less variability, better discriminated between samples that have undergone a maceration during less than 24 hours.

So, we can conclude that, according to the response of the sensors which depends on the affinity and concentration of the molecules in the head space, there was a significant difference between extracts from a maceration of less than one day and those from a maceration of one day or more.

3.6 Microbiological evolution of the plants extract

Microbiological monitoring of the aqueous extract during the maceration of plants focused on the enumeration of the microbial flora on the three culture media: MRS, PCA and PDA.

Fig. 10 shows the evolution of the logarithm of colony-forming units (cfu) per liter of the extract versus time of the plants maceration.

No bacterial growth was observed on MRS medium inoculated with the extract taken immediately after mixing MAPs with water, while the lactic microflora was dominant after 48 h and 72 h of maceration (10.14 and 11.03 Log(cfu/l) respectively). So, the development of the lactic acid bacteria was related to the maceration of MAPs, which has led to fermentation. Populations developed on the others two media (PCA and PDA) were respectively 9.15 Log(cfu/l) in 48 h and 9.92 Log(cfu/l) in 72 h for the mesophilic microflora and 9.60 Log(cfu/l) in 48 h and 10.22 Log(cfu/l) in 72 h for the fungal microflora.

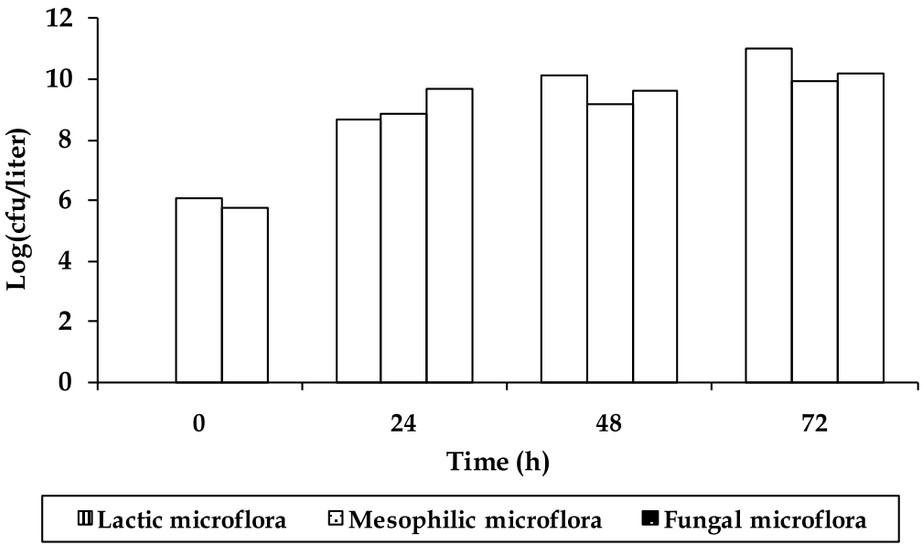

Fig. 10. Evolution of microbial flora (Log (cfu/liter)) during the maceration of medicinal and aromatic plants during 72 hours.

The entire fungal microflora developed in the extract was represented by yeasts. The absence of mold growth in the aromatic extract may be related to aromatic compounds known for their antifungal properties. Extended to three days, the antifungal effect of the aqueous extract has not changed, which may also inform about the release in water of all hydro-soluble active ingredients. It is clearly established that the antifungal activity of the aqueous extract of MAPs was due at least in part to the aromatic compounds they contain. These compounds, particularly those of thyme and oregano, had a marked influence on mold (Beraoud, 1990). The concentration of CO_2 in the jar could also inhibit the mold growth (Desgranges & Durand, 1990).

In addition, the chemical profile of an essential oil is very important due to its antifungal activity. Indeed, essential oil properties are due to all components it contains, especially to the chemical nature of its major components that would affect its fungicide activity in the following decreasing order: Aldehydes > Phenols > Ketones > Oxides > Hydrocarbons > Esters (where the ">" symbol means "more active than") (Beraoud, 1990). All of these chemical classes were found in our aromatic extract.

4. Conclusion

The study of physical, physico-chemical, biochemical and microbiological phenomena occurring during maceration of MAPs for the preparation of the aqueous aromatic extract used in manufacturing of *Tassabount* date juice, showed that the most of the physico-chemical changes took place after two days. The nature of biochemical reactions is fermentative, as indicated by a decrease in soluble solids including sugars, carbon dioxide and lactic acid production, with a predominance of the lactic acid bacteria flora.

According to the qualitative composition of aroma compounds of the MAPs extract, the best expression of the aromatic potential of MAPs was obtained after six hours of maceration, with the identification of 92 compounds. Terpenes and their alcoholic and ketonic derivatives were dominant among the identified aroma compounds.

Global olfactory fingerprint data showed also a very good discrimination between extracts from a maceration of less than one day and extracts from a maceration of one day or more.

Based on the majority of physical, physico-chemical, biochemical and microbiological studied criteria, a period of two days can be considered as an optimum duration of MAPs maceration. The duration limits are the first and third days of maceration.

Moreover, given the presence of bioactive compounds such as carvacrol, α-pinene, thymol and phenolic compunds, aromatic extract has biological activities that are not only related to the aromatization or the preservation (antimicrobial and antioxidant activities), but they are also correlated with functional properties that are potentially useful for pharmaceutical and nutritional applications.

The determination of the antimicrobial activity of the MAPs aqueous extract would be interesting for it exploration in the preservation of *Tassabount* date juice. In addition, the presence of lactic acid in this extract could be exploited for the *Tassabount* stabilization. In fact, its combination with essential oils, as an alternative of chemical preservatives and heat treatment, could be a valuable additive to preserve the nutritional and sensory quality of juice and prevent microbial alteration.

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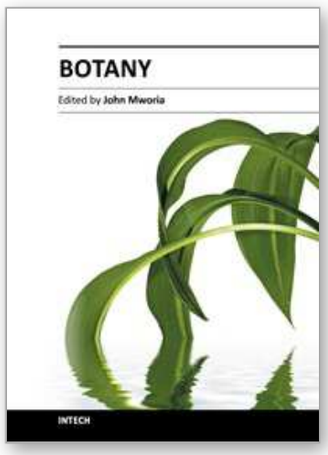

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