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Variation in physicochemical and microbiological characteristics of date palm sap (*Phoenix dactylifera*) during the tapping period in oasian ecosystem of Southern Tunisia

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Abstract

Palm sap tapped from three varieties of date palm *Phoenix dactylifera* was analyzed during two tapping periods: winter and springtime. These samples were collected once-a-week during 14 weeks for each tapping period. Total soluble solids decreased during tapping period; however no significant variation was noted for pH and titrable acidity. The microbial count indicated that after an initial higher presence during 10 weeks, counts of aerobic mesophilic bacteria and yeasts decreased starting from the 11th week. The sugar composition was determined: Sucrose was the only sugar detected in winter (18%). In springtime, in addition to sucrose, small amounts of glucose (1.00-2.47%) and fructose (1.04-1.79%) were detected. Sap harvested in springtime contains more sugars and lower acidity than the one harvested in winter. During the spontaneous fermentation of sap, pH and total soluble solids decreased from 6.80 and 13% in the first day to 3.40 and 6% respectively at the end of fermentation.

Introduction

The date palm *Phoenix dactylifera* L. belonging to the *Arecaceae* family represents an important economical and ecological culture for many countries in the North Africa and in the Arabian Golf (Ahmed *et al.*, 1995). The palm family is one of the economically most important widespread plant families, supplying many needs of man including edible fruits, sugars, sap and building materials, and it enters into commerce as oil, wax, starch and vegetable ivory (Hoyt, 1990).

For a long time, traditional tapping of the date palm has been a common practice. The date palm sap stores the bulk of its reserve of photosynthetically produced carbohydrates in the form of sucrose in solution in the vascular bundles of its trunk. When the central growing point or upper part of the trunk is incised this palm sap will exude as a fresh clear juice consisting principally of sucrose. It is refreshing beverage enjoyed by people in parts of Africa, Asia and South America. Palm sap can be converted by fermentation process into palm wine or vinegar (Mozingo, 1989).

In Tunisia, the date palm sap from date palm (*Phoenix dactylifera* L.) is directly consumed as a fresh drink called "Legmi" or used as an alcoholic beverage after natural fermentation. The fresh sap is purgative, sweet, clear, translucent, and rapidly fermented (Ben Thabet *et al*., 2007). Depending on the region and the palm species, many techniques of sap tapping are used. This is the cause of quantitative and qualitative variation of collected sap. Sap yield varies with the collecting method, the age and the variety of the palm but it can be considered important since a plant can offer until 500 L during one tapping season (Barreveld, 1993). Composition analysis of fresh sap from date palm revealed that sugars are the major components (92–95% dry matter basis) with the dominance of sucrose. It contains also 2.7–5% of proteins and 2.3–2.6% of minerals (Ben Thabet *et al*., 2007; Ben Thabet *et al.*, 2009). Palm sap is rapidly fermented by autochthones microflora composed essentially by yeasts, lactic acid bacteria and acetic acid bacteria. The microflora commonly found in coconut sap (*Cocos nucifera*), palmyra sap (*Borassus flabellifer*) and the oil palm sap (*Elaeis guineensis*) was identified as *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Kloeckera apiculata*, *Schizosaccharomyces pombe*, *Acetobacter aceti*, *Acetobacter rancens*, *Acetobacter suboxydans*, *Leuconostoc dextranicum*, *Micrococcus* sp., *Pediococcus* sp., *Bacillus* sp. and *Sarcina* sp. Of the yeasts, the predominant and best alcoholic fermenter was *Saccharomyces cerevisiae* (Theivendirarajah and Chrystopher, 1987; Shamala and Sreekantiah, 1988; Stringini *et al*., 2009).

In spite of the availability of literature in tapping sap method used overall the world, no data has been

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found on tapping method of date palm sap in Tunisia. As we know, the exploitation of this resource is still traditional. Management and conservation measures are needed both to maximize the economic value of the product and to assure sustained yield from native stands. This study was conducted, as a beginning point for understanding the extent of these activities, with the following objectives: i) to identify the existing method of sap tapping in oasian ecosystem (Mettouia) during two seasons winter (January-February) and springtime (March-April), ii) to study the effect of season on physicochemical and microbiological quality of the sap and iii) following the spontaneous fermentation of the sap.

Materials and Methods

Extraction of palm sap

Study site: Our assessment of the palm sap was conducted in Mettouia: a littoral oasis located on the eastern south of Tunisia. Information collection, interviews with the farmers and tappers, information on harvesting technique, time of harvesting, tool used for harvesting, and overall yield was investigated. Sap samples were collected from three varieties of date palm *Phoenix dactylifera*: *Ammari*, *Bouhattem* and *Felyen*. The local sap collection method was used. It consisted in cutting off the growing point of palm. The juice was collected from a shallow depression scooped out at the top. The samples were immediately stored in an ice box (4°C) during transportation (60 min) to the Arid lands and Oases Cropping Laboratory in the Institute of the Arid Regions (Medenine, Tunisia). The chemical and physical properties of samples were determined within a day. Before analyzing, the sample was filtered by sheet cloth and kept at 4°C until analysis. The microbiological analysis was conducted in the same day. Tapping of the palm sap was conducted in two periods from December to February and from March to May. Sampling was conducted once-a-week and maintained for 14 weeks for each tapping period.

Spontaneous fermentation of palm sap

The fermentation was conducted in 500 ml sterilized Erlenmeyer flask containing 250 ml fresh palm sap. The flask was maintained at ambient temperature and the fermentation is allowed to proceed spontaneously. Samples were withdrawn periodically for microbial counts, pH, titrable acidity and °Brix.

Physicochemical analysis

The pH value was measured at ambient temperature with a pH-meter (Inlab) which calibrated with pH 4.0 and 7.0. Total acidity was estimated by titrating against 0.1 N sodium hydroxide using phenolphthalein as the indicator. Acidity was expressed as a percentage of acetic acid. The total soluble solids of palm sap were determined as degree Brix using hand refractometer (Reichert, Model 10430). Type and concentration of sugars was determined using HPLC (Knauer, Germany) with Eurospher-00NH2 column (7 µm, 250*4.6 mm) and refractive index indicator (RI detectors K-2301). The mobile phase used was the solution of acetonitrile and water (80:20) pumped at a flow rate of 1.5 ml/min and injection volume 20 µl. Before analyzing, all sample solutions were passed through a 0.22 µm filter to remove particulates prior to HPLC analysis. The sugars of D-glucose, D-fructose and sucrose were used as external standards. The calibration curve of each sugar was plotted between peak area and concentrations (Stuckel and Low, 1996).

Microbiological analysis

For microbial enumeration of sap samples collected at the intervals of times indicated above, appropriate decimal dilutions were prepared with sterile peptone water and plated in triplicate on different media. Total count of aerobic mesophilic bacteria was enumerated in standard Plate Count Agar (Oxoid) incubated at 37°C for 48h. Yeasts and moulds were enumerated on Sabouraud dextrose + chloramphenicol agar (Pronadisa) incubated at 22°C for 5 days. After the incubation, plates with 30-300 colonies were counted and the results were expressed as Log_{10} cfu/ml.

Statistical Analysis

Results calculated from triplicate data were expressed as means ± standard deviations. The data were compared by least significant difference test using Statistical Analysis System (SAS, ver. 9.1).

Results and Discussion

Overview on date palm sap tapping

Tapping palm sap is a fastidious activity and the technique used in Tunisia is similar to those used in Ghana for *Elais guineensis* (Amoa-Awua *et al.*, 2007), in Egypt for *Phoenix dactylifera* (Abdelfettah, 2000), in Bangladesh for *Phoenix sylvestris* and *Cocos nucifera*, in India for *Phoenix dactylifera* (Naidu and Misra, 1998) and in Côte d'Ivoire for *Borassus aethiopium* and *Raphia Hookeri* (Theivendirarajah and Chrystopher, 1987). Farmers, with the help of a rope climb the date palm tree, cut the older leaves of

Table 1. General data about tapping date palm sap in the oasis of Mettouia (East southern Tunisia)

Main varieties used for date palm sap	*Ammari* *Bouhattem* *Felyen* *Mermella* *Dokhar (male date palm)* *Hammouri*
Period of tapping	Winter (December to February) Springtime (March to May)
Duration of tapping	10 days to 3 months
Tapping implements	Knives, sickles, earthen pots and climbing rope
Yield of palm sap	Winter: 5-10 l/tree/day Springtime: 10-15 l/tree/day
Age of date palm tree used for tapping sap	10 to 15 years

the tree using the knives and expose the tender part at the tip of the shoot (stem). The tender xylem is then punctured and the sap oozes up. This sap is collected in an earthen pot, hung below, by channeling the sap to the mouth of the pot. Everyday in the early hours, the sap is collected from the hanging earthen pots. Fresh sap (before fermentation) is sweet, if collected before sunrise. When temperature rises, fermentation starts and alcohol is formed. In fact, the influence of temperature on the ethanol fermentation was previously described in several studies (Phisalaphong *et al*., 2006; Rivera *et al*., 2006).

Each day after collecting the sap, a thin end slice of about 2 mm is removed by a sickle. It is important to disinfect the cutting tool since flow is reduced by bacterial growth. Sap flow is low for the first week rising then to about 5 to 10 1 per palm per day depending in the tapping period (Table 1). In Papua New Guinea obtained average yields of 1.3 1 per palm per day, though yield of 1.8 1 was achieved (Paivoke, 1985). Yield is highest in cloudy weather, and it is claimed that transpiration competes with sap yield but is partly compensated by variation in sugar content (Hamilton and Murphy, 1988).

The main date palm varieties used for sap tapping were *Ammari*, *Bouhattem* and *Felyen.* This can be explained by the abundance of these three varieties in Mettouia. The activity of tapping palm sap was conducted in two seasons: winter and springtime and the duration of tapping vary between 10 days to 3 months. This results concords with those showed by Abdelfateh (2000) for *Phoenix sylvestris* with a tapping period of two months. The age of date tree used for tapping is between 10 and 15 years. Abdelfateh (2000) showed that the age of tree used for tapping palm sap ranked between 5 to 30 years.

Changes in microbial counts and physico-chemical characteristics of sap during tapping period

Since the quality and composition of palm sap is found to vary with time and duration of tapping, we study the changes in physicochemical characteristics

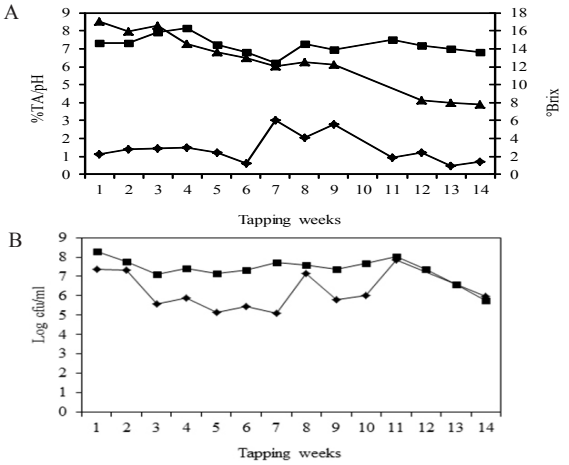

Figure 1. Changes in physicochemical parameters A (pH (■), Titrable acidity (♦), °Brix (▲)) and microbial counts B (Total aerobic mesophilic bacteria (■), yeasts and moulds (♦)) of date palm sap during the tapping period of winter.

and microbial counts during the winter tapping period for 14 weeks. The results are presented in Figure 1A. Variation in pH, titrable acidity and °Brix during the period of tapping was noted. During the first day, palm sap presents a neutral pH and a titrable acidity of 1.16%. Similar results have been found by Ben Thabet *et al.* (2007) for palm sap in the continental oasis of Tozeur.

No significant variation was noted for pH and titrable acidity during tapping period. pH values oscillate around a mean value of 7.19. Titrable acidity varies between 0.5% and 3%. The maximal value was reached in seventh week, corresponding to the middle of tapping period. The titrable acidity is caused essentially by lactic acid produced during the fermentation of sap by lactic acid bacteria in the first hours of tapping day. Similar results have been showed by Amoa-Awua *et al.* (2007) on sap of Elaeis guineensis. Total soluble solids of palm sap samples varied significantly during taping period ($P < 0.05$). The °Brix decrease gradually during the tapping period, from 16% in the first week to 8% at the end of tapping period. The variation of total soluble solids depends on fermentation of sugar caused by microorganisms (Iwuoha and Eke, 1996). Obahiagbon and Osagie (2007) explained the fall in total soluble solids by the exhaustion of the tree caused by the continuous production of sap and the daily practice consisting in removing a thin slice of the stem causing stress to the palm tree which affects the composition of the sap.

Figure 1B shows the changes in the count of total aerobic mesophilic bacteria and yeasts and moulds during the tapping period of date palm sap. Palm sap showed an important microbial load, initial values of aerobic mesophilic bacteria and yeasts and moulds

Table 2. Changes in sugars composition (%) of date palm sap harvested during winter come from the varieties of date palm *Felyen*, *Bouhattem* and *Ammari*[a]

Composition (%)	Variety	W1	W2	W3	W4	W5	W7	W8	W9	W10
Sucrose	*Felyen*	18.52±2.52	-	5.74±0.43	10.18±1.20	12.60±0.78	6.75±1.20	9.92±0.80	4.30±0.40	7.61±0.67
	Bouhattem	18.45±1.98	17.52±1.10	10.80±1.02	11.55±1.70	7.91±0.40	6.75±0.60	6.02±0.38	6.69±0.28	5.96±0.45
	Ammari	15.89±0.68	-	6.29±0.50	8.50±1.89	8.56±0.50	10.38±0.40	10.44±0.34	8.91±0.34	7.06±1.10
Glucose	*Felyen*	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Bouhattem	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Ammari	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fructose	*Felyen*	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Bouhattem	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Ammari	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a]Values are means ± SD (n = 3)
ND: Not detected; W: Week of tapping.

Table 3. Changes in sugars composition (%) of date palm sap harvested in springtime come from the varieties of date palm *Felyen*, *Bouhattem* and *Ammari*[a]

Composition (%)	Variety	W1	W2	W3	W4	W5	W7	W8	W9	W10
Sucrose	*Felyen*	8.86±1.20	11.45±1.50	10.56±0.50	12.94±0.78	7.00±0.40	13.02±2.10	12.88±1.78	10.67±1.00	-
	Bouhattem	11.53±1.01	13.83±0.98	12.17±1.30	14.96±1.45	12.23±1.30	9.04±0.60	10,76±0.85	11.44±1.14	11.35±2.35
	Ammari	17.48±1.20	14.05±2.50	13.22±2.4	11.04±0.90	12.09±1.10	11.02±1.20	9.14±1.10	9.62±0.45	8.68±0.67
Glucose	*Felyen*	ND	1.78±0.19	ND	1.18±0.09	1.76±0.40	ND	ND	ND	ND
	Bouhattem	2.47±0.18	1.34±0.18	1.00±0.12	ND	ND	1.22±0.22	ND	ND	ND
	Ammari	ND	ND	1.27±0.19	ND	ND	ND	ND	ND	ND
Fructose	*Felyen*	ND	ND	1.04±0.30	ND	1.45±0.45	ND	ND	ND	ND
	Bouhattem	1.79±0.25	ND	ND	ND	ND	1.71±0.14	ND	ND	ND
	Ammari	ND	1.34±0.23	1.67±0.15	ND	ND	ND	ND	ND	ND

[a]Values are means ± SD (n = 3)
ND: Not detected; W: Week of tapping.

were around 8.30 $\log_{10}$ cfu/ml and 7.30 $\log_{10}$ cfu/ml respectively. The origin of palm sap microflora is the autochthonous microflora of palm tree, contamination from environment and equipment used for tapping and from birds sheltering the palm tree. The microbial evolution of the tapped palm sap indicated that after an initial higher presence during 10 weeks, total counts of aerobic mesophilic bacteria and yeasts decrease starting from the 11th week. This decrease may be caused by the decrease of nutritive elements at the end of tapping period. This result agrees with the decrease of total soluble solids (Figure 1B).

The microflora of palm sap is generally composed by Lactic Acid Bacteria, Acetic Acid Bacteria and yeasts (Amoa-Awua *et al*., 2007). *Saccharomyces cerevisiae*, *Saccharomyces marxianus* and *Torulopsis* sp. are the only yeasts reported to be isolated from palmyrah toddy (Theivendirarajah and Chrystopher, 1987). The same authors reveal the presence of bacteria isolated belonged to the genus *Bacillus*; the most common were *Bacillus cereus* and *Bacillus sphaericus*. The reason for the abundance of these species is not clear.

Impact of season on quality of palm sap

Sucrose, glucose and fructose contents were analyzed in this study, no significant difference across sample ($P < 0.05$) has been demonstrated (Tables 2 and 3). The unfermented date palm sap harvested in winter containing around 18% w/v sugars represented only by sucrose (Table 2). Fructose and glucose are not detected at the beginning and during the tapping period. A similar result has been demonstrated for unfermented palmyrah sap containing 10-16.5% w/v sugars (mainly in the form of sucrose), *Cocos nucifera* sap and *Raphia Hookeri* sap (Atputharajah *et al.*, 1986; Obahiagbon and Osagie, 2007). Oil palm (*Elaeis guinnensis*) sap is essentially 9-11% w/v sucrose solution and contains very little (much less than 1% w/v each) of glucose and fructose. There is also a very small variable amount (0.35% w/v) of a fourth sugar, raffinose. The level of sucrose fluctuate during the tapping period and rich his minimal value (4.30%) in the 9th week for the varieties *Felyen*. However, we note a decrease for the varieties *Ammari* and *Bouhattem* in the last weeks of tapping. These variations between varieties can be explained by genetic and metabolic characteristics of the tree.

During tapping period of springtime, in addition to sucrose, glucose and fructose were detected in date palm sap (Table 3). Glucose contents were ranged from 1.00 to 2.47%. Fructose contents ranged from 1.04 to 1.79%. Similar result has been shown for the oil palm *Elaeis guinnenesis*. Sucrose contents were found to vary between 7.00% and 17.48% (Eze and Uzoechi Ogan, 1988). The presence of fructose and glucose in sap samples might be due to the inversion reaction caused by invertase activity and acid condition. The occurrence of invertase in palm sap was due to its present naturally and also synthesized by microorganisms. The microorganisms can convert sucrose to glucose and fructose by invertase and finally to organic acids and alcohols in palm sap (Naknean *et al.*, 2010). It is generally known that the primary sources of invertase are yeasts such as *Saccharomyces cerevisae*, *Saccharomyces carlsbergensis* and fungi such as *Aspergillus oryzae* and *Aspergillus niger* (Takano, 2005). Moreover, an

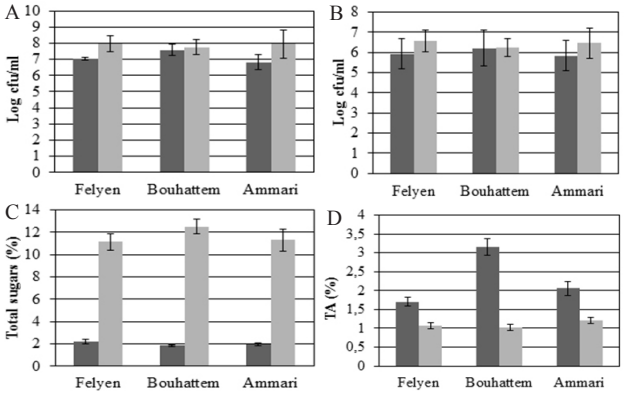

Figure 2. Comparison in microbial counts (total mesophlic bacteria (A), Yeasts and moulds (B)) and physicochemical parameters (Titrable acidity (C), total sugars (D)) between date palm sap harvested in winter (■) and springtime (■) come from the varieties *Felyen*, *Bouhattem* and *Ammari.*

increase in total acidity and decrease in pH are also responsible for the inversion reaction (Naknean *et al*., 2010).

To study the effect of tapping season in total acidity and total sugars and microbial counts, an average value was calculated, results are shown in figure 2. The standard Plate Count Agar counts obtained were not affected by tapping seasons. The average counts of yeasts and moulds were around 6 $\log_{10}$ cfu/ml for the different samples. In spite of the average value for total aerobic mesophilic bacteria and yeasts and moulds are slightly higher in springtime, no significant difference has been noted between seasons ($P < 0.05$). However, microbial counts remain constants, significant changes in total acidity and total sugars are noted ($P < 0.05$). Palm sap harvested in springtime contains more sugars with lower acidity than the one harvested in winter. This fact can be explained by difference in nutritive needs of palm tree that vary between seasons and enhance the degradation or the storage of sugars as reserves. In general, no significant differences ($P < 0.05$) was noted between date palm varieties for microbial counts, titrable acidity and total sugars.

Microbial evolution and main fermentation characters of tapped palm sap

The microbial evolution during the spontaneous fermentation of date palm sap is shown in Fig. 3A and 3B. These data indicated that after an initial presence of 6.0 $\log_{10}$/ml, the yeast population showed a concentration at about 7.5 $\log_{10}$/ml starting from the second day, then decrease until the 10th day only for the varieties *Ammari* and *Bouhattem*, it remains constant for the variety *Felyen*. The yeast population increase again and rich the maximal value in the 17th day when it decrease to the end of fermentation.

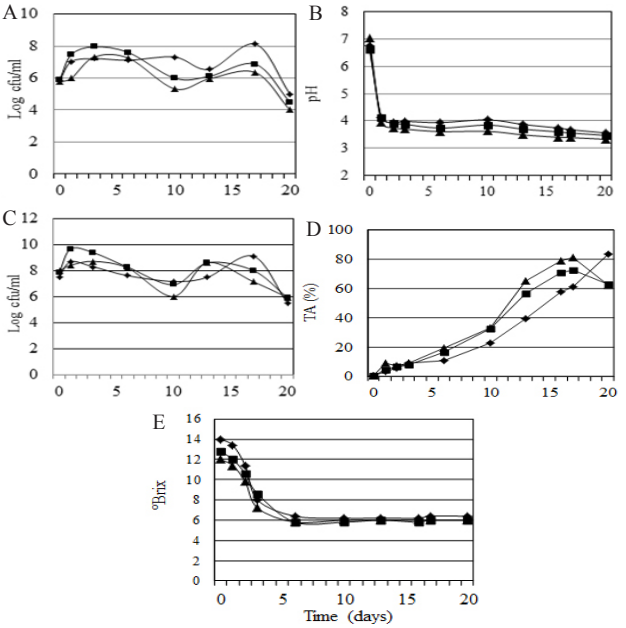

Figure 3. Changes in microbial counts (Yeasts and moulds (A), total mesophlic bacteria (B)) and physicochemical parameters (pH (C), Titrable acidity (D), °Brix (E)) during spontaneous fermentation of date palm sap come from the varieties *Felyen* (♦), *Bouhattem* (■) and *Ammari* (▲).

Total aerobic mesophilic bacteria were also abundant in the sap from the initial (7.5-8.0 $\log_{10}$/ml). This concentration decrease until the 10th day (6-7 $\log_{10}$/ml), then increase again and rich the maximal value in the 13th day for the varieties *Ammari* and *Bouhattem* and the 17th day for the variety *Felyen*. These data are generally in agreement with data obtained by Amoa-Awua *et al.* (2007), where there was a constant presence of yeast, Lactic Acid Bacteria and Acetic Acid Bacteria in the tapped palm wine. In spite of the fermentation behavior of the varieties Felyen defer slightly, no significant variation ($P < 0.05$) were noted between varieties.

We also present data for the evolution of pH, Total acidity and °Brix during the fermentation of the sap (Figure 3C, D and E). The pH of the sap, as shown in figure 3C, decreases steadily from near neutral (around pH 6.8) in pure sap at "zero" time until about the second day when it stabilizes at 3.75-3.92. pH remains constant until 10th day, then decrease slightly with an average value of 3.40 to the end of fermentation. Thus, the stabilization of pH around 3.40 must be due the efficient buffering of the protons by the weak organic acids produced. Some of these acids have been reported to be lactic, tartaric and acetic acids (Rokosu and Nwisienyi, 1980). It is probable that the bacteria and fungi are, at this point metabolizing the alcohol in the sap to organic acids under these conditions. These microorganisms are mainly represented by Lactic Acid Bacteria

(Taiapaiboon, 2004).

Titrable acidity representing total dissociable protons in the sap increases steadily from 0.5% in pure palm sap to 72% and 81% at the 17th day for the varieties *Bouhattem* and *Ammari* respectively (Figure 3D). The total acidity during the fermentation of sap come from the variety Felyen continue to increase until the 20th day of fermentation. Total soluble solids of palm sap samples varied from 12 to 14°Brix in the first day of fermentation, then decrease to around 6° Brix at the 6th day (Figure 3E). From the 6th day to the end of fermentation total soluble solids remain constant. The decrease of total soluble solids depends on fermentation of sugar caused by microorganisms. These microorganisms use sugars in the sap as an energy source and results in fermentation of palm sap. The fermenting microorganisms are dominated by yeasts, particularly *Sacharomyces cerevisae* and Lactic Acid Bacteria (Chantachum and Beuchat, 1997). Since palm sap is rich in sugars (16-17%) and, unless it is collected under hygienic conditions, rapidly fermentation and conversion reactions to acid and alcohol occur (Iwuoha and Eke, 1996).

We noted that throughout the fermentation the sap color has been turned to milky white. In fact, Lactic Acid Bacteria have been shown to be responsible for the consistency and soluble white color of palm sap through their production of gum likely dextran in the early stage of fermentation in the beverage, which change the consistency and the color from transparent to whitish. In addition, a heavy suspension of yeast and bacteria also gave a milky-white appearance (Lasekan *et al.*, 2007).

Conclusions

The results presented the tapping method and the seasonal variations of palm sap quality harvested in southern Tunisia. The method used was similar to those reported in the literature. The variations on sap quality may come from the genetic and metabolic characteristics of the tree, environment factors, the collecting time, microbial load, personal hygiene and sanitary equipment. Microorganisms seem to be the main affect on quality of palm sap due to they can use sugar through the inversion reaction and produce organic acids.

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