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Research Article

Mold-Ripened Soft Cheeses Fortified with Date Palm Fruit Product as Functional Dairy Products

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Abstract

Date fruit based products are gaining popularity among the consumers in almost all date growing countries due to its added nutritional value. Therefore, novel products were developed by combining two types of foods i.e., soft ripened cheeses and date fruit syrups or date powder. This study is the first to report the surface mold-ripened cheese production with date syrup and date powder. Model cheeses were prepared from pasteurized milk inoculated with *Streptococcus thermophilus*, *Penicillium camemberti* and *Geotrichum candidum*. Date syrup-1, date syrup-2, date powder or the date mixture were added at the stage of curdling. Based on the kinetic growth of the microbial groups in all the treatments, there was no change in the growth of these in various date palm product. On the contrary It may be said that addition of the date fruit product supports their growth. After 35 days, the amounts of total poly phenols were 128.3 ± 1.01 , 81.8 ± 1.11 , 33.5 ± 2.19 , 156.23 ± 1.27 mg GAE/100 g in the cheeses support with date syrup-1, date syrup-2, date powder or the date mixture, respectively. Antioxidant activity of date fruits ranged from 80.13 IC₅₀ (date syrup-2) to 82.23 IC₅₀ (date syrup-1). Based on the chemical characteristics and sensory analysis, the study results showed the potential for innovative application of date products for developing new functional dairy products as an ideal medium for the delivery of biological active compounds with beneficial health effects over.

Key words: Mold-ripened cheese, date syrup, date powder, antioxidant, polyphenol

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cheese is one of the most fascinating, complex and diverse foods enjoyed today. Three elements constitute the cheese complex ecosystem (Lessard *et al.*, 2012), ripening agents, consisting of enzymes and microorganisms, the composition of the fresh cheese and the environmental conditions during aging. These factors determine and define not only the sensory quality of the final cheese product but also the vast diversity of cheeses produced worldwide (Almena-Aliste and Mietton, 2014).

Soft ripened cheeses are characterized by the development of white fungi on the cheese surface during ripening (O'Sullivan *et al.*, 2005; Shaw, 1981). The most common commercial ripening strains used to produce Camembert-type cheese from pasteurized milk are *Penicillium camemberti*, *Geotrichum candidum* (teleomorph, *Galactomyces candidus*), *Kluyveromyces lactis* and *Debaryomyces hansenii*. These ripening fungi are the major contributors to the technological and sensory properties of bloomy-rind cheeses due to their biochemical and microbiological changes during ripening (Rousseau, 1984; Nielsen *et al.*, 1998; Leclercq-Perlat *et al.*, 2000, 2013; Spinnler and Gripon, 2004; Amrane and Prigent, 1997; McSweeney, 2004; Boutrou *et al.*, 2006).

A development in Camembert cheese production has been the replacement of traditional mesophilic starter strains with thermophilic mild acid-producing species such as *Streptococcus thermophilus*. In this modified process, the pH of the curd before salting does not decrease below 5.0 (Shaw, 1993). The mold-ripened cheese has a characteristic appearance, a typical aroma and taste. It also possesses more complex ripening patterns than other varieties of cheese with more simple microflora. These cheeses are becoming increasingly popular with consumers thus resulting in higher demand by the consumers. Previously, many French companies alone produced more than 300,000 t of Camembert, Brie, Coulommier and Carré de l'Est per year (Gripon, 2002). According to an estimate by Gripon (2002), the cheeses with white surface mold accounted up to 7-8% of the total cheese produced in Europe and up to 2-3% of total world production. However, cheeses with additives such as herbs, spices and other condiments continue to increase in popularity among consumers looking for variety and robust flavors. Added herbs and spices also give color to the cheese and improve presentation and attractiveness of the cheese to consumers. Besides, some of the herbs and spices are added as a source of health-promoting compounds for consumer health and well-being. For example, Otlu cheese

is a herb-flavored Turkish cheese produced for over 200 years from raw or pasteurized sheep's milk (Hayaloglu and Fox, 2008). In France, «Raclette» is a semi-hard cheese from «Savoie», which can be smoked or contain white wine, pepper, rosemary, thyme, garlic and other herbs. «Munster» from «Alsace» is a cow's milk cheese with caraway seeds and «Le Roulé» is a fresh cheese with a distinctive swirl of herbs and garlic. In this case, the additives may be also salmon, dill, chives and strawberry (Hayaloglu and Farkye, 2011).

Date fruits of date palm (*Phoenix dactylifera* L.) are important for most of the population in Near East and North Africa countries, where most of date fruit production comes from the Arab World (75%). The world production of dates has increased from about 4.6 million tons in 1994 to 7.68 million tons in 2010 and is expected to increase in future (Al-Farsi and Lee, 2008). Many researchers have stressed its importance from nutritional, biochemical and medicinal values (Al-Farsi *et al.*, 2005a, b; Al-Shahib and Marshal, 2003; Myhara *et al.*, 2000; Tang *et al.*, 2013). Also from economic point of view, it is marketed globally as a high-value fruit (Al-Farsi and Lee, 2008).

Several studies indicated that date fruits contain thirteen flavonoid glycosides at different stages of maturity (Hong *et al.*, 2006; Biglari *et al.*, 2008). The polyphenols are of considerable interest to researchers, industrials and consumers due to their antioxidant, hypoglycemic, antihypertensive, antimicrobial, antifungal, anti-inflammatory and anti-atherosclerotic and antiviral, including anti-HIV properties (Furneri *et al.*, 2002; Lee-Huang *et al.*, 2003; Somova *et al.*, 2003; Micol *et al.*, 2005; El and Karakaya, 2009; Barbosa-Pereira *et al.*, 2013). The health effects of polyphenols depend on the quantity consumed and their bioavailability even when present in a food matrix (Lima *et al.*, 2014; Manach *et al.*, 2004). However, their use is limited by poor bioavailability and disagreeable taste (Haratifar *et al.*, 2014).

Date fruits or date syrups can be used as an ingredient for beverage (Keshtkaran and Mohammadifar, 2013), yogurt (Amellal-Chibane and Benamara, 2011), jam, ice cream, bakery products, sesame paste/date syrup blends (Razavi *et al.*, 2007) to obtain healthy and natural products. On the other hand, consumers do not appreciate foods only in terms of their taste and immediate nutritional requirements, but also in terms of their ability to provide specific health benefits. Presently, the functional foods became an important food sector promoting health benefits due to their functional ingredients in these products (Haddadin *et al.*, 2012). Functional food targets improve the balance and activity of the intestinal ecology and currently provide the largest sector of functional food market (Saarela *et al.*, 2002; Verschuren, 2002). Foods can be modified

by addition of phytochemicals, probiotic and/or prebiotic to become functional (Nagai and Inoue, 2004).

On this principle in this study, tests were carried for adding different date based components into soft rind cheeses. To our knowledge, till to-date, no study has been performed with dates as an additive in this type of cheese. Yet as seen from the previous studies, dates are of interest due to their nutritional properties. Taking into consideration all the above mentioned facts, the present study intended to introduce a new functional dairy food to consumers.

MATERIALS AND METHODS

Materials: Date palm syrup-1 (Khulas variety), Date palm syrup-2 (Rzaiz variety) and date powder (Khalas variety) were purchased from local markets in Al-Ahsa city, Kingdom of Saudi Arabia at the beginning 2014 cropping season.

Chemicals and reagents: All the chemicals were purchased from Sigma chemical Co., St. Louis, Mo, unless stated otherwise. All the chemicals and reagents were of analytical grade.

Chemical analysis

Chemical analysis of date syrup-1, date syrup-2 and date powder: The AOAC (2005, 2007) methods were used to determine moisture (AOAC-926.08), ash (AOAC-920.108), total fat (AOAC-933.05), total protein (AOAC-930.33), crude fiber (AOAC-962.09), fructose, glucose, sucrose (AOAC-977.20), total dietary fiber (AOAC-993.21), calcium, potassium, manganese, magnesium, iron, copper, sodium, zinc and phosphorus (AOAC-991.25).

Extraction of antioxidants from date syrup-1, date syrup-2 and date powder: One hundred grams of date syrup-1, date syrup-2 or date powder (100 g) were mixed well in 300 mL methanol-water (4:1, v/v), at 20°C for 5 h using an orbital shaker at 150 rpm. Then, the date syrup extracts were filtered through Whatman 42 paper and centrifuged (Hettich Zentrifugen, Tuttlingen, Germany) at 5300 g for 5 min. The supernatant was concentrated under reduced pressure at 38°C for 3 h using a rotary evaporator (IKA-WERKERV06ML; Staufen, Germany) to obtain the date palm fruit methanolic crude extract (Haddadin, 2010).

Estimation of total phenolics: Total phenolic contents of each extract were determined by the Folin-Ciocalteu micro method (Biglari *et al.*, 2008). Briefly, 40 µL of gallic acid (Merck, Darmstadt, Germany) standard of extract solution

were mixed with 1.8 mL of Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), pre-diluted 10-fold with distilled water and allowed to stand at room temperature for 5 min. Then 1.2 mL of sodium bicarbonate (7.5%) was added to the mixture. After standing for 60 min at room temperature in the dark, its absorbance was measured at 760 nm. Gallic acid standard solution (2.0 mg mL⁻¹) was prepared by accurately weighing 0.01 g and dissolving 50 mL of distilled water. The solution was then diluted to obtain the standard solution concentrations of 1.5, 1.0, 0.5, 0.2 and 0.1 mg mL⁻¹. The phenolic content was expressed as mg Gallic Acid Equivalents (GAE)/100 g sample (Shui and Leong, 2006).

Determination of antioxidant activity: The ability of date extracts to scavenge 1,1-diphenyl-2-picrylhydrazyl or DPPH radicals was determined according to the method described by Lee *et al.* (1998).

Microbial analysis on date syrup-1, date syrup-2 and date powder: The samples were enumerated for counting total microorganisms (standard plate count), yeasts/molds and some potential pathogenic bacteria (Coliforms, *E. coli* and *Staphylococcus aureus*). Ten grams of each additive (date syrup-1, date syrup-2 or date powder) were diluted with 90 g of buffered peptone water and homogenized in a stomacher instrument (AES) for 3 min. Decimal dilutions were made with buffered peptone water. Plate Count Agar (BIOKAR Diagnostics, France), Baird Parker Agar supplemented with egg yolk-tellurite (BIOKAR Diagnostics, France) and Chloramphenicol Glucose Agar (BIOKAR Diagnostics, France) were the selective media for the enumeration of total microorganisms, *Staphylococcus aureus* and Yeasts/molds, respectively. The preparation of dehydrated media and sterilization were prepared according to the manufacturer's instructions. The dilutions were pour-plated on petri-dishes. The incubation time of 48 h and a temperature of 30°C were used for total microorganisms, 48 h and 37°C for *Staphylococcus aureus*, 48 h and 30°C for yeasts, 5 days and 25°C for molds. For the enumeration of *E. coli* and Coliforms, Petrifilm plates were used (3 M Corporation, USA). However, the Petrifilm plates could be more sensitive for the detection of certain bacteria compared to the standard microbiology methods (Schraft and Watterworth, 2005).

Manufacturing of Mold-Ripened Soft Cheeses (MRSCs):

Before starting the preparation of MRSCs, different date palm products (date syrup-1, date syrup-2, date powder and a mixture of date syrup-1 and date powder) were supplied on a

pilot scale. A series of small laboratory scale experiments was carried to determine the best concentration of these additives for use on a pilot scale. Sensorial attributes were the criteria to determine the best concentrations. The criteria for the MRSC were flowing aspect, curd color, firm texture, moisty texture, sticky texture, melting texture, granular texture, richness of aromas, fresh milk taste, creamy taste, rancid taste, fruity taste, cooked taste, ammonia taste, mushroom taste, acid, bitter, salty and sweet.

The concentrations of 10, 5 and 3 g of date syrup-1, date syrup-2 and date powder, respectively were used on a pilot scale for one liter of milk. The date mixture composed of 10 and 3 g L⁻¹ of date syrup-1 and date powder, respectively. The standard cheese was without any additive. The MRSCs were produced on a pilot scale. For each cheese making trial, 200 L of raw cow milk was used. The raw whole milk was purchased from a local dairy farm (Amman, Jordan). Milk was standardized at 62 g of fat/L by adding full-cream milk and at 37 g of total protein/L by adding retentate. 0.26 mL L⁻¹ of CaCl₂ solution was added.

After mixing, the milk was pasteurized for 40 sec at 76±1°C and cooled to 39±1°C. Then the milk was poured into vats and kept at 39°C. The milk was inoculated with lactic acid bacteria (*Streptococcus thermophilus* SSC 100 at 3,2 d/1000 L), *Penicillium camemberti* (TN Freeze-dried at 5 d/1000 L from Cargill, France) and *Geotrichum candidum* (Danisco Choozit Geo 17 at 0,4 d/1000 L).

The pH of milk reached to 6.35 after 30 min due to the activity of lactic acid bacteria. Then the coagulant (rennet containing 520 mg of chymosin/L, Chr. Hansen) was added as 36 mL/100 L. The coagulation time was approximately 6 min and the curd was cut into small cubes (1×1×1 cm) using a sanitized cheese knife after 20 min of hardening. The mixture (whey+curd) was stirred gently two times i.e., 15 and 30 min after adding coagulant. Approximately, 40% of whey was removed after 60 min of coagulation. At this stage of processing, date syrup-1, date syrup-2, date powder or the date mixture were added to the curd. However, the standard cheese was without any date product additive.

The curd was then shaped in polyurethane molds, producing cheeses that weighed approximately 200 g. By taking the cheese through a series of maturation stages where temperature and relative humidity were carefully controlled. This allowed the surface mold to grow and the mold-ripening of the cheese by fungi to occur. The lactic acid bacteria were growing with acidification of the curd and the whey was drained slowly. At the end, the draining occurred approximately 12 h after molding (pH on day 1 was around 5.10±0.05).

On day 1, cheeses were unmolded and then plunged into brine solution with a concentration of 27% NaCl (Leclercq-Perlat *et al.*, 2013), at 12±1°C for 14 min. Cheeses were then transferred to a ripening chamber. They were maintained at 12±1°C and 98% relative humidity for 8 days. They were turned on day 5. The Cheeses were wrapped on day 9 and stored at 3 to 4±1°C.

Physico-chemical analysis of cheese: The Total Solids (TS) were determined by the loss of moisture through drying of cheese samples using the hot air oven method (AOAC., 2005), fat (AOAC-933.05), protein (Nx6.25) (AOAC-2001.14), ash (AOAC-920.108), lactose (AOAC 980-13), OMEGA 3 and OMEGA 6 by using HPLC system: Thermo Scientific Dionex UltiMate 3000 RSLC Dual Gradient. The pH was measured by Jonway 705 pH meter. Titrable acidity was determined according to the methods of AOAC (2007).

Microbial analysis of cheese

***Streptococcus thermophilus* enumeration:** The cheese rind was discarded. The samples of cheese core were diluted (1:9) in buffered peptone water and homogenized in a stomacher instrument. Decimal dilutions were spread on Man Rogosa Sharpe Agar (BIOKAR Diagnostics, France). The incubation plates were prepared in duplicate. The colonies were counted after incubation at 42°C for 24 h under anaerobic conditions. The count determination was done after 2 h 30 min, 3 h 30 min, 7 h, 00 h and on day 1, 5, 9, 25, 35 and 45 after molding.

***Geotrichum and penicillium* enumeration:** The rind of cheese was scraped using a sterile surface template and scalpel. The procedure for the preparation of samples was the same as for the enumeration of *Streptococcus thermophilus*. The dilutions were streak-plate on chloramphenicol glucose agar (YEGC, Biokar, Beauvais, France) medium. Plates were incubated at 30°C for 48 h or at 22°C for 5 days for *Geotrichum* and *Penicillium*, respectively. The determination was done on day 1, 5, 9, 25, 35 and 45 after molding.

Sensory evaluation of cheese: The sensory profiles of the mold-ripened soft cheeses at day 30 (optimum stage of consumption) was described in duplicate by a specially trained test panel of 8 individuals. Twenty descriptive approaches were chosen to allow the identification of organoleptic differences between the mold-ripened cheese supplied with different date products (Delahunty and Drake, 2004). The samples were served blindly labeled with

three-digit codes from a random number table. The sensory descriptors were the odor intensity, flowing aspect, curd color, firm texture, moisty texture, sticky texture, melting texture, granular texture, richness of aromas, fresh milk taste, creamy taste, rancid taste, fruity taste, cooked taste, ammonia taste, mushroom taste, acid, bitter, salty and sweet. The marking for each descriptor was performed on an intensity scale ranging from 0-8. Where 0 = no intensity descriptor and 8 as the maximum intensity in all cases except two special cases. Appreciation of appearance; 0 = degraded surface of the rind, 8 = beautiful white well covering rind. Curd color; 0 = very white curd, 4 = cream curd, 8 = brown color. However, for the evaluation of aromatic notes and flavors, the products were packed in containers. The samples were kept at 20°C for 1 h before serving.

Statistical analysis: The statistical Analysis was performed by using the General Linear Model procedure of Statgraphics (StatPoint Technologies Inc., 2009). The least Significant Difference test (LSD) was used to test differences between means. The levels of significance $p < 0.05$ or ≤ 0.10 were used in this study.

RESULTS AND DISCUSSION

Chemical properties of date syrup-1, date syrup-2 and date powder: The chemical composition of the studied date products such as date syrup-1, date syrup-2 and date powder

is presented in Table 1. The moisture, ash, protein, fat and reducing sugar content of date syrup-1 and 2 ranged between 12.18-13.92, 0.02-0.04, 0.91-1.08, 0.05-0.02 and 65.47-72.37 g/100 g, respectively. These results are in general agreement with those reported previously (Al-Farsi *et al.*, 2007; Baliga *et al.*, 2011; Tang *et al.*, 2013). The analytical tests did not detect dietary fiber or crude fiber contents in date syrup as given in Table 1. The results of this study are consistent with the results obtained by Al-Farsi *et al.* (2007). They found that date syrup of Shahal variety contains very low dietary fiber 0.01 g/100 g on fresh weight basis. Date syrup-1, date syrup-2 and date powder were found to be a good source of macro-elements such as calcium, phosphorus, potassium, magnesium and sodium but were significantly low in iron, zinc, manganese and copper contents (Table 1). However, the results of other researchers reported high concentration of minerals in date syrup (Abbes *et al.*, 2011; Alkhateeb, 2008).

The total phenolic contents in the three products ranged between 1549.55 ± 9.25 to 1842.36 ± 12.2 mg/100 g GAE (Table 1). Whereas, Wu *et al.* (2004) reported that Deglet Nour and Medjol varieties contain 661 and 572 mg of GAE/100 g, respectively. Al-Farsi *et al.* (2007) found the total phenolic content range of 172-246 mg of GAE/100 g in three native sun dried date varieties from Oman.

However, Mansouri *et al.* (2005) reported much lower contents (2.49-8.36 mg of GAE/100 g) of phenolic profile of seven date varieties from Algeria. These variations in total phenolic contents might be subjected to various factors

Table 1: Chemical analysis of date syrup-1, date syrup-2 and date powder

Parameter	Additive		
	Date syrup-1	Date syrup-2	Date powder
Moisture (g/100 g)	12.18±0.02	13.92±0.02	4.31±0.12
Ash (g/100 g)	0.02±0.01	0.04±0.01	1.42±0.10
Total fat (g/100 g)	0.05±0.01	0.02±0.01	0.05±0.02
Crude protein (g/100 g)	0.91±0.03	1.08±0.03	2.22±0.03
Crude fiber (g/100 g)	0.01±0.010	ND	2.80±0.03
Glucose (g/100 g)	34.13±0.10	37.23±0.30	35.26±1.20
Fructose (g/100 g)	31.34±0.12	35.14±0.35	31.95±0.91
Sucrose (g/100 g)	0.31±0.05	0.20±0.01	0.30±0.02
T. Dietary fiber (g/100 g)	ND	ND	17.49±1.05
Potassium (mg/100 g)	500.68±2.10	465.58±3.1	414.59±2.30
Magnesium (mg/100 g)	51.93±0.26	57.93±1.3	66.23±1.88
Phosphorus (mg/100 g)	50.11±1.0	44.16±1.1	70.68±2.16
Calcium (mg/100 g)	22.25±0.70	21.75±1.2	34.25±3.10
Sodium (mg/100 g)	16.62±0.75	18.82±0.95	9.32±0.65
Iron (mg/100 g)	0.62±0.07	1.22±0.32	3.14±0.95
Manganese (mg/100 g)	0.24±0.05	0.30±0.07	0.49±0.04
Zinc (mg/100 g)	0.15±0.01	0.41±0.01	1.49±0.08
Copper (mg/100 g)	0.09±0.01	0.09±0.01	0.48±0.01
Total polyphenol (mg/100 g GAE)	1688.65±10	1842.36±12.2	1449.55±9.25
Antioxidant activity (DPPH, IC ₅₀)	82.23±0.65	80.13±0.91	81.37±0.84

Results are indicated as mean and standard deviation of triplicates for each type of test

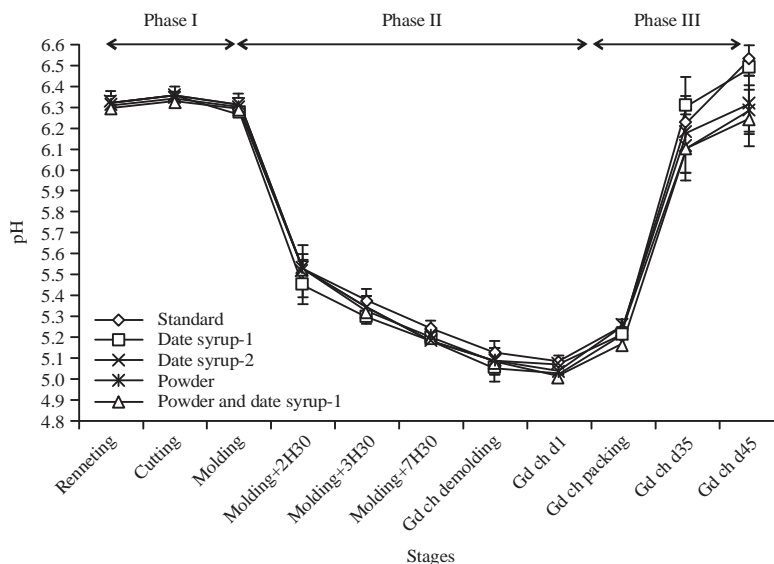


Fig. 1: Evolution of whey pH during ripening stages of the different cheeses with or with out the addition of date products. Results are mean and standard deviation of triplicates. Significant differences at $p < 0.05$. Gd ch: Ground cheese, d: day

such as variety, handling practices, growing conditions, maturity, season, soil type, temperature, humidity and geographic origin etc. From the study results, it seems that date syrups and date powder contain potential antioxidant activity (Table 1). These results are in agreement with the results obtained by Al-Juhaimi *et al.* (2014) and Mansouri *et al.* (2005).

Physico-chemical properties of the mold-ripened soft cheeses: From the results in Fig. 1, three phases of pH profile of whey can be observed from renneting stage to day 45. The first one from renneting to cheese molding where the pH ranged from 6.29-6.35 and without any significant differences ($p < 0.05$). The second phase started from cheese molding to cheese packaging where the pH ranged between 5.03-5.32 and with significant differences ($p < 0.05$ or < 0.1). However, in the third phase, the pH increased and ranged from 6.31 at day 35 to 6.49 at day 45.

In all the treatments, the fortification of cheese with date syrup-1, date syrup-2 and date powder did not cause any significant changes in pH ($p < 0.05$) in the first 2 h (Fig. 1). However, the decrease in pH was significantly higher ($p < 0.05$ or < 0.1) in the treatments than the standard during the second phase ($p < 0.05$). The significant decrease in pH could be attributed to date products that might have enhanced the growth of lactic acid bacteria, *S. thermophilus* (Fig. 1). This enhancement could be attributed to the presence of high contents of sugars and other nutrients needed by the bacteria for their growth as shown in Table 1.

The pH of cheese fortified with date syrup-1 increased from 5.03 at day 1 to 6.49 by day 45 (Fig. 2). The same phenomenon was observed with the other trials. This increase may be due to assimilation of lactic acid and deamination of amino acids by the mold. Thus, as the mold neutralized the acidity of the cheese, the pH increased (Lenoir, 1984). The study results agreed with the results of other authors who reported that an increase in pH could be attributed to an intensive proteolysis during the first ten days (Macej *et al.*, 2001).

Noomen (1978) indicated that there is a positive correlation between pH at an interval of 5.1-6.0 and β -casein breakdown. He suggested that plasmin is probably involved to a greater extent of protein breakdown during ripening in Camembert cheese. Also, Kataoka *et al.* (1987) showed that pH increased to an optimum level of 6.0 during ripening. Similarly, the results of Nukuda *et al.* (1984) found an increase of pH from 4.7-5.2, 6.9-8.0 during ripening for 30-45 days. They also found a good correlation between ripening coefficient and increasing pH. In the surface mold ripened cheeses such as Camembert and Brie, the softening increased from the outside to the center of the cheese during ripening.

The Titrable Acidity (TA) of whey in the treatments and the standard followed the same kinetic profile (Table 2). The increasing in TA started to rise from the molding stage. At this stage, the TA increased significantly ($p < 0.05$). Moreover, 6 h after molding stage, a second increasing stage in TA was observed in all the treatments. At this stage, the increase of TA was significant ($p < 0.1$). The titrable acidity of whey of cheese

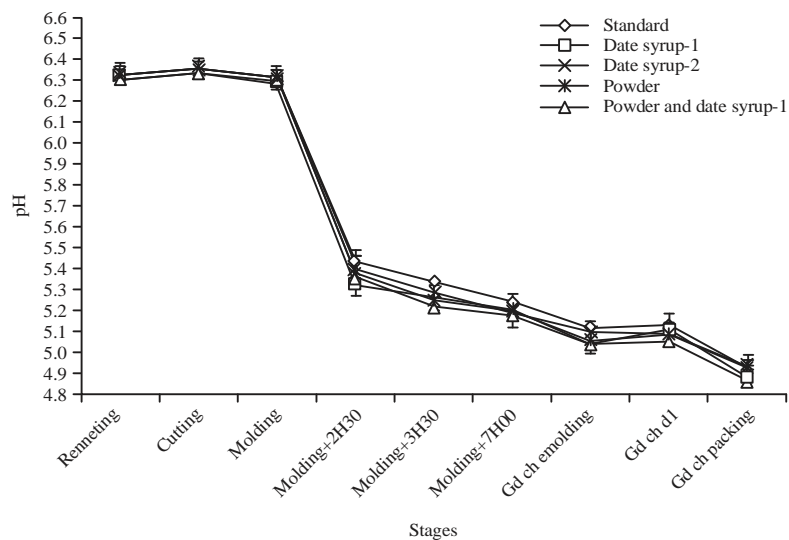


Fig. 2: Evolution of cheese pH during ripening stages of the different cheeses with or without the addition of date products. Results are mean and standard deviation of triplicates. Significant differences at $p < 0.05$. C ch: Core of cheese, d: day

Table 2: Evolution of titrable acidity of whey of cheeses supplied with different date products and standard cheese as control one over different stages

Titrable acidity percentage of lactic acid	Additive					
	Probability (p)	Date syrup-1	Date syrup-2	Powder	Powder+date syrup-1	Standard
Cutting whey	NS	0.126±0.01	0.133±0.02	0.126±0.02	0.133±0.02	0.124±0.01
Molding whey	0.0151 ^a	0.137±0.02	0.139±0.01	0.128±0.02	0.140±0.03	0.124±0.01
Molding whey+2H30	NS	0.382±0.01	0.360±0.02	0.361±0.01	0.363±0.01	0.344±0.03
Molding whey+3H30	NS	0.523±0.02	0.520±0.03	0.513±0.01	0.530±0.03	0.475±0.02
Molding whey+7H00	0.0818 ^b	0.648±0.02	0.648±0.02	0.635±0.01	0.640±0.03	0.600±0.03
Demolding whey	NS	1.130±0.03	1.037±0.03	1.167±0.03	1.093±0.04	1.023±0.02

Results are indicated as mean and standard deviation of triplicates for each type of test. NS: Non significant. a: Significant at $\alpha = 5\%$, b: Significant at $\alpha = 10\%$, TS: Total solid, TP: Total protein. d: day

fortified with date powder at demolding stage was the highest with a mean value of 1.167% lactic acid, while pH was 5.09 (Fig. 1). On the other hand, the cheese that develops high acidity during the first day of ripening will influence faster molds growth and a decrease in cheese moisture content. Further ripening results in increase of both the titrable acidity and pH (Macej *et al.*, 2001).

Keeping in view the study results, It can be concluded that a great part of the lactose was fermented during the first day as indicated by high value of titrable acidity and low pH. But later on, the acidity begins to rise as previously explained. Because, low pH limits the rate of lipase activity, but in some cheeses, e.g., Brie and Camembert, the pH rises close to neutrality as ripening progresses thus making them especially susceptible to lipolysis (Dumont *et al.*, 1977). Moreover, in soft mold-ripened cheeses, the pH increases during ripening, which increases the growth potential of undesirable bacteria (Frank, 2001).

The chemical analysis of all the cheeses manufactured with or without addition of date syrup-1, dates syrup-2 and date powder at day 35 is presented in Table 3. It was observed that the total solids and total protein contents in the treatments supplied with date products were higher than the standard. Boutrou *et al.* (1999) found that the level of total solid increased from 460-523 g kg⁻¹ during the first 13 day of ripening and then stabilized due to wrapping of the cheeses. These findings are in agreement with this study results but the total solids content in the treatments fortified with date syrups and date powder were slightly higher than those found by Boutrou *et al.* (1999). The higher level in total solid could be due to continuous loss of moisture from the curd as a result of lactic acid development which causes curd contraction. Cheeses are good food sources of many minerals, such as calcium, phosphorus and magnesium (Henning *et al.*, 2006). High level of sodium were also found (Table 3), as result of the salting step during cheese processing. Salt has three major

Table 3: Chemical composition of cheeses supplied with different date products and standard cheese as control one at day 35

Parameter at d+35	Probability (p)	Additive				
		Date syrup-1	Date syrup-2	Powder	Powder+date syrup-1	Standard
TS (%)	0.00 ^a	53.10±0.87	52.85±0.66	52.59±0.44	52.700±0.33	51.72±0.37
TP (%)	NS	17.97±0.35	16.97±0.24	16.84±0.16	17.02±0.21	16.28±0.33
Fat (%)	NS	31.85±0.35	31.79±0.39	31.66±0.38	31.69±0.29	32.65±0.36
Ash (%)	NS	2.55±0.17	2.93±0.15	3.10±0.22	3.09±0.13	2.44±0.21
Carbohydrates by difference (%)	NS	0.73	1.16	0.96	0.90	0.35
Lactose (%)	NS	<0.2	<0.2	<0.2	<0.2	<0.3
OMEGA3 (mg/100 g)	NS	222±0.66	223±0.51	216±0.61	222±0.72	211±0.44
OMEGA3 (mg/100 g)	NS	815±0.88	809±0.62	820±0.53	816±0.42	814±0.47
Calcium (mg/100 g)	NS	491.8±12.17	493.1±11.27	490.3±10.90	495.2±10.35	490.8±11.17
Sodium (mg/100 g)	NS	505.1±11.18	506.3±10.12	509.1±10.35	503.4±12.10	501.3±10.55

Results are indicated as mean and standard deviation of triplicates for each type of test. d: day, ND: Not detected, a: α at 0.05

Table 4: Evolution of total polyphenol content of cheeses supplied with different date products and standard cheese as control one over different stages

Parameter	Probability (p)	Additive				
		Date syrup-1	Date syrup-2	Powder	Powder+date syrup-1	Standard
Polyphenol packing (mg/100 g GAE)	0.00 ^a	130.8±1.01	81.3±1.09	34.5±1.35	162.40±1.27	ND
Polyphenol day+35 (mg/100 g GAE)	0.00 ^a	128.3±1.01	81.8±1.11	33.5±2.19	156.23±1.27	ND
Polyphenol day+45 (mg/100 g GAE)	0.00 ^a	127.7±1.01	81.2±1.09	32.5±1.10	155.40±1.11	ND

Results are indicated as mean and standard deviation of triplicates for each type of test. d: day, ND: Not detected, a: α at 0.05

functions in cheese namely acts as a preservative, contributes directly to flavor and a source of dietary sodium. Together with the desired pH, water activity and redox potential, salt assists in cheese preservation by minimizing spoilage and preventing the growth of pathogens (Guinee, 2004). Under French law, Camembert is a non-scalded and non-pressed cheese. The cheese must have at least 110 g of total solids and about 45% fat in dry matter, but there may be variants with lower or higher fat contents (Shaw, 1981). It can be observed that the content of lactose in the treatments was less than <0.2% and less than that in the standard trial (<0.3%). These results are in consistent with the results obtained in Table 2 which indicate that high acidity signify the transformation of lactose to lactic acid. The results in Table 3 indicated that the cheese contained the two essential fatty acids that the body needs all the time and cannot produce OMEGA 3 (alpha-linolenic acid) and OMEGA 6 (linoleic acid).

Since the objective of this study was to fortified cheese with antioxidant compounds, therefore, the follow up of the dynamics of these materials in the cheese is very important. Polyphenols are abundant micronutrients in our diet and evidence for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is emerging. The manufacturing of cheese fortified with the date syrup-1, date syrup-2, date powder and a mixture of date syrup-1 and date powder indicated the presence of total polyphenols compounds until day 45 (Table 4). From the

results in Table 4, it can also be observed that there are significant differences ($p < 0.05$) between treatments regarding the polyphenol contents. The results presented in Table 1 showed that date syrup-2 contained more polyphenols (1842.36 ± 12.2 mg/100 g GAE) as compared to date syrup-1 and date powder. The cheese fortified with date syrup-2 contained polyphenols content of 113.3 ± 1.29 mg/100 g GAE at day 1. Whilst the concentration of polyphenols fell by 28.3% at day 45. Despite a decreasing trend in the concentration of polyphenols, there is still a substantial quantity. The lowest rate of reduction in the polyphenol contents between day 1 and 45 was obtained in the cheese fortified with date powder with a reduction rate of about 20%. The percentage of reduction in the polyphenols content in the cheese fortified with date syrup-1 (27.9%) is comparable to that obtained in the cheese fortified with date syrup-2 (28.3%).

Based on obtained dates, the date syrup-1, date syrup-2 and date powder were analyzed for their antioxidant activity. The mean antioxidant activity for the varieties examined in this study are shown in Table 1. The date syrup-1 showed the highest antioxidant activity (82.23 ± 0.65). These values ranged between 80.13 ± 0.91 IC₅₀ (date syrup-2) to 82.23 ± 0.65 IC₅₀ (date syrup-1). Generally, the antioxidant activities of samples were found similar. No significant difference was found between the various date products. Dates used in this study demonstrated good level of antioxidants. These results are in agreement with previous findings (Al-Juhaimi *et al.*, 2014).

The health effects of polyphenols depend on the amount consumed and on their bioavailability, even when present in a food matrix (Lima *et al.*, 2014; Manach *et al.*, 2004). However, their use is limited by poor bioavailability and disagreeable taste (Haratifar *et al.*, 2014). Moreover, Lamothe *et al.* (2014) indicated that the consumption of polyphenols in green tea is associated with beneficial health effects. Although polyphenols are unstable in the intestinal environment, they may be protected by interactions with dairy proteins during digestion. Lamothe *et al.* (2014) evaluated the effect of a green tea extract on the digestibility of different dairy matrices and monitored the antioxidant activity of these matrices with or without the green tea extract during digestion in a simulated gastrointestinal environment. Milk, yogurt and cheese with similar fat-to-protein ratios were subjected to simulated digestion. Matrix degradation, protein and fat hydrolysis, polyphenol concentration and radical scavenging activity were analyzed during gastric and intestinal digestion phases. They concluded that presence of dairy matrices significantly improved polyphenol stability in the intestinal phase and increased the antioxidant activity by 29% (cheese) to 42% (milk) compared to the control (tea extract dispersed in water). These results suggest that simultaneous consumption of green tea and dairy products helps to maintain the integrity and antioxidant activity of polyphenols during digestion.

Microbiological properties of the mold-ripened soft cheeses: The growth evolution of the inoculated microbial groups namely, *S. thermophilus*, *G. candidum* and *P. camemberti* was investigated during manufacturing and ripening of mold-ripened soft cheeses fortified with date palm products are shown in Fig. 3-5. It can be observed that the three groups of microorganisms followed the same growth profiles with or without the addition of the date palm products.

Regarding the growth profile of *S. thermophilus*, the results indicated that there are no significant differences between the Standard and the treatments fortified with the various date products ($p < 0.05$) (Fig. 3). Moreover, *S. thermophilus* followed the same growth profile in all the treatments as shown in Fig. 3 which indicated that various date palm products did not alter the growth kinetic of the lactic acid bacteria. But it can be said that the addition of date palm products favor the growth of the microbial groups. However in the standard cheese *S. thermophilus* has lower biomass concentration in the first 7 h after molding (Fig. 3). This observation is also consistent with lower acidification of the cheese matrix (Fig. 2).

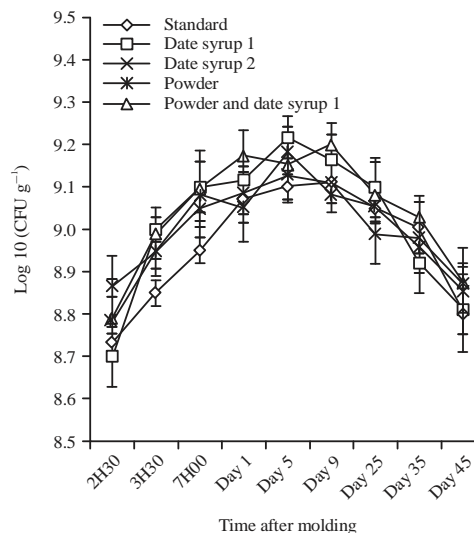


Fig. 3: Growth evolution of the *S. thermophilus* during ripening stages of cheeses with or without the addition of date products. Results are mean and standard deviation of triplicates. Significant differences at $p < 0.05$

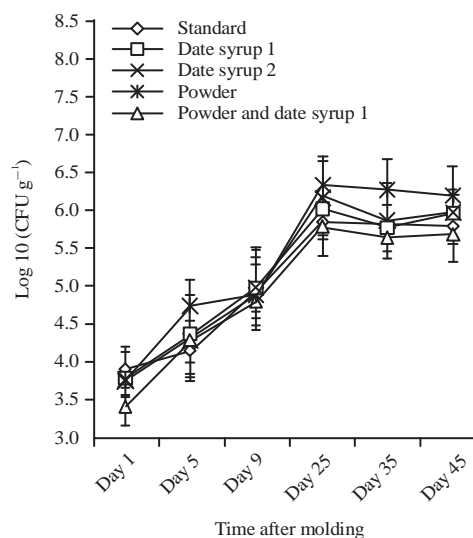


Fig. 4: Growth evolution of the *P. camemberti* during ripening stages of cheeses with or without the addition of date products. Results are mean and standard deviation of triplicates. Significant differences at $p < 0.05$

In all cases (standard and treatments of fortification with various date palm products), the maximum biomass concentration of lactic acid bacteria reached to a maximum average of 1.5×10^9 CFU g^{-1} at day 9. After packaging, the growth rate gradually decreased and reached a biomass concentration of 7.1×10^8 CFU g^{-1} .

Regarding the growth of the other ripening flora, *G. candidum* and *P. camemberti*, there were no significant differences between the standard and the various treatments ($p < 0.05$) (Fig. 4 and 5). The result of the growth profile (Fig. 4) indicated that the *P. camemberti* continues its growth even after packaging (probably it consumes oxygen that is still available in the complex). From day 25, growth was greatly slowed down, then reached to a stationary phase at a biomass concentration of about 1.0×10^6 CFU g^{-1} (Fig. 4).

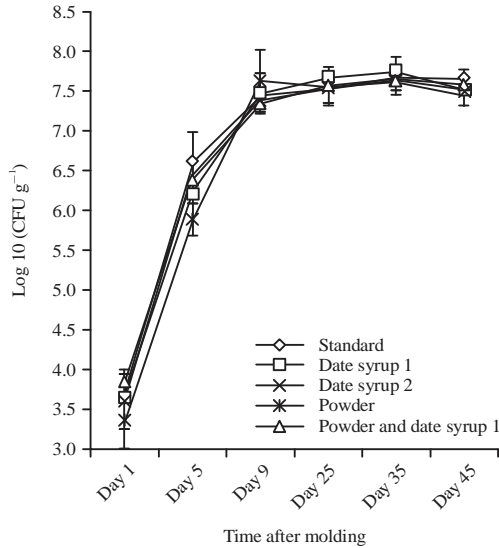


Fig. 5: Growth evolution of the *G. candidum* during ripening stages of cheeses with or without the addition of date products. Results are mean and standard deviation of triplicates. Significant differences at $p < 0.05$

The choice of the *P. camemberti* strain is important in the production of soft surface mold-ripened cheeses. However, the proteolytic activity of the different strains does not vary as much as their lipolytic and β -oxidative activities (Lenoir *et al.*, 1971; Lamberet *et al.*, 1982). The choice of *P. camemberti* strain is also guided by the growth rate, color, density and height of the mycelium, which contribute to the appearance and attractiveness of surface mold cheeses (Spinnler and Gripon, 2004).

The *G. candidum* is in the exponential growth phase between day 1 and 5 and then its growth slows down to packaging, to achieve a maximum biomass concentration of about 3.2×10^7 CFU g^{-1} (Fig. 5). It can be observed from the results that *G. candidum* and *P. camemberti*, by consuming lactate for their growth raised the pH.

Biochemical studies indicated that *G. candidum* is able to reduce bitterness, enhances sulphur flavors due to its very efficient peptidase system and has an impact on rind texture, firmness and thickness. While *P. camemberti* is responsible for the white and bloomy aspect of the rind, produces enzymes involved in proteolysis and lipolysis activities and plays a crucial role in the appearance of bitterness in Camembert (Vassal and Gripon, 1984; Engel *et al.*, 2001; Lessard *et al.*, 2014).

Sensory characteristics of mold-ripened soft cheeses: The sensory profiles of the 5 types of cheeses are presented in Fig. 6. The principal component bi-plot of the descriptive sensory attributes is shown in Fig. 7a and b. It was noticed that cheeses manufactured with the addition of

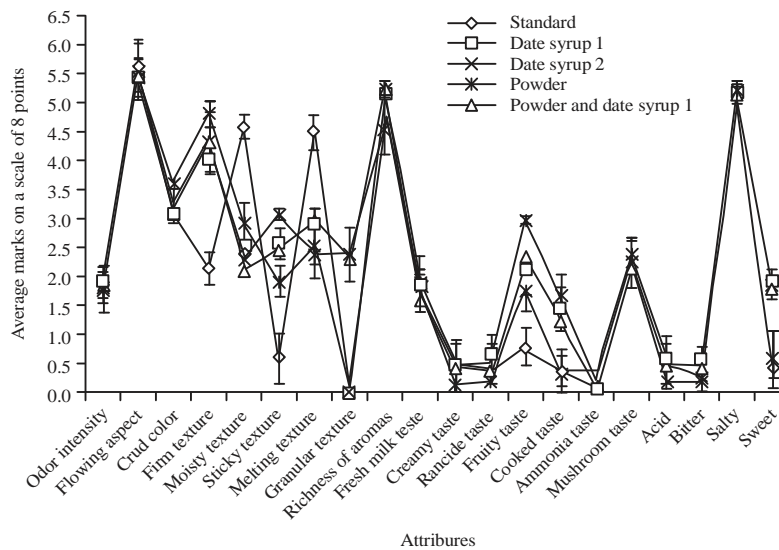


Fig. 6: Sensorial profile of the different mold-ripened cheeses produced with or without the addition of date syrups and date powder. The notes are the mean and standard deviation. Significant differences of the Sensory descriptors were at $p < 0.05$

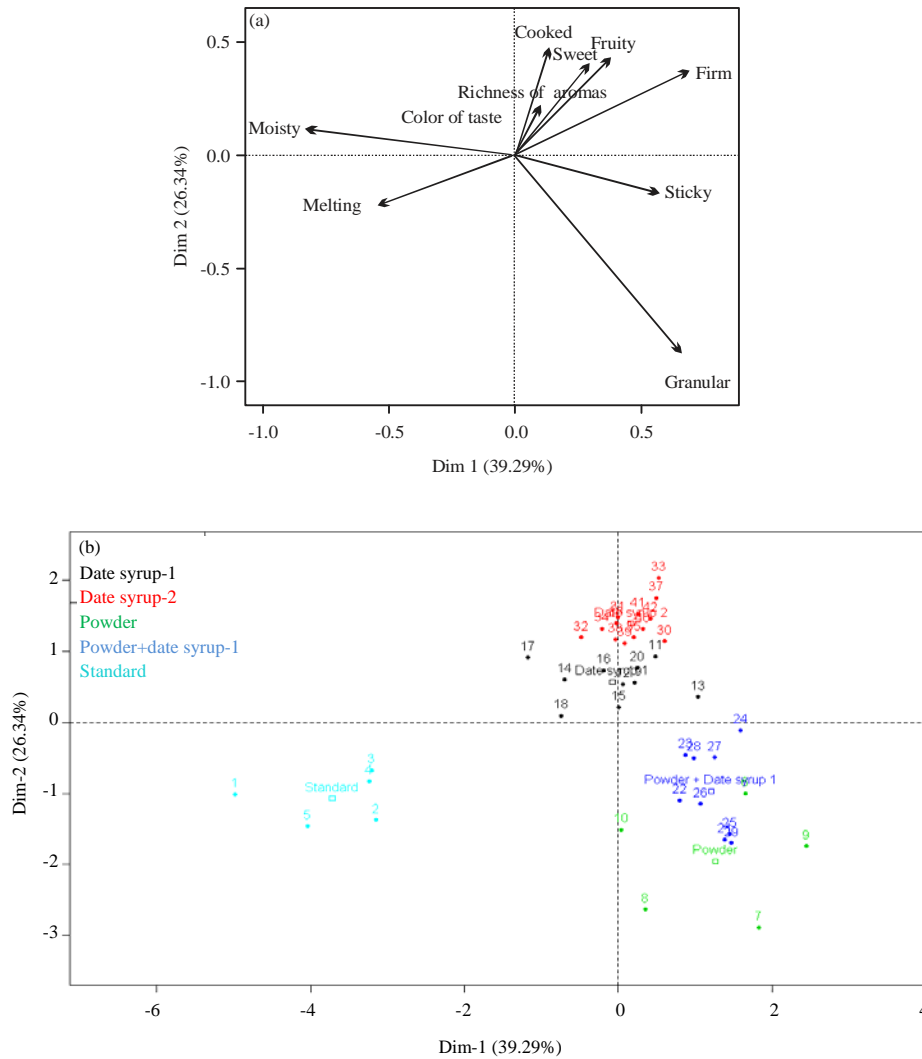


Fig. 7(a-b): Two-dimensional representation of the result of principal components analysis (a) Variables factor map of descriptive analysis sensory data of the different mold-ripened cheeses produced using the different date products (date syrup-1, date syrup-2, date powder and a mixture of date syrup-1 and date powder) and (b) The individuals factor map of different date products (date syrup-1, date syrup-2, date powder and a mixture of date syrup-1 and date powder)

different date products (date syrup-1, date syrup-2, date powder and a mixture of date syrup-1 and date powder) are significantly different in texture from the standard cheese. Indeed textures of cheeses fortified with date products were significantly ($p < 0.05$) higher firmer, sticky, less moistly and less fondant as the texture of standard cheese (Fig. 7a).

The results of chemical analysis supports the results of sensory analysis. Since the cheeses fortified with date products showed highest percentage of total solids as well as higher degree of titrable acidity and lower pH values. Fathollahi *et al.*

(2010) indicated that the cheese pH affects the texture of curd directly by influencing the solubility of the caseins; cheeses with high pH values are softer than cheeses with lower pH values. The cheeses manufactured with the addition of date powder and a mixture date powder and date syrup-1 were characterized by granular textures. In fact this is due to the presence of fine small particles of date powder added to the cheeses.

There is no effect of the addition of dates on the smell of cheese (considered low with an average rating of close to 2/8) and the appearance of the surface flora (judged satisfactory

with an average score of 5.5/8). However, the color of the cheese curd supplied with date syrup-2 was considered out of standard. Indeed, the curd of the cheese was colored "light brown" unappetizing. Therefore, the color of the cheese is significantly ($p < 0.05$) affected by date additive type, while cheeses with date syrup-1 or date powder have the same color as control cheese. Fresno *et al.* (2006), reported that many biochemical changes occur in the ripening process of cheeses. According to Van Boekel (1998) changes in lactose because of series of biochemical reactions result in brown pigmented products such as pyrazines and melanoidins and small acid molecules are also formed during the Maillard reaction. The cheeses fortified with the different date products (date syrup-1, date syrup-2 and the mixture of date powder and date syrup-1) exhibited higher aromatic complexity as the standard cheese. The cheese fortified with the addition of date powder only indicated that the date syrups are more flavoring agents than the date powder. In this case, the aromatic difference is realized at the fruity (highly represented in the case of addition of date syrup-2), cooked and sweet flavor descriptors level. These results may be attributed to proteolytic activity enhanced by the presence of date syrup, which can improve the flavor and texture through increased proteolysis. Moreover, researchers indicated that proteolysis products and free fatty acids produced through lipolysis imparted the characteristic flavor of cheeses (Michalski *et al.*, 2003; Vafopoulou *et al.*, 1989).

With respect to other studied attributes, no significant difference was found between the standard and various treatments with the addition of dates products. The descriptors of concern are fresh milk, creamy, rancid, ammonia and the mushroom Savors acid, bitter, salty.

Figure 7 shows the distribution of the sensory descriptors and products in space. The correlation matrix for the various significant variables is shown in Fig. 7a. Axes-1 and 2 account for about 65.63% of the variability which is a very honorable score. Axis-1 is linked to taste descriptors with cooked, fruity as well as the sweet favor. These three descriptors are strongly correlated. The axis-2 is represented by the cheese texture descriptors. There is no real correlation between the descriptors of the taste and those of texture (orthogonal arrangement of the descriptors of the taste with respect to those of the texture). The graph highlights in particular a negative correlation between the descriptor "moisty" and "sticky", which is quite logical. The variable "moisty" is well correlated with the variable "melting". Conversely, these will not necessarily be related to a product with texture "sticky" and a product with texture "granular". The descriptors richness of aroma and color of curd are too close to the center of the circle, making their interpretation totally hazardous.

Figure 7b shows the distribution of individuals on a 2-dimensional map and allows to identify trends. This analysis confirms entirely the results of the sensory profiles (Fig. 6). In the 4th quadrant, the standard cheese that stands out very clearly from experiments with adding dates by a moist and tender texture. In the right bottom of the map are positioned trials with the addition of only date powder or date syrup-1+date powder characterized by a firm texture and particularly granular. Finally, interesting aromatic notes such as fruity, cooked as well as a sweet flavor been provided by the two syrups.

CONCLUSION

In conclusion of sensory analysis, manufacturing of cheese with syrup-1 was the most interesting test. As compared to the standard cheese, it has a rich aroma brought by the aromatic notes of fruity and cooked. However, the texture of this test was judged too firm, therefore it is necessary to correct this in the future trials. This study results support a new function for cheese as an ideal way for delivery of biological active compounds and opens the horizon to new generation of functional dairy products with more health beneficial effects.

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