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Effectiveness of convective drying to conserve indigenous yeasts with high volatile profile isolated from algerian fermented raw bovine milk (Rayeb)

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Abstract

Yeasts *Candida tropicalis*, *Yarrowia lipolytica*, *Wickerhamomyces anomalus*, *Issatchenkia orientalis*, *Kluyveromyces marxianus*, *Saprochaete suaveolens* and *Trichosporon coremiiforme* were isolated and identified by physiological, biochemical tests with API 20C AUX system and molecular methods by restriction fragment analysis of PCR-amplified 28S-rRNA from Algerian fermented raw bovine milk (Rayeb). Selected yeasts *S. suaveolens*, *I. orientalis*, *K. marxianus* and *W. anomalus* produced esters and higher esters which can exert a pertinent influence on the sensory characteristics of Rayeb. Viability of *S. suaveolens* and *W. anomalus* using three methods of drying (freeze-drying, convective drying, and spray-drying) and during 4 months of storage at 4 °C and 25 °C in the darkness was studied. Immediately after each drying method, high survival was obtained using freeze-drying followed by convective drying in rice cakes and spray-drying respectively. During storage at 4 °C, convective drying provided better survival of yeast cultures of *S. suaveolens* and *W. anomalus* than freeze-drying. At 25 °C of storage, convective and freeze-dried yeast cultures showed no significant loss of viable cells up to 2 months of storage. Spray-dried yeast cultures had the greatest loss of viable count during the 3 months of storage at 25 °C.

Keywords: Rayeb; yeasts; identification; volatile compounds; preservation methods.

Practical Application: Conservation of selected yeasts that produce volatile compounds by convective air drying compared to high cost freeze-drying and spray-drying methods.

1 Introduction

Rural communities of Algeria have, for centuries, produced variety of traditional dairy products such as "Rayeb", "Lben", "Jben" and "Smen". These traditional fermented products from untreated raw milk are manufactured with ancestral methods by rural women. Rayeb is the most appreciated in all rural and urban areas of Algeria, Africa and Mediterranean areas (Samet-Bali et al., 2012). Rayeb, produced by spontaneous fermentation of raw milk at ambient temperature for a period of 24 h to 72 h, has nutritional benefits and plays an important role in the population diet, particularly those of rural population (Idoui et al., 2010). Mixed species of lactic acid bacteria (LAB), *Leuconostoc*, enterococci, yeasts, especially species of the genera *Saccharomyces* and *Candida* as well as moulds are responsible of spontaneous fermentation (Rehaiem et al., 2010). Owing to their proteolytic and lipolytic activities, yeasts represent a significant part in flavoring properties by producing volatile compounds and inducing organoleptic characteristics of Mediterranean areas-fermented milk (Samet-Bali & Attia, 2012). Few studies are conducted on the identification of yeasts from Algerian Rayeb. Besides, previous studies focused mainly on LAB (Marroki et al., 2011; Idoui & Karam, 2008).

Yeasts isolated from fermented milk are with high potential desirable technological properties especially in food applications (Grondin et al., 2015). According to the earlier studies showing high performance of *S. suaveolens* and *W. anomalus* to produce flavors (Grondin et al., 2015; de Oliveira et al., 2013), they were chosen for conservation by freeze-drying, spray-drying and convective air drying respectively. Freeze-drying and spray-drying are used to conserve potential microorganisms with industrial uses (Miyamoto-Shinohara et al., 2006; Abadias et al., 2005; Cerrutti et al., 2000). These methods offers the convenience related to the storage, transport and handling, and they retain viable microorganisms for a long time periods (Zhu et al., 2016). However, they are relatively costly and require sophisticated equipment and adequate power supply (Santivarangkna et al., 2007). In addition, microorganisms undergo many stresses mainly related to freezing and dehydration during freeze-drying (Coulibaly et al., 2009) and high operating temperatures during spray drying (Atalar & Dervisoglu, 2015). The low cost convective air drying is an alternative method for microorganism preservation and appeared to be more efficient on cell viability (Nyanga et al., 2012).

The aim of the present study is to address the following three issues: The first deals with identification of some yeasts isolated from Algerian Rayeb using physiological, biochemical tests with API 20C AUX system and molecular methods by restriction fragment analysis of PCR-amplified 28S rRNA. The second allocates to the identification of volatile compounds produced by

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selected yeasts cultivated in bovine raw milk. The last is dedicated to evaluate the convective air drying method in comparison with freeze-drying and spray-drying one to conserve selected yeasts.

2 Materials and methods

2.1 Microorganisms

Yeasts were isolated from 10 samples of traditional Algerian Rayeb prepared by spontaneous fermentation of whole 10 samples of bovine raw milk from farms located in Algiers region. A volume of 0.1 mL of each sample was diluted in 9.9 mL of peptone water solution (Difco, Detroit, USA). After serial dilutions, 1 mL of aliquots from suitable dilutions was pour-plated in Yeast Extract Glucose agar (YEG agar) prepared using: 10 g/L yeast extract (Himedia, Mumbay, India), 20 g/L glucose (Sigma, Switzerland), and 20 g/L agar (Sigma-Aldrich, Germany). The YEG agar was acidified to pH 3.5 using 100 g/L of tartaric acid solution. After incubation at 25 °C for 5 days, 9 different colonies were randomly selected and purified on YEG agar and stored on the same medium at 4 °C until their identification.

2.2 Yeast identification on the basis of physiological and biochemical properties

The following tests were used: urea hydrolysis, assimilation of different carbon compounds with API 20 C AUX test strips (bioMérieux, Canada Inc.), fermentation of glucose, galactose, maltose, lactose, sucrose and trehalose in Durham tubes containing Yeast Extract Peptone broth (YEP broth) with 2% of the appropriate sugars, growth at 37 °C, and osmotolerance in 500 g/L of glucose and 100 g/L of NaCl.

2.3 DNA extraction

Yeast cells were grown in YEG broth overnight at 25 °C and under agitation at 200 rpm. Genomic DNA of yeast was extracted with a Miniprep E.Z.N.A® Yeast DNA Kit (Omega Bio-tek, Norcross, USA) according to the manufacturer's instructions. In the final step, DNA was eluted in 50-100 µL elution-Buffer preheated to 65 °C then stored at –20 °C until PCR amplification.

2.4 PCR amplification

For amplification and sequencing of the nuclear large subunit (LSU) 28S rRNA region, forward primer pairs ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Gardes & Bruns, 1993) were used. Reverse primer pairs LROR (5'-ACCCGCTGAACTTAAGC-3') and LR5 (5'-TCCTGAGGGAAACTTCG-3') were used to amplify D1/D2 region of 28S RNA large subunit (White et al., 1990). The amplification was carried out in 50 µL reaction mixture containing 25 µL of Ready PCR Mix (×1) (Amresco, USA). 1 µL of each of couple forward and reverse primers (ITS1/ITS4 and LROR/LR5), 10 µL gDNA at 5 ng/µL and 13 µL of high purity HPLC-grade water and amplification was performed with a total of 30 PCR cycles in a thermal cycler (Mastercycler, Eppendorf, Germany). The cycling program was started with an initial cell lysis at 95 °C for 4 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30s and elongation at 72 °C for 1 min. The PCR was ended with a final extension at 72 °C for 10 min and the amplified product was stored at –20 °C. A negative control was performed with each run by replacing the PCR mixture with high purity HPLC-grade water. The quality of PCR products was verified by electrophoresis in 8 g/L agarose gel under 90 V during 45 min, detected by ethidium bromide (EtBr) staining and photographed under UV light with a charge coupled device camera (Sony, Japan). Fragment sizes were determined by using a standard molecular size marker (100 bp ladder, Amresco).

2.5 Sequencing analysis

PCR products were purified by adding 150 µL of PB buffer (Qiagen, Mississauga ON), and transferred in the wells of a Whattman GF/C filter plate. Amplified DNA was then washed three times with 80 µL of 80% ethanol/20 mM Tris (pH 7.5) and eluted in 45 µL of HPLC-grade water. Samples were quantified using the Quant-iTPicoGreen® dsDNA quantification kit (Invitrogen, Carlsbad, CA, USA) using the manufacturer's instructions. DNA sequencing was performed on Applied Biosystems Gene Amp PCR system 9700 (96 or 384 wells) using the BigDye Terminator V.3.1 kit (Applied Biosystems, Foster City, CA, USA). DNA was first denatured by an initial heating step at 96 °C for 30 s, then cycled using a protocol of 25 cycles of denaturation (96 °C for 10 s) and annealing (53 °C for 5 s), followed by one last step of elongation (59 °C for 3 min.). Sequencing reactions were purified by ethanol-EDTA precipitation and re-suspended in HiDiformamide. Samples were then run on an Applied Biosystem Prism 3730xl automated genetic analyzer using 50 cm capillaries. Sequences were analyzed and edited by Stadden Package version 4.11.2 Primer 3 software (Rosen & Skaletsky, 1998). The code is available at https://www.broadinstitute.org/genome_software/other/primer3.html (Broad Institute, 2015). Then, consensus sequences were compared to the GenBank database of NCBI with algorithm BLAST (Blast, 2015).

2.6 Preparation of strains to GC-MS, extraction and analysis of volatile compounds

Raw bovine milk was inoculated with preculture of selected yeasts and incubated for 48h at 27 °C. Raw bovine milk, fermented raw bovine milk for 24 h, Rayeb-Batch 1 and Rayeb-Batch 2 were used as controls. For each medium, 2 mL-sample of preparation was saturated with sodium chloride (0.3 g/mL) for its analysis for volatile compounds. Volatile compounds were analyzed using G1888 Headspace sampler coupled to a HP6890 GC and a 5973N quadrupole MS detector (Agilent Technologies, Wilmington, DE) according to Corcuff et al. (2011).

2.7 Conservation of selected yeasts

Production of yeasts

Production of yeasts was done according to Hamoudi et al. (2007) method. Viable counts before each preservation methods were 6×10^7 CFU/mL for *S. suaveolens* and 2×10^8 CFU/mL for *W. anomalus*.

Freeze-drying

After centrifugation at 3000 × g for 15 min at 5 °C, harvested cells by were suspended in the protectants solutions D (+) dihydrate sucrose prepared at 7% w/v with Hydroxyethyl Starch (HES) prepared at 12% w/v (Sigma Aldrich, St. Louis, Mo., U.S.A.) with demineralized water, and sterilized at 121 °C for 15 min. After freezing in a freezer for 16h at –25 °C, mixed cells and protectants were freeze-dried in a Unitop 400 L (Virtis, Gardiner, N.Y., U.S.A.) drying chamber connected to a Freeze-mobile 35 L (Virtis, Gardiner, N.Y., U.S.A.) during 24 h under vacuum (less than 1 Pa).

Convective air drying

Rice cakes were made by drying rice dough at 40 °C in a convective air drying oven for 5 h to reach a moisture content of about 4-5% w/w according to Dung et al. (2005).

Spray-drying

Cells harvested by centrifugation at 3000 × g for 15 minutes and at 5 °C were mixed with whey permeate (20% w/w), previously sterilized at 80 °C in a water bath for 20 min. Spray-drying was performed in a pilot scale spray-dryer (Niro Atomizer, Denmark). Mixing cells / whey permeate was introduced under sterile conditions into the dryer using a feed pump. The flow rate of the mixture was set at 1 kg/h; the temperature of the inlet air was at 80 and 90 °C and the temperature of the outlet air was at 45 and 50 °C.

Storage conditions and enumeration of survivors

Dried cells from each preservation method were stored at 4 °C and at 25 °C in desiccators on silica gel in order to avoid samples rehumidification, in a dark cabinet during 3 months. Two replications were done for each experiment. After each drying method, enumeration of survivors was done according to Hamoudi et al. (2007) protocole.

Determination of the residual moisture of the dried products

Residual moisture of the dried products from each drying method was determined in duplicate by the gravimetric method in the vacuum oven at 55 °C for 48 h and in the presence of phosphorus pentoxide (P_2O_5).

2.8 Statistical analysis

The experimental data were analyzed using Sigma plot 7 (2001) and Microsoft Office Excel 2007.

3 Results and discussion

3.1 Physiological, biochemical and molecular identification of yeasts

Plate counts from ten samples of Rayeb showed a yeast load of 2.7×10^7 CFU m/L. Table 1 shows the test results of the identified yeast isolates on the basis of urea hydrolysis, assimilation of different carbon compounds using API 20 C AUX, fermentation of chosen sugars, growth at 37 °C, and osmotolerance.

Molecular identification of randomly chosen yeasts from Algerian Rayeb was performed by restriction fragment analysis of PCR-amplified LSU 28S rRNA using forward primers ITS1/ITS4, and D1/D2 region of 28S RNA LSU using reverse primers LROR/LR5. PCR amplification profile of yeasts was verified by electrophoresis in agarose gel. Results showed 100% similarity with sequences in GenBank. Yeasts identified were: *Candida tropicalis*, *Yarrowia lipolytica*, *Wickerhamomyces anomalus*, *Issatchenkia orientalis*, *Kluyveromyces marxianus*, *Saprochaete.*

Table 1. Identification of yeast species isolated from Rayeb by physiological, and biochemical tests with API 20C AUX system.

	Character	*C. tropicalis*	*Y. lipolytica*	*W. anomalus*	*I. orientalis*	*K. marxianus*	*S. suaveolens*	*T. coremiiforme*
Growth	Urea hydrolysis	-	-	-	-	-	-	+
	100 g/L of NaCl	+	-	+	+	-	-	-
	500 g/L of Glucose	+	-	+	+	-	-	-
Fermentation	Glucose	+	-	+	+	+	+	-
	Galactose	+	-	+	-	+	+	-
	Sucrose	-	-	+	-	+	-	-
	Maltose	+	-	+	-	-	-	-
	Lactose	-	-	-	-	+	-	-
	Trehalose	+	-	-	-	-	-	-
Assimilation	Glucose	+	+	+	+	+	+	+
	2-Methyl glucoside	+	+	+	+	-	-	+
	Cellobiose	+	-	+	+	+	-	+
	Galactose	+	+	+	-	-	+	+
	Lactose	-	-	-	-	-	-	+
	Maltose	+	-	+	+	+	-	+
	Raffinose	-	+	+	+	+	-	-
	Mannitol	+	+	+	-	+	-	-
	Erythritol	-	+	-	-	-	-	+
	2-Ketogluconate	+	+	-	+	-	-	+

suaveolens and *Trichosporon coremiiforme. C. tropicalis* fermented glucose, galactose, sucrose, maltose and trehalose but not lactose. Lachance et al. (2011) found same results earlier. This yeast has been reported in Ghanaian fermented milk (Akabanda et al., 2013) and Tunisian Leben (Samet-Bali & Attia, 2012). *Y. lipolityca* is strictly oxidative, assimilates glucose, galactose, erythritol (Kurtzman et al., 2011) and it was reported as one of the most largely occurring yeast in fermented milk (Johnson & Echavarri-Erasun, 2011). *I. orientalis*, that assimilate glucose, is usually found in fermented dairy products because of it proteolytic and lipolytic activities; besides it has been reported to inhibit the growth of *Colletotrichum capsicion* on the surface of fruits and vegetables (Chanchaichaovivat et al., 2007). *W. anomalus* that ferments glucose but not disaccharides such as lactose, trehalose and maltose, produced ethyl acetate with antifungal activity against spoilage yeasts (Muccilli & Restuccia, 2015). *K. marxianus*, that able to ferment lactose and hydrolyze milk fat, enhanced the survival of *Lactobacillus bulgaricus* in yoghurt (Liu & Tsao, 2009).

It is important to highlight the presence of *S. suaveolens* and *T. coremiiforme* in Algerian Rayeb because few papers have reported their presence in fermented milk (Bai et al., 2010). Besides, no studies were carried out on the identification of yeasts from Algerian Rayeb. To the best of the authors' knowledge, this is the first comprehensive work on the molecular identification of yeasts prevailing in Algerian Rayeb.

3.2 Volatile compounds of selected yeasts

14 volatile compounds were identified by GC-MS and grouped according to chemical families (Table 2). They included 1 alcohol, 4 branched acids, 1 branched aldehyde, 7 esters and 1 terpene. As an example, Figure 1 show a total ion chromatogram (TIC) corresponding to the headspace profiles obtained for fermented raw bovine milk with *S. suaveolens*.

The number of volatile compounds produced by *S. suaveolens* is higher compared to *K. marxianus*, *I. orientalis* and *W. anomalus*. Indeed, *S. suaveolens* exhibited about 13 different volatile compounds belonging to branched acids and esters. The number of these volatiles was reduced to ~ 5 with *K. marxianus* and to ~3 with *I. orientalis,* or *W. anomalus* (Table 2). *K. marxianus* was characterized by the production of branched acids, esters and little amount of higher esters; however, *S. suaveolens* was characterized by the production of butanoic acid, ethyl ester and 3-methyl-butanoic acid, ethyl ester. Acetic acid was instead produced by *S. suaveolens*. Some of these volatile compounds were also produced by *S. suaveolens* in cassava wastewater (Damasceno et al., 2003). Table 3 shows the relative peak area (relative abundance) of the volatile compounds detected in raw bovin milk inoculated with the investigated yeasts. In general, the volatile compounds were characterized by a high proportion of butanoic acid, ethyl ester, followed by 3 methyl-1-butanol, and 3-methyl butanoic acid, ethyl ester. Peak areas of some volatile compounds produced were small except for butanoic acid, ethyl ester produced by *S. suaveolens* and 3-methyl 1-butanol

Table 2. Volatile compounds produced by raw milk inoculated with *S. suaveolens, I. orientalis, K. marxianus, W. anomalus*, raw milk, raw milk after 24h of fermentation, Rayeb-Batch 1 and Rayeb-Batch 2 identified by GC retention time and GC-MS analysis.

Peak number	Retention time (min)	Compounds	*S. suaveolens*	*I. orientalis*	*K. marxianus*	*W. anomalus*	Raw milk	Raw milk (24h of fermentation)	Rayeb-Batch 1	Rayeb-Batch 2
		Primary alcohols								
1	3.63	1-Butanol, 3-methyl	+	+	+	+	-	+	+	+
		Branched acids								
2	4.68	Butanoic acid	+	-	-	-	-	-	-	-
3	2.58	Acetic acid	+	+	+	+	-	+	+	+
4	9.94	Hexanoic acid	+	-	-	-	-	-	-	-
5	15.71	Octanoic acid	+	-	-	-	-	-	-	-
		Esters								
6	2.28	Ethyl acetate	+	+	+	+	-	-	-	+
7	3.41	Butanoic acid, methyl ester	+	-	-	-	-	-	-	-
8	4.34	3-methyl-butanoic acid, methyl ester	+	-	-	-	-	-	-	-
9	3.94	2-methyl-propanoic acid ethyl ester	+	-	-	-	-	-	-	-
10	4.84	Butanoic acid, ethyl ester	+	-	-	-	-	-	-	-
11	6.06	3-methyl-butanoic acid, ethyl ester	+	-	-	-	-	-	-	-
12	12.02	Butanoic acid, 3 methyl butyl ester	+	-	-	-	-	-	-	-
		Aldehydes								
13	2.67	3-methyl-butanal	-	-	-	-	-	-	-	+
		Terpenes								
14	11.28	Limonene	+	-	+	-	-	-	-	-

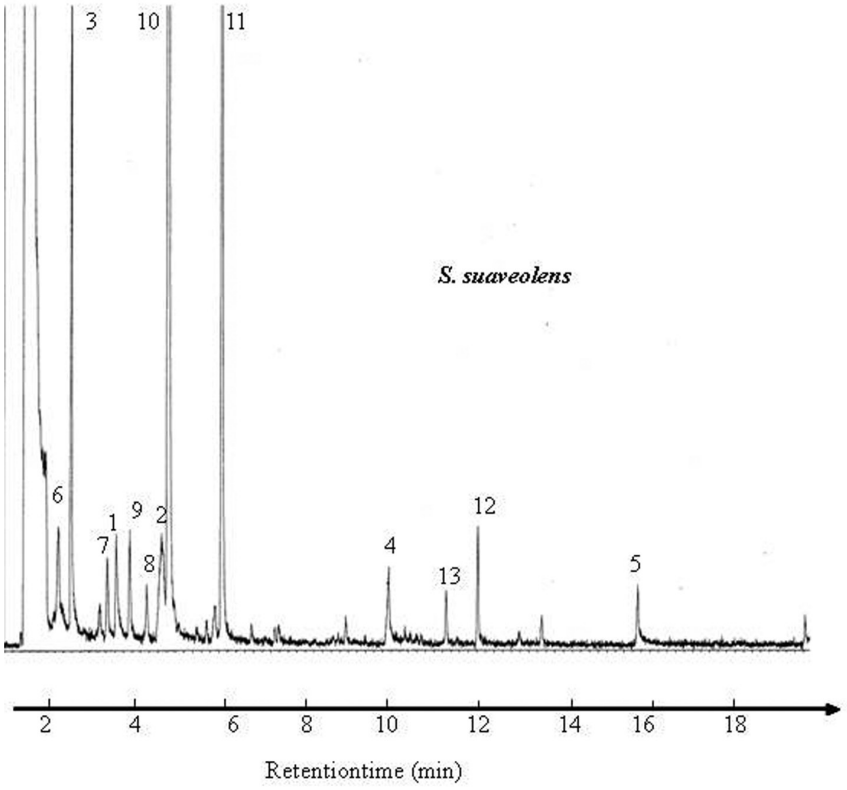

Figure 1. Total ion chromatograms corresponding to the headspace of fermented raw bovine milk with *S. suaveolens*: (1) 1-Butanol, 3-methyl; (2) Butanoic acid; (3) Acetic acid; (4) Hexanoic acid; (5) Octanoic acid, 3 methylbutyl ester; (6) Ethyl acetate; (7) Butanoic acid, methyl ester; (8) 3-methyl-butanoic acid, methyl ester; (9) 2-methyl-propanoic acid ethyl ester; (10) Butanoic acid, ethyl ester; (11) 3-methyl-butanoic acid, ethyl ester; (12) Butanoic acid, 3 methyl butyl ester; (13) Limonene.

Table 3. Relative peak area (%) of volatile compounds detected in raw milk inoculated with *S. suaveolens*, *I. orientalis*, *K. marxianus*, *W. anomalus*, raw milk, raw milk after 24h of fermentation, Rayeb-Batch 1 and Rayeb-Batch 2.

Volatile compounds	*S. suaveolens*	*I. orientalis*	*K. marxianus*	*W. anomalus*	Raw milk	Raw milk (24h of fermentation)	Rayeb-Batch 1	Rayeb-Batch 2
Primary alcohols								
1-Butanol, 3-methyl	1.33 ± 0.02[a]	33.42 ± 2.01[a]	61.86 ± 3.10	34.37 ± 2.10[a]	-	33.16 ± 2.10[a]	54.15 ± 2.20[a]	67.29 ± 3.50[a]
Branched acids								
Butanoic acid	4.34 ± 0.20	-	-	-	-	-	-	-
Hexanoic acid	1.46 ± 0.10	-	-	-	-	-	-	-
Esters								
Ethyl acetate	1.63 ± 0.10	26.96 ± 2.01	7.37 ± 0.90	35.35 ± 2.10	-	-	-	4.45 ± 0.95
Butanoic acid, methyl ester	1.29 ± 0.10	-	-	-	-	-	-	-
3-methyl-butanoic acid, methyl ester	0.58 ± 0.02	-	-	-	-	-	-	-
2-methyl-propanoic acid ethyl ester	1.30 ± 0.10	-	-	-	-	-	-	-
Butanoic acid, ethyl ester	53.80 ± 2.10	-	-	-	-	-	-	-
3-methyl-butanoic acid, ethyl ester	24.03 ± 1.00	-	9.70 ± 1.00	-	-	-	-	-
Butanoic acid, 3 methyl butyl ester	1.46 ± 0.10	-	-	-	-	-	-	-
Aldehydes								
3-methyl-butanal	-	-	-	-	-	-	-	10.53 ± 1.60
Terpenes								
Limonene	0.74 ± 0.02		3.22 ± 0.90					

[a]Mean and standard deviation of three repetitions.

by *K. marxianus*. Also, 3-methyl-1-butanol was higher in Rayeb issued from Rayeb-Batch 2 sample. This alcohol could be produced by microbial milk spoilage such as *Bacillus cereus*, *Pseudomonas fragi*, *P. perolens* and *B. pumilus* (Magan et al., 2001).

In general, high levels of butanoic acid, ethyl ester (53.767%), and 3-methyl-butanoic acid, ethyl ester (24.026%) were produced by *S. suaveolens*. These volatiles are mostly responsible for the flowery and fruity aroma. Grondin et al. (2015) also described this yeast for production of alcohol and esters compounds. During fermentation, intracellular enzyme catalyzed reactions responsible of the formation of esters (Verstrepen et al., 2003). *S. suaveolens* produced 3-methyl-butanoic acid, ethyl ester (ethylisovalerate) after partial metabolism of leucine and isoleucine by oxidative deamination and esterification by ethanol (Farbood et al., 1987). It is worth observing that little amount of limonene was produced in raw milk with *S. suaveolens* or with *K. marxianus*. Henssen et al. (1984) reported that yeast developed specific fruity odors when associated with bacteria. Aldehydes, such as 3-methyl-butanal were detected in Rayeb. Biosynthesis of this aldehyde is favorable when yeasts are associated with bacteria like *Brevibacterium linens* (Arfi et al., 2005). According to the results obtained, *I. orientalis*, *K. marxianus* especially *S. suaveolens* and *W. anomalus* with relatively low fermentative activity have high capacity to form volatile compounds such as esters and branched acids as well as higher esters with pleasant aroma.

3.3 Drying of selected yeasts

Figure 2 shows the viability of yeast cells of *S. suaveolens* and *W. anomalus* immediately after freeze-drying, convective air drying and spray respectively.

HES in combination with sucrose provided a light and porous structure of freeze-dried product that made rehydration easy and with moisture content 2.45% ± 1.2. High levels of viability were observed for freeze-dried *S. suaveolens* and *W. anomalus* (68% and 74% respectively). Combination of HES with non-reducing disaccharide sucrose protect algae, protozoa, fungi and bacteria during freeze-drying; often at concentrations ranging from 5 to 15% (Hubálek, 2003). The mechanism of direct interaction between the sugar molecules and membrane phospholipids is among the main protection mechanisms during freeze-drying (Crowe et al., 1998).

S. suaveolens and *W. anomalus* with moisture content 4.5% ± 0.5 shows high levels of viability (60% and 65% respectively). These results are in broad agreement with earlier studies showing that convective drying in rice cakes is very effective to conserve *S. cerevisiae* and *I. orientalis* yeast strains (Nyanga et al., 2012).

S. suaveolens and *W. anomalus* with moisture content of 3% ± 0.2 gave poor survival values, up to 20% and 29% respectively after spray drying. Although, authors reported that, the survival after spray drying depends on the types and concentrations of the encapsulating agent and the inlet air temperature (Atalar & Dervisoglu, 2015). In this work, processing conditions of spray-drying constant inlet were 80-90 °C. High inlet air temperature causes excessive rapid moisture evaporation, resulting diminution in water activity and cracks in the polymeric membrane of microorganisms (Brun-Graeppi et al., 2011; Telang & Thorat, 2010).

3.4 Storage of drying yeasts

Figure 3 shows the survival results of *S. suaveolens* and *W. anomalus* after 3 months of storage at 4 °C (Figure 3A, B) and 25 °C (Figure 3C, D) in the darkness and on silica gel.

At 4 °C, a drastic decrease in viability of freeze-dried selected yeasts was found at the 1rst 30 days of storage, followed by a stabilization period after 40 days. At the end of storage, the

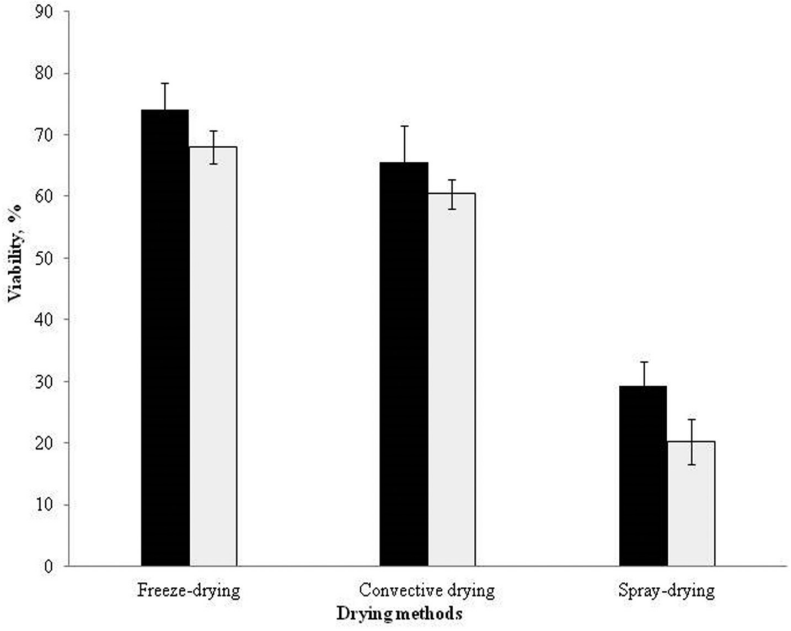

Figure 2. Viability of *W. anomalus* and *S. suaveolens* immediately after freeze-drying, convective drying and spray-drying.

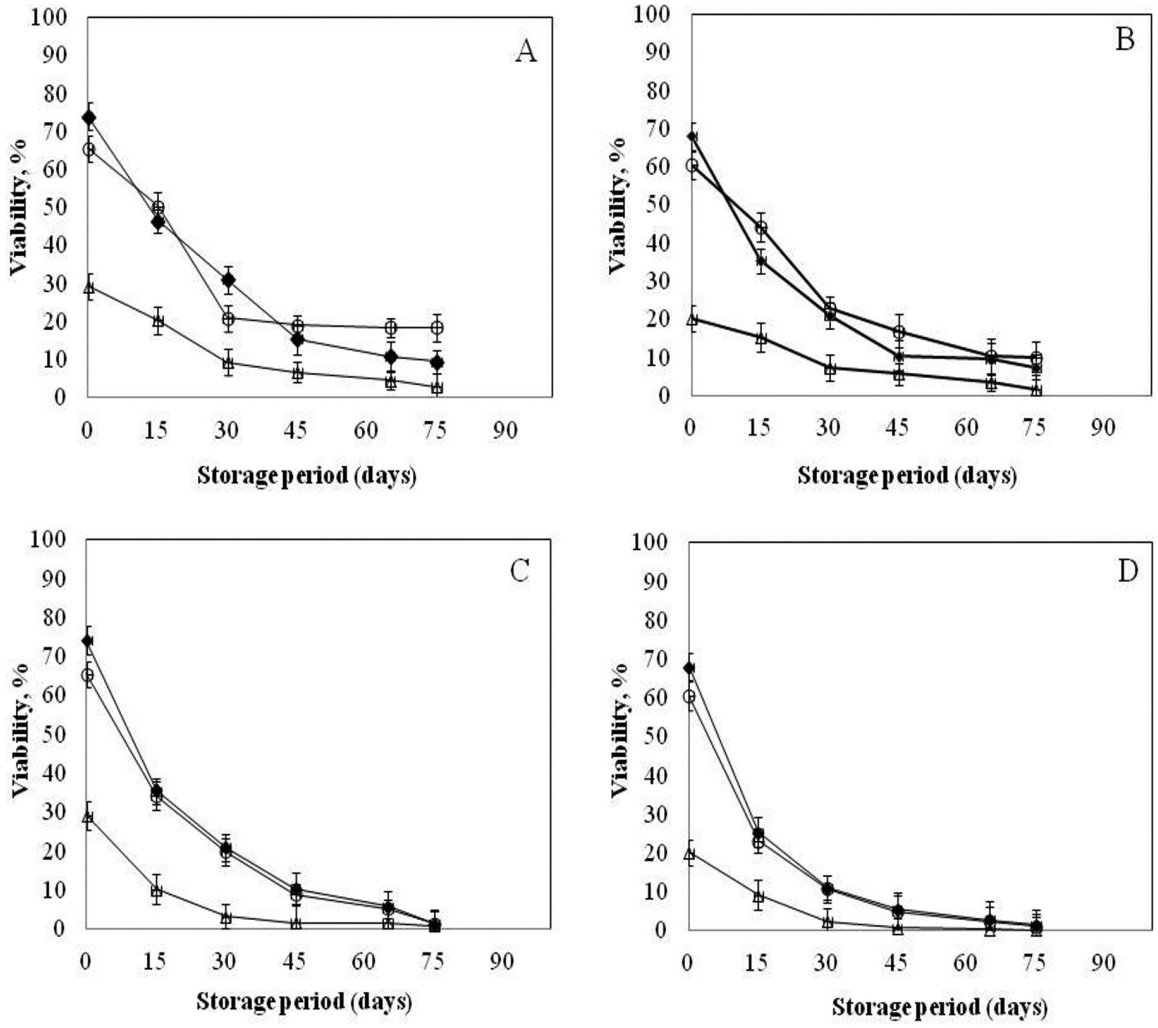

Figure 3. Cell viability of *W. anomalus* and *S. suaveolens* during storage for 3 month at 4 °C (A, B), and 25 °C (C, D) on silica gel. *W. anomalus* and *S. suaveolens* cells were dried using (♦) freeze-drying, (○) convective drying, and (Δ) spray-drying.

viability of *S. suaveolens* and *W. anomalus* was stabilized at less than 10% and 20% respectively.

As can be seen from Figure 3A, B, a gradual decrease in viability of convective air dried cells was observed; and at the end of storage, viability was maintained at 10% and 18%. These results demonstrated that, convective air dried yeast cultures retained viability with regard to freeze-drying. Rice contains starch, comprising amylopectin and amylose, could provide the hydroxyl groups in order to fix the yeast cells, and possibly by the formation of a glassy structure, and thus protect the yeast cells damage associated with drying (Nyanga et al., 2012). Spray-dried yeast cells of *S. suaveolens* and *W. anomalus* showed a drastic loss of viability during storage and at the end of storage the viability values were less than 5% approximately for the two yeast strains.

At 25 °C of storage, the viability decreased at, ultimately reaching at the end of storage values less than 5%, whatever the strain or the drying method being analysed. Costa et al. (2002) found that viability of *P. agglomerans* CPA-2 was maintained for 4 weeks of storage at 4 °C than at 25 °C. These authors suggested that low temperatures kept the metabolic activities at their lowest level and contribute to stability during storage. Garzon-Rodriguez et al. (2004) found that remaining moisture contribute also to protein degradation.

4 Conclusion

Yeasts *C. tropicalis*, *Y. lipolytica*, *W. anomalus*, *I. orientalis*, *K. marxianus*, *S. suaveolens* and *T. coremiiforme* were randomly isolated and identified from Algerian fermented Rayeb. Selected yeasts *S. suaveolens*, *I. orientalis*, *K. marxianus* and *W. anomalus* produced different volatile compounds such as branched acids, esters and higher esters with pleasant aroma. Viability of *S. suaveolens* and *W. anomalus* was better immediately after freeze-drying followed by convective air drying in rice cakes and spray drying in whey permeate respectively. During storage at 4 °C, convective air drying provided better survival of selected yeast cultures than freeze-drying. The use of convective air drying to preserve yeasts proves a promising economic alternative to freeze-drying and spray-drying.

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