GRASAS Y ACEITES 72 (1)
January-March 2021, e396
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.1258192

Nutritional, functional and microbiological characteristics of Jordanian fermented green Nabali Baladi olives

M.N. Ahmad ✉, G.F. Mehyar and G.A. Othman

Department of Nutrition and Food Technlolgy. University of Jordan, Amman 11942, Jordan

✉ Corresponding author: mosnuman@ju.edu.jo

Submitted: December 21, 2019; Accepted: February 21, 2020; Published online: February 24, 2021

SUMMARY: The quality characteristics of green olives produced by the traditional spontaneous fermentation method in Jordan have never been studied. We investigated the nutritional, functional, and microbiological characteristics of Jordanian fermented green Nabali Baladi olives (GNBFO). Proximate composition, fatty acids, and total polyphenols were determined by standard protocols. Cultivable microflora was monitored over 3 months of fermentation. Isolated microorganisms were identified by molecular sequencing and *in vitro* probiotic traits were tested. GNBFO contained fiber (3g⁄100g), total polyphenols (306mg⁄100g), oil (19.3g ⁄100g), and oleic acid (70%). Yeast strains (*Candida diddensiae* and *Candida naeodendra*) were predominant and showed acid (pH=2.5) and bile salt (0.1% and 0.3%) resistant and high adhesion ability (ca∼10^7CFU/ml) to intestinal cell lines; they were positive to catalase and negative to lipase and none possessed antimicrobial activity against selected pathogens. Lactic acid bacteria were not detected. In conclusion, the GNBFO have promising functional characteristics as they contain valuable nutrients, antioxidants, and yeast strains with potential probiotic traits.

KEYWORDS: *Oleic Acid; Olive; Polyphenols; Probiotics; Spontaneous Fermentation; Yeast*

RESUMEN: ***Características nutricionales, funcionales y microbiológicas de las aceitunas verdes jordanas fermentadas Nabali Baladi.*** Hasta la fecha, no se han estudiado las características de calidad de las aceitunas verdes producidas por el método tradicional de fermentación espontánea en Jordania. En este trabajo, investigamos las características nutricionales, funcionales y microbiológicas de las aceitunas Nabali Baladi verdes fermentadas jordanas (GNBFO). La composición proximal, los ácidos grasos y los polifenoles totales se determinaron mediante protocolos estándar. La microflora cultivable se controló durante 3 meses de fermentación. Se identificaron microorganismos aislados por secuenciación molecular y se probaron los rasgos probióticos in vitro. GNBFO contenía fibra (3g/100g), polifenoles totales (306mg/100g), aceite (19,3g/100g) y ácido oleico (70%). Las cepas de levaduras (*Candida diddensiae* y *Candida naeodendra*) fueron predominantes y mostraron acidez (pH=2.5) y sales biliares (0,1% y 0,3%) resistentes y alta capacidad de adhesión (ca ∼ 10^7CFU/ml) a las líneas celulares intestinales; fueron positivos para catalasa y negativos para lipasa y ninguno poseía actividad antimicrobiana contra patógenos seleccionados. No se detectaron bacterias del ácido láctico. En conclusión, GNBFO tiene características funcionales prometedoras, ya que contienen valiosos nutrientes, antioxidantes y cepas de levaduras con posibles rasgos probióticos.

PALABRAS CLAVE: *Ácido Oleico; Fermentación Espontánea; Levadura; Oliva; Polifenoles; Probióticos*

Citation/Cómo citar este artículo: Ahmad MN, Mehyar GF, Othman GA. 2021. Nutritional, functional and microbiological characteristics of Jordanian fermented green Nabali Baladi olives. *Grasas Aceites* **72** (1), e396. https://doi.org/10.3989/gya.1258192

1. INTRODUCTION

The olive (*Olea europaea* L.) is an ancient cultivated human plant food. It originates in the Middle East and the Mediterranean region and is central to the local diet (Al-Ismail *et al*., 2011; Ghanbari *et al.,* 2012). Despite the global spread of olive cultivation, most of its production comes from the Mediterranean basin, (Ghanbari *et al.,* 2012). In Jordan, almost 80% of the olives are used for oil production and 20% for fermentation to produce table olives (MOA, 2016). In 2016, table olive consumption in Jordan reached 28 thousand tons (MOA, 2016). Consumption of olives is related to higher-quality diets, including higher intakes of dietary fiber, proteins, a number of vitamins, mineral elements, phenolic compounds, organic acids, pigments, and phytosterols, monounsaturated oleic acid, and lower intakes of saturated fat (López *et al.,* 2007; Ghanbari *et al.,* 2012). These components are known to possess multiple biological, medicinal, and therapeutic effects such as antioxidant, antihyperlipidemic, antimicrobial, anti-inflammation, antihypertensive, anticarcinogenic, and antithrombotic (Lanza *et al.,* 2010). In essence, the intake of olives and their oil is associated with several health benefits, particularly reduced risks of cardiovascular disease, insulin resistance, and cancer (Saibandith *et al.,* 2017). Nevertheless, the salt content in table olives greatly varies depending on the preparation process (López *et al.,* 2008), a matter that may represent a health concern. This is especially important in populations which consume high amounts of olives and subsequently salt, because of its association with hypertension (Pino *et al.,* 2018).

The olive's functional nutrients, bioactive, and health-promoting components have been shown to vary greatly with olive genotype, maturity, product quality, postharvest handling and storage conditions, agricultural practices, and methods of processing and analysis (Bleve *et al*., 2014). Worldwide, about two thousand olive varieties have been identified (Ghanbari *et al.,* 2012); several of these varieties inhabit Jordan, mainly Nabali Baladi, Muhasan, Rassei, Shami and Nasohi (Al-Ismail *et al.,* 2011). The Nabali olive is the major olive genotype grown in Jordan and is widely used in traditional fermentation and oil production as it is naturally adapted to the dry conditions in Jordan and provides a high oil yield (Humeid *et al.,* 1991). In fact, the available literature on the nutritional and health benefits of olives and olive oil is basically derived from results of studies investigating olive varieties other than those grown in Jordan (Lanza *et al.,* 2010; Aponte *et al.,* 2010; Issaoui *et al.,* 2011).

Furthermore, a number of technological methods to produce table olives are available (Issaoui *et al.,* 2011) and yield olives with varied chemical and microbiological characteristics (Abriouel *et al.,* 2011). These methods of olive fermentation greatly influence the competitive activities of the natural microflora that lead to spontaneous fermentation (Abriouel *et al.,* 2011). Several Lactic acid bacteria (LAB) and yeast species are the main natural microbes engaged in olive fermentation (Bleve *et al*., 2014). In Jordan and eastern Mediterranean countries, the traditional fermentation method of green olives depends on spontaneous fermentation in high brine concentration after debittering the green olives by soaking in water for 3 days (NCARE, 2000). This method results in fermented olives with a unique sour taste that differs remarkably from Greek, Italian or Spanish methods. To the best of our knowledge, the nutritional, functional and microbiological characteristics of the fermented Nabali olive have not yet been elucidated. Hence, this study was performed to identify some of such characteristics of table olives produced by the traditional fermentation method in Jordan.

2. MATERIALS AND METHODS

2.1. Olives fermentation

Green Nabali Baladi olives (15 kg) were purchased from a local farm in Jerash, Jordan, in two successive seasons (November 2017 and November 2018) and processed according to the traditional method of green olive fermentation in Jordan (NCARE, 2000). Olives of each season were crushed under mechanical pressure to make a scratch in the flesh, soaked in water for three days, placed in two glass jars and the brine solution (10% wt/vol) was added. The brined olives were kept in a dark, dry place at room temperature (17-20 °C) to allow natural fermentation to take place (NCARE, 2000). The fermentation lasted 3 months.

2.2. Microbiological analysis

A 30 g sample of olive flesh was taken from each jar after 30, 60 and 90 days of fermentation. Samples were washed with sterile saline (0.9% NaCl wt/vol) solution (30 ml) and homogenized by a Stomacher lab blender (Bag Mixer® 400W, Interscience, France). A 10g sample of the homogenized olive paste was diluted with 90 ml peptone water, and then 10-fold serial dilution was carried out. Lactic acid bacteria (LAB) and yeasts were quantified as follows: samples of 100 µl of each dilution were applied to agar plates that contained Man, Rogosa and Sharpe Agar (MRS, Oxoid, England), followed by anaerobic incubation at 30 °C for 48-72 hours (Bleve *et al.,* 2014) and Potato Dextrose Agar (Oxoid, England) supplemented with chloramphenicol and chlortetracycline (Dar Al Dawa, Amman, Jordan), followed by incubation at 25 °C for 2-4 days (FDA, 2001). Gram staining and viable counts were performed to identify the isolated microorganisms. The pH of the brine solution was measured (Hanna Instrument, USA). Analysis of brine concentration was also performed by using Mohr's titration method (AOAC, 1995). The experiments were performed in triplícate.

2.3. Analysis of proximate composition

A proximate composition analysis was performed on the fermented olives according to the reference Weende method (AOAC 1995). For moisture content determination, samples of olive pulp were dried at 105 °C using the air oven (Memmert, Karlklob-West Germany) until a constant weight was reached. The dried olive pulp was used to determine the fat content following the solvent (ether 40 -70 °C) extraction procedure for 6 hours using the Soxhlet apparatus. The ether was evaporated and the residual oil was then weighed (AOAC, 1995). Crude protein in the pulp of the dried olives was measured using the micro Kjeldahl method (AOAC, 1995). The pre-weighed samples of olives were digested in 10 ml sulfuric acid in the presence of catalyst tablets containing K_2SO_4, $CuSO_4$, and selenium. The reduced ammonia was liberated by boiling with 50% sodium hydroxide and distilled (Rapid Distillation Unit, Lanconco Corporation, Kansas City, USA) into a boric acid solution to form ammonia borate that was titrated directly using a standard sulfuric acid solution. The resultant nitrogen content was multiplied by the factor 6.25 to determine the crude protein content.

Crude ash was analyzed by the dry ashing method (AOAC, 1995). The pre-weighed samples were ignited in a muffle furnace (Naber Model D2804, Bremen) at 550 °C for 6 hours. The inorganic ash residue was then weighed. The crude fiber of dried and defatted olive pulp was determined according to the Van Soest method (AOAC, 1995). In pre-weighed filter bags, a sample of 1 g of dried and defatted olives was weighed. The sample-containing bag was sealed and placed in the bag suspender trays of the fiber analyzer (ANKOM, USA). The sample was digested using a standard sulfuric acid solution (1.25%) for 30 minutes, and then in a sodium hydroxide solution (1.25%) for 30 minutes. The bags were then dried in an air oven at 105 °C and cooled before weighing. The sample-containing bag was ashed in pre-weighed crucibles for 2 hours at 550 °C, cooled in a desiccator, and then weighed. The crude fiber content was calculated by subtracting the weight before and after ashing (AOAC, 1995). The carbohydrate content of olive pulp was estimated as the resulting difference from subtracting the content of each of moisture, protein, fat, ash, and fiber from 100% (Bleve et al., 2014).

2.4. Analysis of fatty acid composition

The extraction of fat from fermented olives was performed according to Issaoui *et al*., (2011). The olive pulp was well ground to form a paste from which fat was extracted using a methanol/water/chloroform (1/1/1; v/v/v) solvent mixture. The fat-containing mixture was centrifuged, the solvent layer was evaporated at 60 °C, and the EC Regulation no. 2568/91 method was used to prepare the fatty acid methyl esters. In brief, a pre-weighed sample of fat extract (50 mg) was well mixed with hexane (2 ml, GC grade) and a freshly-prepared solution (200 µl) made up of 2 M-potassium hydroxide in anhydrous methanol was added and thoroughly mixed to form a clear solution, and then acetic acid (200 µl) was added with continuous mixing. Soon after esterification was complete, the capillary GC column (Restek, Rtx-225, USA, cross bond 50%-cyanopropyl-methyl 50%-phenylmethyl polysilo-xane, 60 m, 0.25 mm/D, 0.25 µm df) was used to determine the fatty acid methyl esters. The latter was identified using the chromatogram of the corresponding fatty acid methyl ester standards (Supelco Inc, Bellefonte, USA) as described elsewhere (Al-Ismail *et al.,* 2011).

2.5 Analysis of total polyphenols

The Folin-Ciocalteu assay was used to quantify the total polyphenols in olive pulp. The method is essentially based on the ability of phenols to reduce phosphomolybdic acid in aqueous alkali media. Methanol solvent was used to extract (3 times) olive pulp (5 g) and (0.5 ml) of this extract was well mixed with (0.5 ml) Folin-Ciocalteu's phenol reagent (Sigma-Aldrich, Buchs, Switzerland). A volume of (3 ml) of Na_2CO_3 saturated solution was added to the batter and brought up to (10 ml) with distilled wáter, and then allowed to stand for 30 minutes at room temperature. The final solution was centrifuged (10 minutes, 3000 g) and the supernatant absorbance was read (765 nm) by the spectrophotometer (LABOMED, Los Angeles, USA). The olive pulp's total polyphenols were expressed as mg of gallic acid/100g of fresh fruits (Bleve *et al.,* 2014).

2.6. Identification of yeast isolates

The isolated yeast from the fermented olives after 90 days of fermentation was named as Y1 and Y2 for the two yeast strains isolated in the 2017 season, and Y3 and Y4 for the two strains isolated in the 2018 season. Y1, Y2, Y3, and Y4 and the reference strain of *Candida albicans* ATCC 10231 were grown overnight in the YPG medium (Yeast extract 1%: oxoid, England, peptone 2%: oxoid, England, glucose 2%) at 30 °C. The manufacturer's instructions of Wizard Genomic DNA purification (Promega, Madison, WI, USA) were strictly followed in the extraction of the experimental genomic DNA. Sequencing the 5′ end of the 26S rDNA encompassing the D1 and D2 expansion domains using the primers NL1 (5′-GCATATCAATAAGGGGA GGAAAAG-3′) and a reversed primer NL4 (5′-GGT CCG TGT TTC AAG ACGG-3′) were then performed for the identification of experimental genomic DNA. Amplification was performed for 36 PCR cycles with annealing at 52 °C, extension at 72 °C for 2 minutes, and denaturation at 94 °C for 1 minute. The products of PCR were purified and sequenced for species identification (Macrogen, Korea). The National Center for Biotechnology Information BLAST online program was used to determine the DNA sequence similarity (BLAST, 2019) and as given by Wang et al., (2008).

2.7. *In Vitro* probiotic characterization of yeast

2.7.1. Tolerance of yeast to low pH and bile salt

An overnight culture of yeast isolates was tested for viability as affected by low pH and bile salt. Fresh yeast culture (100 µl; ca $1x10^8$ CFU/ml) of each yeast isolate was inoculated in sterile test tubes containing either: 1 ml of acidified YPG medium (5 M HCl, pH 2.5), 1 ml of YPG containing 0.1% bile salts, 1 ml of YPG containing 0.3% bile salts, or 1 ml of YPG alone. A viable count of yeast isolates in acidified YPG medium was measured at 0, 1, 2 and 3 hours of incubation at 37 °C by taking samples of 100 µl, serially diluted and plated onto YPG agar plates. Yeast isolates' survival in bile salts was measured after incubation for 24 hours at 37 °C. A yeast culture (one loopful of 2 mm in diameter) was streaked onto YPG agar to check viability. Yeast isolates inoculated in YPG medium alone was used as the positive control. The test was performed in duplicate (van der Aa Kuhle *et al.,* 2005).

2.7.2. Adhesion of yeast strains to intestinal cell lines

The colonocyte-like cell lines Caco-2 were used to determine the adhesion ability of the yeast isolates. Caco-2 cell line was kindly supplied by Prof. Y. Bustanji of the University of Jordan. The culture and maintenance of the cell lines were carried out following standard procedures using DMEM medium High Glucose (EuroClone, Italy) supplemented with fetal bovine serum (10%) and with a mixture of antibiotics (10 U/ml penicillin, 10 µg/ml streptomycin). Intestinal cells were seeded in 24-well plates and cultivated until a confluent differentiated state was reached (monolayers). Yeasts were grown in YPG media for 48 hours at 30 °C, then 1 ml of yeast culture was centrifuged and the pellet was washed twice with PBS solution. The yeast pellet was suspended in the DMEM media without antibiotics to reach an inoculum size of ca~10^8 CFU/ml. Cellular monolayers were also carefully washed with PBS solution, and yeast suspensions were added. Adhesion experiments were carried out for 90 minutes at 37 °C, 5% CO_2 and, afterward, wells were gently washed to release unattached yeasts before proceeding with the lysis of cellular monolayers using a 0.25%

Trypsin- EDTA solution (Sigma, USA). Dilutions of samples, before and after adhesion, were made in PBS solution and yeast counts were performed on YPG agar plates. The adhesion was calculated as: % CFU adhered yeasts/ CFU added yeasts. Experiments were carried out in two replicated plates and in each plate, two wells were used per sample (Zivkovic *et al*., 2015).

2.7.3. Antimicrobial activity of yeasts

A few common food-borne pathogens were selected, *Escherichia coli* (ATCC 8739), *Salmonella typhimurium* (02:8432), *Salmonella enteridis* (CRIES1016), *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus aureus* (ATCC 6538P); they were cultured on Trypticase Soy Soft (TSS) agar (20 mL, with 8 g/L agar; Oxoid, England). The capacity of each yeast strain to inhibit the previous bacterial pathogens was determined using the TSS agar medium. One loopful (2 mm in diameter) of yeast was streaked as a line (2-3 cm) onto the soft-agar surface; the plates were then incubated at 27 °C for 24 hours and screened for inhibition zones around the bacterial colonies (Silva *et al.,* 2011).

2.8. Technical characteristics of yeast isolates

The lipolytic activity of yeast isolates was qualitatively tested with 5% (v/v) olive oil, emulsified by vigorous shaking. The plates were inoculated by streaking once across and incubated at 25 °C for 10 days. The plates were then flooded with a saturated copper sulphate solution and allowed to stand for 10 minutes. Where growth occurred, the appearance of a bluish color was taken as being indicative of positive lipolytic activity. The blue zones were intensified if the plates were kept in the refrigerator after pouring off the developer (Kurtzman *et al.,* 2011). Catalase activity was determined by adding drops of H_2O_2 (3%) to the cultured colonies; the release of gas was indicative of a positive result (Silva *et al.,* 2011).

2.9. Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences programme version 20 (SPSS®), Chicago, Il, USA). Values are given as the mean ± SD. Each datum represents six replicates for each season; i.e. three sample replicates for each of the two jars each season were measured. Appropriate data were tested using the independent sample t-test for significant differences. The statistical significance level was fixed at $p < 0.05$ (Issaoui *et al.,* 2011)

3. RESULTS

3.1. Microbiological Characteristics of fermented olives

After 3 months of olive fermentation, yeast was the predominantly isolated microorganism from the olive samples. As shown in Figure 1, the yeast count reached its highest level after 2 months of fermentation (ca~ $3x10^4$ CFU/ gram olive in the 2017 season). A significant (p=0.004) reduction in yeast count was detected by the end of the third month compared to the yeast count during the second month. The pH of the brine measured at zero time fermentation was 5.2. pH values and were significantly (p=0.001) reduced after 60 days of fermentation (pH= 4.45 in season 2017, pH=4.26 in season 2018) compared to values after 30 days of fermentation (Figure 1). At the start of fermentation, the brine concentration was 100 g/L. It markedly (p=0.001) decreased after 30 days of fermentation (90 g/L in season 2017 and 91 g/L in season 2018). A further, but stable drop in this variable, was noticed from 60 days till the end of fermentation (89 and 86 g/L for in the two seasons, respectively). In the 2018 season, lower yeast counts were detected after 3 months of fermentation compared to the yeast count in the 2017 season; the yeast count reached ca~ $5x10^2$ CFU/g olives on the 30^{th} day, $8.8x10^3$ CFU/g olives on the 60^{th} day and 7.8 x 10^3 CFU/g olives on the 90^{th} day of fermentation. By the end of 90 days of fermentation, the LAB was not detected at any level. Figure 1 clarifies the dynamics between yeast count and pH values during the olive fermentation process.

3.2. Proximate composition and total polyphenols in fermented olives

The analysis of proximate composition of fermented green Nabali Baladi olives revealed a high content in oil (19% wet weight). Table 1 shows the detailed proximate composition analysis results for fermented olive samples taken from 2 separate jars in two different seasons. The results of the two seasons' analysis were comparable. Crude fiber content was 3 g/100g edible part of olives. A low amount of protein was

detected in fermented olives (0.9 g/100g edible part). The total polyphenol content in green Nabali Baladi olives ranged from 290-310 mg gallic acid equivalent/100g of fermented green olive pulp (Table 1).

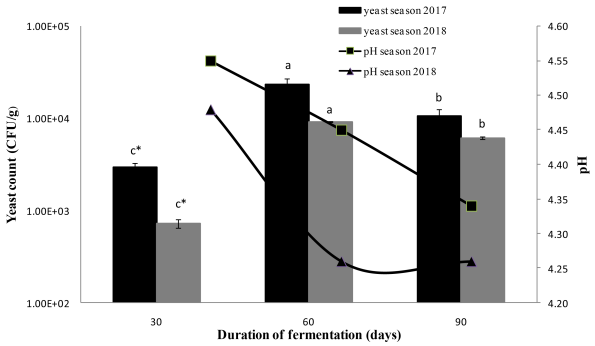

FIGURE 1. Viable count of yeasts (CFU/g) and brine pH changes during green Nabali Baladi olive fermentation.
Lactic acid bacteria were not detected during 90 days of the experiment. Values are given as mean ± SD (Based on three samples replicaes for each of the two jars each season; i.e. six replicates for each season). Independent sample t-test was used. Bars with different superscripts are significantly different ($p<0.05$). * Significantly different ($p<0.05$) in the same time duration.

3.3. Fatty acid composition of fermented olives

Table 2 presents the fatty acid composition of green Nabali Baladi fermented olives. Oleic acid was the major fatty acid (about 70% of total fatty acids). Other fatty acids such as palmitic acid, linoleic acid and stearic acid account for about 25% of the total fatty acids. Total saturated fatty acids compose 17% of the fatty acid, whereas total polyunsaturated fatty acids were only 9%.

3.4. Identification of yeast isolates

The identification of yeast isolates (Y1, Y2, Y3, and Y4) was performed by the sequencing of the 26S rDNA encompassing the D1/D2 expansion domains. BLAST identified the two DNA sequences as *Candida diddensiae*, *Candida naeodendra* strain MB14804, *Candida diddensiae,* and *Candida diddensiae* as 100%, 99%, 99%, and 99%, respectively (Table 3).

3.5. *In Vitro* probiotic characterization of yeast

3.5.1. Tolerance to low pH and bile salt and adhesion to intestinal cell lines

Table 3 shows the results of Y1, Y2, Y3 and Y4 *in vitro* probiotic characteristics testing. Y1 showed higher tolerance to HCl than Y2. On another hand, isolates Y3 and Y4 isolated in the second season had comparable tolerance to acidic media. Collectively, ca~ 10^7 CFU/ ml of yeasts were able to survive acidic conditions after 3 hr of incubation. In addition, all yeast isolates showed comparable tolerance to bile salt at different concentrations (0.1 and 0.3%). Y1, Y2, Y3, and Y4 also showed high adhesion ability to Caco-2 cell line, ca~ 10^7 CFU of yeast isolates were able to attach to intestinal cell lines after 90 min of incubation. Y3 and Y4 had higher adhesion ability to the Caco-2 cell line than Y1 and Y2, but it was considered low adhesion ability (Table 3).

TABLE 1. Proximate composition analysis of fermented green Nabali Baladi olives per 100 g of edible part [1-4]

Nutrient	g/ 100 g of edible part	
	Season 2017	Season 2018
Moisture	67.9±0.3[a]	67.5±0.6[a]
Fat	19.27±0.4[a] (60.0±0.3)	19.83±0.2[a] (61.0±0.2)
Carbohydrate	4.9±0.6[a] (15.26± 1.2)	4.3±0.4[a] (13.2±0.3)
Crude Protein	0.9±0.05[a] (2.8±0.3)	0.9±0.02[a] (2.8±0.3)
Crude Fiber	3.0±0.1[a] (9.5±0.7)	3.13±0.06[a] (10.2±0.1)
Ash	4.1±0.1[a] (12.5±1.9)	4.2±0.1[a] (12.9±1.3)
Total Polyphenols*	306.3±42.3[a] (954.2±130.8)	295.4±14.6[a] (908.9±45.5)

1. Edible part of fermented olive is 75.27 g/ 100 g of whole olives
2. Values between parenthesis are calculated based on dry weight
3. Values are given as mean ± SD (Based on three samples replicates for each of the two jars each season; i.e. six replicates for each season). Independent sample t-test was used.
4. Values with different superscripts within the same row are significantly different ($p<0.05$)
*Total polyphenols was expressed as mg of gallic acid/100 g edible olive

3.5.2. Antimicrobial activity of yeasts

The yeast isolates (Y1, Y2, Y3, and Y4) did not show any inhibition zone surrounding their colonies in the antimicrobial tests, which indicated that the isolates did not have antimicrobial activity against the selected pathogenic bacteria.

3.6. Technical characteristics of yeast isolates

Yeast isolates were tested for lipase and catalase production. They were found negative for lipase production and positive for catalase (Table 3).

TABLE 2. Fatty acid composition of oil extracted from fermented olives[1, 2]

Fatty acid	g/ 100 g of total fatty acid	
	Season 2017	Season 2018
C 16:0	14.3±0.06 [a]	14.3±0.06 [a]
C 16:1	1.5±0.02 [a]	1.6±0.02 [a]
C 18:0	2.6±0.08 [a]	2.6±0.08 [a]
C 18:1	70.6±0.3 [a]	69.8±0.3 [a]
C 18:2	8.2±0.1[a]	8.2±0.1[a]
C 18:3	0.73±0.02 [a]	0.74±0.02 [a]
C 20:0	0.4±0.01 [a]	0.4±0.01 [a]
C 20:1	0.23±0.01[a]	0.22±0.01[a]
C22:0	0.1±0.01[a]	0.1±0.01 [a]
Σ SFA	17.4	17.3
Σ MUFA	72.33	71.57
Σ PUFA	8.94	8.89

1. Values are given as mean ± SD (Based on three sample replicates for each of the two jars each season; i.e. six replicates for each season). Independent sample t-test was used.
2. Values with different superscripts within the same row are significantly different ($p<0.05$)

4. DISCUSSION

The Nabali Baladi olive is one of the major local olive varieties in Jordan; it is widely used for fermentation and oil production (Humeid *et al.,* 1991). A limited number of studies investigated the qualitative characteristics of fresh Nabali Baladi olives with an emphasis on oil production purposes. To our knowledge, this is the first study investigating the nutritional, functional and microbiological characteristics of fermented green Nabali Baladi olives in Jordan.

The results demonstrated that the oil content of fermented olives ranged from 19-20% (wet weight) of flesh (60% based on dry weight) after 3 months of fermentation in two different seasons (2017 and 2018). It has been reported that the oil content of ripened Nabali Baladi olives without fermentation was 60% of flesh dry weight in Jordan (Al-Ismail *et al.,* 2011) and 55% (dry weight) in Palestine (Ebiad and Abu-Qaoud, 2014). These results are comparable to those of the present study. However, the oil content of fermented green olives is widely variable depending on the olive variety. In Tunisia, one of the major olive producer in the region, the oil content of green table olives ranged from 29% (dry weight) in the *Sayali* olive variety (Sakouhi *et al.,* 2008) to 35% (dry weight) in the *Meski* olive variety (Issaoui *et al.,* 2011). Moreover, Turkish olive varieties such as the *Domat* cultivar had low oil content amounting to 23% based on dry weight (Savas and Uylaser, 2013), while *Gemlike* and *Edincik* varieties had high oil content reaching 38 and 50%, based on dry weight, respectively (Borcakli *et al.,* 1993). High oil content (68% of dry pulp) was found in the Italian *Intosso d'Abruzzo* cultivar table olives (Lanza *et al.,* 2010). Accordingly, Nabali Baladi olives can be considered a rich source of oil before and after fermentation compared to other olive varieties in the region.

In this study, it was demonstrated that 100 g of edible fermented green Nabali Baladi olives contained 0.9 g protein, 4.6 g carbohydrate and 3 g of crude fiber. Similar to our results, the Italian green *Intosso d'Abruzzo* fermented olives were found to contain 1 g protein, 2.6 g fiber and 2.8 g carbohydrates in 100 g fermented olives (Lanza *et al.,* 2010). However, it has been documented that the olive fresh fruit's average composition includes water (50%), protein (1.6%), oil (22%), carbohydrate (19.1%) and cellulose (5.8%) (Ghanbari *et al.,* 2012).

Indeed, the analysis of fatty acid profile in Nabali Baladi olive oil revealed that oleic acid is the main fatty acid followed by palmitic acid and linoleic acid (70, 14.3 and 8.3%, respectively). It was found that olive processing and fermentation did not cause any significant changes in the fatty acid profile; instead, the fatty acid profile was dependent on olive varieties (López-López *et al.,* 2015). Previous studies analyzed the fatty acid profile of olive oil extracted from Nabali Baladi olives cultivated in different regions of Jordan and Palestine and the results were highly variable (Humeid *et al.,* 1991; Al-Ismail *et al.,* 2011; Ebiad and Abu-Qaoud, 2014). It was found that oleic acid accounted for 67% of the oil extracted from Nabali Baladi olives collected from Bani Kenaneh in north Jordan, followed by linoleic acid (14%) and palmitic acid (12.5%) (Al-Ismail *et al.,* 2011). Oleic acid represented (66%) of total fatty acids in the oil extracted from Nabali Baladi olives in Palestine, followed by palmitic acid (15%) and linoleic acid (12.8%) (Ebiad and Abu-Qaoud 2014). On another hand, oleic acid represented a higher percentage (73-76%) and lower linoleic acid (8.5-9.7%) and palmitic acid (8.8-11.8%) in oil extracted from Nabali Baladi olives collected from Amman, Jordan (Humeid *et al.,* 1991). As in olive oil, fermented green Nabali Baladi olives are a valuable source of

TABLE 3. Yeast identification, HCl (pH 2.5) and bile salt tolerance, adhesive ability and technical characteristics for the yeast isolates from fermented green Nabali Baladi olives

	Identification by sequencing	Identity (%)	Viability of yeast				Adhesion ability CFU/ml		Technical characteristics	
			HCl (pH2.5) CFU/ml		Bile salt (%)		Time (min)		Lipase	Catalase
			0 h	3 h	0.1	0.3	0	90		
Y1	*Candida diddensiae*	100	$1.07x10^{8}$±$2x10^{7}$	$5.9x10^{7}$±$1.5x10^{6}$	+*	+	$1.6x10^{8}$±$3.1x10^{7}$	$3.5x10^{7}$±$1.1x10^{6}$	-	+
Y2	*Candida naeodendra* strain MB14804	99	$3.25x10^{7}$±$4.1x10^{6}$	$9.49x10^{6}$±$6.1x10^{5}$	+	+	$3.9x10^{8}$±$1.1x10^{7}$	$6.32x10^{7}$±$2.2x10^{6}$	-	+
Y3	*Candida diddensiae*	99	$5.35x10^{7}$±$5x10^{6}$	$2.43x10^{7}$±$3.6x10^{6}$	+	+	$1.35x10^{8}$±$5.2x10^{7}$	$4.34x10^{7}$±$1.6x10^{6}$	-	+
Y4	*Candida diddensiae*	99	$6.25x10^{7}$±$2.5x10^{6}$	$3.2x10^{7}$±$2x10^{6}$	+	+	$2.5x10^{8}$±$6.5x10^{7}$	$7.68x10^{7}$±$9.2x10^{5}$	-	+

*+ No inhibitions were observed compared to the control

Values are given as mean ± SD (Based on three sample replicates for each of the two jars each season i.e. six replicates for each season).

monounsaturated fatty acids (oleic acid), which is considered one of the health benefits of olives and olive oil (Ghanbari *et al.,* 2012).

Fermented olives have a wide range of functional properties besides high oil and oleic acid contents. Olive fruits are considered a rich source of polyphenols and other bioactive compounds (Ghanbari *et al.,* 2012). A clinical study demonstrated that olive intake increases polyphenols and total antioxidant potential in plasma, thus indicating that olive polyphenols have good bioavailability (Kountouri *et al*., 2007). In this study, the total polyphenol content in green Nabali Baladi olives was found to be moderate (300 mg gallic acid equivalent/100 g). Previous studies showed highly variable total polyphenol contents in fermented olives. The Italian green *Intosso d'Abruzzo* fermented olive total polyphenol content was 167 mg caffeic acid equivalent /100 g (Lanza *et al.,* 2010). In Tunisia, green *Meski* fermented olives contained 1170 mg hydroxytyrosol equivalent/100 g (Issaoui *et al.,* 2011). However, the total polyphenol content in fermented green olives has been reported to be dependent on olive variety, time of harvest and treatment method (Othman *et al.,* 2009; Bouaziz *et al*., 2010). As is well known, the debittering step in olive fermentation reduces the polyphenol content, mainly oleuropein, by either dilution or chemical hydrolysis (Issaoui *et al.,* 2011). The total polyphenol content in the green *Chetoui* olive variety was decreased by 58% after spontaneous fermentation for 67 days (Othman *et al.,* 2009). Polyphenol content was increased from 346 to 576 mg gallic acid equivalent/100 g in green *Chetoui* olives during 1 month of tree maturation (Bouaziz *et al*., 2010). Moreover, it was found that olive varieties with small drupe size have a higher quantity of oleuropein (Morello *et al.,* 2005). Green Nabali Baladi olives have a large fruit size and high oil content (Al-Ismail *et al.,* 2011), thus they are expected to have low total polyphenol content compared to other olive varieties.

Upon fermentation, olives became a rich source of beneficial microorganisms including LAB and yeasts (Aponte *et al.,* 2010; Hurtado *et al.,* 2012; Argyri *et al.,* 2013). Different isolates of LAB and yeasts were found to possess probiotic properties *in vitro* (Van der Aa Kuhle *et al.,* 2005, Zivkovic *et al.,* 2015). Traditional fermentation methods vary among countries; the Spanish-style green olives are characterized by treatment with NaOH before the addition of brine (brine concentration usually ranges between 6-8% w/v) and starter culture may be added (Abriouel *et al.,* 2011). Green olive fermentation according to the Sicilian method is characterized by repeated washing of the olives with water as a debittering mechanism and brining in a 6-10% salt solution (Aponte *et al.,* 2010). The Turkish traditional method uses either scratched or cracked green olives which are first placed in water to reduce bitterness and then transferred to the fermentation tanks where the brine-to-salt ratio is increased progressively and reaches 5-6% (Cillidag, 2013). Two famous traditional olive fermentation methods are reported in Tunisia. The first is a simple process and consists of a modest addition of a balanced amount of water and salt to the olive fruits that must be harvested unripe and kept in jars made of clay or glass (Issaoui *et al*., 2011). The second process is the oldest and consists of adding oil and salt to the olive fruits which must be kept in a fabric bag with a large opening. In the latter method, olives ripen faster and lose their color rapidly due to direct contact with air and temperature (Issaoui *et al*., 2011).

In Jordan, the traditional fermentation method is characterized by debittering olive fruit through soaking in water for three days followed by spontaneous fermentation in brine (> 10% w/v of raw salt) under low-temperature conditions (NCARE, 2000). Vegetable fermentation is a natural and spontaneous process that is achieved through the activity of indigenous microbiota, particularly LAB and yeast on the raw material (Bautista-Gallego *et al.,* 2011). It is well documented that LAB is the leading micro-organism in different vegetable fermentations including olives (Aponte *et al.,* 2010, Hurtado *et al.,* 2012). However, olive fermentation could be affected by different factors that would encourage specific microorganisms to dominate the fermentation process such as brine concentration, olive variety, previous alkali treatment and temperature during the fermentation process. Most green olive fermentations are dominated by LAB species (Hurtado *et al.,* 2012). Previous studies revealed that the Spanish-style treated olive fermentation is due to LAB, whereas black olives were fermented primarily by yeasts, and LAB represents a small proportion of the total microflora (Aponte *et al.,* 2010).

Indeed, our results showed that green Nabali Baladi olive fermentation was dominated by yeast throughout the 90 days of fermentation. The identification of isolated yeasts by sequencing of the D1/D2 region of the 26S rDNA region revealed that *Candida diddensiae* and *Candida naeodendra* were the predominant yeasts in the green Nabali Baladi fermented olives. High brine concentrations and low pH values at the start of fermentation would favor yeast growth and inhibit lactic acid bacteria. When brine concentration was maintained above 80g/L, yeast growth dominated the black olive fermentation for 90 days (Bleve *et al.,* 2014). In this study, the brine concentration started at 100 g/L and ended at 86 g/L after 90 days of fermentation. Furthermore, the fermentation of green cracked olives in brine concentrations (90-110 g/L) was dominated by yeast for 6 months of fermentation with little or no lactic acid bacteria growth (Abriouel *et al*., 2011).

Nevertheless, different yeast species were isolated from fermented green and black olives. Green Sicilian olive fermentation was dominated by *Candida parapsilosis*, *Pichia guilliermondii,* and *Pichia kluyveri* (Aponte *et al.,* 2010). The exploration of the biological diversity of yeasts in the mass fermentation activities of green table olives in Spain revealed that *Candida diddensiae*, *Saccharomyces cerevisiae,* and *Pichia membranifaciens* were the most abundant yeast species directly isolated from brined Aloreña olives; whereas for Gordal and Manzanilla cultivars, they were *Candida tropicalis*, *Pichia galeiformis* and *Wickerhamomyces anomalus*. In the case of Gordal and Manzanilla green olives which were prepared according to the Spanish method, the prevailing yeasts were *Debaryomyces etchellsii, C. tropicalis, P. galeiformis,* and *Kluyveromyces lactis* (Bautista-Gallego *et al.,* 2011). *Candida naeodendra* is a species of the *Candida diddensiae* group that has been frequently identified in olive fermentation processes (Bautista-Gallego *et al.,* 2011). Moreover, different strains of *Candida diddensiae* were shown to produce a wide range of lipase, esterase or β- glucosidase activities that qualify them to be used as a potential starter in olive fermentation (Ciafardini and Zullo, 2015).

On the other hand, the probiotic characteristics of the *Candida* species have been scarcely studied. However, strains of yeasts isolated from fermented olives can have some beneficial criteria that may improve the technical and functional properties of fermented olives (Hatoum *et al.,* 2012). Accordingly, *Candida diddensiae* and *Candida naeodendra* were tested for their acid and bile tolerance and adhesion to intestinal cell lines *in vitro*. The results presented in Table 3 demonstrate that the two isolates can tolerate gastric conditions and potentially survive in the gastrointestinal tract. Neither isolate showed antimicrobial activities against a group of pathogenic bacteria. It is possible that yeast isolates have positive immunomodulatory activity as documented previously (Smith *et al.,* 2014). In essence, the isolated yeasts were positive for catalase production. This character is important for olive preservation due to its high content of unsaturated fatty acids; in fact, it is, therefore, possible to effectively prevent oxidation and peroxide formation (Silva *et al.,* 2011).

5. CONCLUSIONS

Fermented green Nabali Baldi olives are a rich source of oleic acid, polyphenols, and dietary fiber. The fermentation of green Nabali Baladi olives according to the traditional fermentation method in Jordan is dominated by yeast strains, primarily *Candida diddensiae,* with no detection of LAB.This yeast strain can tolerate HCL and

bile salt and has a medium ability of adhesion to intestinal cell lines. Thus, *C. diddensiae* isolated from fermented green Nabali Baladi olives may represent a good probiotic candidate. In this respect, further *in vitro* and *in vivo* studies are required.

ACKNOWLEDGMENTS

This study was supported by the Deanship of Scientific Research, The University of Jordan.

REFERENCES

Association of the Official Analytical Chemists (AOAC). 1995. Official Methods of Analysis. Arlington, Virginia, USA.

Abriouel H, Benomar N, Lucas R ,Galvez A. 2011. Culture-independent study of the diversity of microbial populations in brines during fermentation of naturally-fermented Aloreña green tableolives. *Int. J. Food Microbiol.* **144**, 487-96. https://doi.org/10.1016/j.ijfoodmicro.2010.11.006

Al-Ismail KM, Ahmad R, Al-Dabbas M, Ajo RY, Rababah T. 2011. Some physiochemical properties of olive and olive oil of three jordanian olive varieties. *Riv. Ital. Sostanze Grasse* **88**, 191-198.

Aponte M, Ventorino V, Blaiotta G, Volpe G, Farina V, Avellone G, Lanza CM, Moschetti G. 2010. Study of green Sicilian table olive fermentations through microbiological, chemical and sensory analyses. *Food Microbiol.* **27**, 162-70. https://doi.org/10.1016/j.fm.2009.09.010

Argyri AA, Zoumpopoulou G, Karatzas KA, Tsakalidou E, Nychas GJ, Panagou EZ, Tassou CC. 2013. Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. *Food Microbiol.* **33**, 282-291. https://doi.org/10.1016/j.fm.2012.10.005

Bautista-Gallego J, Rodriguez-Gomez F, Barrio E, Querol A, Garrido-Fernandez A, Arroyo-Lopez FN. 2011. Exploring the yeast biodiversity of green table olive industrial fermentations for technological applications. *Int. J. Food Microbiol.* **147**, 89-96. https://doi.org/10.1016/j.ijfoodmicro.2011.03.013

BLAST: Basic Local Alignment Search Tool. Last retrieved, September 2019. https://blast.ncbi.nlm.nih.gov/Blast.cgi

Bleve G, Tufariello M, Durante M, Perbellini E, Ramires FA, Grieco F, Cappello MS, De Domenico S, Mita G, Tasioula-Margari M, Logrieco AF. 2014. Physico-chemical and microbiological characterization of spontaneous fermentation of Cellina di Nardò and Leccino table olives. *Front. Microbiol.* **5**, 570. https://doi.org/10.3389/fmicb.2014.00570

Borcakli M, Özay G, Alperden I, Özsan E, Erdek Y. 1993. Changes in chemical and microbiological composition of two varieties of olive during fermentation. *Grasas Aceites* **44**, 253-258. https://doi.org/10.3989/gya.1993.v44.i4-5.1075

Bouaziz M, Jemai H, Khabou W, Sayadi S. 2010. Oil content, phenolic profiling and antioxidant potential of Tunisian olive drupes. *J. Sci. Food Agric.* **90**, 1750-1758. https://doi.org/10.1002/jsfa.4013

Ciafardini G, Zullo BA. 2015. Effect of lipolytic activity of *Candida adriatica*, *Candida diddensiae* and *Yamadazyma terventina* on the acidity of extra-virgin olive oil with a different polyphenol and water content. *Food Microbiol.* **47**, 12-20. https://doi.org/10.1016/j.fm.2014.10.010

Cillidag S. 2013. Table olive processing technologies. Options Méditerranéennes. Séries A: Mediterranean Seminars. CIHEAM-IAMZ; IOC, International Olive Council.

Ebiad R,Abu-Qaoud H. 2014. Morphological and biochemical characterization of three olive" *Oleaeuropaea* L." cultivars in Palestine. *Jordan J. Agric. Sci.* **10**, 130-143.

Food and Drug Adiminstration. Bacteriological Analytical Manual on line. 2001. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

Ghanbari R, Anwar F, Alkharfy KM, Gilani AH, Saari N. 2012. Valuable nutrients and functional bioactives in different parts of olive (*Olea europaea* L.)-A review. *Int. J. Mol. Sci.* **13**, 3291-340. https://doi.org/10.3390/ijms13033291

Hatoum R, Labrie S, Fliss I. 2012. Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. *Front. Microbiol.* **3**, 421. https://doi.org/10.3389/fmicb.2012.00421

Humeid MA, Takruri HR, Daqqaq RF. 1991. Nabali olive oil ripening and oil properties. *Nutr. Health.* **7**, 151-154. PMID:1923072

Hurtado A, Reguant C, Bordons A, Rozes N. 2012. Lactic acid bacteria from fermented table olives. *Food Microbiol.* **31**, 1-8. https://doi.org/10.1016/j.fm.2012.01.006

Issaoui M, Dabbou S, Mechri B, Nakbi A, Chehab H, Hammami M. 2011. Fatty acid profile, sugar composition, and antioxidant compounds of table olives as affected by different treatments. *Eur. Food Res.Tech.* **232**, 867-876. https://doi.org/10.1007/s00217-011-1455-3

Kountouri A, Mylona A, Kaliora A, Andrikopoulos N. 2007. Bioavailability of the phenolic compounds of the fruits (drupes) of *Olea europaea* (olives): impact on plasma antioxidant status in humans. *Phytomedicine* **14**, 659-667. https://doi.org/10.1016/j.phymed.2007.06.001

Kurtzman CP, Fell JW, Boekhout T, Robert V. 2011. Methods for isolation, phenotypic characterization and maintenance of yeasts. *The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam* **,** 87-110.

Lanza B, Di Serio MG, Iannucci E, Russi F, Marfisi P. 2010. Nutritional, textural and sensorial characterisation of Italian table olives (*Olea europaea* L. cv. 'Intosso d'Abruzzo'). *Int. J. Food Sci. Tech.* **45**, 67-74. https://doi.org/10.1111/j.1365-2621.2009.02104.x

López A, Garrido A, Montaño A. 2007. Proteins and aminoacids in table olives: relationship to processing and commercial presentation. *It. J. Food Sci.* **19**, 217-228.

López A, García P, Garrido A. 2008. Multivariate characterization of table olives according to their mineral nutrient composition. *Food Chem.* **106**, 369-378.

Lopez-Lopez A, Cortes-Delgado A, Garrido-Fernandez A. 2015. Effect of green Spanish-style processing (Manzanilla and Hojiblanca) on the quality parameters and fatty acid and triacylglycerol compositions of olive fat. *Food Chem.* **188**, 37-45. https://doi.org/10.1016/j.foodchem.2015.04.080

Ministry of Agriculture (MOA). Agricultural Statistical Yearbook. 2016. Amman, Jordan.

Morelló JR, Vuorela S, Romero MP, Motilva MJ, Heinonen M. 2005. Antioxidant activity of olive pulp and olive oil phenolic compounds of the Arbequina cultivar. *J. Agric. Food Chem.* **53**, 2002-2008. https://doi.org/10.1021/jf048386a

National Center of Agricultural Research and Education (NCARE). 2000. Pickled Green Olive Production. Publication of NCARE no **62**. Amman, Jordan.

Othman NB, Roblain D, Chammen N, Thonart P, Hamdi M. 2009. Antioxidant phenolic compounds loss during the fermentation of Chétoui olives. *Food Chem.* **116**, 662-669. https://doi.org/10.1016/j.foodchem.2009.02.084

Pino A, De Angelis M, Todaro A, Van Hoorde K, Randazzo CL, Caggia C. 2018. Fermentation of Nocellara Etnea table olives by functional starter cultures at different low salt concentrations. *Front. Microbiol.* **9**. https://doi.org/10.3389/fmicb.2018.0112

Saibandith B, Spencer JPE, Rowland IR, Commane DM. 2017. Olive polyphenols and the metabolic syndrome. *Molecules* **22**, E1082. https://doi.org/10.3390/molecules22071082

Sakouhi F, Harrabi S, Absalon C, Sbei K, Boukhchina S, Kallel H. 2008. α-Tocopherol and fatty acids contents of some Tunisian table olives (*Olea europea* L.): changes in their composition during ripening and processing. *Food Chem.* **108**, 833-839. https://doi.org/10.1016/j.foodchem.2007.11.043

Savas E, Uylaser V. 2013. Quality improvement of green table olive cv.'domat' (*Olea europaea* L.) grown in Turkey using different de-bittering methods. *Notulae. Botanicae Horti. Agrobotanici. Cluj-Napoca.* **41**, 269

Silva T, Reto M, Sol M, Peito A, Peres CM, Peres C, Malcata FX. 2011. Characterization of yeasts from Portuguese brined olives, with a focus on their potentially probiotic behavior. *LWT-Food Sci. Tech.* **44**, 1349-1354. https://doi.org/10.1016/j.lwt.2011.01.029

Smith IM, Christensen JE, Arneborg N, Jespersen L. 2014. Yeast modulation of human dendritic cell cytokine secretion: an in vitro study. *PloS One* **9**, e96595. https://doi.org/10.1371/journal.pone.0096595

van der Aa Kuhle A, Skovgaard K, Jespersen L. 2005. In vitro screening of probiotic properties of *Saccharomyces cerevisiae* var. *boulardii* and food-borne *Saccharomyces cerevisiae* strains. *Int. J. Food Microbiol.* **101**, 29-39. https://doi.org/10.1016/j.ijfoodmicro.2004.10.039

Wang SY, Chen HC, Liu JR, Lin YC, Chen MJ. 2008. Identification of yeasts and evaluation of their distribution in Taiwanese Kefir and Viili starters. *J. Dairy Sci.* **91**, 3798-805. https://doi.org/10.3168/jds.2007-0468

Zivkovic M, Cadez N, Uroic K, Miljkovic M, Tolinacki M, Dousova P, Kos B, Suskovic J, Raspor P, Topisirovic L, Golic N. 2015. Evaluation of probiotic potential of yeasts isolated from traditional cheeses manufactured in Serbia and Croatia. *J. Intercult. Ethnopharmacol.* **4**, 12-8.