SHORT COMMUNICATION

Identification of Some Yeasts by Fatty Acid Profiles

BASSAM ALOKLAH[1*], ANWAR ALHAJALI[2] and SABAH YAZIJI[2]

[1] Department of Industrial and Food Technique, National Commission for Biotechnology, Syria
[2] Department of Food Science, Faculty of Agriculture, Damascus University, Syria

A b s t r a c t

178 yeasts isolated from various foods were identified using ID32 C strip; *Saccharomyces cerevisiae* (47), *Rhodotorula mucilaginosa* (19), *Candida kefyr* (33), *Candida krusei* (37), *Candida lusitaniae* (28), *Cndida lipolytica* (14). The yeasts were harvested, saponified, derivatized, and extracted, and fatty acid analysis was performed by Gas Chromatography Mass Spectrometry. The yeast lipids contained Decanoic acid (C10:0), Luaric acid (C12:0), Myristoleic acid (C14:1), Myristic acid (C14:0), Pentadecanoic acid (C15:0), Palmitoleic acid C16:1), Pamitic acid (C16:0), Heptadecenoic acid (C17:1), Linoleic acid (C18:2), Linolenic acid (C18:3), Oleic acid (C18:1) and Stearic acid (C 18:0). The differences in the composition of these fatty acids could be used to identify the species as an accurate, efficient and relatively rapid method.

K e y   w o r d s: fatty acids, gas chromatography-mass spectrometer (GC-MS), identification, yeast

Yeasts are defined as microscopic unicellular fungi, which reproduce asexually by budding or fission (Suh *et al.*, 2006). There are approximately 100 genera and 800 described species of yeasts (Kurtzman and Fell, 1998). Yeasts are known for the contamination and spoilage of foods and dairy products (Mushtaq *et al.*, 2006). They have been used by the food industry principally for the production of ethanol and carbon dioxide, which are important to the brewing, wine distilling and baking industries, Yeasts are rich in proteins, lipids and vitamins (Kutty and Philip, 2008). Over the years, morphological, biochemical and physiological characteristics have been used to identify yeasts (Barnett *et al.*, 1990). This conventional methodology requires the evaluation of some 60 to 90 tests, resulting in a complex (Arias *et al.*, 2002). These systems are however laborious and at times give ambiguous results (Sebolai, 2004). Identification of yeast strains is routinely performed by time-consuming commercially available systems such as the ID 32 C system, Vitek Biochemical Card, BBL Chromagar API 20C and the Minitek system (El Menyawi *et al.*, 2000) (Maquelin *et al.*, 2002). Alternatively phenotypic methods and genotypic methods developed for more accurate microbial identification PCR based methods or DNA sequencing are usually time-consuming and involve extensive sample preparation. Therefore, considerable efforts have focused on the invention of new techniques that can provide rapid, sensitive and informative characterization of microorganisms (Li *et al.*, 2011).

Fatty acid methyl esters (FAME) analysis using gas-liquid chromatography has been successfully applied to the identification of yeast species (Botha *et al.*, 1993). Fatty acids (FAs) are a source of cellular energy and also serve as building blocks for a number of complex membrane lipids. Both fatty acids and sterols have the capacity to act as signaling molecules and to affect gene expression via upstream promoter elements (Garbarino and Sturley, 2005).

The spectrum of fatty acids in yeasts is rather simple, consisting mostly of C16 and C18 fatty acids. Cellular fatty acids (CFAs) are derived from three different sources: external supply, endogenous lipid (protein) turnover, and de novo synthesis and elongation (Tehlivets *et al.*, 2007). Primary fatty acids, ranging from C14 to C20, have been detected in yeasts and have been used in the classification and identification of these organisms (Smitt *et al.*, 1988). The most abundant FA species in yeast cells are Palmitoleic acid (16:1) and Oleic acid (18:1) followed by Palmitic acid (16:0), Stearic acid (18:0), with minor amounts of Myrictic acid (14:0) are also found in yeast (Tuller *et al.*, 1999) (Chumnanpuen, 2012).

Kock *et al.* (1985) have published a number of papers dealing with the CFA of a large number of yeast species. A rigorously standardized experimental technique

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  * Corresponding author: B. Aloklah, Department of Industrial and Food Technique, National Commission for Biotechnology, Syria;
e-mail: bassam78alk@gmail.com

makes comparison of data for different genera and species possible. Various studies showed that fatty acid compositions were useful in distinguishing between species within a genus as seen in *Candida*, *Kluyveromyces*, *Pichia*, *Saccharomyces* and *Schwanniomyces* (Cottrell and Kock, 1989; Van der Walt, 1992; Brondz *et al.*, 1989), (Brondz and Olsen, 1990; Marumo and Aoki, 1990; Moss *et al.*, 1982). Van der Westhuizen (1993) could distinguish between other yeast genera of clinical importance namely *Cryptococcus*, *Malassezia*, *Rhodotorula* and *Trichosporon* which are all characterized by the presence of C:18:2 and C18:3 (ω 3) FAs as opposed to *Geotrichum* and *Kloeckera* which only have C:18:2 but not C:18:3 (ω 3). *Candida glabrata* in turn, produces neither C:18:2 nor C:18:3 FAs but only C:18:1 FA (Van der Westhuizen, 1993).

Gangopadhyay *et al.,* (1979) studied the cellular long-chain fatty acid contents of the anamorphic genera *Candida*, *Cryptococcus* and *Torulopsis* and obtained significant results which allowed them to differentiate between 38 isolates. Gunasekaran and Hughes (1980) examined 85 strains of Candida representing seven species. They found each species produced a distinctive fatty acid fingerprint, characterized by the presence or absence of certain fatty acids.

The use of long chain FA profiles for yeast identification is well reported (Botha and Kock 1993). Augustyn *et al.* (1991) demonstrated that minor fatty acids were useful for discrimination of 46 of the 50 *Saccharomyces cerevisiae* strains studied. In addition, they found that it was also possible to separate *S. cerevisiae* according to the range of its CFA profiles. CFA profiles discriminated between various yeasts associated with wine spoilage (Malfeito-Ferreira *et al.*, 1989). CFA analysis alone clearly distinguished *Candida albicans* from *C. galabrata*, *C. holmii*, *C. parapsilosis*, *Cryptococcus albidus*, *Exophiala Jeanselmei*, *Lecythophara multabilis and S. cerevisiae* (Prillinger *et al.*, 2011). Kock *et al.* (1986) recommended estimation of fatty acid profiles as a reliable and rapid alternative to laboratory tests for distinguishing between species of *Saccharomyces* cultured under standard conditions. The aim of the present

research is to evaluate the CFA profiles of yeast isolates obtained from various Syrian foods, using GC-MS as a rapid methodology to identify yeasts compared with the ID 32 C system.

In recent years, several identification methods have been proposed as alternative ways to compare some classical yeast identification techniques. Among these methods were commercial miniaturized systems such as, ID32 C system, bioMérieux (Arias *et al.*, 2002) which is a very effective way to identify yeast. A total number of 178 yeast strains were obtained from various food samples (sausage, yogurt, cheeses, jams, honey and juices) collected from various Syrian markets, especially from Damascus. In this study, isolates of yeasts were obtained from different foods (raw sausage, traditional prepared yogurt, white brined cheeses, canned jams, honey and pasteurized juices). Table I illustrates the total number of isolates. *S. cerevisiae* was predominant in jam, honey and juice whereas *R. mucilaginosa*, *C. kefyr*, *C. krusei and C. lusitaniae* were predominant in yogurt and cheese, but *C. lipolytica* was predominant in sausage. These results were in agreement with Arias *et al.* (2002) who studied yeasts associated with orange juice and with Mushtaq, *et al.* (2006) in detection of yeast micro-flora from milk and yogurt in Pakistan.

The yeasts were grown on Malt Extract Agar (MEA) medium consisting. The isolates were incubated on (MEA) at 30°C for 24 h, and identified using ID 32 C system (bioMérieux, Marcy-l'Etoile, France) which consists of 31 assimilation tests (Table II) according to manufacturer's instructions. The yeast isolates which were identified by ID 32 C system were *S. cerevisiae*, *R. mucilaginosa*, *C. kefyr*, *C. krusei*, *C. lusitaniae* and *C. lipolytica,* the results showed that a combination of tests (CEL, 2KG, MDG, RHA, SBE, PLE, GLN) are positive in *C. lusitaniae*, (ARA, RIB, ESC) are positive in *R. mucilaginosa*, LAC is positive in *C. kefyr*, and ERY is positive in *C. lipolytica*.

The isolates used for FAMEs were grown on MEA at 30°C for 24 h, 0.4 g of wet cells was harvested and transferred into a 20 mL glass screw-cap test tube. The

Table I
Yeast species isolated from different foods

Food	*S. cerevisiae*	*R. mucilaginosa*	*C. kefyr*	*C. krusei*	*C. lusitaniae*	*C. lipolytica*
Sausage	7	3	4	6	5	9
Yogurt	9	6	16	13	9	4
Cheese	6	5	10	11	8	1
Jam	14	1	1	–	1	–
Honey	3	–	–	–	–	–
Juice	8	4	2	7	5	–
Total	**47**	**19**	**33**	**37**	**28**	**14**

Table II
Physiological diagnostic results by ID 32 C system

Test	S. cerevisiae	R. mucilaginosa	C. kefyr	C. krusei	C. lusitaniae	C. lipolytica
GAL	–	+	+	–	+	–
ACT	–	–	+	+	–	+
SAC	+	+	+	–	+	–
NAG	–	+	–	+	+	+
LAT	–	–	+	+	–	+
ARA	–	+	–	–	–	–
CEL	–	–	–	–	+	–
RAF	+	+	+	–	–	–
MAL	+	+	–	–	+	–
TRE	–	+	–	–	+	–
2KG	–	–	–	–	+	–
MDG	–	–	–	–	+	–
MAN	–	–	–	–	+	+
LAC	–	–	+	–	–	–
INO	–	–	–	–	–	–
SOR	–	–	+	–	+	–
XYL	–	+	+	–	–	–
RIB	–	+	–	–	–	–
GLY	–	+	–	–	–	+
RHA	–	–	–	–	+	–
PLE	–	–	–	–	+	–
ERY	–	–	–	–	–	+
MEL	–	–	–	–	–	–
GRT	–	–	–	–	–	–
MLZ	–	–	–	–	+	+
GNT	–	–	–	–	–	–
LVT	–	–	–	–	–	–
GLU	+	+	+	+	+	+
SBE	–	–	–	–	+	–
GLN	–	–	–	–	+	–
ESC	–	+	–	–	–	–

suspension was boiled for at C in a water bath (Memmert, Germany) to extract FA from cellular lipids and saponify them to sodium salts. The FAMEs derivatives were prepared by adding 6N HCl solution in methanol and heating at 80°C. FAMEs were extracted from the aqueous phase with a hexane-methyl-tert-butyl ether reagent. A diluted solution of NaOH in water was used to remove residual reagents from the organic extracts, The upper solvent phase was then transferred into 2 ml glass vials (Sasser, 2001). FAMEs analysis was performed on a GC-MS (Agilent technology 7890A, USA) with a capillary column DB-1 (30 m × 0.25 mm × 0.25 μm). The temperature was programmed from 125°C (held for 2 min) to 150°C at a rate of 5°C/min, then from 150°C to 250°C at a rate of 4°C/min. The carrier gas was helium with a split ratio of 1: 50. FAMEs (C12:0,

C14:0, C15:0, C16:1, C16:0, C18:1(9), C18:1(11), C18:2, C18:0) were identified by the standard solution standard Supelco-47080-U (Sigma-Aldrich inc, Germany) and the FAMEs (C10:0, C14:1, C17:1, C18:3) were compared with NIST library in MS (Agilent 5975A, USA), with the following settings; ionization voltage 70 eV, quadrupole temperature 150°C, ion source temperature 230°C, scan mass range 15–450 amu.

The procedure for the determination of fatty acids by GC-MS consists of several steps such as esterification of lipids, sample injection, separation, identification and quantification (Eder, 1995). The accuracy of total fatty acids quantification is generally influenced by a number of experimental factors. Esterification of lipids is the most critical step among those. Acid-catalyzed transesterification is the most common used method

Table III
Average percentage fatty acid composition of isolated yeasts

Fatty acids	S. cerevisiae	R. mucilaginosa	C. kefyr	C. krusei	C. lusitaniae	C. lipolytica
C10:0	6.15 ± 1.18	–	0.06 ± 0.01	0.03 ± 0.02	–	–
C12:0	7.59 ± 1.35	–	0.22 ± 0.02	0.04 ± 0.01	0.28 ± 0.02	–
C14:1	0.98 ± 0.04	–	0.24 ± 0.05	–	–	–
C14:0	1.90 ± 0.05	1.83 ± 0.11	2.05 ± 0.13	0.44 ± 0.06	1.06 ± 0.04	–
C15:0	–	–	0.25 ± 0.06	0.79 ± 0.04	–	0.87 ± 0.11
C16:1	51.21 ± 2.25	5.69 ± 0.38	27.46 ± 2.48	17.69 ± 1.67	10.21 ± 1.05	17.22 ± 1.12
C16:0	12.72 ± 1.45	17.56 ± 1.88	20.06 ± 1.55	15.26 ± 1.55	18.41 ± 2.13	11.99 ± 2.23
C17:1	–	0.40 ± 0.06	0.08 ± 0.01	1.65 ± 0.18	–	2.71 ± 0.43
C18:2	–	11.85 ± 1.75	19.41 ± 2.13	22.21 ± 2.14	33.15 ± 2.17	23.01 ± 2.15
C18:3	–	4.60 ± 0.98	4.01 ± 0.66	13.61 ± 1.78	9.30 ± 1.44	–
C18:1(9)	18.50 ± 1.33	55.89 ± 2.55	24.61 ± 2.38	25.51 ± 2.15	25.66 ± 2.33	42.85 ± 3.65
C18:1(11)	–	–	0.40 ± 0.02	1.94 ± 0.11	0.54 ± 0.04	0.58 ± 0.04
C18:0	0.95 ± 0.02	2.18 ± 0.07	1.15 ± 0.04	0.83 ± 0.03	1.39 ± 0.06	0.77 ± 0.02
Total %	100	100	100	100	100	100
SFA	29.32	21.57	23.79	17.39	21.14	13.63
MUFA	70.69	61.98	52.79	46.79	36.41	63.36
PUFA	–	16.45	23.42	35.82	42.45	23.01
Total %	**100**	**100**	**100**	**100**	**100**	**100**

for preparation of FAMEs, which is found in various applications (Khoomrung *et al.*, 2008; Glaser *et al.*, 2010)

FAME analysis using gas-liquid chromatography is a method widely available in microbiological laboratories and which has successfully been applied to the identification of clinically important yeast species (Kutty and Pilip, 2008).

It has been reported that long chain FAs of C16:0 and C18:0 chain lengths predominate in yeasts and include Palmitic (16:0), Palmitoleic (C16:1), Stearic (C18:0), Oleic (C18:1), Linoleic (C:8:2), Linolenic acids (C18:3)) Prillinger *et al.*, 2011)

Various studies have shown that some variations in the mean relative percentages of FAs present in the cellular material from different strains within the same species can occur (Kock and Botha, 1998). Thus, to obtain a representative FA profile of a particular yeast species, as many representative strains as possible must be examined.

Within genera, long-chain FA composition seems to be of more value. For instance, within *Kluyveromyces*, *Lipomyces*, *Nadsonia*, *Rhodosporium*, *Saccharomyces* and *Schizosacchaomyces*. It was possible to distinguish between a selection of species using FA composition (Prillinger *et al.*, 2011). In the present study FAMEs were analyzed by means of GC-MS. The results revealed the existence of thirteen fatty acids in the yeasts species in this study. Table III illustrates the average FA composition of isolated yeasts in hundred percent. These fatty acids consisted of C10:0, C12:0, C14:1, C14:0, C15:0,

C16:1, C16:0, C17:1, C18:2, C18:3, C18:1(9), C18:1(11) and C18:0. The FA of yeasts species varied in composition and in quantity between them in this study.

The six species of yeasts linking at Euclidian Distance could be separated into five groups as shown in the dendogram cluster analysis based on fatty acid profiles (Fig. 1).

Saccharomyces cerevisiae had the highest content of C10:0 and C12:0 (6.15 and 7.59%, respectively), for this reason it had the highest content of SFAs (29.23%), and so the content of C16:1 (51.21%), which increased the MUFAs content (70.68%); while the PUFAs such as C18:2 and C18:3 were not detected and seem to be absent. These results were in agreement with Avery *et al.* (1996), who noticed that the content of C16:1 were 55.7% and Torijia *et al.* (2003) who found that the content of medium-chain FAs of C10:0 and C12:0 were 15.61%. The absence of PUFAs was also reported by Frerreira *et al.* (2004) and Rozez *et al.* (1992) in their studies on strains of *S. cerevisiae*.

Cis-9-octadecenoic [C18:1(9)] was The predominant FA in *R. mucilaginosa* (55.89%) when compared with other species, while C10:0, C12:0, C14:1, C15:0, and C18:1(11) were not detected. These results corresponded with those recorded by Schneider *et al.* (2013), which revealed the predominance of Palmitic (16:0) and Oleic (18:1) acids in this species.

C. kefyr contained all thirteen FAs from C10:0 to C18:0. The predominant FAs were C16:1, C18:1(9), C16:0, C18:2 (27.46%, 24.61%, 20.06% and 19.41%,
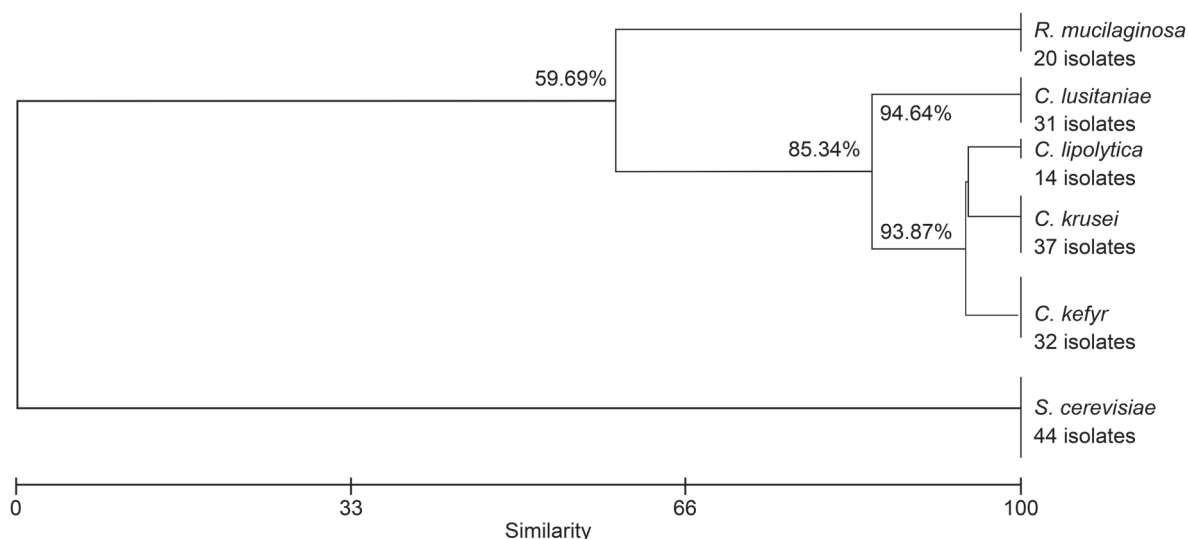
Fig. 1. Dendrogram cluster analysis based on fatty acids of yeasts

respectively). The percent of C16:1 corresponded with that found by Viljone *et al.* (1987) who reported that C16:1 was 27.9%. Moreover, the SFAs and PUSFAs were almost the same (23.79% and 23.42%, respectively); this feature seems to be characterized more in this species compared with the others.

The major FAs in *C. krusei* were C18:1(9), C18:2, C16:1, C16:0 and C18:3 (25.51%, 22.21%, 17.69%, 15.26% and 13.61%, respectively), with the highest amount of C18:3 when compared with the other yeast species in this study. This content was much higher than the value recorded by Blignaut *et al.* (1996) who found that the content of this FA was ranged between 5.1 and 6.3%.

C. lusitiniae showed that it contained nine fatty acids with the absent of C10:0, C14:1, C15:0 and C17:1. This species was recognized by the highest content of C18:1(9) (33.15%), that interoperates the high amount of total PUFAs (42.45%).

The predominant FA in *C. lipolytica* was C18:1(9) (42.85%).This result was in agreement with Viljone *et al.*, (1987) who reported that the content C18:1(9) was the dominating FA in this species with o 55.89% percent. Apart from C18:1(9), the major FAs were C18:2, C16:1 and C16:0 (23.01%, 17.22% and 11.99%, respectively). Both *C. lipolytica* and *S. cerevisiae* can be recognized by the absence of C18:3.

In summary, fatty acids were extracted from whole yeast cells of *S. cerevisiae*, *R. mucilaginosa*, *C. kefyr*, *C. krusei*, *C. lusitaniae* and *C. lipolytica* isolated from a variety of foods (sausage, yogurt, cheeses, jams, honey and juices) and identified by ID 32 C system and using GC-MS. The results of thde ID32 C system showed close agreement with fatty acids results.

The FAMEs were identified and quantified by using GC-MS, which is a rapid and sensitive procedure to distinguish between different species of yeasts, while the ID32 C system was accurate but expensive, labor-intensive and time-consuming.

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