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Low-temperature thermal drying of *Saccharomyces cerevisiae* starter culture for food production

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Abstract

The production of low-temperature thermally-dried cells of *Saccharomyces cerevisiae* at 32 °C is examined in the present investigation. Cells are obtained in a thin layer after heating for 4 h in an incubator. This thermally-dried starter culture showed acceptable fermentation efficiency, proved by repeated batch fermentations of glucose. The starter culture, when compared with freeze-dried cells, showed better fermentation ability. Storage of thermally-dried *S. cerevisiae* for one month showed resistance to loss of fermentation efficiency. The chemical composition of volatiles produced during fermentation were similar to those obtained with freeze-dried and wet starter culture fermentations. The thermally-dried cells effectively ferment at low temperatures.

Keywords: Yeast; Thermal drying; Glucose; Fermentation; *Saccharomyces cerevisiae*; Volatiles; GC–MS

1. Introduction

Drying methods in general are nowadays well developed and widely used in the food industry. However, thermal processes have not been applied to produce dried microbial starter cultures in bulk quantities, due to problems of reduced cell viability and starter culture efficiencies. Starter cultures and dried baker's yeast *Saccharomyces cerevisiae* are produced by high cost industrial freeze drying processes. In recent years researchers have focused on much cheaper thermal drying of starter cultures. The spray drying of microorganisms, has been recently reported with relatively low viability, even for mesophilic lactic acid bacteria (Corcoran, Ross, Fitzgerald, & Stanton, 2004; Desmond, Ross, O'Callaghan, Fitzgerald, & Stanton, 2002; Gardiner et al., 2000; Silva, Carvalho, Teixeira, & Gibbs, 2002). Moisture levels, drying rates and yeast cells' viabilities were determined by Bayrock and Ingledew (1997a, 1997b), using a fluidised bed dryer, to produce a dry baker's yeast product. They found that the viabilities of pressed yeast were not affected by the drying temperature, when moisture contents were higher than 15%. Furthermore, the size of granules plays an important role in the time that is required for drying and in energy that is consumed. Therefore, thermal drying of granules was studied by Turker, Kanarva, Yuzgec, Kapucu, and Senalp (2006). They developed a drying model for production-scale fluid bed drying of granular baker's yeast and indicated that diffusive transport of moisture inside particles plays an important role in the overall drying performance of the granules. This is the reason that in our drying method the sample was applied as a thin layer. Furthermore, moisture levels lower than 5–8% in active dry yeasts are not seen, since irreversible damage to metabolic functions occurs, due to the removal of chemically bound water (Koga, Echigo, & Nunomura, 1966).

A cost-effective method for producing dry baker's yeast is necessary; this could involve drying under mild conditions, to avoid overheating of cell biomass, which could cause alteration and reduction of fermentation activity. Applications of relatively high temperatures were applied for convective drying of the thermo-tolerant yeast *Kluyveromyces marxianus* (Papapostolou, Bosnea, Kanellaki, & Koutinas, 2007). However, such high temperatures cannot

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be employed to dry heat-sensitive baker's yeast *S. cerevisiae*. The aim of this investigation was to produce fermentative low-temperature thermally-dried baker's yeast *S. cerevisiae,* as a starter culture for food production.

2. Materials and methods

2.1. Yeast strain and fermentation medium

A strain of commercial baker's yeast *Saccharomyces cerevisiae* produced by ZANAE (Sindos-Thessaloniki, Greece) was used in the present study for all experiments. Fermentations were performed in synthetic media, containing various concentrations of glucose in the range 10–30%, $(NH_4)_2$ SO_4 (0.1%), KH_2PO_4 (0.1%), $MgSO_4 \cdot 7$ H_2O (0.5%) and yeast extract (0.4%). Agar (2% w/v) was added to the aforementioned complete medium, containing 2% glucose in de-ionised water, to prepare solid culture. Fermentation liquid medium had a pH 5.5–5.7 and it was sterilised in an autoclave at 130 °C and 1.5–2 bar.

2.2. Thermal drying of biomass

Pressed baker's yeast (20 g) was placed in a thin layer on glass plate and drying was performed by heating in a chamber at 32 °C. Kinetics of drying were performed by weighing the glass plate at various time intervals and the thermal drying was continued until the weight of samples stabilised. The percentage of weight lost was always 62%. The dried baker's yeast was stored in the refrigerator. To compare the fermentation efficiency of this thermally-dried baker's yeast product with that produced by freeze drying, a freeze-dried baker's yeast product was produced from the same biomass (Bekatorou, Kaliafas, Koutinas, & Kanellaki, 2001) using a Labconco Freeze Dry System/Freezone 4.5 (Labconco Corporation, Kansa City, MO).

2.3. Repeated batch fermentations using thermally-dried biomass

After drying of baker's yeast *S. cerevisiae*, fermentations of synthetic media containing glucose were performed, in order to study the effect of temperature and also the effect of initial glucose concentration. To study the effect of temperature, experiments were carried out in Erlemeyer flasks of 1 l each, contained 500 ml of synthetic liquid medium with 12% w/v glucose. In each flask were added 3.8 g dried *S. cerevisiae* and three replicate flasks incubated separately at 30, 25, 20, 15, 8 °C and allowed to ferment. Repeated batch fermentations were performed at each temperature with the same cell concentration. Cells were obtained after centrifugation of fermented liquids at the end of each fermentation and inoculation of cell biomass in the next fermentation broth.

To compare the low-temperature thermally-dried starter culture with the commercial pressed baker's yeast of wet cells, fermentations were performed in parallel at 15, 10 and 5 °C, with the fermented liquids to contain equal biomass concentration with fermentations containing thermally-dried cells. Using thermally-dried *S.cerevisiae,* to study the effect of initial glucose concentration, repeated batch fermentations were carried out at 30 °C and initial glucose concentrations of 14%, 18%, 22% and 28%.

Kinetics were performed by measuring the ^{0}Be density at various time intervals. Samples of fermented liquids were analysed for ethanol and residual sugar concentrations. The centrifuged biomass was weighed and an appropriate amount of it was inoculated in the next fermentation broth.

2.4. Comparative study on fermentations

To compare low-temperature thermally-dried *S. cerevisiae* with wet cells and freeze-dried cells and with those low-temperature thermally-dried cells stored for 1 week and 1 month, fermentations were carried out in parallel using each type of cell. All fermentations were performed at 30 °C, using glucose synthetic medium of 22%.

2.5. Analytical assays

Ethanol and residual sugar were determined by high performance liquid chromatography, using a Shimadzu chromatograph with an SCR-101N stainless steel column, an LC-9A pump, a CTO-10A oven at 60 °C and an RID-6A refractive index detector (Shimadzu, Kyoto, Japan). Three times distilled water was used as mobile phase with a flow rate of 0.8 ml/min and 1-pentanol was used as internal standard. Samples of 0.5 ml fermented liquid and 2.5 ml of a 1% (v/v) solution of 1-pentanol was diluted, and 50 and 40 μl were injected directed onto the column. Ethanol and residual sugar concentrations were calculated using standard curves and expressed as %v/v for ethanol and grams of residual sugar per litre.

2.6. Analysis of volatiles by GC–MS

Headspace analysis of volatiles in samples after glucose fermentations were carried out by GC–MS analysis. A solid-phase microextraction (SPME) sampling method was used, employing a DVB/CAR/PDMS fibre (Supelco, Bellefonte, PA) for adsorption of volatiles. A liquid sample of 10 ml and 2.2 g NaCl were transferred into a 20 ml glass vial, sealed with a rubber septum. The contents were magnetically stirred for 5 min at 50 °C, and the fibre was exposed to the headspace for 45 min at 55 °C. Desorption of volatiles took place in the injector of the gas chromatograph in splitless mode, at 240 °C for 5 min. Before each analysis, the fibre was conditioned in the injection port for 10 min, to remove any volatile contaminants. Separation of volatiles was performed on a Shimadzu GC-17A gas chromatograph (SUPELCO WAX-10 column), connected with a GCMS-QP505A Mass Spectrometer (70 eV ionisation energy; 29–400 m/z mass range). A Supelco-

wax-10 column was used with helium as carrier gas (2 ml/min). Oven temperature was programmed at 35 °C for 5 min, and then it was raised to 60, 200, and 250 °C with rates of 2.0, 5.0 and 25.0 °C/min, respectively. Identification was carried out by comparison of retention times and MS data with those of standard compounds and data obtained from NIST libraries. 4-Methyl-2-propanol was used as internal standard.

2.6. Electron microscope investigation

Wet cells of pressed baker's yeast and thermally-dried cells were coated with gold in a Balzers SCD 004 sputter coater (Oerlikon-Balzers, Balzers, Leichtenstein) for 3 min, to increase the electron conductivity. Then the sample was studied in a Jeol model JSM-6300 scanning electron microscope (JEOL Ltd., Tokyo).

3. Results and discussion

3.1. Rational of the investigation

Baker's yeast *S. cerevisiae* is globally produced in high capacities as pressed wet product and freeze-dried product. Likewise, strains of *S. cerevisiae* are also produced as freeze-dried wine and brewery yeasts. However, the freeze-dried starter cultures are added value commercial products, of high production cost, due to the energy demand and expensive freeze drying machinery necessary. As strains of *S. cerevisiae* employed as baker's yeast and wine and brewer yeasts are thermally sensitive, thermal drying at a low-temperature is necessary, to develop a promising cost-effective drying process.

To ensure the production of a cost-effective and fermentative dried *S. cerevisiae* starter culture, drying of wet cells at 32 °C, in a thin layer on a glass plate was performed in this investigation.

3.2. Low-temperature thermal drying of S. cerevisiae

The idea of drying the thermosensitive yeast *S. cerevisiae,* at the low-temperature of 32 °C, was based on usual practice, where dried yeast cells of grape flora are dried at summer temperatures and provide vital cells. Therefore, in order to accommodate drying of yeast wet biomass, a thin layer of pressed yeast on a glass plate was created and the whole incubated. Kinetics of drying were measured and the results are presented in Fig. 1. This figure shows that a drying time of about 4 h is necessary to remove 90% of moisture. This percentage of moisture removal is desired, because Koga et al. (1966) have reported that moisture contents in dried products of at least 5–8% are needed, in order to produce active cells. The good quality of thermally-dried cells at 32 °C is also shown by the electron micrograph in Fig. 2.

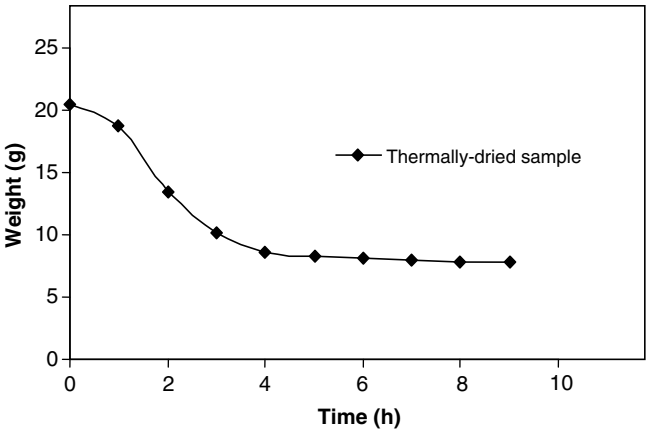

Fig. 1. Kinetics of thermal drying of yeast biomass.

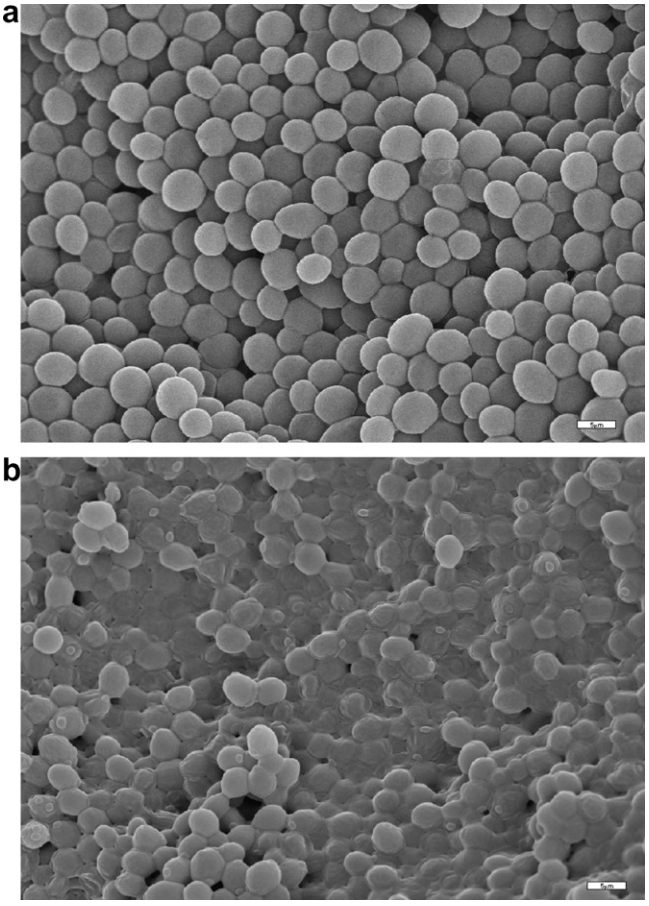

Fig. 2. Electron micrographs of (a) low-temperature thermally-dried cells and (b) wet cells at 1400×.

3.3. Fermentation efficiency of low-temperature thermally-dried S. cerevisiae

To examine the fermentation activity of thermally-dried cells, fermentations of synthetic media containing various initial glucose concentrations and the same dry biomass concentration were performed. The results show in Fig. 3 that all fermentations were fast, with fermentation times

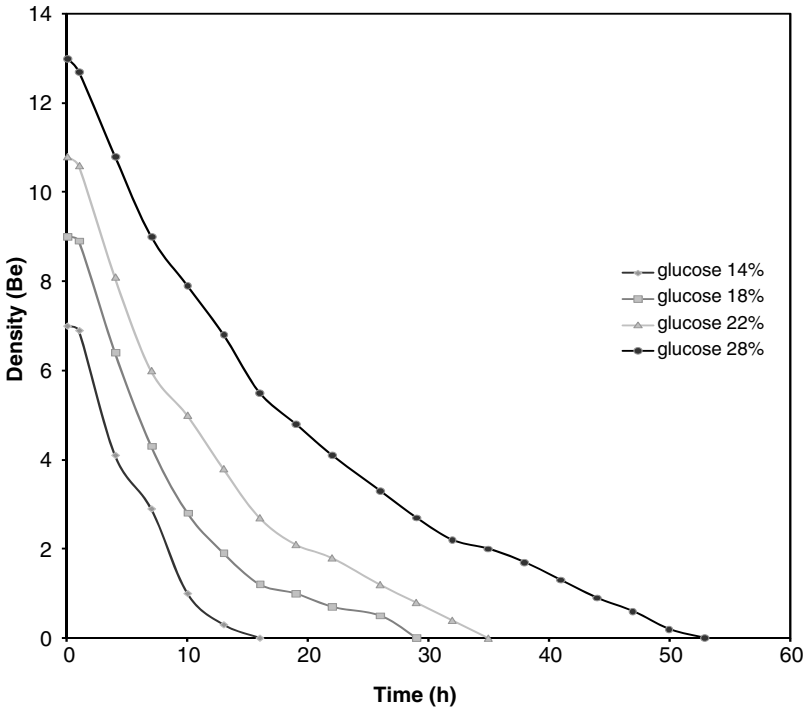

Fig. 3. Effect of initial sugar concentration on kinetics of glucose fermentations by low-temperature thermally-dried yeast cells.

of 15–55 h, corresponding to 14–28% w/v glucose concentrations. This range of glucose concentrations create an osmotic pressure similar to that which exists (1) in the bioreactors of potable alcohol production using molasses and raisin extracts and (2) in fermentors of grape must fermentations for wine making. In addition, low-temperature thermally-dried biomass of *S. cerevisiae* will be also effective in brewing, in the fermentation of wort, as wort contains less than 10% glucose. The start-up temperature of the fermentation is very low, indicating rapid hydration of thermally-dried cells (Fig. 4). The dry starter culture ferments at the low-temperatures of 10–15 °C at normal kinet-

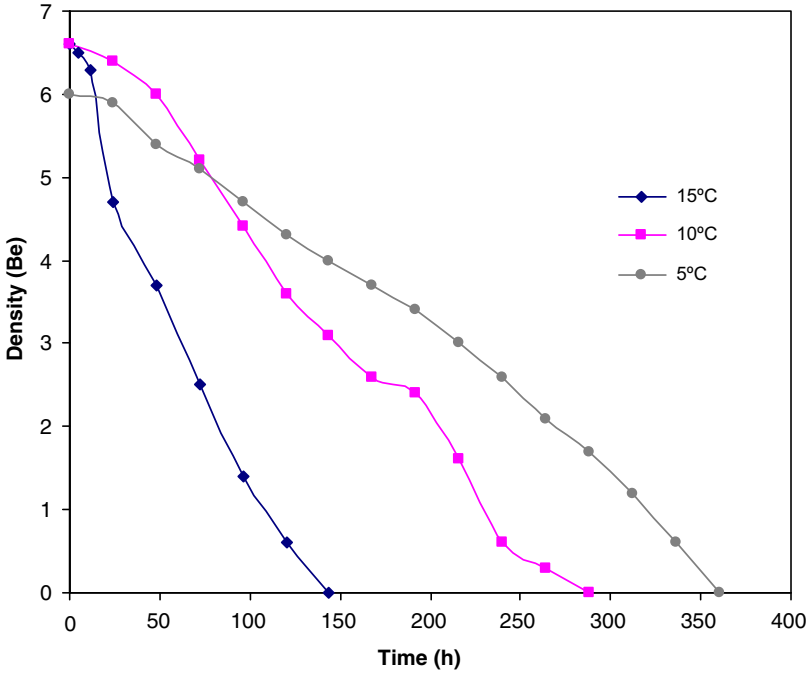

Fig. 4. Effect of temperature on kinetics of glucose fermentation by low-temperature thermally-dried cells, starting in each batch with a new yeast culture.

ics and 140–280 h fermentation time, as do wet cells in industrial brewing. Furthermore, fermentation at 5 °C, is completed in 20 days, which could be accepted by manufacturers, provided that new beer products could be brought to the market. Indeed fermentation of wort at 5 °C means extremely low-temperature brewing and a new chemical composition of volatiles in beer could be formed. It should be stressed here that repeated batch fermentations using low-temperature thermally-dried cells were done and showed stability of starter culture fermentation efficiency for a number of fermentation batches (Fig. 5).

3.4. Comparative study

In order to demonstrate thermally-dried cells of *S. cerevisiae* are competitive with wet and freeze-dried cells, a series of fermentations were carried out in parallel. The results are presented in Fig. 6 and Table 1. The figure shows that low-temperature thermally-dried and wet cells exhibited similar kinetics and fermentation times. Freeze-dried cells gave 35% higher fermentation time than thermally-dried cells, as well as higher residual sugar and lower conversion. These results show thermally-dried cells are more active than freeze-dried cells at 32 °C. The higher alcohol yield

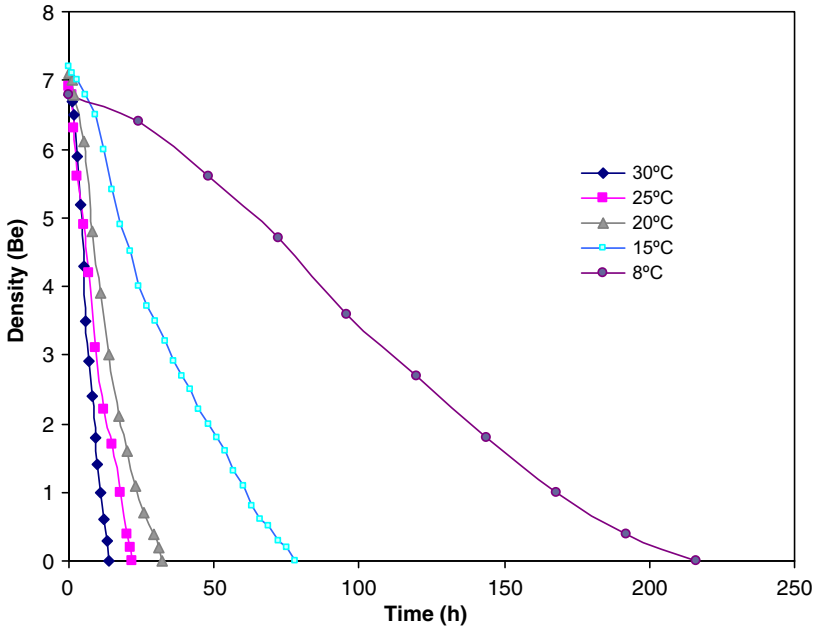

Fig. 5. Effect of temperature on kinetics of repeated batch fermentations by thermally-dried yeasts.

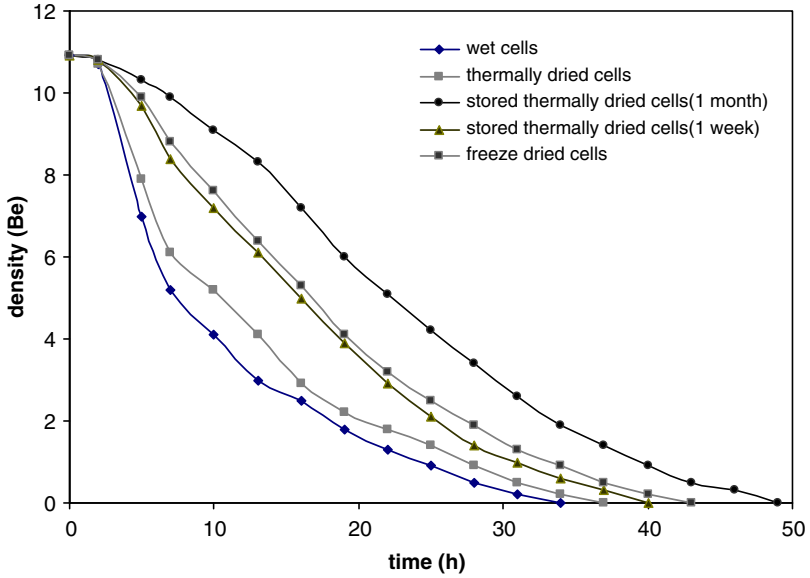

Fig. 6. Kinetic of wet, low-temperature thermally-dried and freeze-dried yeast cells.

Table 1
Comparison of kinetic parameters observed in glucose fermentations by low-temperature thermally-dried, freeze-dried and wet cells of *Saccharomyces cerevisiae*

Sample	Fermentation time (h)	Residual sugar (g/l)	Alcohol concentration (%v/v)	Alcohol yield factor (g/g)	Alcohol productivity (g/ld)	Biomass concentration (g/l)	Conversion (%)
Wet yeast	34	0.37	10.73	0.43	67.81	1.87	99.8
Freeze-dried yeast	49	29.39	10.39	0.48	45.81	0.98	86.6
Low-temperature thermally-dried yeast	37	9.24	10.62	0.44	54.42	1.90	96.3
Stored, low-temperature thermally-dried yeast (1 week)	40	8.51	10.41	0.43	49.34	1.45	96.1
Stored, low-temperature thermally-dried yeast (1 month)	43	8.11	9.71	0.40	37.57	0.69	95.8

Table 2
Volatiles determined by GC–MS analysis in glucose fermentation by wet, freeze-dried and low-temperature thermally-dried cells of *S. cerevisiae*, at 15 °C

Retention time (min)	Compound	Wet cells (ppm)	Freeze-dried cells (ppm)	Low-temperature thermally-dried cells (ppm)
Esters				
5.600	Ethyl Acetate	37.012	24.747	22.684
10.883	Butanoic acid, ethyl ester	2.194	3.549	1.581
15.733	3-Methyl-1-butanol-acetate	18.783	15.918	17.239
22.083	Hexanoic acid, ethyl ester	–	55.213	12.927
30.250	Octanoic acid, ethyl ester	57.512	26.377	17.601
30.467	Acetic acid, octyl ester	–	1.130	–
33.325	Nonanoic acid, ethyl ester	–	1.069	0.366
36.300	Decanoic acid, ethyl ester	40.545	61.074	20.212
37.608	4-dbecenoic acid, ethyl ester	14.641	8.289	8.709
40.883	Acetic acid, 2-phenylethyl ester	8.193	15.663	4.099
41.400	Dodecanoic acid, ethyl ester	1.864	1.334	1.218
46.608	1,2-Benzenedicarboxylic acid, butyl octyl ester	–	2.362	–
	Total	190.812	219.567	108.701
Alcohols				
7.508	Ethanol	[a]	[a]	[a]
11.242	1-Propanol	3.116	11.124	2.193
14.900	2-Methyl-1-propanol	12.524	8.470	7.306
21.690	2-Methyl-1-butanol	–	62.763	11.647
21.900	3-Methyl-1-butanol	171.353	134.396	110.610
32.267	2-Octen-1-ol	–	0.857	–
33.992	1-Octanol	0.540	1.603	0.198
43.158	Phenylethyl alcohol	52.008	38.585	47.958
45.742	3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	0.471	1.242	0.253
	Total	240.01	259.04	180.165
Aldehydes				
3.275	Acetaldehyde	–	–	1.913
33.183	Benzaldehyde	–	2.754	0.189
	Total	–	2.754	2.102
Lactones				
47.733	Furanone,5-hexyldihydro	1.226	2.718	1.137
	Total	1.226	2.718	1.137
Organic acids				
38.217	2-Methyl,butanoic acid	0.391	0.826	0.812
39.458	Hexanoic acid	–	1.334	0.163
46.700	Octanoic acid	94.603	90.369	51.636
49.900	Decanoic acid	2.979	3.172	4.411
	Total	99.536	97.43	58.433
	Total	531.584	578.755	350.538

[a] Ethanol concentrations were determined by HPLC analysis.

factor for freeze-dried cells can be attributed to the lower final biomass concentration obtained, in comparison with wet cells and low-temperature thermally-dried cells. Thermally-dried cells stored for one month without inert atmosphere, did not show any significant change in fermentation kinetic parameters. This result indicates that thermally-dried cells could be commercialised as a packaged product.

3.5. Volatile by-products formation

The formation of volatile by-products showed the effect of low-temperature thermally-dried starter culture on the quality of wine and beer, which could be produced employing this starter culture. Therefore, volatiles formed in fermented liquids produced by low-temperature thermally-dried *S. cerevisiae*, were analysed by GC–MS and compared with freeze-dried and wet cells. The results are presented in Table 2. These results clearly show low-temperature thermally-dried cells form all the compounds that are formed in fermented liquids by wet cells of *S. cerevisiae*. The percentage of esters of total volatiles for both is about 28%. Total higher alcohols were lower for low-temperature thermally-dried cells. This reduction may improve products from a low-temperature thermally-dried starter culture. Ketones and aldehydes were formed in about similar concentrations for wet, freeze-dried and thermally-dried cells.

3.6. Technological consideration

The potential of dried yeast *S. cerevisiae* could be determined by its use in potable and fuel-grade ethanol production, winemaking, brewing, baking and single cell protein (SCP) to feed animals. However, although winemaking, brewing and baking could employ an added value starter culture of relatively high cost, such as that from the freeze drying process, potable and fuel-grade ethanol and SCP could not afford a high cost starter culture, due to their bulk production capacities. Drying of *S. cerevisiae* at the low-temperature of 32 °C was done in a relatively thin layer, ensures high viability of cells proved by the stability of the fermentation efficiency of thermally-dried cells, as compared with wet cells. The thin layer allows drying at the low-temperature of 32 °C and creates the potential for industrial machinery for drying starter cultures, at low-temperatures. The preservation of thermally-dried cells for at least one month without an inert gas atmosphere, suggests that this thermally-dried starter culture could be commercialised without packaging, further reducing purchase price. Also, drying at 32 °C avoids the removal of chemically-bonded water, preventing loss of cell viability (Koga et al., 1966).

The result of higher activity obtained for low-temperature thermally-dried cells in comparison with freeze-dried cells, is a further argument supporting the industrialisation of low-temperature thermally-dried starter culture of *S. cerevisiae*. This higher activity can be attributed to the improved ellipsoidal shape compared with wet cells (Fig. 2). Thermally-dried cells of *S. cerevisiae* without packaging could be employed in bakeries as a competitive alternative product, substituting the wet-pressed baker's yeast product. That product could predominate due to higher shelf-life, in comparison with wet baker's yeast. Here it should be stressed that the commercial freeze-dried product due to high production cost is not in competition with wet-pressed baker's yeast.

The analysis of volatiles showed no significant differences from wet and freeze-dried starter cultures. This ensures low-temperature thermally-dried starter culture will not impair the quality of wine and beers. Finally, low-temperature thermally-dried *S. cerevisiae* starter culture production, will reduce the energy demand in the production plant, compared with freeze-drying and spray-drying.

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