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Evaluation of thermally-dried *Kluyveromyces marxianus* as baker's yeast

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ABSTRACT

The aim of the present study was to evaluate the use of thermally-dried *Kluyveromyces marxianus* as baker's yeast. Bread samples produced by thermally-dried *K. marxianus* were compared with samples produced by wet *K. marxianus* culture and by commercial baker's yeast. The type of the culture had no effect on loaf weight, loaf volume, specific loaf volume, density or moisture loss, in contrast to pH, total titrable acidity (TTA), and moisture content. The use of thermally-dried *K. marxianus* resulted in lower pH values and higher TTA, while the bread samples showed higher resistance to spoilage as counts of moulds and yeasts were significantly lower during preservation. The SPME GC/MS analysis of volatiles and the preliminary sensory evaluation showed no significant differences in the profile of aroma-related compounds or overall quality of the tested samples.

1. Introduction

Nowadays, an upsurge of interest in providing suitable starter and adjunct cultures for food production has been observed. Many researchers have proposed a variety of cultures as alternatives to baker's yeast, including *Kluyveromyces marxianus* (Plessas, Bekatorou, et al., 2008; Plessas, Fisher, et al., 2008), kefir grains (Esteller, Zancanaro, Palmeira, & Da Silva Lannes, 2006; Filipcev, Simurina, & Bodroza-Solarov, 2007; Plessas et al., 2007), and mixed cultures (Gobbetti et al., 1995; Paramithiotis, Chouliaras, Tsakalidou, & Kalantzopoulos, 2005; Plessas, Fisher, et al., 2008), aiming at quality improvement and extension of shelf-life.

In an effort to obtain technically and commercially feasible starter cultures, attention has been paid to the selection of the most suitable drying process. The observed worldwide preference for freeze-dried (Palmfeldt & Hahn-Hägerdal, 2000; Palmfeldt, Rådström, & Hahn-Hägerdal, 2003) and spray-dried (Desmond, Ross, O' Callaghan, Fitzgerald & Stanton, 2002; Gardiner et al., 2000) cultures is due to the long preservation times, but their industrial application usually suffers from a high installation cost of the production unit, accompanied by a need to use cryoprotectants in the case of freeze-drying, and the decrease of the cell viability.

Thermal-drying, recently applied for preparation of dried cultures (Dimitrellou et al., 2008; Kopsahelis, Panas, Kourkoutas, & Koutinas, 2008; Tsaousi, Dimitrellou, & Koutinas, 2008), was a promising alternative. However, the dried cultures have not yet been tested in food production. The aim of the present study was thus to evaluate thermally-dried *K. marxianus* as a baker's yeast.

2. Materials and methods

2.1. Microorganism

Kluyveromyces marxianus (DSMZ, strain IFO 288) was grown at 30 °C in nutrient broth containing 1 g/l of KH_2PO_4 (Fluka, Buchs, Switzerland), 1 g/l of $(NH_4)_2SO_4$ (Fluka), 4 g/l of yeast extract (Fluka), 5 g/l of $MgSO_4$ (Fluka), and 50 g/l of glucose (Fluka). The synthetic medium was sterilised at 130 °C for 15 min prior to use. The wet cells were harvested by centrifugation at 5000 rpm for 10 min and then were used for bread making and thermal-drying.

2.2. Flour

Commercial flour, made from hard type wheat (Allatini, Elbisco Industrial & Commercial S.A., Pikermi Attikis, Greece), containing 13.0% proteins, 69.0% carbohydrates, 1.5% fat and 2.5% fibre, was used for bread making.

2.3. Thermal-drying process

The centrifuged cells were dried in a chamber equipped with air circulation (J.P. Selecta, Spain) at 38 °C (Dimitrellou et al., 208; Kopsahelis et al., 2008; Tsaousi et al., 2008). The drying process was monitored by determining the exact weight of the cultures at various intervals and was carried out up to constant weight.

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2.4. Bread making

Thermally-dried *K. marxianus* (1.5 g) were suspended in 320 ml of tap water for 30 min at 30 °C, mixed with 500 g of wheat flour and 4 g of salt and, subsequently, straight-dough bread making was carried out. Mixing of the ingredients was performed manually for 15 min and the dough was allowed to ferment at 30 °C for 30 min, proofed at 46 °C for 30 min and baked at 230 °C for 40 min. In parallel, breads made with 5 g (wet weight) of *K. marxianus* and 5 g (wet weight) of commercial baker's yeast were also produced for comparison reasons, as described above. In all cases, the dough was moulded manually in 1.5 l baking pans, prior to fermentation. Samples were collected at various intervals (1, 3, 5, 7 days after preparation) and tested by chemical and microbiological analyses, as described below.

2.5. pH and total titratable acidity (TTA)

In total, 15 g of breadcrumbs and 100 ml of distilled water were placed in a clean dry container, which was sealed and stirred until the bread was dispersed into a semi-liquid mixture. The pH was recorded using a Cyberscan 10 pH-meter. TTA was determined by the volume of 0.11 N NaOH solution consumed until the pH reached 6.6 and expressed as ml NaOH consumed (Gélinas, McKinnon, & Pelletier, 1999).

2.6. Loaf volume and moisture loss

Loaf volume was measured by the rapeseed displacement method (Hallen, Ibanoglu, & Ainsworth, 2004). Each loaf was put into a container and covered with rapeseed to totally fill the container. Then, the loaf was removed and the volume of the rapeseed noted. Loaf volumes were calculated by deducting the rapeseed volume from the container volume. After cooling, the loaves were weighed and specific loaf volume was calculated as ml/g (Hallen et al., 2004), while the bread density was calculated as g/ml (Shogren, Mohamed, & Carriere, 2003). Moisture loss (g) was measured by deducting the weight of the bread from the initial weight of the dough before baking.

2.7. Ash, moisture, and total protein

Ash content of bread samples was determined according to AOAC (1995). Moisture content was determined by drying at 105 °C to constant weight and total protein in dry matter (TP-DM) by using the Kjeldahl procedure.

2.8. Microbiological analysis

Representative 25 g portions of duplicate bread samples were homogenised in 225 ml of phosphate buffer (1.25 ml of 0.25 M solution of KH_2PO_4/l distilled water) (Soares, Rutishauser, Melo, Cruz, & Andrade, 2002), and subjected to serial dilutions. Moulds were determined on acidified (0.05% tartaric acid) potato dextrose agar (PDA) (Fluka) after incubation at 25 °C for 5 days (Soares et al., 2002). Yeasts were determined on malt agar (Fluka) after incubation at 30 °C for 3 days and lactobacilli on MRS agar (Fluka) after incubation at 37 °C for 3 days.

2.9. Solid phase microextraction (SPME) gas chromatography/mass spectrometry (GC/MS) analysis

After baking, the bread samples were left for ≈3–4 h to reach ambient temperature and then subjected to SPME GC/MS analysis. The bread samples (≈2 g each, both crust and crumb) were placed in 20 ml headspace vials fitted with a teflon-lined septum, sealed with an aluminium crimp seal, through which the SPME syringe needle (bearing a 2 cm fibre coated with 50/30 mm divinylbenzene/carboxen on poly-dimethyl-siloxane bonded to a flexible fused silica core, Supelco, Bellefonte, PA, USA), was introduced. The container was then thermostatted at 60 °C for 60 min. The absorbed volatile analytes were then analysed by GC/MS (Shimadzu GC-17A, MS QP5050, capillary column Supelco CO Wax-10 60 m, 0.32 mm i.d., 0.25 μm film thickness). Helium was used as carrier gas (linear velocity of 2 ml/min). Oven temperature was set at 35 °C for 5 min, increased by 5 °C/min to 50 °C and held for 5 min, then increased by 5.5 °C/min to 230 °C. A final extension was applied at 230 °C for 5 min. The injector was operated in splitless mode. Injector and detector temperatures were 280 and 250 °C, respectively. The mass spectrometer was operated in the electron impact mode with the electron energy set at 70 eV. The identification was effected by comparing the retention times with those of authentic compounds, by mass spectra of these authentic compounds, generated in the laboratory, by mass spectra obtained from NIST107, NIST21, and SZTERP libraries, and by determining Kovats' retention indices (KI) and comparing them with values reported in the literature (Bianchi, Careri, Chiavaro, Musci, & Vittadini, 2008; Bianchi, Careri, Mangia, & Musci, 2007; Kandylis & Koutinas, 2008). Kovats' retention indices were determined by injection of a standard mixture containing the homologous series of normal alkanes (C_8–C_{22}) in pure hexane under exactly the same experimental conditions as described above. All authentic compounds used were obtained from Sigma–Aldrich, Poole, UK. For quantification of volatiles, 4-methyl-2-pentanol (Sigma–Aldrich) diluted in pure hexane was used as an internal standard (IS) at 400 μg/100 g of bread. The volatile compounds were quantified by dividing the peak areas of the compounds of interest by the peak area of the IS and multiplying this ratio by the initial concentration of the IS (expressed as μg/100 g). The peak areas were measured from the full scan chromatograph using total ion current (TIC). Each determination was carried out in triplicate and the mean data are presented (standard deviation for all values was about ±10% in most cases).

2.10. Preliminary sensory evaluation

To assess bread quality, a consumer blind sensory evaluation (preference) test was carried out. Immediately after production, the bread samples produced by wet and thermally-dried *K. marxianus* were compared with bread produced by commercial baker's yeast. In total, 10 randomly selected tasters were asked to evaluate the samples, giving scores between 0 (unacceptable) and 10 (exceptional) for attributes of flavour, taste, appearance and overall quality.

Spoilage was determined macroscopically and by using sensory tests. A scoring scale with three categories was used: class 1 corresponded to high quality bread without any off-odour or off-flavour, class 2 corresponded to bread with slight off-odours or off-flavours but still acceptable and class 3 corresponded to bread of unacceptable quality. The shelf-life limit was defined as the point when 50% of the panellists rejected the bread samples.

2.11. Statistical analysis

In the experiments conducted, the effects of the nature of the culture and the preservation time were studied. All treatments were carried out in triplicate and the mean values are presented (standard deviation for all values was about ±5% in most cases). The experiments and the preliminary sensory evaluation were designed and analysed statistically by ANOVA. Duncan's multiple range test was used to determine significant differences among results (coefficients, ANOVA tables and significance ($P < 0.05$) were computed using Statistica v.5.0).

3. Results and discussion

3.1. General

Recently, thermal-drying was proposed as an alternative method for production of dried cultures (Dimitrellou et al., 2008; Kopsahelis et al., 2008; Tsaousi et al., 2008). In addition, it was previously suggested that the use of *K. marxianus* in baking resulted in improved quality and extension of shelf-life (Plessas, Fisher, et al., 2008). Hence, the strategy adopted in the present study was to investigate the suitability of thermally-dried *K. marxianus* as baker's yeast. Thus, bread, using the straight-dough method, was produced and compared with bread produced by wet *K. marxianus* culture and commercial baker's yeast. The results concerning quality characteristics and shelf-life are presented in Tables 1 and 2.

The type of culture had no effects on loaf weight, loaf volume, specific loaf volume, density or moisture loss ($P > 0.05$). However, pH, TTA, and moisture content were significantly affected by both the type of the culture and the preservation time ($P < 0.01$). In contrast, neither factor had any effect on ash content or total protein in dry matter (TP-DM) ($P > 0.05$).

In general, thermally-dried *K. marxianus* resulted in lower pH values and higher TTA. Bread produced by the thermally-dried culture showed higher resistance to moisture loss during preservation and thus to staling (Table 1) (Arendt, Ryan, & Dal Bello, 2007).

3.2. Microbiological analysis

During preservation, the samples were subjected to microbiological analysis, in order to determine the evolution of the most significant microbial groups usually appearing in bread. The results are summarised in Table 3.

The type of the culture and the preservation time significantly affected counts of moulds, yeasts, and lactobacilli ($P < 0.01$). A strong interaction between the two factors affecting numbers of moulds and yeasts was also observed ($P < 0.01$).

In bread samples produced by *K. marxianus*, counts of moulds and yeasts were significantly lower, showing increased resistance to spoilage, which was also confirmed macroscopically (Table 1).

3.3. SPME GC/MS analysis

In order to evaluate the suitability of thermally-dried *K. marxianus* in baking, breads produced by thermally-dried and wet *K. marxianus* were compared to bread produced by commercial baker's yeast, in terms of volatile compounds. Quantitative results of the volatile compounds are presented in Table 4. In total, 75 compounds were detected, 58 in bread produced by thermally-dried *K. marxianus*, 49 in bread produced by wet *K. marxianus*, and 59 in bread produced by commercial baker's yeast. The most important compounds identified were esters, organic acids, alcohols and carbonyl compounds. Most of these compounds are well known to affect bread flavour and their origin is associated with yeast, or they are produced during the baking process. Their contribution to bread aroma has been widely reviewed (Hansen & Hansen, 1994; Hansen & Schieberle, 2005; Kirchhoff & Schieberle, 2001, 2002; Rehman, Patterson, & Piggot, 2006).

Table 2
Effect of thermally-dried *K. marxianus* and preservation time on bread characteristics.

Microorganism used	Days	pH	TTA (ml)	Moisture (%)	Ash in DM (g/100 g)	TP-DM (g/100 g)
Baker's yeast (control)	1	6.0 ± 0.1	0.60 ± 0.2	37.4 ± 1.5	2.12 ± 0.04	11.4 ± 0.5
	3	6.1 ± 0.1	0.60 ± 0.1	31.6 ± 1.2	2.20 ± 0.05	11.2 ± 0.5
	5	6.4 ± 0.1	0.50 ± 0.1	25.2 ± 1.0	2.17 ± 0.06	11.0 ± 0.6
	7	6.7 ± 0.1	0.40 ± 0.1	18.6 ± 0.9	2.15 ± 0.05	10.8 ± 0.4
Wet *K. marxianus*	1	6.1 ± 0.1	0.65 ± 0.3	39.6 ± 2.1	2.22 ± 0.10	11.6 ± 0.6
	3	6.0 ± 0.1	0.65 ± 0.3	34.2 ± 2.0	2.25 ± 0.04	11.5 ± 0.5
	5	6.4 ± 0.1	0.50 ± 0.1	29.1 ± 1.5	2.23 ± 0.04	11.3 ± 0.5
	7	6.5 ± 0.1	0.45 ± 0.1	21.0 ± 1.2	2.31 ± 0.09	11.0 ± 0.5
Thermally-dried *K. marxianus*	1	5.8 ± 0.1	0.80 ± 0.4	40.4 ± 2.4	2.21 ± 0.06	11.8 ± 0.8
	3	5.7 ± 0.1	0.75 ± 0.3	35.1 ± 1.2	2.23 ± 0.08	11.5 ± 0.6
	5	6.0 ± 0.1	0.60 ± 0.2	30.7 ± 1.1	2.28 ± 0.09	11.6 ± 0.5
	7	6.2 ± 0.1	0.60 ± 0.2	22.2 ± 0.8	2.22 ± 0.06	11.3 ± 0.3

TTA: total titrable acidity, TP: total protein, DM: dry matter.

Table 3
Effect of thermally-dried *K. marxianus* and preservation time on growth of moulds, yeasts, and lactobacilli.

Microorganism used	Days	Moulds (log cfu/g)	Yeasts (log cfu/g)	Lactobacilli (log cfu/g)
Baker's yeast (control)	1	2.57 ± 0.1	3.01 ± 0.1	2.15 ± 0.1
	3	4.38 ± 0.2	5.03 ± 0.1	3.65 ± 0.1
	5	6.17 ± 0.2	6.59 ± 0.2	4.01 ± 0.1
	7	6.93 ± 0.2	7.15 ± 0.2	4.63 ± 0.1
Wet *K. marxianus*	1	2.61 ± 0.1	2.85 ± 0.1	2.31 ± 0.1
	3	3.65 ± 0.2	4.03 ± 0.1	3.79 ± 0.1
	5	4.19 ± 0.2	4.95 ± 0.1	4.85 ± 0.1
	7	5.64 ± 0.3	6.13 ± 0.2	4.77 ± 0.2
Thermally-dried *K. marxianus*	1	2.53 ± 0.1	2.81 ± 0.1	2.29 ± 0.1
	3	3.73 ± 0.2	3.85 ± 0.1	3.93 ± 0.1
	5	4.28 ± 0.2	4.63 ± 0.1	4.97 ± 0.2
	7	5.15 ± 0.2	5.95 ± 0.2	4.89 ± 0.2

However, the presence of certain volatiles in bread does not consequently imply a positive contribution to the overall aroma. To correlate the occurrence or amounts of volatile compounds with pronounced aroma notes or a more intense overall aroma, extended knowledge of the key aroma compounds among the bulk of odourless volatiles is a prerequisite in flavour characterisation (Hansen & Schieberle, 2005).

Many esters were detected which are known for their positive impact on bread aroma, due to their low aroma threshold values (Hansen & Hansen, 1994), although they usually evaporate during baking. The most abundant ester detected, in all samples, was ethyl 9-hexadecenoate (149, 117 and 98.9 μg/100 g in breads produced by baker's yeast, wet, and thermally-dried *K. marxianus*, respectively).

Organic acids identified included octanoic, nonanoic, decanoic, and undecylenic acid. Octanoic acid was previously detected in many bread types (Plessas, Bekatorou, et al., 2008; Plessas, Fisher, et al., 2008). Although octanoic acid was the major acid in breads produced by baker's yeast and by thermally-dried *K. marxianus* (69.9 and 34.0 μg/100 g, respectively), the predominant acid in bread produced by wet *K. marxianus* was decanoic acid (58.5 μg/100 g).

Table 1
Effect of thermally-dried *K. marxianus* on characteristics and shelf-life of bread.

Microorganism used	Loaf weight (g)	Loaf volume (ml)	Specific loaf volume (ml/g)	Density (g/ml)	Moisture loss (g)	Shelf-life (days)
Baker's yeast (control)	748.3 ± 8.2	1220 ± 35	1.63 ± 0.3	0.61 ± 0.1	135 ± 9	4
Wet *K. marxianus*	747.5 ± 6.4	1271 ± 41	1.70 ± 0.4	0.59 ± 0.2	119 ± 11	5–6
Thermally-dried *K. marxianus*	751.3 ± 6.1	1352 ± 57	1.80 ± 0.6	0.56 ± 0.2	115 ± 7	5–6

Table 4
SPME GC/MS analysis of aroma-related compounds (μg/100g) extracted from bread samples produced using baker's yeast, wet and thermally-dried cells of *K. marxianus*.

Compound	Identification method	Calculated KI	KI by literature data	Microorganism used		
				Baker's yeast (μg/100 g)	Wet *K. marxianus* (μg/100 g)	Thermally-dried *K. marxianus* (μg/100 g)
Esters						
Ethyl acetate	RT, KI, MS	897	896[a], 893[c]	9.5 ± 3.1	14.3 ± 1.7	9.2 ± 1.8
Ethyl 2-hydroxypropanoate	KI, MS	1371	1386[a]	1.5 ± 0.2	2.3 ± 0.2	3.1 ± 0.9
Ethyl octanoate	RT, KI, MS	1439	1438[c]	4.4 ± 0.6	Nd	10.1 ± 1.8
Ethyl decanoate	RT, KI, MS	1643	1647[c]	13.0 ± 1.8	9.6 ± 1.3	15.6 ± 2.6
Diethyl butanedioate	KI, MS	1683	1700[b]	2.8 ± 0.9	6.0 ± 0.5	6.8 ± 0.9
Ethyl 9-decenoate	KI, MS	1697	1709[b]	11.5 ± 1.5	Nd	19.6 ± 1.4
2-Phenylethyl acetate	RT, KI, MS	1835	1847[b]	44.4 ± 4.4	29.0 ± 2.4	41.4 ± 8.1
Ethyl dodecanoate	KI, MS	1848	1850[b]	6.9 ± 2.5	12.8 ± 1.1	13.4 ± 3.9
Dibutyl phthalate	KI, MS	2183	2185[b]	Nd	9.1 ± 0.8	28.0 ± 5.7
Ethyl hexadecanoate	KI, MS	2268	2271[b]	29.3 ± 5.6	31.0 ± 1.9	32.2 ± 2.8
Ethyl 9-hexadecenoate	KI, MS	2291	2292[b]	149 ± 9.8	117 ± 21.3	98.9 ± 11.6
Diethyl phthalate	KI, MS	2363	2365[b]	11.4 ± 2.6	12.2 ± 4.1	18.0 ± 2.0
3-Methyl-2-phenylethyl butanoate	MS	2385	–	2.9 ± 0.2	Nd	Nd
Ethyl octadecanoate	KI, MS	2410	2416[b]	2.0 ± 0.3	Nd	3.0 ± 1.1
Ethyl 9-octadecenoate	KI, MS	2429	2435[b]	15.1 ± 2.7	11.2 ± 1.7	8.9 ± 0.9
Organic acids						
Octanoic acid	RT, KI, MS	2150	2156[b]	69.9 ± 14.4	37.9 ± 8.3	34.0 ± 4.7
Nonanoic acid	RT, KI, MS	2212	2213[b]	27.2 ± 5.1	Nd	Nd
Decanoic acid	RT, KI, MS	2338	2336[b]	46.4 ± 7.5	58.5 ± 9.4	6.9 ± 0.5
Undecylenic acid	MS	2378	–	6.0 ± 2.6	Nd	Nd
Alcohols						
Ethanol	RT, KI, MS	938	942[a], 937[c]	434 ± 17.7	584 ± 63.7	625 ± 132
1-Propanol	KI, MS	1044	1045[a]	Nd	Nd	1.9 ± 0.2
1-Butanol	RT, KI, MS	1165	1170[a], 1152[c]	1.5 ± 0.2	Nd	2.1 ± 0.4
3-Methyl-1-butanol	RT, KI, MS	1218	1226[a], 1212[c]	73.2 ± 2.7	17.0 ± 2.1	7.0 ± 1.1
1-Pentanol	KI, MS	1265	1267[a]	Nd	Nd	2.6 ± 0.5
1-Hexanol	RT, KI, MS	1361	1366[a], 1359[c]	5.3 ± 0.4	22.5 ± 1.9	23.8 ± 5.2
1-Octen-3-ol	RT, KI, MS	1454	1456[c]	2.2 ± 0.3	7.3 ± 0.6	5.2 ± 0.5
1-Heptanol	RT, KI, MS	1462	1460[c]	1.0 ± 0.2	0.8 ± 0.1	0.6 ± 0.2
2-Ethyl-1-hexanol	RT, KI, MS	1492	1492[c]	1.7 ± 0.5	10.2 ± 0.7	Nd
1-Octanol	RT, KI, MS	1560	1561[c]	Nd	Nd	4.9 ± 0.8
2-Furanmethanol	KI, MS	1669	1680[b]	57.1 ± 10.3	47.7 ± 5.3	13.8 ± 0.9
α-Terpineol	MS	1706	–	12.1 ± 4.1	Nd	3.7 ± 0.4
3-(Methylthio)-1-propanol	KI, MS	1727	1730[b]	3.5 ± 0.4	Nd	Nd
Benzyl alcohol	KI, MS	1905	1916[b]	Nd	Nd	6.6 ± 0.5
2-Phenylethanol	KI, MS	1926	1933[b]	121 ± 10.5	95.4 ± 8.7	111 ± 22.5
Phenol	MS	2040	–	1.4 ± 0.2	Nd	3.4 ± 0.6
Carbonyl compounds						
acetone	KI, MS	815	814[a], 813[c]	Nd	92.1 ± 7.2	79.5 ± 12.7
3-Methyl-butanal	KI, MS	917	917[a], 916[c]	27.6 ± 6.5	19.3 ± 5.4	1.2 ± 0.2
2-Methyl-butanal	KI, MS	922	921[a]	Nd	8.4 ± 3.0	Nd
2-Butenal	KI, MS	1002	992[a]	0.6 ± 0.2	Nd	1.0 ± 0.5
2,3-Butanedione	KI, MS	1040	1041[c]	Nd	4.1 ± 0.8	Nd
Hexanal	KI, MS	1086	1089[a], 1085[c]	Nd	9.9 ± 0.4	14.7 ± 2.1
Heptanal	KI, MS	1192	1194[a], 1191[c]	Nd	3.8 ± 0.2	4.0 ± 0.3
Octanal	KI, MS	1293	1296[a], 1292[c]	1.7 ± 0.2	2.5 ± 0.2	3.4 ± 1.1
2-Heptenal	RT, KI, MS	1334	1331[c]	Nd	2.0 ± 0.1	3.2 ± 0.6
6-Methyl 5-hepten-2-one	KI, MS	1348	1349[a]	1.3 ± 0.2	2.0 ± 0.1	4.3 ± 0.8
Nonanal	KI, MS	1396	1396[c]	7.9 ± 1.8	13.5 ± 2.4	26.1 ± 7.3
2-Octenal	KI, MS	1435	1432[c]	1.1 ± 0.2	Nd	4.0 ± 0.7
Furfural	RT, KI, MS	1476	1486[b]	179 ± 9.4	64.1 ± 5.8	13.6 ± 2.7
Decanal	RT, KI, MS	1499	1502[c]	3.3 ± 0.2	3.7 ± 0.5	4.6 ± 2.1
1-(2-Furanyl)-ethanone	KI, MS	1513	1539[a]	20.8 ± 1.7	13.6 ± 1.2	Nd
Benzaldehyde	RT, KI, MS	1537	1539[a]	25.9 ± 4.1	138 ± 21.9	73.5 ± 13.1
2-Nonenal	KI, MS	1544	1546[c]	7.0 ± 1.4	16.1 ± 3.3	20.3 ± 6.4
5-Methyl-furfural	KI, MS	1582	1578[b]	33.0 ± 2.2	Nd	4.4 ± 0.5
1-(2-Pyridinyl)-ethanone	MS	1610	–	5.5 ± 1.1	Nd	Nd
Benzeneacetaldehyde	MS	1658	–	Nd	9.2 ± 2.4	4.7 ± 0.9
1-Phenyl-2-butanone	MS	1820	–	6.0 ± 1.5	24.2 ± 3.1	18.5 ± 2.1
Geranyl acetone	MS	1827	–	12.3 ± 2.1	21.3 ± 3.7	33.2 ± 2.7
2,4,5-Trimethyl-benzaldehyde	MS	1884	–	8.7 ± 0.9	30.7 ± 2.5	Nd
γ-Nonalactone	MS	2055	–	Nd	15.0 ± 1.8	22.4 ± 2.4
Miscellaneous compounds						
Toluene	KI, MS	1043	1043[c]	7.4 ± 0.5	76.7 ± 6.9	62.9 ± ± 5.2
Undecane	RT, KI, MS	1100	1100[c]	Nd	8.5 ± 1.4	7.0 ± 2.8

Table 4 (*continued*)

Compound	Identification method	Calculated KI	KI by literature data	Microorganism used		
				Baker's yeast (μg/100 g)	Wet *K. marxianus* (μg/100 g)	Thermally-dried *K. marxianus* (μg/100 g)
Dodecane	RT, KI, MS	1200	1200[c]	6.7 ± 0.4	20.6 ± 4.4	15.3 ± 1.9
Limonene	RT, KI, MS	1201	1197[c]	Nd	Nd	1.2 ± 0.3
Methyl-pyrazine	MS	1245	–	9.7 ± 1.2	Nd	Nd
1,3,5-Trimethyl-benzene	MS	1254	–	2.0 ± 0.4	2.5 ± 0.3	2.2 ± 0.3
2,5-Dimethyl-pyrazine	KI, MS	1299	1318[c]	2.5 ± 0.2	Nd	Nd
Tridecane	RT, KI, MS	1300	1300[c]	Nd	31.7 ± 6.2	28.4 ± 4.1
2,6-Dimethyl-pyrazine	KI, MS	1322	1325[c]	2.1 ± 0.3	Nd	Nd
2,3-Dimethyl-pyrazine	KI, MS	1342	1344[c]	1.2 ± 0.1	Nd	Nd
2-Ethyl-5-methyl-pyrazine	KI, MS	1391	1406[c]	1.1 ± 0.2	Nd	Nd
2-Ethyl-6-methyl-pyrazine	KI, MS	1396	1402[c]	0.6 ± 0.1	Nd	Nd
Tetradecane	RT, KI, MS	1400	–	11.6 ± 1.0	37.8 ± 5.1	36.2 ± ± 5.0
Naphthalene	MS	1760	–	8.4 ± 0.7	25.4 ± 4.1	46.9 ± 3.2
2-Methyl-naphthalene	MS	1879	–	7.2 ± 0.3	23.8 ± 2.9	16.0 ± 1.5
2,3-Dihydro-benzofuran	MS	2370	–	3.1 ± 0.2	Nd	Nd

RT: positive identification by retention times that agree with authentic compounds and by mass spectra of authentic compounds generated in the laboratory, KI: tentative identification by Kovats' retention index, MS: tentative identification by mass spectra obtained from NIST107, NIST21, and SZTERP libraries, Nd: not detected.

[a] Bianchi et al. (2008).

[b] Kandylis and Koutinas (2008).

[c] Bianchi et al. (2007).

Alcohols can be formed by transamination of amino acids into the corresponding α-keto acids, followed by decarboxylation and reduction (Hansen & Schieberle, 2005). Factors favouring growth of yeasts are considered to result in higher contents of alcohols. Ethanol was the dominating volatile compound in all cases, as yeasts are primary contributors. Many fusel alcohols were also detected which could be the result of the metabolism of amino acids or carbohydrates. 3-(Methylthio)-1-propanol, present only in bread produced by commercial baker's yeast, might derive from methionine, through *Strecker* degradation and subsequent reduction (Hansen & Schieberle, 2005). Benzyl alcohol, identified only in bread produced by thermally-dried *K. marxianus*, provides spicy notes and may originate from benzaldehyde (Hansen, Lund, & Lewis, 1989). Characteristic was the peak of 2-phenyl ethanol present in all samples, as the second most abundant alcohol after ethanol (Table 4). It is among the most odourous compounds usually generated during dough proofing (Frasse, Lambert, Richard-Molard, & Chiron, 1993).

Carbonyl compounds identified included mainly aldehydes, ketones, and lactones. 3-Methyl-butanal, present in all samples, can be formed from leucine by a chemical degradation during baking (*Strecker* reaction) (Hansen & Schieberle, 2005). 2,3-Butanedione (diacetyl), identified only in bread produced by wet *K. marxianus,* provides a buttery-like note (Frasse et al., 1993) and it is a by-product of yeast metabolism (Hansen & Schieberle, 2005). Furfural, detected in all cases, is known for its sweet, bread-like odour (Frasse et al., 1993). γ-Nonalactone, present only in bread produced by *K. marxianus*, is known for its coconut-like odour (Frasse et al., 1993).

Several miscellaneous compounds, such as hydrocarbons and pyrazines, were also detected. Hydrocarbons do not make a major contribution to cheese aroma, while pyrazines were identified among the major volatiles of many types of flour (Rehman et al., 2006).

The most important compounds, from a quantitative point of view, were alcohols, carbonyl compounds, esters, and organic acids. However, the amounts of total volatiles in the samples tested were not significant different (1573, 1856, 1714 μg/100 g in breads produced by baker's yeast, wet, and thermally-dried *K. marxianus*, respectively).

3.4. Preliminary sensory evaluation

The preliminary sensory evaluation revealed no statistical differences on flavour, taste or overall quality among the bread samples. However, the tasters showed a significant ($P < 0.01$) preference concerning appearance for bread samples produced by wet and thermally-dried *K. marxianus* (see Table 5).

Table 5
Preliminary sensory evaluation of bread samples produced by wet and thermally-dried *K. marxianus* compared with bread produced by commercial baker's yeast.

Bread	Flavour	Taste	Appearance	Overall quality
Baker's yeast (control)	7.6 ± 0.70	8.0 ± 0.41	7.8 ± 0.42	7.8 ± 0.42
Wet *K. marxianus*	7.8 ± 0.79	8.2 ± 0.35	8.4 ± 0.39	8.1 ± 0.39
Dry *K. marxianus*	7.9 ± 0.21	8.4 ± 0.46	8.4 ± 0.39	8.2' ± 0.35

4. Conclusions

Thermally-dried *K. marxianus* proved to be a suitable alternative culture for use in bread making, offering the possibility of development of a marketable process, for industrial applications. Avoidance of cryoprotectants, which are widely used during freeze-drying, implies significant reduction of cost in industrial practice but, more importantly, complete lack of risk of cryoprotectant residues, which may be transferred to the final product and may result in quality deterioration.

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