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Thermal Drying of *Lactobacillus delbrueckii* subsp. *bulgaricus* and its Efficient Use as Starter for Whey Fermentation and Unsalted Cheese Making

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Abstract *Lactobacillus bulgaricus* grown on whey was dried by a simple thermal drying method at the range 35–55°C and its efficiency for lactic acid fermentation of whey was evaluated. Drying of cells in whey suspension in the examined temperature range did not affect significantly their viability (82–87% survival), indicating a protective effect of whey as both growth and drying medium. The kinetics of fermentation of whey and mixtures of whey/molasses using the dried culture were comparable to those of non-dried cells, and only low pH had a detrimental effect on the fermentation ability of the dried cells. Furthermore, dried *L. bulgaricus*, free or immobilized on casein coagulates, was used as starter for the production of unsalted hard-type cheese. The effects of the amount of starter culture and the immobilization technique, the evolution of microbial counts, and the sensory properties of the produced cheeses were evaluated during ripening at various temperatures.

Keywords *Lactobacillus delbrueckii* subsp. *bulgaricus* · Thermal drying · Whey · Lactic acid fermentation · Volatiles · Unsalted hard-type cheese · Immobilization

Introduction

Lactic acid bacteria (LAB) are associated with the production of the majority of fermented foods worldwide, at commercial or traditional level. The nutritional significance and organoleptic superiority of fermented foods has been reviewed [1]. The use of defined, mixed-starter cultures containing LAB, novel or inspired from traditional wild cultures, is lately being extensively studied, due to the increased interest in foods with probiotic properties, improved flavor, extended shelf life, as well as for the production of novel products [2–4]. Therefore, the need for production of dried commercial starter cultures containing LAB, surviving production process, and maintaining viability and fermentation activity after drying, storage, and rehydration/reactivation has led to extensive investigation

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for optimization of existing drying methods as well as the development of new processes, which ideally should be of low cost. Drying methods involved in LAB starter culture production include freeze-drying, spray drying, osmotic drying, vacuum drying, fluidized-bed drying, as well as mixed dried systems [5, 6]. The production of active dried LAB starter cultures can be influenced by several process factors including bacterial species and intrinsic tolerance of cultures, growth media and conditions, osmotic stresses, cell density, protective agents, rehydration and storage conditions, etc. [5, 7].

Lactobacillus delbrueckii subsp. *bulgaricus* (LDSB) is a thermophilic LAB able to ferment lactose, glucose, fructose, and mannose. It is commonly found and used in dairy starter cultures, such as those involved in the production of yogurt and cheese, therefore is of great interest to industrial applications [8, 9]. Various researchers have dealt with the optimization of LDSB drying processes for active commercial dry cultures production [8, 10–12]. LDSB is also part of kefir microflora. Kefir and selected LAB have been recently investigated for the production of novel starter cultures for use in food production such as sourdough bread making, cheese making, and fermented products from whey [13–17].

Whey is the main liquid waste of the dairy industry, which is discarded in large amounts annually creating serious environmental problems. However, intense recent research attempts try to deal with the problem focusing on the development of technologies that employ whey as raw material to produce foods or chemicals of added value [18]. Whey, molasses, citrus and starchy residues, as well as other by-products of the food industries, which are rich in carbohydrates, can be utilized using suitable microorganisms to produce added value and reduce their pollution potential. Specifically, the fermentation of various whey-based media by LDSB has been studied for the production of exopolysaccharides, lactic acid, peptides, biomass, etc. [9, 19–22]. The aim of this study was to evaluate (a) the efficiency of LDSB grown on milk whey and dried by a simple method of low cost (thermal drying) for lactic acid fermentation of whey and whey/molasses at varying conditions and (b) the suitability of the dried LDSB as starter culture for unsalted hard-type cheese making.

Materials and Methods

Microorganism and Media

LDSB (*L. d.* subsp. *bulgaricus* DSMZ 20081), isolated from Bulgarian yogurt, was supplied from Deutsche-Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Germany. It was initially inoculated and grown at 37°C in medium containing 51 g/L MRS broth, 0.1% *v*/*v* Tween-80 and 0.05% *w*/*v* L-cysteine hydrochloride (Sigma-Aldrich GmbH), following repeated subculturing in milk whey. Cells were harvested by centrifugation at 5,000 rpm for 15 min. MRS agar was used for culture maintenance and viability count measurements. Whey was prepared from cow's milk after rennin coagulation, filtration to separate casein proteins, and subsequent heat treatment at 90°C for 15 min for removal of whey proteins. Preparation of coagulated casein from commercial pasteurized bovine skim milk (0% fat) and immobilization of cells in casein were performed as described by Dimitrellou et al. [17]. Molasses was obtained from BG Spiliopoulos SA alcohol distillery (Patras, Achaia, Greece). It was diluted with water to a hydrometer density of 10°Be (10.34 g total fermentable sugar). Phosphate KH_2PO_4/K_2HPO_4 buffer (Sigma-Aldrich GmbH) was used for pH adjustments. Ringer's solution

(RS; Merck) was used for cell rehydration and viability counts. Pure lactose, sucrose, glucose, galactose, and fructose (Sigma-Aldrich GmbH) were used for addition to media or HPLC standard preparation. All media were sterilized by autoclaving at 121°C for 15 min.

Drying Process

Drying of cultures was carried out in an oven equipped with air circulation (J.P. Selecta, Spain). Drying of LDSB suspensions in whey was carried out without the use of other protective media. Specifically, aliquots of 1 mL of cell suspensions containing approximately 0.03 g of LDSB cells per milliliter were spread in a thin layer in 5 cm petri dishes and were dried in a laboratory convective oven at 35°C, 45°C, and 55°C for 10 h. After drying, the retained moisture content was determined by further drying at 102°C, and the degree of survival of the dried cultures was determined after rehydration in RS. Experiments were carried out in triplicate and the results are given as average values (Table 1). Samples of dried cultures were stored at 4°C and their viability was examined again after 1 and 2 month periods. LDSB cultures used for cheese making were dried at 35°C.

Whey Fermentation

The effects of (a) drying temperature, (b) initial lactose concentration (ILC), (c) pH, and (d) addition of molasses on the ability of dried LDSB to ferment whey were evaluated. All fermentation experiments were carried out at 37°C for a period of 3 days. Specifically, in a first set of experiments (a), each of the cell cultures dried at 35°C, 45°C, or 55°C was resuspended in 16 mL of whey (approximately 4% *w*/*v* ILC, pH 5.5) and the systems were allowed to ferment in order to examine the effect of drying temperature on their lactose conversion ability. In a similar way (b), the fermentation of 16 mL whey containing different ILCs (approximately 4%, 6%, or 8% *w*/*v*) and pH 5.5 by cells dried at 55°C was also examined. To evaluate the effect of pH (c) on lactose conversion by dried LDSB, fermentations of 16 mL whey/buffer solutions having pH 4, 5, or 6 and containing approximately 1% *w*/*v* ILC were carried out using LDSB cells dried at 55°C. Finally (d), the ability of LDSB cells dried at 55°C to ferment mixtures of whey (4% *w*/*v* ILC) and molasses (10^{o}Be) was tested. Specifically, fermentations of 16 mL of mixtures of 25:4 or 25:8 whey to molasses ratios were carried out as described above (37°C, pH 5.5) using LDSB cells dried at 55°C. In all cases, samples were collected and analyzed for residual sugar, produced lactic acid (for evaluation of fermentation activity), and volatiles by solid-phase micro-extraction method (SPME) GC-MS (for evaluation of possible effect on the quality of whey-based foods).

Moisture and Survival Rates

The residual moisture content of the dried cultures was determined by convective drying at 102°C until constant weight. The survival rate of LDSB after drying was determined by the drop-count method [8]. Specifically, the dried samples were rehydrated with 2 mL of sterile RS and 1 mL of this cell suspension was diluted to 9 mL of sterile RS. Ten successive dilutions were made in the same manner, the last two of which were drop plated (100 μL) in triplicate on MRS agar. The plates were examined after incubation at 37°C for 48 h, and viability was estimated as colony forming units per

Table 1 Moisture and viability before and after drying of LDSB cells at 35, 45 and 55°C.

Storage at 4°C	Drying temperature	Weight before drying	Weight after drying	Moisture loss after drying	Residual moisture in dried culture	Viability before drying	Viability after drying	Survival rate
Month	°C	g	g	% *w/w*	% *w/w*	CFU/mL	CFU/mL	%
0	35	1.045	0.030	97.1	23.3	5.98×10^9	5.22×10^9	87.3
1	35	-	-	-	-	-	5.03×10^9	84.1
2	35	-	-	-	-	-	4.16×10^9	69.6
0	45	1.040	0.031	97.0	35.5	5.98×10^9	4.92×10^9	82.3
1	45	-	-	-	-	-	3.92×10^9	65.6
2	45	-	-	-	-	-	3.56×10^9	59.5
0	55	1.015	0.025	97.5	20.0	5.98×10^9	5.12×10^9	85.6
1	55	-	-	-	-	-	4.68×10^9	78.3
2	55	-	-	-	-	-	3.53×10^9	59.0

milliliter of initial cell suspension (colony forming units per milliliter). Survival rates were calculated as percentage of bacteria after drying on the number of bacteria before drying.

Fermentation kinetics Fermentation kinetics were performed by measuring the residual sugar (sum of lactose, glucose, galactose, sucrose, and fructose) by HPLC at various time intervals. Fermentation rates were calculated by linear regression of the lactose time course data over the first 10 h needed for initiation of fermentation. Residual sugar and ethanol were determined on a Shimadzu LC-9A HPLC system. A Shim-pack (SCR-101 N) column, a refractive index detector, three times distilled and filtered water as mobile phase (0.8 mL/min), and 1-butanol (0.05% *v*/*v*) as internal standard, were used. Column temperature was 60°C. Sample dilution was 1% *v*/*v* and injection volume was 40 μL. The total titratable acidity (TTA), expressed as %*w*/*v* lactic acid, was determined by a titrating 5 mL of the fermented liquids with standard 0.1 N NaOH solution using phenolphthalein as indicator [23].

GC-MS Analysis of Aroma Profile

The headspace volatiles in samples of whey, mixture of whey/molasses, fermented whey, and fermented whey/molasses were isolated by the SPME. The fiber used was a 2 cm–50/30 mm DVB/Carboxen/PDMS StableFlex for manual holder (Supelco, USA). The conditions of headspace-SPME sampling used were as follows: 1 mL sample and 0.3 g NaCl (saturated solution ~30%) were transferred into a 10 mL glass vial sealed with a rubber septum. The SPME fiber was exposed to the headspace and the vial was immersed in a water bath at 60°C for 1 h for absorption of volatiles. Desorption of volatiles took place in the injector of the gas chromatograph in splitless mode at 250°C for 3 min. GC/MS analysis was performed on a Shimadzu model GC-17A chromatograph coupled to a GC-MS QP5050A mass spectrometer. A Supelcowax-10 column (60 m–0.32 mm i.d., 0.25 μm film thickness) was used. The GC temperature program was 35°C held for 5 min, then increased by 5°C/min to 50°C, where it was held again for 5 min, then increased by 5.5°C/min to 230°C, where it was held again for 5 min, for a total run time of 52 min. The carrier gas was helium with a column flow of 2 mL/min. The injector temperature was at 280°C. The interface temperature was 230°C. Mass spectra were recorded by electronic impact at 70 eV. The scan mode was used to detect all the compounds in the range *m*/*z* 33–300. Identification of compounds was done by comparing the retention times and MS data with those of standard compounds and by MS data obtained from NIST107, NIST21, and SZTERP libraries.

Cheese Making and Analysis

Cheese was made adopting the industrial practice of hard-type cheese production (AVIGAL S.A., Achaia, Greece) [16]. Nine types of unsalted cheeses were prepared, without addition of starter culture (rennet cheeses), or with rennet as well as the addition of 0.5 g/L dried LDSB and 0.5 g/L dried LDSB immobilized on casein [17]. All cheeses were ripened at 5°C, 18°C, or 22°C. Chemical, physicochemical, sensory, and microbial analysis was carried out as described by Katechaki et al. [16]. Taste, aroma, textural characteristics, and degree of openness (hole formation) were assessed on a scale of one to seven. Results were the means of three repetitions. One-way analysis of variance was used to test the significance of

differences among results in terms of type and amount of starter, temperature, and stage of ripening.

Results and Discussion

In this study, LDSB, a common food LAB, was grown on whey, and suspensions of the culture in whey were dried by a simple thermal drying method at mild conditions (35–55°C). The efficiency of the dried culture for lactic acid fermentation of whey and for hard-type cheese making was evaluated.

Thermal Drying of LDSB

The thermal drying of LDSB cells in whey suspension in the examined temperature range did not affect significantly their viability (82–87% survival). This indicated a protective effect of whey as both growth and drying medium. Specifically, LDSB cell suspensions, containing about 0.03 g of cells/mL (wet weight), were dried at 35°C, 45°C, and 55°C. The moisture contents of the dried cultures and the numbers of viable cells, before and after drying, are shown in Table 1. It can be observed that most of the moisture content (~97%) of all samples was removed during the 6–8 h of the drying process. The moisture retained in the remaining dried cultures (residual cellular or extracellular water) was determined by further drying at 102°C and it was 20–36%. The survival rates under these drying conditions were maintained at high levels (82–87%) and were not affected significantly by the drying temperature within the studied range of temperatures. After storage at 4°C, the survival rates were reduced to 70% for cells dried at 35°C and about 60% for cells dried at 45–55°C (Table 1). The maximum standard deviations recorded for moisture of dried cultures, cell viability, and survival rate after drying were 3.0, 0.14×10^9, and 2.3, respectively.

The purpose of developing suitable drying methods for food microorganisms is to exploit the possibility of producing commercial dried starter cultures easy to handle and preserve. The successful application of low temperature thermal drying techniques is attractive due to the increased cell viabilities and lower cost for equipment and energy demand as in other expensive techniques like freeze-drying [5]. Nevertheless, most researchers have dealt with the optimization of LDSB freeze-drying, spray drying, or vacuum drying processes for active commercial dry cultures production [6, 8, 10–12]. In most cases, research focuses on process conditions, survival after drying and during storage, and effect of the drying medium. The results of this study regarding the simplicity and cost of the technique used are very encouraging for commercial application. Moreover, the use of whey as growth and drying medium is important since whey is low cost and seriously polluting waste. Therefore, any effort regarding its utilization is significant [18, 24]. Similar thermal drying techniques were recently applied successfully for the production of dried kefir, *Kluyveromyces marxianus*, *Lactobacillus plantarum*, and *Saccharomyces cereviasiae* for use in various food and feed applications [24–28].

Whey Fermentations

The effect of drying temperature was evaluated in terms of fermentation activity of the dried cells (Table 2 and Fig. 1). As expected by the observed survival rates, no significant differences were observed in the times for completion of fermentation (about 40 h), the

Table 2 Effects of drying temperature, initial sugar, pH, and addition of molasses on whey fermentation at 37°C by dried LDSB cells.

Substrate	Drying temperature °C	pH	Total initial sugar % *w/w*	Total residual sugar % *w/w*	Sugar conversion %	TTA (as lactic acid) % *w/w*
Whey	-	5.5	4.19	2.13	49.8	1.67
Whey	35	5.5	4.17	2.99	28.3	1.01
Whey	45	5.5	3.96	2.89	27.0	0.98
Whey	55	5.5	4.08	3.12	23.5	0.67
Whey	55	5.5	4.19	2.90	30.8	1.12
Whey	55	5.5	5.99	3.75	37.4	1.38
Whey	55	5.5	7.52	4.98	33.8	1.53
Whey	55	4	1.07	0.95	11.2	0.07
Whey	55	5	1.36	0.21	84.6	1.19
Whey	55	6	0.94	0.03	96.8	1.21
Whey/molasses (25:4)	55	5.5	5.41	4.76	12.0	1.48
Whey/molasses (25:8)	55	5.5	6.91	5.56	19.5	1.29

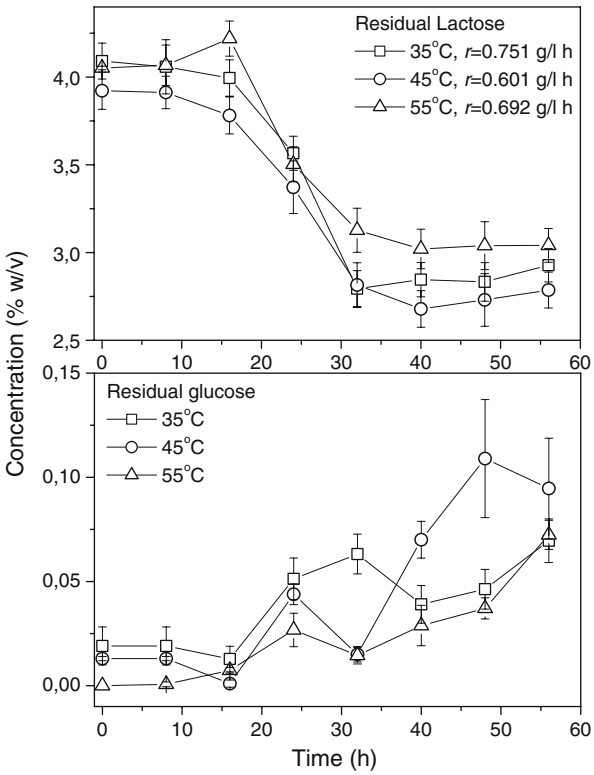

Fig. 1 Effect of drying temperature (35°C, 45°C, and 55°C) on the kinetics of whey fermentation at 37°C by dried LDSB

levels of residual sugar and lactose conversions (23.5–28.3%) during the 3-day fermentations of whey (4% lactose) at 37°C. All these parameters were slightly better in the case of cultures that were dried at 35°C. Therefore, this temperature was preferred for the drying of LDSB for use as starter culture in the cheese making experiments. In Fig. 1, the profiles of glucose generation and consumption are shown. Subsequently, the effects of ILC (1%, 4%, 6%, and 8% *w/v*), pH (4, 5, and 6), and the addition of molasses (solutions of 25:4 and 25:8 whey:molasses) on the fermentation of whey using LDSB dried at 55°C for 24 h were studied. Total residual sugar (sum of residual lactose, glucose, galactose, fructose, and sucrose) and TTA (as %*w/v* lactic acid) in all the studied systems were assayed after fermentation at 37°C for a 3-day period. The results are shown in Table 2 and Figs. 2, 3, and 4. The increase of ILC did not seem to have an effect on the fermentation ability of dried LDSB (Fig. 2). Fermentation times were about 40 h (after which fermentation rate was reduced or ceased), and lactose conversion was not complete as observed in all the other cases. Yet, it was noticed that although the residual sugar was higher when the ILC was higher, the lactose conversions were improved (Table 2). The pH on the other hand had a more significant effect on whey fermentation by dried cells. Fermentation of whey/buffer mixtures were carried out at constant pH 4, 5, or 6 values. From the results shown in Table 2 and Fig. 3, it is obvious that the best fermentation kinetics were observed at pH 6 followed by pH 5, while at pH 4 the lactose conversion within 3-days was minimum (11.2%). At pH 6 the fermentation rate was higher (Fig. 3), and as observed in the previous cases, fermentation ceased after 40 h, but with very small

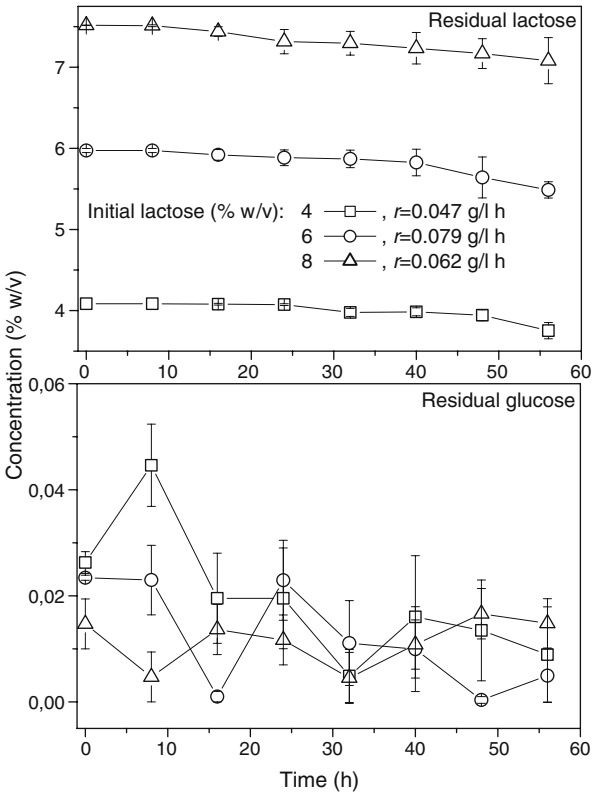

Fig. 2 Effect of initial lactose concentrations (4°C, 6°C, and 8% *w/v*) on the kinetics of whey fermentation at 37°C by LDSB dried at 55°C

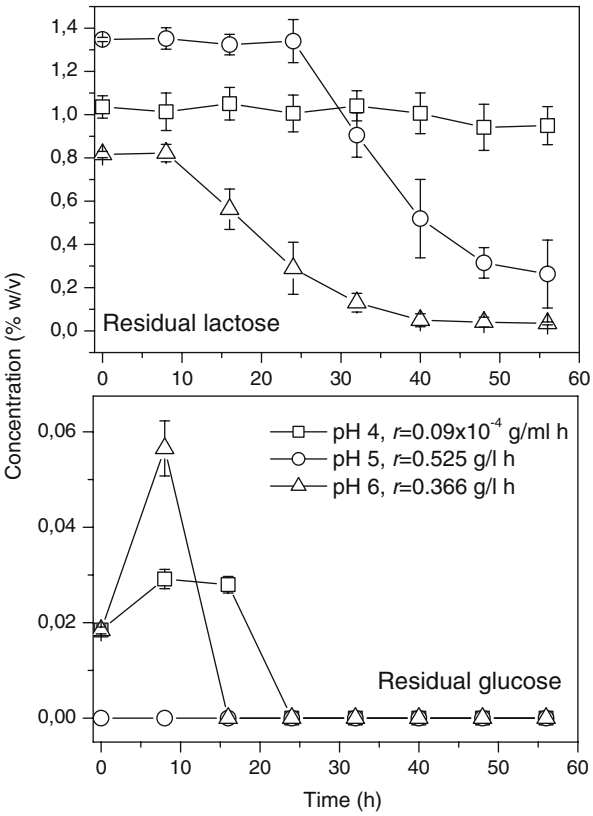

Fig. 3 Effect of pH (4, 5, and 6) on the kinetics of whey fermentation at 37°C by LDSB dried at 55°C

amounts of residual sugar and the highest obtained conversion of lactose (97%). Finally, the addition of molasses reduced the lactose conversion rate within the same time limits (~40 h) compared to the previous cases and led to higher residual sugar, mainly galactose and fructose (Table 2 and Fig. 4). The lower fermentation rates in the presence of molasses may be attributed to the lower rates of sucrose utilization (Fig. 4). The TTA values were at about the same levels for all the studied systems (1.23±0.28% *w/v* lactic acid) except the case of pH 4 where only very low amounts of lactic acid were formed (Table 2). The respectable values of developed acidity in all the studied systems prove the presence of active LAB. The formation of lactic acid and subsequent increase of acidity are also desirable in many types of fermented foods, such as in cheese making, for improvement of flavor and extension of shelf life.

Volatiles

Although all samples were allowed to ferment for only 3 days using a very small amount of dried biomass, and although only about 1 g of sugar was utilized in almost all cases, the fermentation of whey or mixture of whey and molasses led to different profiles of headspace volatiles. From Fig. 5 and Table 3, it can be observed that more compounds were identified in the headspaces of the fermented substrates compared to the non-fermented ones. Moreover, more compounds were identified in non-fermented molasses compared to non-fermented whey, justifying the higher number of compounds identified in the fermented mixture of whey and molasses. Specifically, 26, 32, 34, and 48 compounds

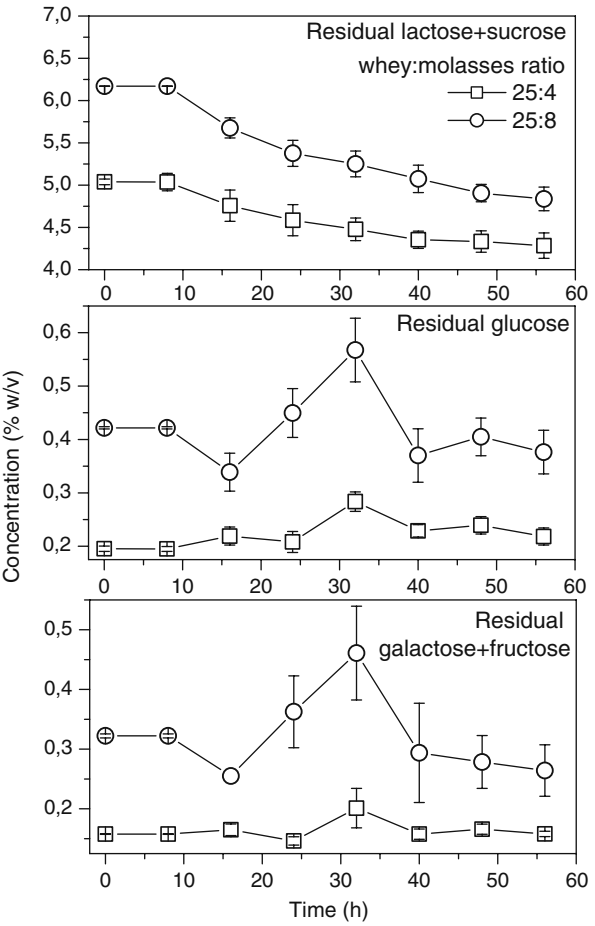

Fig. 4 Effect of addition of molasses on the kinetics of whey fermentation (25:4 and 25:8 ratios of whey:molasses) at 37°C by LDSB dried at 55°C

were identified in non-fermented whey and molasses and in fermented whey and mixture of whey/molasses by dried LDSB, respectively. Most of the identified compounds were alcohols, carbonyl compounds, organic acids, alkanes, alkenes, and only a few esters (mainly methyl and ethyl esters of organic acids).

Hard-type Cheese Making

LDSB, thermally dried at 35°C, in free cell form or immobilized on casein coagulates, was used as starter culture for the production of unsalted hard-type cheese. The moisture levels of all cheese samples immediately after preparation (day 1) were similar (significance level $p=0.05$), and at the end of the ripening period, they were reduced by 24.8%, 26.8%, and 68.7%, at 5°C, 18°C, and 22°C, respectively (Table 4). The type of starter did not affect the rate of moisture loss ($p=0.05$). In rennet cheeses, the conversion of lactose was little, while in cheeses made with starters, only traces of total residual sugar were found after the first week of ripening (Table 5). Low and similar levels of ethanol ($p=0.05$) were found in all samples, therefore, sugar was converted mainly to lactic acid and to a lesser extend to microbial biomass (Table 5). The presence of ethanol indicates heterofermentative metabolism by LDSB or the

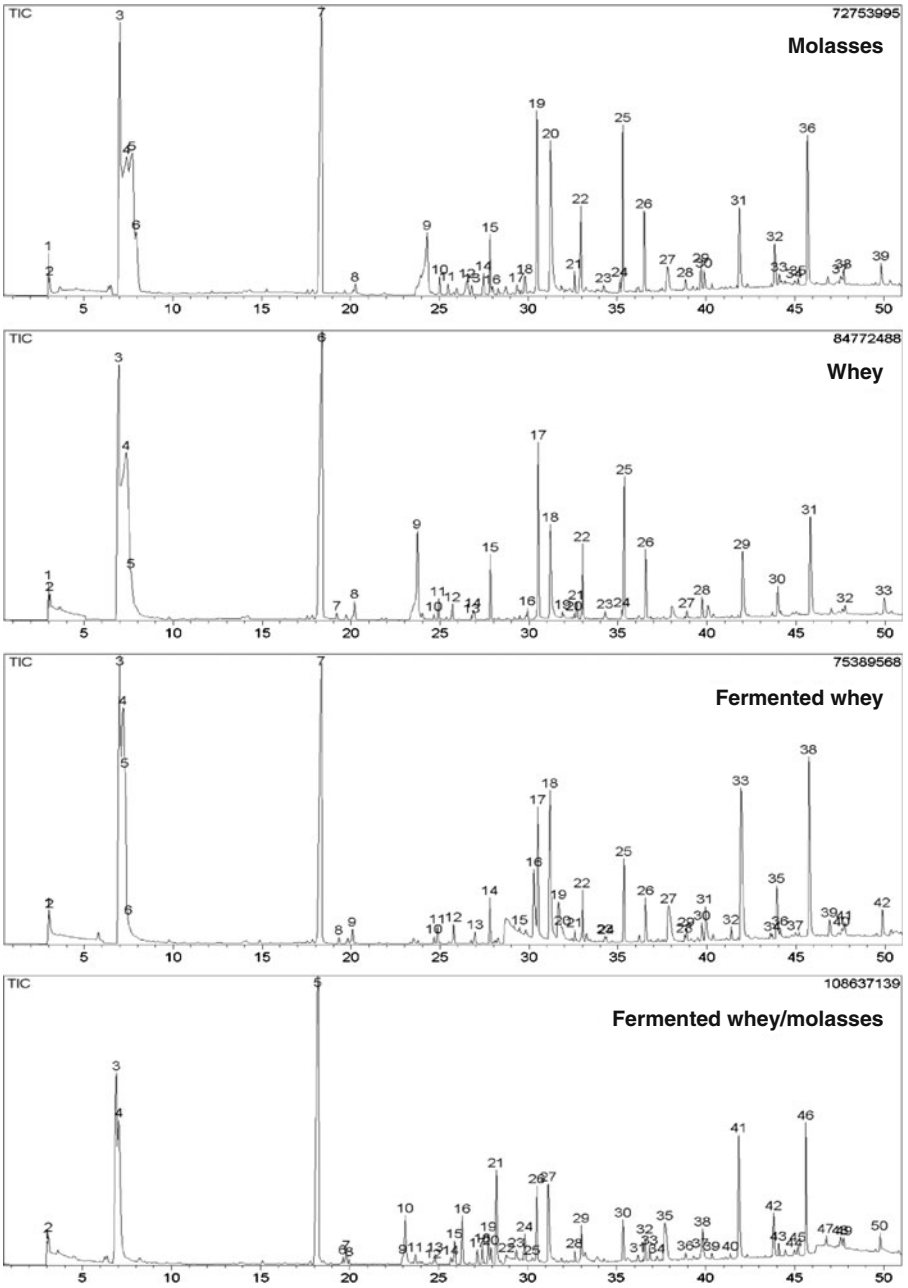

Fig. 5 SPME GC-MS analysis of volatiles in non-fermented whey and molasses and in whey and mixture of whey/molasses fermented at 37°C by LDSB dried at 55°C

wild microflora developed in rennet cheeses. LDSB, free or immobilized, led to lower pH values of cheeses indicating fast lactose conversion, followed by proteolysis and lipolysis.

No differences were observed on microbial counts (total aerobic counts, yeasts and molds, *Lactococci* and *Lactobacilli*) among rennet cheeses, ripened at different temperature

Table 3 SPME GC-MS analysis of volatile aroma profile in whey and molasses before and after fermentation at 37°C by dried LDSB cells.

Rt	Compound	Non-treated		Fermented	
		Whey	Molasses	Whey	Molasses
3.592	Acetaldehyde	-	a	a	a
4.534	Acetone	-	a	a	a
7.15	Ethanol	a	a	a	a
18.35	2-Hydroxy-4-methyl-pentanoic acid, methyl ester	b	-	a	a
18.37	5-Methyl-1-hepten-4-ol	b	b	b	b
18.983	2-Pentenol	-	-	-	a
19.759	2-Methyl-1-butanol	-	-	-	b
19.995	1,2,3-Trimethyl-benzene	-	-	-	b
20.019	3-Methyl-1-butanol	b	b	b	b
23.736	3-Hydroxy-2-butanone	a	b	-	b
23.758	2,5-Dimethyl-pyrazine	-	-	-	b
24.808	1-Hexanol	a	a	a	a
25.696	2-Ethyl-6-methyl-pyrazine	-	-	-	b
25.911	2,6,8-Trimethyl-4-nonanone	a	a	a	a
26.337	Trimethyl-pyrazine	-	b	-	b
26.843	2-Octenal	-	b	-	-
27.025	Octanoic acid, ethyl ester	-	-	b	a
27.192	3-Hexadecene	b	-	b	b
27.468	3-Ethyl-2,5-dimethyl-pyrazine	-	b	-	b
27.809	1-Heptanol	a	a	a	a
27.820	Formic acid, heptyl ester	-	-	b	b
27.953	2-Ethyl-3,5-dimethyl-pyrazine	-	b	-	b
28.260	Furfural	-	-	-	b
29.047	Benzaldeheyde	-	-	-	b
29.380	3,5-Diethyl-2-methyl-pyrazine	-	b	-	-
29.476	Acetic acid	-	-	b	-
29.925	2-Nonenal	b	-	-	-
30.529	1-Octanol	a	a	a	a
31.169	2,3-Butanediol	a	a	a	a
31.658	Propylene glycol	-	-	b	-
31.900	2-Octen-1-ol	a	-	-	-
32.550	1-Heptadecene	b	-	-	-
32.621	2-Decenol	b	b	b	b
32.657	1-Octadecene	-	-	-	b
33.022	1-Nonanol	a	a	a	a
34.258	2-Nonen-1-ol	b	b	b	b
35.175	2-Undecenol	b	b	-	b
35.379	1-Decanol	b	b	-	b
36.217	1-Hexadecane	-	-	-	a
36.608	9-Decyn-1-ol	b	b	b	b
36.867	3-Methyl-2-thiophenecarboxaldeheyde	-	-	-	b

Table 3 (continued)

Rt	Compound	Non-treated		Fermented	
		Whey	Molasses	Whey	Molasses
37.281	2-Undecanol	-	-	-	b
37.724	Hexanoic acid	-	b	b	a
38.885	Phenylethyl alcohol	a	a	a	a
39.750	Dodecanol	b	a	b	b
39.841	Heptanoic acid	-	b	b	b
40.367	Heptadecane	-	-	-	b
41.869	Octanoic acid	b	a	a	a
43.675	1-Tetradecanol	-		b	-
43.831	Nonanoic acid	a	b	a	a
44.117	Octadecane	-	b	-	b
44.120	Nonadecane	-	-	b	-
45.186	Decanoic acid, ethyl ester	-	-	b	a
45.639	n-Decanoic acid	a	a	a	a
46.908	Undecylenic acid	-	-	a	-
47.200	Tetradecanoic acid, ethyl ester	-	-	a	a
47.766	2-Tetradecanol	-	-	-	b
47.768	Heneicosane	-	a	b	-
49.858	Dodecanoic acid	b	a	b	a

a positive identification from MS data and retention times, *b* positive identification from ms data only

(p=0.05). A twofold increase of all counts was observed during the first week of ripening. In LDSB cheeses, counts slightly increased after the first week but no differences (p=0.05) were observed by the use of free or immobilized cells or by ripening at different temperature.

Table 4 Moisture, shelf life, and sensory evaluation of unsalted hard-type cheeses.

Ripening temperature (°C)	Dried starter	Moisture (%)		Shelf life (days)	Sensory evaluations			
		Day 1	Day 60		Taste	Aroma	Texture	Degree of openness
5	Rennet	50.4	38.1	28	3.16	3.42	5.78	1.10
5	LDSB	50.5	37.9	42	5.20	5.12	6.03	5.15
5	Immobilized LDSB	50.2	38.0	40	4.78	4.90	6.00	6.05
18[a]	Rennet	50.4	37.0	15	1.26	1.20	5.10	1.00
18	LDSB	50.4	36.8	15	4.92	4.90	5.93	5.06
18	Immobilized LDSB	50.3	36.9	15	4.81	4.97	5.83	5.95
22	Rennet	50.5	15.9	36	3.12	3.50	2.76	1.00
22	LDSB	50.4	15.5	44	5.33	5.40	3.40	5.02
22	Immobilized LDSB	50.2	15.7	33	4.91	5.12	3.32	6.10

[a] Cheeses ripened at 18°C were evaluated until 15 days after production

Table 5 Residual sugar, ethanol, and acidity of unsalted hard-type cheeses.

Day of ripening	Ripening temp. (°C)	Lactose (g100 g^{-1})		Ethanol (g100 g^{-1})		pH		Acidity (lactic acid; g100 g^{-1})	
Rennet									
1	5,18, 22	1.52		0.02		6.55		0.09	
7→60	22	1.03→0.75		0.04→0.21		5.90→6.05		0.58→0.41	
7→15	18	1.13→1.10		0.03→0.02		6.29→6.14		0.45→0.40	
7→60	5	1.43→1.05		0.02→0.14		6.45→5.99		0.47→0.22	
LDSB									
		F	IM	F	IM	F	IM	F	IM
1	5,18,22	0.69	0.60	0.09	0.04	6.30	5.98	0.14	0.23
7→60	22	*Traces*	*Traces*	0.11→0.05	0.09→0.04	5.55→5.16	5.43→5.25	0.36→0.40	0.34→0.39
7→15	18	*Traces*	*Traces*	0.18→0.06	0.08→0.10	5.90→5.28	5.56→5.09	0.27→0.38	0.34→0.41
7→60	5	*Traces*	*Traces*	0.11→0.04	0.06→0.03	5.95→5.37	5.77→5.21	0.27→0.37	0.29→0.39

F free, *IM* immobilized

The mean scores for taste and aroma of all tested cheeses were similar (p=0.05; Table 4). Higher scores were achieved for cheeses made with starter compared to rennet cheeses at a specific ripening temperature. Finally, the textural characteristics including development of holes (size and shape) were affected (p=0.05) by the ripening temperature (lower scores at 22°C) and the use of starter. In total, the best scores of sensory evaluations were achieved in the case of cheeses made with free cells of LDSB with the exception of openness degree that was improved by the immobilization technique. Finally, the shelf life of cheeses was affected by the addition of starter but not by the ripening temperature (p= 0.05; Table 4). Rennet cheeses had the shortest shelf life and cheeses made with free cells of LDSB and ripened at 22°C had the highest (44 days). Recently, freeze-dried and thermally dried kefir culture were also found suitable as starters for whey cheese and soft and hard-type cheese making affecting positively both quality and shelf life [29, 30].

Conclusions

The thermal (convective) drying of LDSB cell suspensions in milk whey in the temperature range 35–55°C led to removal of most of the moisture content without affecting significantly the viability of cells. The choice of drying method should also take into account the overall process costs (milder conditions in shorter time frames). The dried cultures were found efficient for lactic acid fermentation of whey, although low conversion rates and high residual sugar where observed since the tested time periods were small. The low conversion rates should be attributed to the high amounts of initial sugar and high volumes of fermented media compared to the small amounts of inocula used, since survival rates after drying were very high, and the fermentation kinetics in all cases were comparable to those of the non-dried LDSB cells (Table 2). The high retention of cell viability may indicate a protective effect of whey during drying. The dried LDSB cells were able to affect the qualitative composition of volatiles in the fermented substrates indicating potential effect on the flavor of whey-based fermented foods or beverages. Cheeses made with dried LDSB presented good quality and increased shelf life compared to rennet cheeses. Specifically, unsalted hard-type cheeses produced using dried LDSB can be readily accepted by consumers as healthier products. Thermal drying is a much simpler and less cost demanding method compared to freeze-drying. Therefore, the possibility to produce dry active starters using simple, mild, and cost-effective drying methods is of great interest to food technology.

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References

1. Steinkraus, K. H. (1994). *Food Research International, 27*, 259–267.
2. Ammor, M. S., & Mayo, B. (2007). *Meat Science, 76*, 138–146.
3. De Vuyst, L., & Neysens, P. (2005). *Trends in Food Science & Technology, 16*, 43–56.
4. Ross, R. P., Stanton, C., Hill, C., Fitzgerald, G. F., & Coffey, A. (2000). *Trends in Food Science & Technology, 11*, 96–104.
5. Santivarangkna, C., Kulozik, U., & Foerst, P. (2007). *Biotechnology Progress, 23*, 302–315.

6. Morgan, C. A., Herman, N., White, P. A., & Vesey, G. (2006). *Journal of Microbiological Methods, 66*, 183–193.
7. Linders, L. J. M., Kets, E. P. W., de Bont, J. A. M., & Van't Riet, K. (1998). *Biotechnology Progress, 14*, 537–539.
8. Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., & Gibbs, P. (2004). *Biotechnology Progress, 20*, 248–254.
9. Shene, C., & Bravo, S. (2007). *Enzyme and Microbial Technology, 40*, 1578–1584.
10. Tymczyszyn, E. E., Gomez-Zavaglia, A., & Disalvo, E. A. (2007). *Journal of Applied Microbiology, 102*, 845–851.
11. Huang, L., Lu, Z., Yuan, Y., Lü, F., & Bie, X. (2006). *Journal of Industrial Microbiology & Biotechnology, 33*, 55–61.
12. Silva, J., Carvalho, A. S., Ferreira, R., Vitorino, R., Amado, F., Domingues, P., et al. (2005). *Journal of Applied Microbiology, 98*, 775–782.
13. Plessas, S., Pherson, L., Bekatorou, A., Nigam, P., & Koutinas, A. A. (2005). *Food Chemistry, 93*, 585–589.
14. Plessas, S., Trantallidi, M., Bekatorou, A., Kanellaki, A., Nigam, P., & Koutinas, A. A. (2007). *Food Chemistry, 105*, 187–194.
15. Kourkoutas, Y., Kandylis, P., Panas, P., Dooley, J. S. G., Nigam, P., & Koutinas, A. A. (2006). *Applied and Environmental Microbiology, 72*, 6124–6135.
16. Katechaki, E., Panas, P., Kandilogiannakis, L., Rapti, K., & Koutinas, A. A. (2008). *Journal of Agricultural and Food Chemistry, 56*, 5316–5323.
17. Dimitrellou, D., Kourkoutas, Y., Banat, I. M., Marchant, R., & Koutinas, A. A. (2007). *Journal of Applied Microbiology, 103*, 1170–1183.
18. Koutinas, A. A., Athanasiadis, I., Bekatorou, A., Iconomopoulou, M., & Blekas, G. (2005). *Biotechnology and Bioengineering, 89*, 788–796.
19. Giorno, L., Chojnacka, K., Donato, L., & Drioli, E. (2002). *Industrial & Engineering Chemistry Research, 41*, 433–440.
20. Gandhi, D. N., Patel, R. S., Wadhwa, B. K., Neena, B., Manjeet, K., & Ganesh-Kumar, C. (2000). *J Food Sci Tech Mys, 37*, 292–295.
21. Ashar, M. N., & Chand, R. (2004). *Milchwissenschaft, 59*, 14–17.
22. Bury, D., Hajsmanova, M., & Jelen, P. (1999). *Milchwissenschaft, 54*, 610–612.
23. AOAC. (1995). Chapter 33. Dairy products. Method 947.05. In: AOAC (Eds.) *Acidity in milk. Official methods of analysis of AOAC International, 16th Edition, vol. II*.
24. Koutinas, A. A., Papapostolou, H., Dimitrellou, D., Kopsahelis, N., Katechaki, E., Bekatorou, A., et al. (2009). *Biores Technol, 100*, 3734–3739.
25. Tsaousi, K., Koutinas, A. A., Bekatorou, A., & Loukatos, P. (2009). *Applied biochemistry and biotechnology*, In press. doi:10.1007/s12010-009-8848-9.
26. Dimitrellou, D., Kandylis, P., & Kourkoutas, Y. (2009). Koutinas, Kanellaki, M. *Food Chemistry, 115*, 691–696.
27. Papapostolou, H., Bosnea, L. A., Koutinas, A. A., & Kanellaki, M. (2008). *Biores Technol, 99*, 6949–6956.
28. Bucio, A., Hartemink, R., Schrama, J. W., & Rombouts, F. (2005). *Journal of General and Applied Microbiology, 51*, 221–227.
29. Dimitrellou, D., Kourkoutas, Y., Koutinas, A. A., & Kanellaki, M. (2009). *Food Microbiology, 26*, 809–820.
30. Katechaki, E., Panas, P., Kourkoutas, Y., Koliopoulos, D., & Koutinas, A. A. (2009). *Biores Technol, 100*, 3618–3624.