Bioresource Technology 100 (2009) 3734–3739

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Whey valorisation: A complete and novel technology development for dairy industry starter culture production

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ARTICLE INFO

Article history:
Received 15 September 2008
Received in revised form 29 January 2009
Accepted 31 January 2009
Available online 28 February 2009

Keywords:
Whey
Starter cultures
Dairy
Drying

ABSTRACT

Whey is the major by-product of the dairy industry, produced in large quantities and usually disposed off causing major environmental pollution, due to its high organic load that makes treatment cost prohibitive. This paper comprises a contribution on the valorisation of this high polluting liquid waste of the dairy industry, based on research for the production of novel dairy starter cultures using whey as raw material. Starter cultures are used for cheese ripening in order to: (i) accelerate ripening, (ii) improve quality and (iii) increase shelf-life. The developed technology involves biomass production from whey followed by thermal drying of cultures. Specifically, *Kluyveromyces marxianus, Lactobacillus bulgaricus* and kefir yeasts were thermally dried, and their efficiency in lactose and milk whey fermentations was studied. The most suitable culture regarding its technological properties was kefir, which was used for cheese ripening in freeze-dried and thermally dried form. Besides the reduction of production cost, which is an essential requirement for the food industry, the use of thermally dried kefir displayed several other advantages such as acceleration of ripening, increase of shelf-life, and improvement of hard-type cheese quality.

1. Introduction

Whey is the major by-product of the dairy industry and its disposal without expensive sewage treatments represents a major source of environmental pollution due to the bulk quantities and its high organic load. Considerable efforts have been made over the past years to explore new outlets for whey utilization and reduce environmental pollution (González-Martínez et al., 2002). Lactose, the major component of whey solids, contributes to its high biochemical and chemical oxygen demand (BOD, COD). The lactose carbohydrate reservoir of whey and the presence of other nutrients essential for microbial growth, make whey a potential raw material for the production of various bio-products through biotechnological means (Panesar et al., 2007). Several methods have been proposed for whey valorisation (Porro et al., 1992; Rech et al., 1999; Barinotto and Benedet, 2000; Dominguez et al., 2000; Zohri, 2000; Domingues et al., 2001; Ferrari et al., 2001; Gomes et al., 2003; Domingues et al., 2003). In this respect, lactose converting microorganisms have been evaluated for the production of potable and fuel-grade alcohol (Athanasiadis et al.,1999, 2001, 2002; Kourkoutas et al., 2002a,b; Petsas et al., 2002), kefir-like whey drinks (Paraskevopoulou et al., 2003), lactic acid (Elezi et al., 2003; Kourkoutas et al., 2005), baker's yeast (Harta et al., 2004; Plessas et al., 2004; Plessas et al., 2007a,b), single cell protein (SCP) as livestock feed (Plessas et al., 2008), probiotic starter cultures for fermented milk products (Kourkoutas et al., 2005) and cheese ripening (Kourkoutas et al., 2006; Dimitrellou et al., 2007; Papavasiliou et al., 2008). The above research efforts for whey valorisation resulted in significant organic load reduction and consequently a scale-up process was developed for potable and fuel-grade alcohol production employing whey in a 12000 L multi stage fixed bed tower (MFBT) bioreactor system (Koutinas et al., 2007). In addition, a second scale-up process for SCP and baker's yeast production from whey was also developed in a 3000 L semi-industrial scale bioreactor (Koutinas et al., 2005). An economic evaluation of these industrial scale microbial growth efforts employing whey is available (Kourkoutas et al., 2007).

In the frame of extensive efforts to reduce starter culture and bakers yeast production costs, and valorisation of the majority of global whey production, research on microbial thermal drying techniques was organized. The aim of this study was to develop an integrated technology for starter culture production from whey for use in cheese ripening.

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0960-8524/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2009.01.058

2. Rationale

The development of this environmentally friendly technology aimed to the valorisation of the total whey production through the production of a large number of products such as bulk chemicals (potable and fuel-grade alcohol) and various other foodstuffs of high nutritional and added value (baker's yeast, protein enriched animal feedstock, whey drinks simulating kefir, and starter cultures for cheese ripening or as probiotic food additives). The production of potable and fuel-grade alcohol and kefir-like whey drink was based on the following features: (i) alcoholic fermentation of whey lactose by kefir yeasts promoted by black raisin extracts (Athanasiadis et al., 2002), (ii) formation of kefir biomass in granular form, (iii) immobilization of kefir cells on delignified cellulosic materials, and (iv) the development of the MFBT bioreactor, suitable for both batch and continuous fermentation.

In order to develop a technology for low cost production of starter cultures for cheese ripening, the strategy adopted was to: (a) successfully produce biomass using whey, (b) examine fermentation ability of dried biomass, (c) select the most suitable lactose converting microorganism, and (d) evaluate the dried biomass of the selected microorganism as starter in cheese production. The successfully developed technology is presented below.

Starter cultures were produced through propagation of *Kluyveromyces marxianus, Lactobacillus bulgaricus* and the mixed kefir culture in whey, and for use at industrial scale lactose conversion processes, a feasible and low cost preservation method (e.g., drying), was necessary. In order to reduce investment and production costs of starter culture drying; a low temperature thermal drying process was examined. The thermally dried microorganisms were evaluated for fermentation activity on both lactose containing synthetic media and whey, and in regard to potential problems that may arise during SCP production (e.g., harvesting). Specifically, in the case of kefir the formation granular biomass led to easy collection of biomass by precipitation, avoiding the use of high cost centrifugal separators. Finally, freeze-dried and thermally dried starter cultures were evaluated as starters for cheese ripening.

3. Biomass production, thermal drying and selection of microorganisms

The thermotolerant strain of *K. marxianus* was used due its capability to tolerate relatively high temperatures in order to facilitate the development of a thermal drying process. The biomass production was performed according to Papapostolou et al. (2007). Fig. 1A illustrates that convective drying at 35 °C was effective without any significant differences in viability and fermentation activity compared to cultures dried at 55 °C (Fig. 1B).

The suitability of kefir yeasts, including non thermotolerant strains, for thermal drying at lower temperatures was also

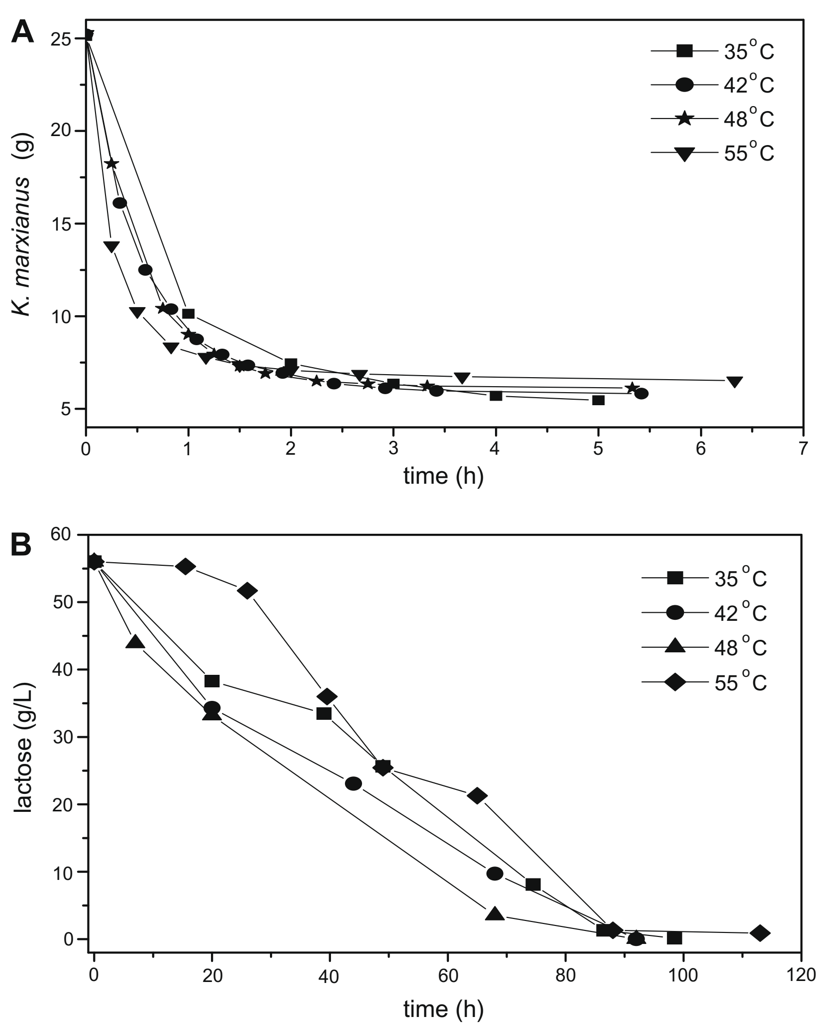

Fig. 1. (A) Kinetics of convective drying of *K. marxianus* at various temperatures. (B) Kinetics of fermentations of whey at 33 °C using convectively dried *K. marxianus* at various temperatures (Papapostolou et al., 2007).

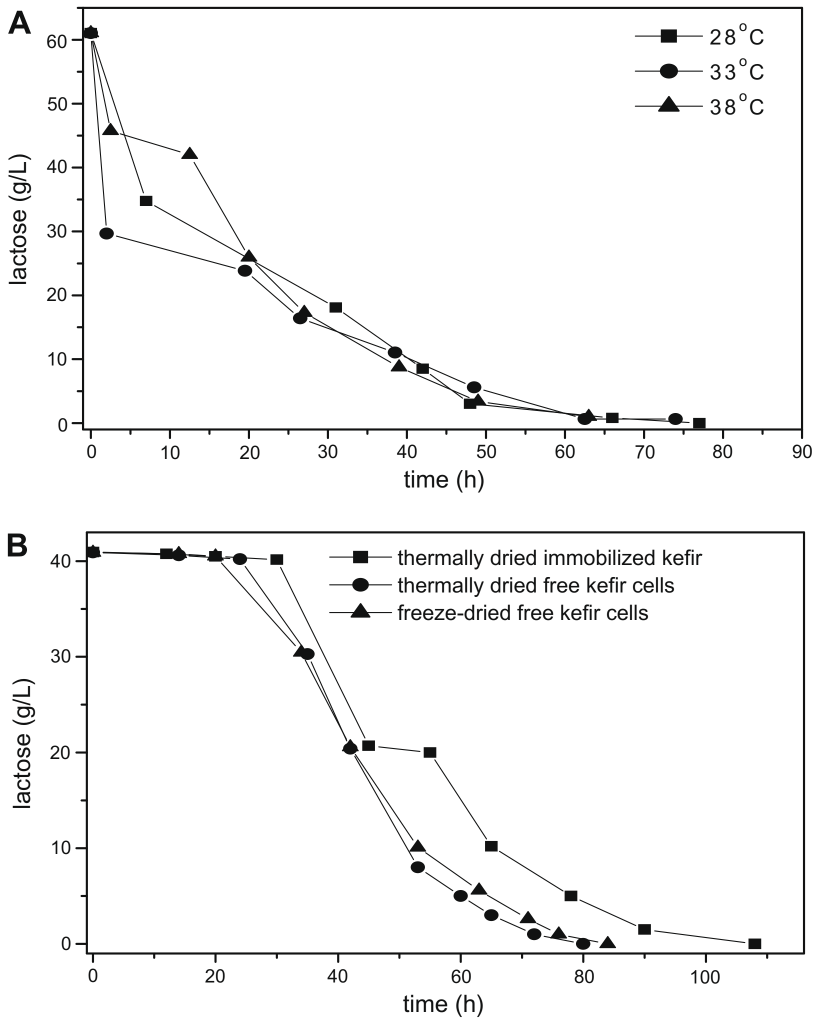

Fig. 2. (A) Fermentation kinetics of whey by convectively dried kefir at 28 (♦), 33 (■) and 38 °C (▲). (B) Kinetics of whey fermentation by thermally dried and freeze-dried free kefir cells, and thermally dried kefir immobilized on casein (Papapostolou et al., 2008; Dimitrellou et al., 2008).

examined. The biomass production was performed according to Dimitrellou et al. (2008). Thermal drying at low temperatures was successful and kefir maintained its fermentation activity in whey without significant differences among cells dried at different temperatures (28, 33, 38 °C) (Fig. 2A). For comparison reasons, fermentations of whey with thermally dried kefir cells free and immobilized on casein coagulates, were performed. Fig. 2B shows that the fermentation kinetics for the 1st fermentation batch of thermally dried or freeze-dried free kefir cells, were better compared to those of thermally dried kefir immobilised on casein. This could be attributed to a possible hindrance of re-hydration of the dried immobilized cells by the immobilization matrix. However, after the 1st batch, the fermentation kinetics were equated in all cases. In addition, an improvement of fermentation time was observed, probably due to adaptation of the thermally dried kefir cells in the fermentation conditions (Dimitrellou et al., 2008). This result encouraged the authors to conduct research on applying thermal drying on other microorganisms as well.

Lactobacillus bulgaricus was the 3rd microorganism evaluated for its fermentation efficiency after thermal drying. Biomass production was performed according to Kopsahelis et al. (2007). Several temperatures (37–55 °C) were studied for thermal drying of *L. bulgaricus* and fermentation efficiency was evaluated. Fermentation efficiency was similar in all studied temperatures proving that thermal drying up to 55 °C was effective (data not shown). However, thermal drying at 37 °C was selected as the most cost effective. Furthermore, *L. bulgaricus* was immobilized on apple pieces, freeze-dried and thermally dried and the dried biocatalyst was examined for its fermentation efficiency. The immobilized biocatalyst led to high lactic acid concentrations at all the studied fermentation temperatures (37–50 °C), which indicates high fermentation efficiency. Moreover, the biocatalyst retained operational stability for 12 repeated fermentation batches (Table 1),

Table 1
Kinetics of repeated batch fermentations of whey at various temperatures, using wet, freeze-dried and thermally dried cells of *L. delbrueckii* subsp. *bulgaricus* immobilized on apple pieces.

Biocatalyst	Fermentation temperature (°C)	Batch	Fermentation time (h)	Lactic acid concentration (g/L)	Conversion (%)
Wet	37	1–4	52.00 ± 7.12	42.70 ± 3.37	95.83 ± 3.07
	45	5–8	65.75 ± 11.30	42.83 ± 1.92	80.45 ± 3.98
	50	9–12	92.50 ± 12.34	28.18 ± 4.79	60.73 ± 12.54
Freeze-dried	37	1–4	52.50 ± 11.70	45.63 ± 3.14	99.55 ± 0.37
	45	5–8	64.75 ± 11.90	40.70 ± 2.83	80.35 ± 10.20
	50	9–12	92.50 ± 12.34	26.35 ± 2.80	59.50 ± 9.89
Thermally dried	37	1–4	51.25 ± 10.50	44.05 ± 2.36	99.85 ± 0.19
	45	5–8	65.75 ± 11.30	36.35 ± 6.76	72.38 ± 10.11
	50	9–12	92.25 ± 12.18	25.50 ± 5.00	43.08 ± 9.40

Table 2
Impact of freeze-dried kefir, free and immobilized on casein, on physicochemical characteristics of hard-type cheeses during ripening at 18 °C.

Cheese sample	Ripening time days	Lactose (g/100 g)	Glucose (g/100 g)	Galactose (g/100 g)	Ethanol (g/100 g)	pH	Acidity (lactic acid) (g/100 g)
Rennet cheese	0	1.84	-	-	0.02	6.58	0.11
	15	0.98	-	-	0.03	5.70	0.41
	30	0.45	-	-	0.01	5.26	0.52
	60	0.41	-	-	-	5.45	0.29
	90	0.39	-	-	-	5.46	0.25
1.0 g/L kefir	0	0.65	0.77	0.60	0.01	6.53	0.16
	15	-	-	-	0.01	5.01	0.76
	30	-	-	-	0.02	5.11	0.68
	60	-	-	-	0.01	5.27	0.50
	90	-	-	-	-	5.13	0.63
1.0 g/L immobilized kefir	0	1.48	0.51	0.47	0.01	5.87	0.34
	15	-	-	-	0.01	5.11	0.70
	30	-	-	-	-	5.25	0.55
	60	-	-	-	0.01	5.26	0.54
	90	-	-	-	-	5.19	0.61

(-): traces.

and therefore can be used as starter in various food processing or as additive to increase preservation time by pH reduction. Immobilized cells were found more efficient compared to free *L. bulgaricus* cells (Kopsahelis et al., 2007).

The above results indicate that *K. marxianus,* kefir yeasts and *L. bulgaricus* thermally dried by this low cost mild drying process, remained active and capable for whey fermentation. However, the use of kefir seems to be more profitable, due to its ability to form granular biomass during growth in whey, which facilitates separation from the fermented liquid, and due to its better productivity in general. In addition, kefir contains yeasts like *K. marxianus* and *Saccharomyces cerevisiae* that can lead to rapid hole formation during cheese ripening, which is desired for some types of cheeses. Lactic acid bacteria that reduce pH and produce metabolites capable to increase cheese shelf-life are also present in the mixed kefir culture.

4. Cheese ripening by starter cultures produced in whey

In order to evaluate the use of thermally dried kefir as starter culture for cheese production, experiments with thermally dried kefir, free and immobilized on casein, were carried out. Having in mind that freeze-drying still remains the most popular method in microbiology due to conveniences it offers in shipping and handling, preservation of cell integrity and viability etc., comparative experiments using freeze-dried kefir, free and immobilized on casein, were also performed.

4.1. Freeze-dried kefir starter culture

In order to study the effect of freeze-dried cells on hard-type cheese production, the physicochemical characteristics, gas hole formation, shelf-life and spoilage were examined. Lactose was consumed during the first week of ripening in cheeses where freeze-dried starter culture was added, while in rennet cheeses lactose was present up to the last day of ripening (Table 2). Furthermore, freeze-dried kefir increased the acidity and reduced pH thus improving the shelf-life of hard-type cheeses. Free cells exhibited improved results compared to cells immobilized on casein, because the immobilization matrix contained lower cell populations (approximately 10% kefir) (Katechaki et al., 2008). Extended shelf-life, better taste, aroma and textural characteristics were obtained when starter culture was added at a concentration of 1g/L milk (Table 3). Gas hole formation is an important parameter in hard cheese production because it provides an indication of cheese ripening. Hole formation during hard cheese ripening is considered desirable by the dairy manufacturers depending on the type of cheese. The increased hole formation can be attributed to the accelerated lactose consumption and is related to the increase of shelf-life and improvement of taste and aroma. The results showed that hole formation and shelf-life were improved when starter culture was added at a concentration up to 1 g/L milk, while these features as well as sensory characteristics where downgraded with the addition of 2 g of starter/L milk as shown in Table 3 (Katechaki et al. 2008). These results were promising for the application of thermally dried starter cultures in cheese production.

4.2. Thermally dried kefir starter culture

Unsalted hard-type cheeses were produced using thermally dried kefir, free and immobilized on casein, and were compared with rennet chesses. Rapid pH decrease and increase of acidity were obtained during ripening of cheeses made with addition of thermally dried starter in both free and immobilized form. Cheeses made with thermally dried kefir had better organoleptic properties (aroma, taste, suitable hole formation, etc.) and extended shelf-life. The effect of cell immobilization, the amount of starter, the evolution of microbial counts, and the sensory properties of cheeses were evaluated during different stages of ripening at various temperatures. The best results were obtained when 1 g/L of starter culture was used in unsalted hard-type cheese making (Tables 4 and 5).

Furthermore, the effect of thermally dried kefir on dry whey cheese production was studied (Table 6). Whey cheese was studied due to its faster contamination in comparison with other cheeses. Whey cheese left to mature without addition of starter culture, had an increasing microbiological load of pathogens, such as coliforms, enterobacteria and staphylococci, as the ripening proceeded. The addition of thermally dried starter culture, in free cell form or immobilized on casein, resulted to a drop of microbial counts in the same ripening time (coliform decrease was up to 35%).

Table 3
Effect of freeze-dried kefir, free and immobilized on casein, on the sensory characteristics, gas hole formation and shelf-life of hard-type cheeses after a 90-day ripening period at 18 °C.

Cheese sample	Shelf-life (days)	Taste	Aroma	Textural characteristics	Gas hole formation
		0–10	0–10	0–10	0–10
Rennet cheese	7	1.88	2.04	4.37	1.15
0.5 g/L kefir	28	6.00	6.14	6.05	6.51
1.0 g /L kefir	30	6.16	6.73	6.03	6.85
2.0 g/L kefir	15	5.06	5.43	5.96	6.20
1.0 g/L immobilized kefir	10	5.90	6.05	5.68	4.30

Table 4
Shelf-life and sensory characteristics of rennet cheeses and cheeses made with 0.5 or 1 g/L thermally dried kefir, free or immobilized on casein, and ripened at 5 and 22 °C.

Cheese sample	Ripening temperature (°C)	Shelf-life (days)	Taste 0–10	Aroma 0–10	Textural characteristics 0–10	Hole formation 0–10
Rennet cheese	5	28	3.16	3.42	5.78	1.10
0.5 g/L kefir	5	34	5.40	5.21	6.50	4.97
1.0 g/L kefir	5	47	6.34	5.81	6.73	6.10
1.0 g/L immobilized kefir	5	45	6.30	5.25	5.90	6.41
Rennet cheese	22	36	3.12	3.50	2.76	1.00
0.5 g/L kefir	22	47	5.36	5.32	3.42	4.36
1.0 g/L kefir	22	>60	6.40	6.04	3.58	6.05
1.0 g/L immobilized kefir	22	>60	6.32	5.55	3.06	6.50

Table 5
pH, acidity and microbial counts of rennet cheeses and cheeses made with 0.5 or 1 g/L thermally dried free kefir cells, or 1 g/L thermally dried kefir immobilized on casein, after 60 days of ripening at 5 and 22 °C.

Cheese sample		Temperature (°C)	pH	Acidity (lactic acid) (g/100 g)	Total aerobic counts (log CFU/g)	Yeasts and moulds (log CFU/g)	Lactococci (log CFU/g)	Lactobacilli (log CFU/g)
RC[a]	–	5	5.99	0.22	10.08	9.87	9.85	9.90
	–	22	6.05	0.41	9.90	9.69	9.55	9.53
TDKC[b]	0.5 g/L	5	5.63	0.57	9.50	9.37	9.31	9.39
	0.5 g/L	22	5.45	0.59	9.72	9.59	9.61	9.52
	1.0 g/L	5	5.71	0.57	9.62	9.39	9.28	9.22
	1.0 g/L	22	5.40	0.60	9.80	9.67	9.69	9.58
TDIKC[c]	1.0 g/L	5	5.33	0.23	9.70	9.65	9.59	9.53
	1.0 g/L	22	5.30	0.60	9.73	9.60	9.56	9.55

[a] Rennet Cheese.
[b] Thermally dried kefir starter culture.
[c] Thermally dried Immobilized kefir on casein starter culture.

Table 6
Effect of thermally-dried kefir immobilized on casein and ripening temperature on the microbiological characteristics of dry whey cheese.

Analysis	Ripening stage (days)	DWC[a]	DWCFDK[b]	DWCIDK[c]
		Ripening temperature		
		20 °C	20 °C	20 °C
Coliforms	1	4.73	4.78	4.80
	4	5.35	5.12	5.00
	15	6.15	6.08	5.80
	30	6.49	5.75	5.68
	45	5.90	4.80	4.58
	60	5.05	3.42	3.20
Enterobacteria	1	4.72	4.93	4.80
	4	5.45	5.05	5.05
	15	6.08	5.58	5.40
	30	6.49	5.50	5.30
	45	5.45	4.23	4.05
Staphylococci	1	4.30	4.18	4.00
	4	5.69	4.89	4.22
	15	5.78	5.14	4.28
	30	6.30	5.10	4.30
	45	5.72	4.05	3.35
	60	5.28	3.25	2.95

[a] Dry whey cheese with no starter culture.
[b] Dry whey cheese made with thermally dried free kefir cells.
[c] Dry whey cheese made with by thermally dried kefir immobilized on casein.

5. Technological consideration of results

The selection of kefir yeasts among lactose converting microorganisms was mainly based on its ability to form granular biomass during growth in whey, which can reduce the investment cost since no use of centrifugals separators is necessary. Furthermore, kefir is a mixed natural culture consisting of yeasts and lactic acid bacteria that facilitate hole formation and increase acidity, respectively. Gas hole formation is necessary in most hard-type cheeses while the reduction of pH leads to increase of their shelf-life. Furthermore, the shelf-life of cheeses may be improved by the potential production of metabolites with antimicrobial properties (e.g., bacteriocins) by lactic acid bacteria that are present in kefir microflora. The increase of shelf-life will make the production of unsalted cheese feasible, without the need for addition of chemical preservatives, thus improving nutritional value.

Thermal drying of biomass at 38 °C was considered an effective drying technique due to both good activity observed during whey fermentations and ripening of hard-type cheeses. Cell immobilization on casein also resulted to an effective thermally dried biocatalyst, yielding similar or better results compared to thermally dried free cells. Moreover, the use of immobilized cells will reduce biomass production cost and will contribute to controlled gas hole formation and distribution within the cheese matrix. The best results were obtained when 1 g of thermally dried biocatalyst/L milk was used in all cases. The main advantage of thermally dried kefir was the reduction of pathogens in cheeses during ripening. It should be stressed that thermal drying of biomass is feasible by supplying air of 38 °C on layers of biomass, spread on stainless steel horizontal plates. The air should pass through a sterile bacteriostatic filter, while the plates could be placed in an airtight closed room with stainless steel walls of $10 m^2$.

6. Conclusions

Kefir yeasts are more suitable for lactose conversion compared to conventional dairy *L. bulgaricus* and lactose converting *K. marxianus*. Thermal drying of kefir at 38 °C was an effective drying method. Addition of 1 g dried starter culture/L milk led to increased shelf-life of cheeses, better gas hole formation, and improved sensory characteristics. Furthermore, counts of coliforms, enterobacteria and staphylococci were reduced during ripening in the presence of the dried starter. It is therefore, concluded that the proposed technology offers the possibility for commercialisation of a process for the production and preservation of dried starter cultures for use in a series of dairy industrial applications, such as cheese production, leading to improved quality and nutritional value. However, further research and scale-up efforts for optimisation of the process are required.

Acknowledgements

This work has been performed within the framework of the Regional Operational Program (ROP) of Western Greece and was co-funded by the European Regional Development Fund and the Region of Western Greece with final beneficiary the Greek General Secretariat for Research and Technology.

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