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Scale-up of Thermally Dried Kefir Production as Starter Culture for Hard-Type Cheese Making: An Economic Evaluation

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Abstract This paper concerns the effect of thermal-drying methodology on the investment cost for dried kefir cells production in order to be used as starter culture in cheese manufacturing. Kefir cells were produced at pilot plant scale using a 250-L bioreactor and whey as the main substrate. Kefir cells were subsequently dried in a thermal dryer at 38 °C and used as a starter culture in industrial-scale production of hard-type cheeses. The use of thermally dried kefir as starter culture accelerated ripening of cheeses by increasing both lipolysis and fermentation rate as indicated by the ethanol, lactic acid, and glycerol formation. Additionally, it reduced coliforms and enterobacteria as ripening proceeded. This constituted the basis of developing an economic study in which industrial-scale production of thermally dried kefir starter culture is discussed. The industrial design involved a three-step process using three bioreactors of 100, 3,000, and 30,000 L for a plant capacity of 300 kg of thermally dried kefir culture per day. The cost of investment was estimated at 238,000 €, which is the 46% of the corresponding cost using freeze-drying methodology. Production cost was estimated at 4.9 €/kg of kefir biomass for a 300-kg/day plant capacity, which is the same as with the corresponding cost of freeze-dried cells. However, the estimated added value is up to 10.8×10^9 € within the European Union.

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Introduction

The last two decades of research interest on cheese production was mainly focused on the improvement of quality for a healthier product. Especially, the production of the popular hard-type cheeses has been widely investigated, and the results showed that the use of appropriate starter cultures plays a substantial role in cheese manufacture and quality [1]. The main role of starter cultures is the conversion of milk lactose to lactic acid, which is a substantial technological feature, affecting both flavor and preservation of the product. The flavor of the final product is also strongly dependent on the microbial associations of mixed starter cultures and wild microflora during cheese production and ripening [2–4]. A great variety of cultures consisting of *Bifidobacteria*, *Lactococci*, *Lactobacilli*, *Leuconostoc*, and *Enterococci* species have been proposed as novel starters in cheese production [5–10]. Among these, the natural mixed culture kefir has revealed great potential for use in a variety of cheese products, including hard-type, feta-type, and whey cheese, mainly due to its advantageous effects on quality, shelf life, and safety properties of the final products [11–13]. In the above studies, kefir culture was used in freeze-dried form and was found to increase the shelf-life of the final products and improve their sensory characteristics by altering the aroma profile and increasing the concentrations of esters, free fatty acids, alcohols, and carbonyl compounds.

In a recent study, the economic features of an industrial-scale production of freeze-dried kefir starter culture were presented, based on laboratory-scale results [14]. The conclusion was that the investment cost required for freeze-dried kefir was 4-fold higher than that of pressed wet kefir production, mainly due to the cost of the freeze-drying machinery. The production of various types of unsalted hard-type cheeses has also been recently reported, using thermally dried kefir, free or immobilized, in order to evaluate the possibility of application of thermal drying as a cost-effective drying process for starter cultures at commercial level [13]. These results were encouraging for further research to scale-up the process, and the present work presents an economic evaluation of a semi-industrial-scale thermally dried kefir production plant for use as starter culture for hard-type cheese ripening.

Materials and Methods

Kefir culture and Media The kefir culture used in this study was isolated from commercial Russian kefir and is available at the Department of Chemistry of the University of Patras [15]. The culture was routinely grown in lab scale at 30 °C in cheese whey supplemented with 1 g/L $(NH_4)_2SO_4$ and 1 g/L $NH_4H_2PO_4$. Kefir cells were harvested at the late-exponential phase by centrifugation at 5,000 rpm for 10 min at 20 °C and were further used for biomass production. Cheese whey was supplied by the dairy company AVIGAL S.A. (Elliniko, Farres, Achaia).

Preparation of Kefir Inoculants Kefir inocula used in the pilot plant operations were prepared through a four-step laboratory-scale process, which led to a total biomass of 300 g. The process involved aerobic growth of kefir cells in 2- and 5-L Plexiglas tower reactors at

30 °C. Air was supplied by an air pump equipped with a bacteriostatic filter. Initially, 10 g (wet weight) of kefir biomass was collected by centrifugation and transferred to a 2-L Plexiglas bioreactor, and 1.5 L of whey enriched with 1.6 g/L KH_2PO_4 and 7.5 g/L $(NH_4)_2SO_4$ was added. Aerobic fermentation was carried out at 30 °C. The pH was adjusted to 5.5 and kept constant during fermentation by addition of 10% *w*/*v* NaOH solution. The process was repeated twice, and the produced kefir was harvested and used to pitch a 5-L bioreactor containing 4 L of enriched whey. Two more batches were carried out until 300 g of kefir biomass were produced, which were used to inoculate the 250-L bioreactor that was installed at AVIGAL S.A.

Production of Kefir Biomass Kefir biomass at pilot scale was produced in a 250-L bioreactor made from stainless steel and equipped by an automatic cooling and heating system. Tap water or steam was supplied into the bioreactor's mantle to control the incubation temperature. Air was supplied through an industrial bacteriostatic filter and was spread into the bioreactor through a perforated pipe fitted 20 cm above the bioreactor bottom. Mixing of culture medium was done by a top entry impeller agitator assembly. The process was initiated by inoculating 50 L of enriched whey with 300 g of kefir cells. The pH was adjusted to 5.5 by the addition of Na_2CO_3 solution when necessary. The system was allowed to ferment for 3 days. At the end of each day, 50 L of fresh enriched whey was added in the bioreactor, until a final volume of 150 L. The fermentation kinetics were monitored by measuring the density (°Be) of the fermenting liquid at various time intervals, and samples were analyzed for produced biomass and residual sugar. The fermented liquid was allowed to stand until the granular kefir biomass was precipitated at the bottom of the bioreactor, the supernatant was then rejected, and the biomass was collected.

Thermal Drying of Kefir Biomass Thin layers of the produced wet kefir biomass were spread on stainless steel shelves in an industrial drying chamber where air stream of 38 °C was supplied. The drying process was monitored by weighing the culture at various intervals until a constant weight.

Cheese Manufacture Cheese making was performed at pilot scale, adopting the industrial technology of *Kefalograviera* (Greek hard cheese) production (dairy factory AVIGAL. S.A., Farres, Achaia). Bulk ewes' milk was standardized, by separation, to 6.0% fat. Thermally dried kefir was added to milk as starter culture, and after agitation, the liquid was allowed to stand at 33 °C for 30 min. Subsequently, commercial rennet enzyme was added at a concentration of 0.01% *w*/*w*, and the mixture was left undisturbed for 25 min at 33 °C for curd formation. The curd was then cut into 2.5–3.0-cm^3 cubes, allowed to rest for 2–3 min, and further cut into corn-sized pieces. It was then stirred very gently and slowly for 20 min. Curd particles and whey were gradually scalded from 33 to 48 °C over a period of 25 min with continuous stirring. Stirring was continued at 48 °C for 20 min in order to obtain curd particles of the desired firmness. The curd was then wrapped in a cheese cloth, placed into molds, and then pressed at 0.22 kg/cm^2 for 20 min. Afterward, the cheeses were removed from the molds, the cheese cloth was changed, and the cheeses were inverted and pressed again. After 60 min, the cheeses were similarly overturned and pressed for another 60 min. When pressing was completed, the cheese cloth was removed, and the cheeses were left in the mold overnight at 14–16 °C. The next day, the cheeses were salted in brine of 19°Be at 12 °C for 24 h. They were then transferred to a clean board where they remained for 10 days at 12 or 18 °C. During this time, the cheeses were inverted every second day, wiped with a dry cheese cloth, and rubbed with dry salt. Cheese ripening was completed at 18 °C.

Results and Discussion

Production of Thermally Dried Kefir Biomass Thermally dried kefir cells were used as starter culture for hard-type cheese production. The final products were characterized by longer preservation time, improved aroma, taste, and texture, and bigger size and number of

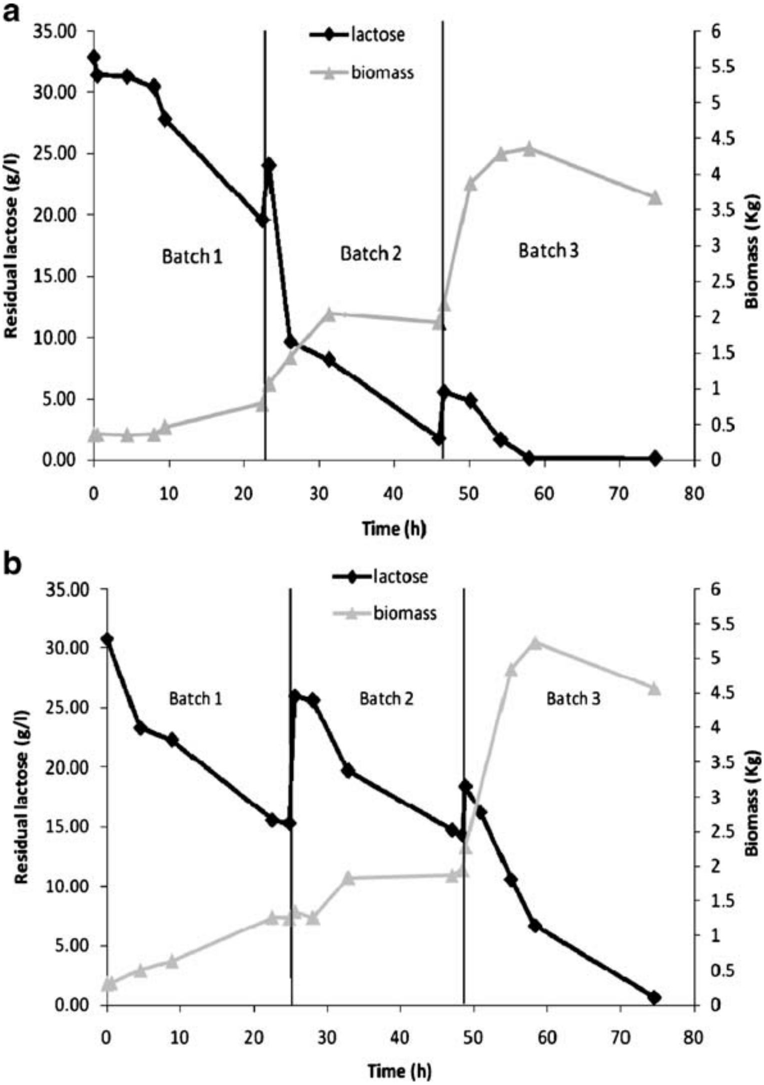

Fig. 1 Kinetics of agitated aerobic propagation of kefir in whey collected **a** after cheese making and **b** after whey cheese making

holes. Production of hard-type cheese at pilot scale involved the use of a 250-L bioreactor for aerobic cultivation of kefir cells and an industrial drying chamber for the thermal drying process. Whey was the main substrate used for kefir propagation, collected after the hard cheese making or the whey cheese-making process (further deproteinization) [12]. Both types of whey led to the production of more than 3.5 kg of kefir biomass after the third fermentation batch, when almost all lactose had been utilized (Fig. 1). Although the whey collected after whey cheese making had lower lactose concentration, it yielded kefir biomass levels as high as 4.5 kg after the third fermentation batch. This constitutes an important observation as it encourages exploitation of whey produced even after whey cheese making. The production of kefir starter culture was completed after cells were dried in a thermal dryer. While 86% of moisture was removed in 5 h, the drying process was completed after 22 h.

Production of Hard-Type Cheese The dried kefir culture was used for the production of hard-type cheese ripened either at 12 or at 18 °C. Chemical analysis of cheese products during ripening revealed higher rates of glycerol formation, especially in those ripened at 18 °C (Fig. 2). Lactic acid concentration was reduced in cheese ripened at 12 °C and slightly increased when cheese was ripened at 18 °C. Increased amounts of alcohol were produced in cheese ripened at 12 °C compared to cheese ripened at 18 °C, possibly due to ethanol evaporation at 18 °C.

The rapid increase of glycerol observed in cheeses ripened at 18 °C was an indication of the contribution of the starter to lipid hydrolysis and suggested early ripening. Acceleration of ripening could also be suggested for cheese ripened at 12 °C during the first 22 days but at lower levels. Lactic acid was also high during the first 2 days of ripening and remained relatively stable.

Microbial counts of cheese products showed a significant reduction of enterobacteria and coliforms during ripening at both 18 °C and 12 °C (Table 1). Decrease was also observed in counts of lactobacilli, lactococci, yeast, and molds. However, high numbers of staphylococci strains were observed at the start of the ripening process possibly due to cross-contamination by personnel.

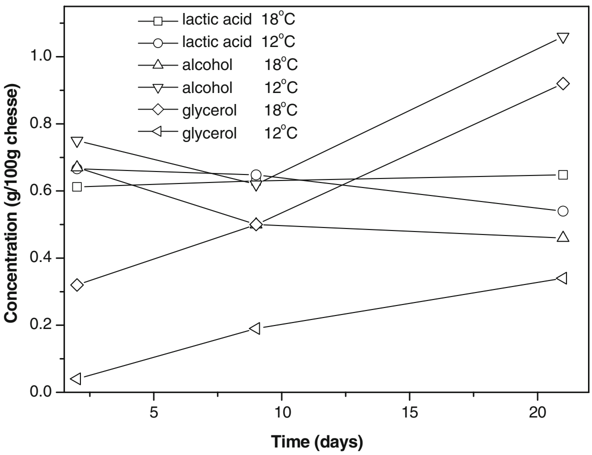

Fig. 2 Lactic acid, alcohol, and glycerol content of hard-type cheese made with 1.0 g/L thermally dried kefir during ripening at 18 and 12 °C

Table 1 Microbiological composition during ripening (log cfu/g cheese) of hard-type cheeses made using thermally dried kefir at industrial scale.

Ripening temperature	Ripening time (days)	Total aerobic counts	Yeasts/molds	Lactococci	Lactobacilli	Staphylococci	Coliforms	Enterobacteria
18°C	2	9.36	10.00	10.79	9.70	5.84	7.78	7.70
	9	8.60	7.70	8.96	8.95	5.25	6.48	6.48
	21	10.09	9.20	9.84	8.86	7.56	5.78	5.82
12°C	2	9.46	9.15	9.25	9.00	5.93	6.70	6.60
	9	8.78	8.00	8.77	8.88	5.60	6.78	7.00
	21	9.20	8.48	10.45	8.78	7.25	5.00	5.14

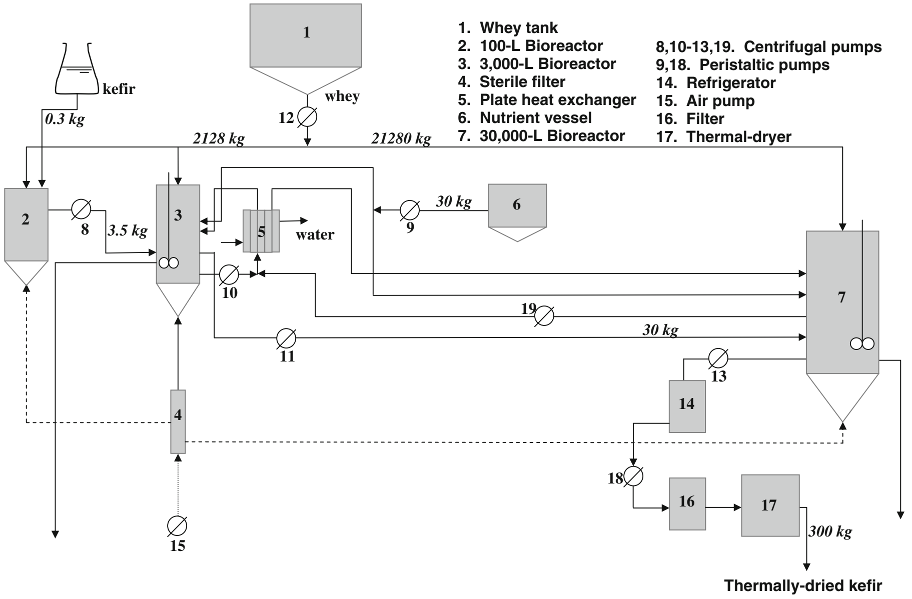

Fig. 3 Process flow sheet with mass balance (kg/day) for a production capacity of 300-kg/day (dry weight basis) thermally dried kefir starter culture

Table 2 Estimated investment cost of an industrial unit for a daily production of 300 kg thermally dried kefir using whey.

Machinery	Quantity	Cost (€)
Centrifugal pump (2 m^3/h)	5	5,000
Centrifugal pump (5 m^3/h)	1	1,500
Peristaltic pump (400 L/h)	2	14,000
Pipes	–	3,000
Plate heat exchanger	1	10,000
Sterile filter	1	3,000
Air pump	1	1,500
Whey tank (30,000 L)	1	5,000
Stainless steel bioreactor (100 L)	1	300
Stainless steel bioreactor (3,000 L)	1	5,000
Stainless steel bioreactor (30,000 L)	1	20,000
Refrigerator	1	10,000
Vacuum filter	1	30,000
Thermal dryer of 350 kg capacity	2	20,000
Engineering	–	50,000
Royalties	–	60,000
Total investment cost	–	238,300

Table 3 Estimated cost for a daily production of 300 kg thermally dried kefir using whey.

Parameter	Quantity	Production Cost (€)
Whey	23,408 L	–
Labor cost	20 workers	800
Consumables	30 kg $(NH_4)H_2PO_4$	30
Water requirements		6
Washing	20 m^3	–
Cooling	250 m^3	–
Steam 5 tn	oil 0.5 tn	250
Electricity		150
Liquidation of debt		170
Total daily cost		1,460

Proposed Process Sheet The proposed industrial flow diagram is illustrated in Fig. 3. Kefir biomass production is proposed to employ three bioreactors that would enable aerobic fermentations via an agitation and an air supply system. Bioreactors of 100, 3,000, and 30,000 L should be connected with a heat exchanger for cooling or heating of the fermenting medium as well as with an air pump for sterile air provision through an appropriate filter. Other equipment necessary for the completion of the process include centrifugal and peristaltic pumps, pipes, whey collection tank, refrigerator for preservation of the wet kefir biomass, vacuum filter, and a thermal dryer for drying of kefir biomass.

Investment Cost All calculations made to estimate the total investment cost were based on a production capacity of 300 kg/day of thermally dried kefir. For this capacity, a total mass of 23,408 kg of whey and an amount of 0.3 kg of wet kefir biomass for the initial inoculation of the 100-L bioreactor are needed (Fig. 3). Total cost was estimated according to the process flow and the mass balance at 238,300€ (Table 2). Investment cost is dependent on the plant capacity reaching levels up to 500,000€ for a 900-kg/day plant capacity and down to 87,000€ for a 30-kg/day capacity. Thus, the estimated cost per 100 kg of biomass for a 900-, 300-, and 30-kg/day plant capacity would be 55,555€, 79,433€, and 290,000€, respectively.

Production Cost The main expenses of thermally dried kefir biomass production were estimated to be mainly due to labor costs constituting 55% of the total production cost

Table 4 Cost estimation for wet, freeze-dried, and thermally dried kefir production.

Type of kefir culture	Investment cost (€)	Production cost (€/kg)	EU added value ($\times 10^9$ €)	WDC[a] added value ($\times 10^9$ €)
Wet kefir	127,300	1.4	–	–
Freeze-dried kefir	518,300	4.9	10.8	27.0
Thermally dried kefir	238,300	4.9	10.8	27.0

For a daily production of 300 kg kefir (dry wt)

[a] World Developed Countries

(Table 3). The rest was due to steam consumption, liquidation of debt, and electricity consumption. The main substrate for kefir production, whey, might have a negligible effect on the production cost if transportation expenses were not involved. At this basis, production costs were estimated at 4.9€/kg of kefir biomass.

Cost Comparison with Freeze-Dried and Wet Kefir Biomass The investment cost in a similar study focusing on the development of a plant for the production of freeze-dried kefir was found to be 4-fold higher than that of pressed wet kefir, mainly due to the cost of the freeze-drying machinery [14]. This investment cost could however be reduced to 46% for the production of thermally dried cells according to the plant proposed in this study, while the production cost was estimated at 4.9€/kg same as that of freeze-dried cells and 3.5-fold higher than that of wet biomass production (Table 4).

Technological Considerations Kefir culture has been shown to constitute a potent starter culture for producing a variety of cheese products, hard-type, feta-type, and whey cheese, mainly due to its effect on quality, shelf life, and safety properties of the final product [11–13]. This culture can be used either in freeze-dried or thermally dried form, facilitating its transport and storage, therefore commercialization.

According to this study, the production cost of thermally dried kefir culture was estimated at 4.9€/kg for a 300-kg/day plant with an investment cost of 218,300 €. This cost can however be further reduced if a 900-kg/day plant was considered. Given that the market price of freeze-dried baker's yeast is about 30€/kg, a high added value can be created, which is estimated to 10.8×10^9€ within the European Union and to 27.0×10^9€ for the World Developed Countries (Table 4). However, this low production cost was estimated on the basis of negligible transportation costs. On the other hand, the high added value can counterbalance transportation expenses leading to a profitable investment.

Conclusions

Thermal drying of kefir biomass by supplying air at 38 °C is feasible at industrial scale. Investment cost was estimated about 46% lower than that of freeze drying. The advantageous nature of kefir as starter in cheese manufacturing and the reduced investment and production costs of a kefir biomass production plant increase the potential for industrialization. Business risk could be further reduced by increasing the variety of commercial products that could be developed.

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