La Prensa Medica Argentina

Research Article

Milk Whey- From a Problematic Byproduct to a Source of Valuable Products for Health and Industry: An Overview from Biotechnology

Chanfrau JMP[1*], Pérez JN[1], Fiallos MVL[1], Leonor Rivera[2,3] Abril VH[4], Guerrero MJC[1] and Toledo LET[4*]

Abstract

Esmeraldas, Carchi, Ibarra and Sucumbíos are Ecuadorian provinces that produce daily more than 407 m^3 of milk. Almost a third of all this production is used to produce different types of cheeses, generating near to 122 m^3 of whey in the provinces of Carchi and Imbabura. An important part of whey is used in animal feed, but unfortunately, a vast amount is poured into rivers, streams, etc.; polluting natural sources of water. More stringent environmental regulations joined to producers' awareness, highlights the need to transform whey into less polluting effluents. If, as a result of this transformation, it is possible to obtain a range of new products with higher added value than the whey itself, the resources used in the conversion would be partially amortized. In the present review, some of the available technologies are explored from the biotechnology point of view to overcome this problem in the context of Zone 1 of Ecuador.

Keywords

Milk whey; Whey permeate; Single-cell protein; Lactic acid; Kefiran; Galactooligosaccharides

Introduction

Carchi, Imbabura, Esmeraldas and Sucumbíos, are Ecuadorian provinces in which agricultural and livestock activities constitute a significant part of the economy and jobs source of these territories. The majority of the population in these zones is dedicated to the production and commercialization of milk, being the Carchi province the most outstanding productive region, reaching 4.8% of the national milk production. A daily delivery of Ecuador is around 79.8 m^3 with a production value of about US $ 3.5 million per year [1].

After whole milk, the most consumed dairy products in Ecuador are fresh cheeses like creole, mozzarella, kneading and curds [2]. In eight years (2006-2014) the per capita consumption of cheese in Ecuador was doubled, from 0.75 kg per person per year in 2006 to 1.57 kg in 2014 [2]. Sales of the cheese industry increased 3.4 times between 2006 and 2016, from US$ 71.4 million to US$ 243.1 million [2].

About one-third of dairy production in the region is devoted to the cheese manufacture [2]. As a result of the production of different types of cheeses, milk whey (sweet (SW) or acid (AW) whey) is obtained. For every 100 kg of milk used to produce cheeses, 9.3 ± 0.7 kg of fresh cheese [3] and around 90.7 kg of SW/AW [4,5] are obtained. The SW/AW retains a large part of the nutrients contained in the milk so; biotechnology has proposed some possible ways to use this by-product [4,6-9] useful as animal food [10-12] and in biological treatment with sludge to produce organic fertilizers for soil improvement [13,14].

In this sense, due to its high lactose levels, the SW/AW is a significant pollutant with values of 30-50 kg m^{-3} of biological oxygen demand (BOD5) [15], and its dumping to lakes, rivers, and soils should be avoided.

One of the most attractive uses of SW/AW is the development of products based on whey proteins [6,16-19], providing a protein concentrate of excellent quality for human and animal consumption [16]. However, as a result of the whey protein isolation process, a whey permeates (PW) of milk with a high lactose content is obtained, and therefore with high pollutant load values [20,21]. Various uses have been proposed for PW, ranging from the production of unicellular microbial protein for animal feed [10,12], the production of organic acids [22,23], alcohols [24,25], also the production of probiotics [9] and different prebiotic substances [26-28].

The objective of this review is to explore various technical solutions from the biotechnology point of view that contributes to the use and valorization of SW/AW or PW encouraging the continued growth of the Dairy Industry in Ecuador.

Milk whey

A byproduct of cheese production, the main constraint to the growth of the dairy sector

According to Association of Livestock of the Sierra and East of Ecuador (AGSO), in 2017 an average of 5.4 million liters of milk was registered daily at the national level [29]. In the "Sierra" region, milk production reaches 77% of the total, whereas Imbabura and Carchi produce 7.4% of the national milk production, which represents more than 407 m^3 of whole milk per day. From those, near of 135.7 m^3 is destined to cheese production, and near of 122 m^3 of milk whey daily is generated in Carchi and Imbabura provinces.

Milk whey (SW/AW) represents 85-95% of the volume of milk and retains 55% of all the nutrients contained in milk [30,31]. SW/AW represents the main by-product and the highest contaminant in dairy production [15,32,33], reaching values of chemical oxygen demand (COD) and biochemical oxygen demand (BOD5) of 60-80 and 30-50 kg m^{-3}, respectively [34,35].

Globally, around 180-190 million metric tons (TM) of whey were produced in 2013 [36,37]. For this, 40% of this production is used in direct feed, as fertilizer, or discarded, while the rest is industrially transformed basically in the production of whey powder, lactose and whey protein concentrates as shown in Figure 1.

As Kosikowski rightly points out, "dispose of" is not the same thing that to "use" the milk whey [5]. Even today, significant amounts

***Corresponding author:** Luis E Trujillo Toledo, Industrial Biotechnology and Bio products Research Group, Center for Nanoscience and Nanotecnology, CENCINAT, Universidad de las Fuerzas Armadas (ESPE), Ave. Rumiñahui s/n. Quito, Pichincha, Ecuador, E-mail: letrujillo3@espe.edu.ec

Received: August 01, 2017 **Accepted:** August 12, 2017 **Published:** August 17, 2017

of SW/AW are discharged into rivers and streams or are sprayed directly into cultivated fields after dilution. The latter, in spite of the apparent advantages that could be observed, after long periods of shedding, the high salt content in the SW/AW tends to salinize the soils, thus diminishing agricultural yields.

Also, increasing awareness of these adverse effects is becoming increasingly prevalent, and states' environmental laws and regulations tend to prohibit such practices. As a consequence, small and medium-sized cheese producers could find it difficult to compete in the market with large companies, given the need to have adequate waste treatment plants to treat their whey effluent. This environmental and regulatory "pressure" would become a constraint to the continued growth of small and medium-sized cheese producers.

Different SW/AW uses have been published anywhere [4,7,8,38-41]. As stated above, SW is very popular for animal feeding due to the important amounts of lactose (4.5-5% (m/v), proteins (0.6-0.8% (m/v)), 0.5% (m/v)) and mineral salts (8-10% on dry basis) [28]. However, as shown in Figure 2, SW/AW has other important uses because it constitutes an important source of proteins isolation [6,16-18,42] one of the most promising SW/AW use nowadays. Several properties such as the high quality of these proteins and their beneficial effects on human and animal health [11,35,36] joined to the simplicity of the process to get them (Figure 3), reinforce the potential of this SW/AW application.

Membrane technology is the most used in SW/AW concentration to produce whey protein concentrates [7,16,20,21,40,43,44]. A typical process is based on successive ultrafiltration steps (UF) and reverses

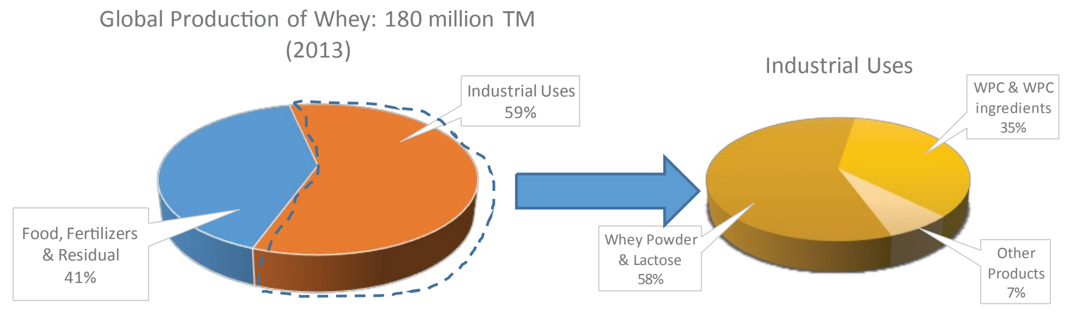

Figure 1: Global production of whey (2013) and its uses.

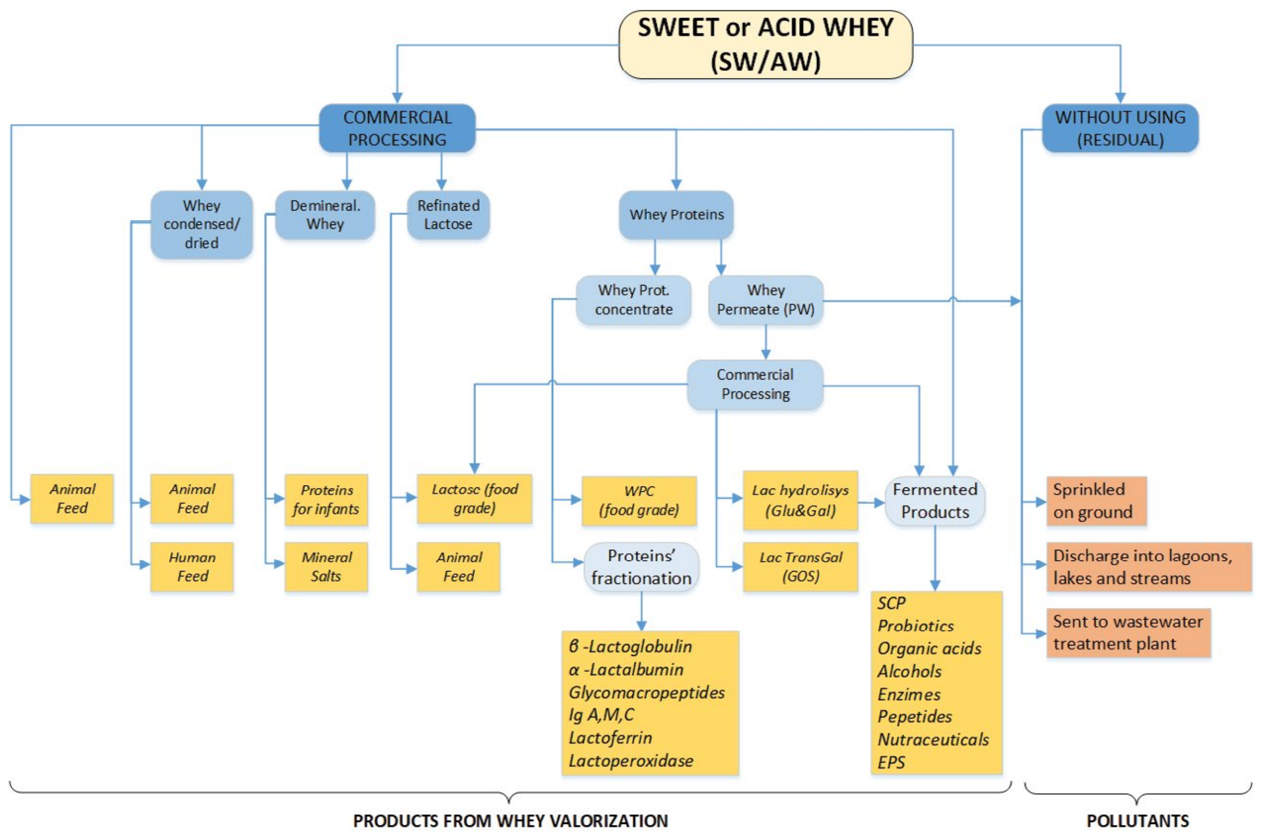

Figure 2: Some uses that may be done to milk whey [15].

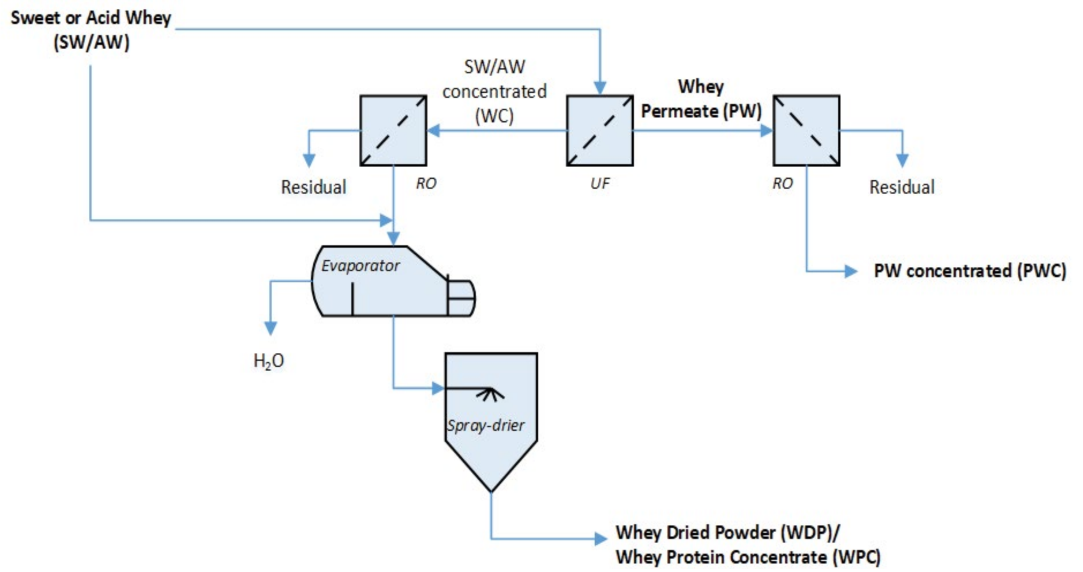

Figure 3: The typical membrane-based process to obtain whey protein concentrates or whey powder.

osmosis (RO), followed by concentration by evaporation and a final spray-drier step.

However, using this technology, a deproteinized milk whey permeate lactose-rich is obtained from the ultrafiltration effluent. This lactose –rich byproduct (3.9-4.8% (m/v)) also contains mineral salts, ash (0.3-0.8% (m/v)) and protein traces (0.1-0.3% (m/v)) (39), which gives BOD5 >30 kg m^{-3} [45] and therefore constitutes a high pollutant which must be treated or used as raw material for other processes. The main cause of its high BOD5 value is lactose, a disaccharide that has several uses in the chemical, food and pharmaceutical industries [46-48].

Microbial-based biotechnological processes to biotransform permeate of whey in valuable products

Despite that lactose excess present in SW/AW is considered as waste, an interesting group of products can be obtained by lactose biotransformation using certain lactose-degrading bacteria and yeast, to produce a broad range of bio products with higher income potentialities than that offered by the original whey [8,15,34,49-52]. By this way, not only can diminish the pollution load of the whey or its permeate but also can increase the business portfolio of dairy companies.

One of them is lactic acid ($C_3H_6O_3$, CAS L (+): 79-33-4) that has several applications in the food and cosmetic industry [53-56]. L(+) - lactic acid can be obtained from the biotransformation of lactose by Lactobacillus spp. strains, such as Lactobacillus casei [49,53,55,57-60]. This process can be performed by direct microbial transformation in submerged cultures [61-63], or by immobilizing the microbial cells and using a continuous culture in a packing-bed bioreactor with immobilized cells [22,55,64,65], as shown in Figure 4.

The conventional process consists briefly of a fermentation stage where the PW is supplemented with salts and vitamins to achieve the growth of the selected lactic-acid bacteria (LAB) strain (e.g., Lactobacillus casei), keeping the pH close to its optimal growth conditions [56,63]. Subsequently, the biomass obtained and the calcium lactate is separated and washed by differential centrifugation. Calcium lactate is then converted to lactic acid by the addition of sulfuric acid and further separated from the sparingly soluble calcium sulphate. Finally, lactic acid is concentrated by evaporation to about 50% (v/v) (Figure 4A). Another alternative procedure consists in in calcium alginate LAB cells immobilizing in a continuous enzymatic bioreactor to convert the lactate present in PW to lactic acid [22,55,64,66] (Figure 4B).

Also, the lyophilized biomass of *L. casei*, a recognized probiotic [67], whose intake gives benefits to human health [67-69], could be obtained by a similar process.

Lactose present in SW/AW, PW or PWC could also be used to obtain microbial single-cell protein (SCP), using, for example, the recognized as safe (GRAS status) yeast Kluyveromyces marxianus [10,11]. This yeast can metabolize lactose and reach high cell densities and also is capable of producing significant levels of ethanol [30,70-72], widely used throughout the industry.

A proposed process flow scheme for the production of single-cell protein (SCP) and ethanol from SW/AW, PW or PWC and K. marxianus is shown in Figure 5. Briefly, the process consists of a continuous or semi-continuous fermentation step where the lactose consuming yeast, for example, K. marxianus, growth and secretes ethanol to medium. Subsequently, after thermal inactivation of the microorganism in the culture, the biomass is separated and washed from the supernatant. Finally, the biomass is spray dried, while the ethyl alcohol (technical grade) obtained from the broth supernatant can be recovered by atmospheric distillation [24,72-76].

Other organic acids, such as propionic acid [23,77,78], butyric acid [79] and citric acid [80] have also been produced by the microbial biotransformation of whey.

Similarly, other alcohols such as butanol have been bio synthesized from certain microbial strains and whey [25,81,82].

Other valuable chemicals derived from whey can be obtained by microbial fermentation such as hydrogen [83-86], polyhydroxyalkanoates [87-89], lipids [90-92], ribonucleotides [93,94], exopolysaccharides [95-97], etc.

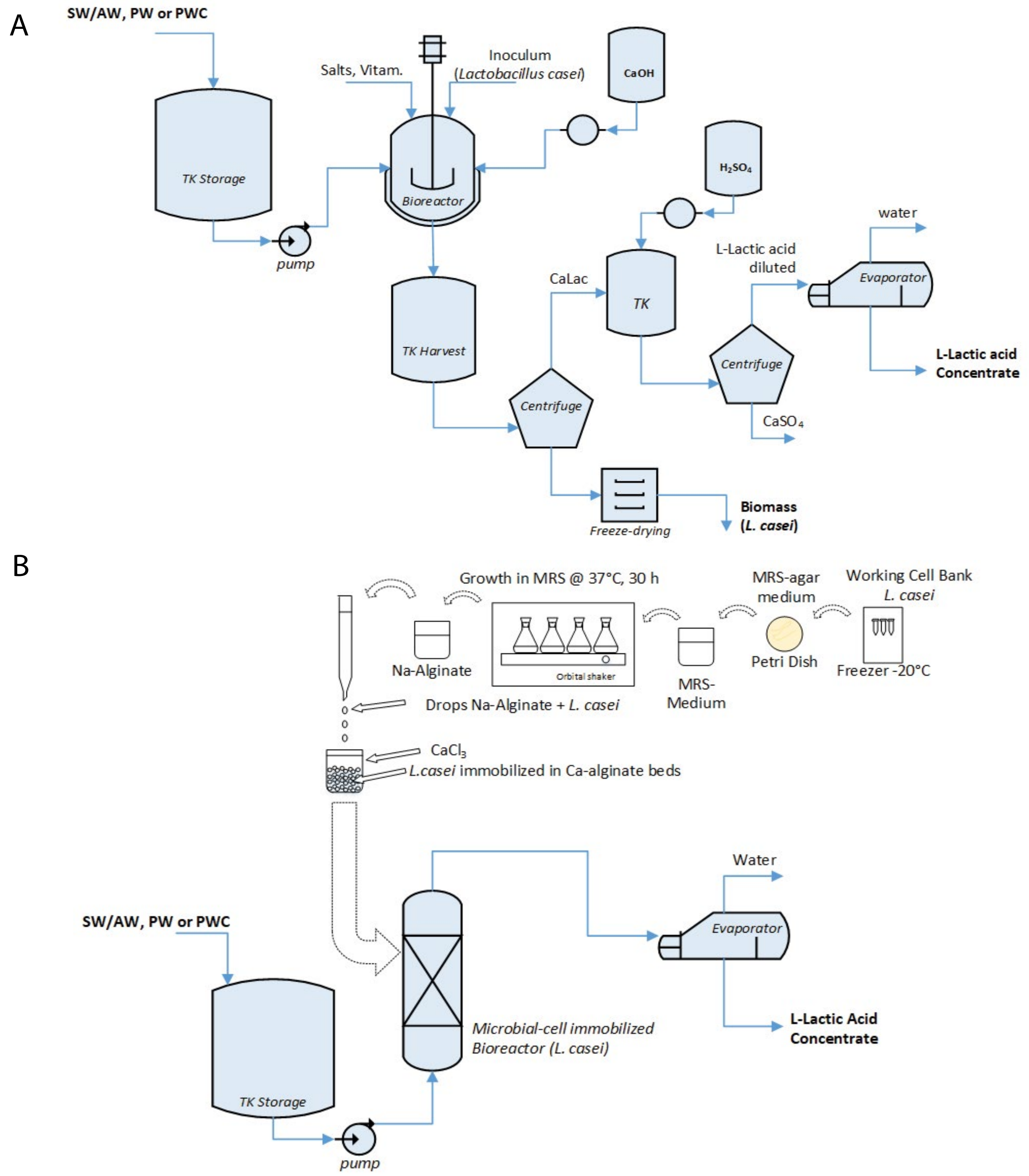

Figure 4: Lactic acid production scheme from SW/AW, PW, or PWC by (A) submerged microbial fermentation; (B) immobilized microbial-cells.

Finally, a consortium made up by lactic acid bacteria (LAB) and yeasts, which cohabit within a polymer called kefiran, and which is called a kefir granule, has been used since ancient times to produce a fermented milk called kefir of high nutritional values [98,99] beneficial for human health.

Kefir granules could be used to biotransform the SW/AW, PW or PWC, rich in lactose, to produce kefiran, an edible biopolymer, consisting of units of glucose and galactose in approximately equal proportions [100,101]. Recent studies [102-104] have demonstrated several health-beneficial properties of this biopolymer that make it attractive in drug formulation [104-107] and food preservation [108]. Due to these interesting properties, this edible and biodegradable biopolymer has attracted the researcher's attention and so, the design of different production process [109]. A proposed process scheme for kefiran production from SW/AW, PW or PWC and kefir granules is shown in Figure 6. Briefly, the process consists in the biotransformation of the lactose present in the PW or PWC, by the existing consortium LAB in the kefir granule. After 48 h fermentation, the culture is homogenized and pasteurized to inactivate the

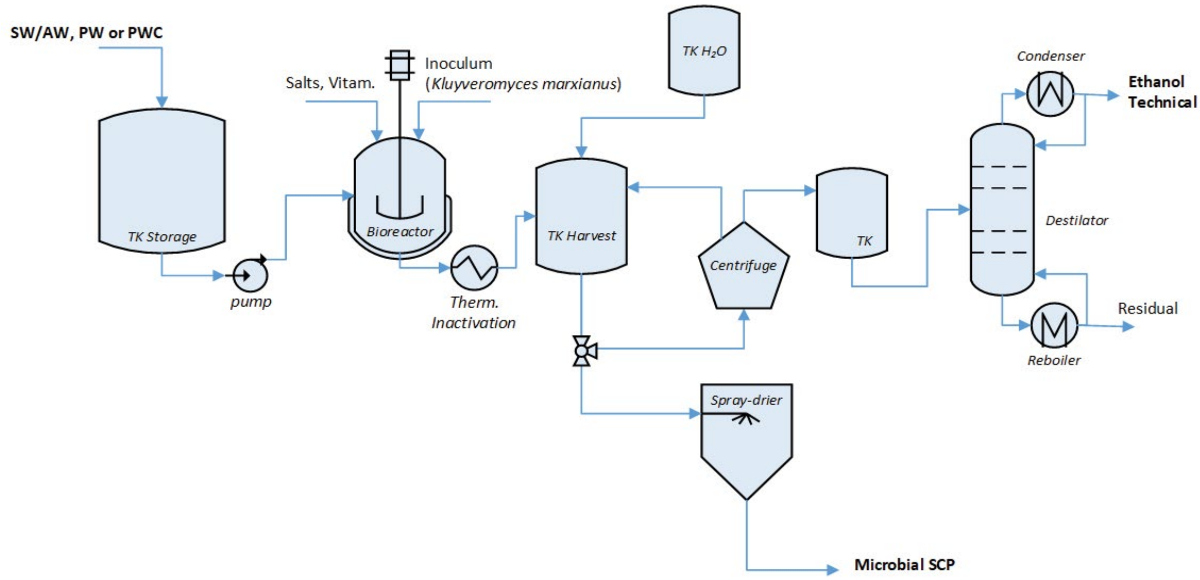

Figure 5: Process flow scheme for the SCP and ethanol production from SW/AW, PW, or PWC.

hydrolytic enzymes, the cell debris is subsequently separated, and the soluble kefiran is precipitated with ethanol, the mixture is centrifuged and the precipitate separated, which is subjected to several washes with hot water until the lactose residues are removed. Finally, the kefiran can be dried at 60°C for 24 h [105].

Enzyme-based biotechnological processes to biotransform permeate of whey in valuable products

Some hydrolytic enzymes, such β-galactosidase (EC 3.2.1.23), could be used to hydrolyze or polymerize lactose, present in SW/AW, PW or PWC. At concentrations of lactose below 30% (m/v), the hydrolysis of lactose in glucose and galactose predominates [106]; while at higher concentrations the trans-galactosylation reaction and the formation of galacto-oligosaccharides (GOS) could be favored [107,110-112].

Some authors highlight the beneficial effects of GOS on human health [108,109] since they are considered as prebiotic substances [113,114].

In Figures 7A and 7B, the proposed production schemes for both, the hydrolysis of lactose and the formation of GOS, respectively, are shown. Both processes can be performed by immobilizing the enzyme β-galactosidase, which would allow reusing the enzyme and lowering production costs.

In the case of lactose hydrolysis (Figure 7A), it is favored at concentrations below 30% (w/v) of lactose [26,106,107,113,114]. In this case, the enzymatic hydrolysis reaction is favored yielding the expected products:

Lac⇋Glu+Gal

Where Lac, Glu y Gal represent Lactose (Glu-Gal), glucose and galactose, respectively.

A nano-filtration system can also be coupled to the collector tank to allow the lactose to separate from glucose and galactose. Lactose can be reused, while the glucose and galactose mixture can be concentrated to marketable levels by evaporation.

If lactose concentration is >30-35% (m/v), the trans-galactosylation of lactose would be favored (Figure 7B), and galactooligosaccharides of different polymerization degrees (PD) could be obtained (PD ≥ 2), depending on the number of different species of GOS and the conditions of the enzymatic reaction. For example, if three GOS with different DPs is produced, we would have product yields as follow:

3(n-1)·Lac ⇌Glu-(Gal)_n+Glu-(Gal)_(n-1)+Glu-(Gal)_(n-2)+3(n-2)·Glu

where Glu-(Gal)_n,Glu-(Gal)_(n-1),Glu-(Gal)_(n-2)represent the GOS and n represents the DP of GOS.

Notes for economic feasibility analysis

The valorization of whey is a strategic decision for dairy companies because it eliminates the "bottleneck" that the high volumes generated SW/AW impose on the continuous growth of dairy production. However, it is always desirable that the investment necessary for the implementation of some of the technologies described above, can be amortized and yields gains in reasonable terms.

For the complete economic analysis, each valuation technology, in particular, the predictions of product and sector growth, market demand and the financial availability of dairy companies should be taken into account [115,116].

From 100 kg of whole fresh milk and an average yield of 9.30 ± 0.7 kg in the production of the different kinds of cheeses and the generation of about 90.70 kg of sweet whey [3]. Table 1 compares some of the reported yields and reference prices for a group of available whey technologies.

[a] Average value [3] . A price is assumed for the cheese curd US$3 764/TM (taken from: http://www.clal.it/en/?section=ricotta, July 24th 2017)

[b]Assuming a yield Yxs=0.52 TM DB TM lactose^{-1} [117], so: 0.52*(90.70/1.04)*(48/1000)=2.18 TM and a reduction of 80-90% of COD

[c]Assuming a 36 h process where 95.6% of the lactose present in the serum (4%) was consumed and a lactic acid concentration of

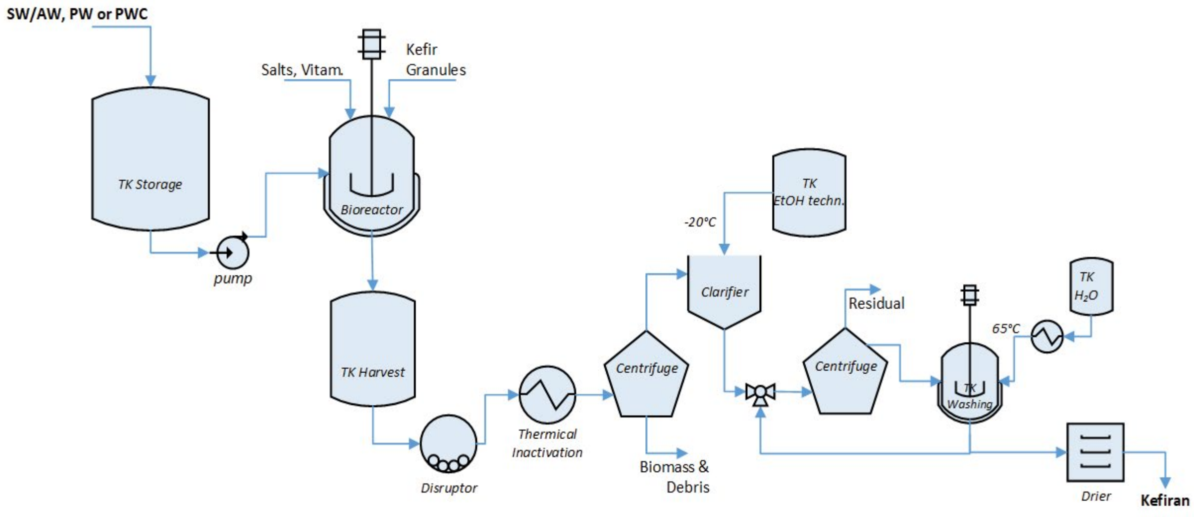

Figure 6: Process flow scheme for the production of kefiran from kefir grain and SW/AW, PW, or PWC.

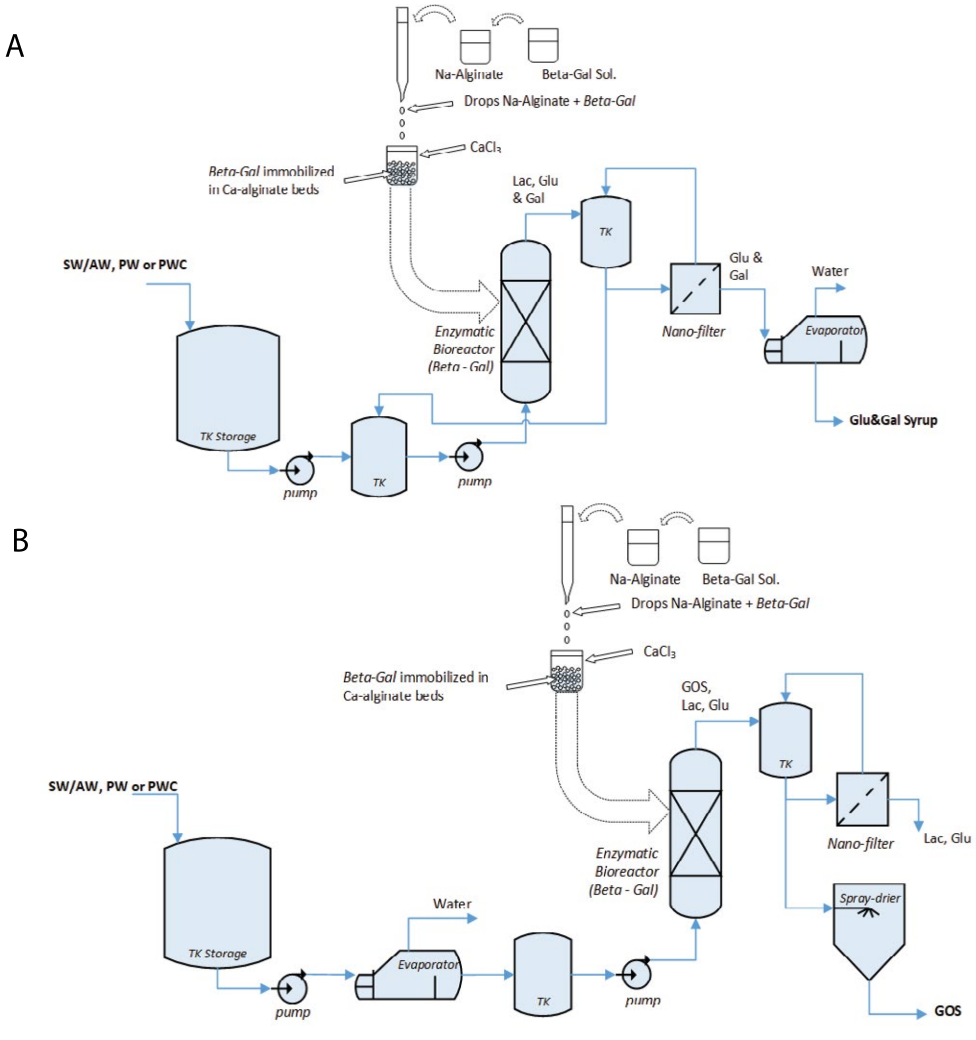

Figure 7: Process flow scheme for the enzymatic transformation of SW/AW, PW, or PWC in (A) Hydrolysis of lactose to glucose and galactose; (B) Trans-galactosylation of lactose to galactooligosaccharides of different degree of polymerization.

33.73 g L^{-1} [63]. So: Y_{PX}=33.73/(0.956*40)=0.88 TM Lactic Acid TM lactose^{-1} and density. @ 80% = 1.2 TM m^{-3}; Vol. syrup @ (80% (m/v)) = 0.88*90.70/0.8 = 99.77m^3 syrup.

[d] Unpublished Result.

[e] Taken from: http://www.clal.it/en/?section=sieroproteine (July 7th 2017)

[f] Taken from: https://wholesaler.alibaba.com/product-detail/Manufacture-wholesale-price-instant-dry-yeast_60614513305.html?spm=a2700.7782932.1998701000.11.0W8xnR (July 14th 2017)

[g] Taken from: http://www.clal.it/en/?section=whey (July 7th 2017)

[h] Taken from: https://www.alibaba.com/product-detail/Fructose-Glucose-Maltose-rice-syrup-Factory_60669804468.html?spm=a2700.7724838.2017115.231.87bIGj&s=p (17/7/2017)

[i] Values assumed in proportion to the lactose concentration in the whey permeate (PW) of milk.

The products prices as described below vary from those that are similar to that which would be obtained from the commercialization of the fresh milk itself. It means 8.3 kg fluid milk kg whole milk powder^{-1}, therefore: 100 TM fluid milk equivalents to 12 TM of approximately whole milk powder and whose price is US $ 3 200 per TM, to those that exceed two digits to that of milk (Figure 8).

These values allow classifying whey recovery technologies, in those that provide low, medium and high income (Figure 8).

Also, during the valorization process of whey, it is possible to reduce significantly the contaminant load of the effluents, which is one of the main reasons for the implementation of these technologies. From effluents requiring a primary, secondary and tertiary treatments (for the treatment of SW/AW and PW), to less polluting effluents, presumably requiring only primary treatment, which would reduce the costs of treatment in the dairy companies.

Conclusion Remarks

Zone 1 of Ecuador and specifically the provinces of Carchi and Imbabura are purely livestock and cheese producers. An important part of the economically active population of these provinces is dedicated to the production and commercialization of cheeses, prevailing small and medium producers. With the increment of environmental regulations together with the gradually ecological conscience of producers, it becomes more difficult to pour, dairy surplus and significant amounts of whey in rivers, lakes and streams. Therefore, the growth of the dairy and cheese sector of small and medium producers is limited by the high volumes of milk whey this would bring, due to the high COD and BOD values of this by-product.

An alternative is to seek to reduce the impact of effluents by searching for technologies that use whey as the starting raw material, as discussed below in this review.

However, to implement these technologies, it is essential to undertake an investment process and to have certain financial resources that are hardly available in small and medium milk and

Table 1: It is comparative data of some of the whey valorization technologies available. From 100 TM of whole fresh milk.

Process	Not treat.	WPC	SCP	WP	Glu & Gal	Lactic Ac.	GOS	KEF
Cheeses, TM	9.30 ± 0.7 [a]							
Valorized Product	-	WPC-34 (34% Prot.)	Single-cell protein	Whey dried powder	Glucose & Galactose	Lactic Acid	Galacto-oligosaccharides	Kefirán
Qty.[2,3], TM	-	2.06	2.18 [b]	5.80	81.63	79.82 [c]	81.63	0.31 [d]
Ref. Price	-	US$1974 /TM [e]	US$1950 /TM [f]	US$1100 /TM [g]	US$600-790 / TM [h]	US$1000 /TM	US$20-200 /kg	US$30 /g
Residual	SW/AW	PW	waste water	waste water	waste water	waste water	waste water	waste water
Qty., TM	90.70	88.64	>88.43	84.90	9.07	>10.88	9.07	>90.38
Lac, %(m/v)	4.8	4.7	0.5	0.1	0.4	0.2	0.2	0.2
COD, kg/m^3 [i]	70	55	6.0	1.2	4.6	2.3	2.3	2.3

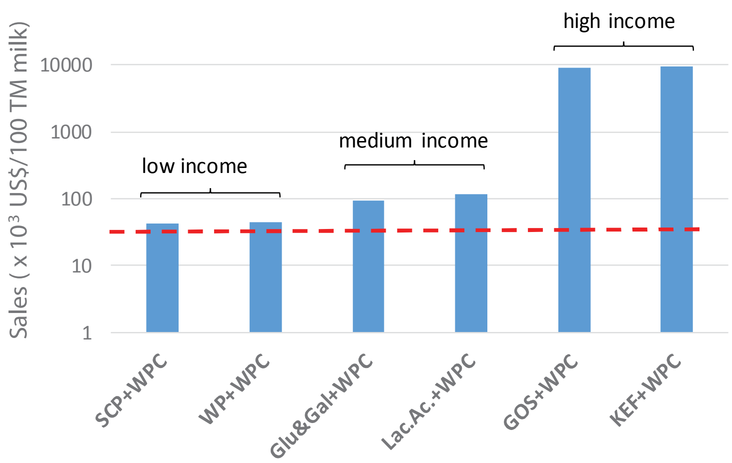

Figure 8: It is a comparison of some of the existing technologies for the valorization of whey. The dashed line represents the price of whole milk powder obtained from processing 100 TM of whole fresh milk.

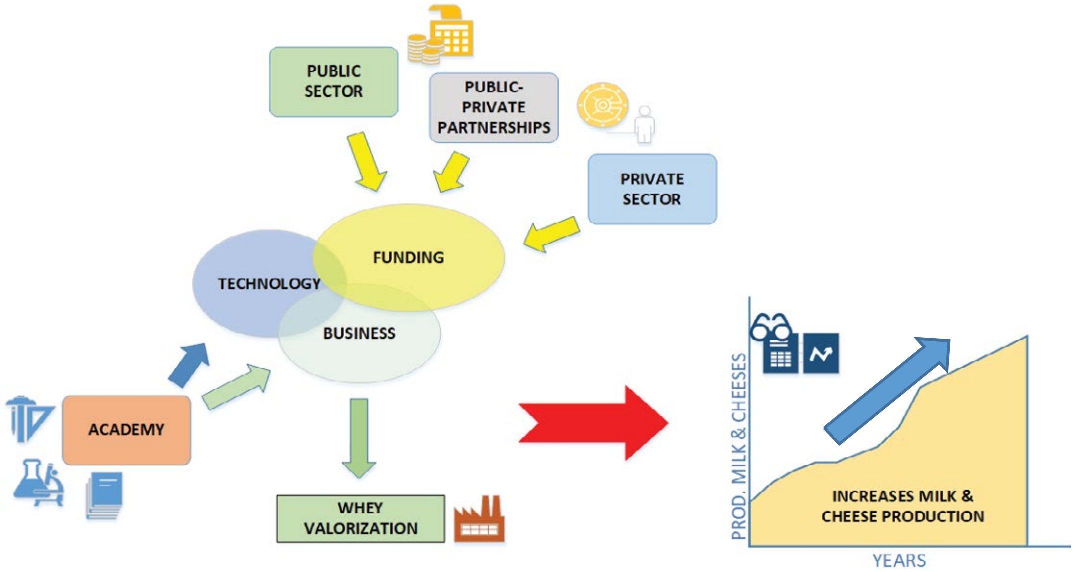

Figure 9: Interrelations that will enable the continued growth of Dairy production to small and medium-sized producers by eliminating the limitations that the high volumes of milk whey may exercise on growth in the production of milk and cheeses.

cheese producers, even if they may eventually be associated in cooperatives for these purposes.

A possible solution is to find out external funds from the private or state sector. Also, to establish public-private partnerships that allow access to financial resources and encourage entrepreneurship, and so that, it can stimulate and foster increases in future of milk production among small and medium producers in Zone 1 and all over Ecuador.

The Academy including Universities, Technical Institutes and Research Centers, on the other hand, could contribute to the required studies, the developments and the most appropriate technologies, as well as in the formation of the entrepreneurs that can undertake these projects. All these efforts, as a whole, could be combined to guarantee the continuous growth of milk production in Zone 1 of Ecuador in the forthcoming years, in the way shown in Figure 9.

Also, with the new facilities, new job sources would be generated, current imports would be replaced, and the productive matrix of Ecuador would be changing and expanding.

References

1. Requelme N, Bonifaz N (2012) Characterization of dairy production systems in Ecuador. Univ Polytechnic Sales Ecuador Rev La 15: 55-68.
2. Orozco M (2015) A third of dairy production is devoted to cheese. Leaders 1.
3. Dalla Costa CA (2015) Theoretical and real questional performance of the milk of the villa maría Basin, Córdoba. Catholic University of Cordoba, Argentina
4. Parra R a (2009) Whey : importance in the food industry. Rev Fac Nac Agropecu Medellín 62:4967-4982.
5. Kosikowski FV (1979) Whey Utilization and Whey Products. J Dairy Sci 62:1149-1160.
6. Smithers GW, Ballard JF, Copeland AD, de Silva KJ, Dionysius DA, et al. (1996) New Opportunities from the Isolation and Utilization of Whey Proteins. J Dairy Sci 79: 1454-1469.
7. Fuquay JW, Jelen P (2011) Whey processing | Utilization and Products. Encycl Dairy Sci 731-737.
8. Ryan MP, Walsh G (2016) The biotechnological potential of whey. Rev Environ Sci Biotechnol 15: 479-498.
9. Yadav JS, Yan S, Pilli S, Kumar L, Tyagi RD, et al. (2015) Cheese whey: A potential resource to transform into bioprotein, functional/nutritional proteins and bioactive peptides. Biotechnol Adv 33: 756-774.
10. Somaye F, Marzieh M, Lale N (2005) Single Cell Protein (SCP) production from UF cheese whey by Kluyveromyces marxianus. 18 Th Natl Congr Food Technol 8-13.
11. Babu M (2014) Production of single cell protein using kluveromyces marxianus isolated form paneer whey. Int J Biomed Adv Res 5: 79-80.
12. Yadav JSS, Yan S, Ajila CM, Bezawada J, Tyagi RD, et al. (2016) Food-grade single-cell protein production, characterization and ultrafiltration recovery of residual fermented whey proteins from whey. Food Bioprod Process 99: 156-165.
13. Pesta G, Pittroff MR, Russ W (2007) Utilization of whey. Util. By-Products Treat. Waste Food Indb 193-207
14. Grosu L, Fernandez B, Grigoraş CG, Patriciu OI, Grig ICA, et al. (2012) Valorization of whey from dairy industry for agricultural use as fertiliser: Effects on plant germination and growth. Environ Eng Manag J 11: 2203-2210.
15. Marwaha SS, Kennedy JF (1988) Whey-pollution problem and potential utilization. Int J Food Sci Technol 23: 323-336.
16. Jayaprakasha HM, Brueckner H (1999) Whey Protein Concentrate: A Potential Functional Ingredient for Food Industry. J Food Sci Technol 36: 189-204.
17. Jelen P (2009) Dried Whey, Whey Proteins, Lactose and Lactose Derivative Products, Dairy Powders and Concentrated Products Society of Dairy Technology. John Wiley & Sons, USA.
18. Boland M (2011) Whey proteins. In: Phillips GO, Williams PA, Handbook of Food Proteins. Elsevier, USA.
19. Jeewanthi RKC, Lee NK, Paik HD (2015) Improved Functional Characteristics of Whey Protein Hydrolysates in Food Industry. Korean J Food Sci Anim Resour 35: 350-359.
20. Hanemaaijer JH (1985) Microfiltration in whey processing. Desalination 53: 143-155.
21. Atra R, Vatai G, Molnar BE, Balint A (2005) Investigation of ultra- And nanofiltration for utilization of whey protein and lactose. J Food Eng 67: 325-332.

22. Norton S, Lacroix C, Vuillemard JC (1994) Kinetic study of continuous whey permeate fermentation by immobilized Lactobacillus helveticus for lactic acid production. Enzyme Microb Technol 16: 457-466.
23. Colomban A, Roger L, Boyaval P (1993) Production of propionic acid from whey permeate by sequential fermentation, ultrafiltration, and cell recycling. Biotechnol Bioeng 42: 1091-1098.
24. Gabardo S, Rech R, Rosa CA, Ayub MAZ (2014) Dynamics of ethanol production from whey and whey permeate byimmobilized strains of Kluyveromyces marxianus in batch andcontinuous bioreactors. Renew Energy 69: 89-96.
25. Qureshi N, Friedl A, Maddox IS (2014) Butanol production from concentrated lactose/whey permeate: Use of pervaporation membrane to recover and concentrate product. Appl Microbiol Biotechnol 98: 9859-9867.
26. Golowczyc M, Vera C, Santos M, Guerrero C, Carasi P et al. (2013) Use of whey permeate containing in situ synthesised galacto-oligosaccharides for the growth and preservation of Lactobacillus plantarum. J Dairy Res 80: 374-381.
27. Padilla B, Frau F, Ruiz-Matute AI, Montilla A, Belloch C et al. (2015) Production of lactulose oligosaccharides by isomerisation of transgalactosylated cheese whey permeate obtained by β-galactosidases from dairy Kluyveromyces. J Dairy Res 82: 356-364.
28. Geiger B, Nguyen HM, Wenig S, Nguyen HA, Lorenz C et al. (2016) From by-product to valuable components: Efficient enzymatic conversion of lactose in whey using ??-galactosidase from Streptococcus thermophilus. Biochem Eng J 116: 45-53.
29. Peralta A (2017) Efficiency in milk production. VIII Forum of Sect. Milk. Ecuadorian. Strategies for Better. The Consum. Dairy in the population., Quito: Center of the Milk Industry (CIL) 20.
30. Guimarães PMR, Teixeira JA, Domingues L (2010) Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey. Biotechnol Adv 28: 375-384.
31. Kosikowski F V 1979 Whey Utilization and Whey Products. J Dairy Sci 62: 1149-1160.
32. Peters RH 2005 Economic aspects of cheese making as influenced by whey processing options. Int. Dairy J 15: 537-545.
33. Koutinas AA, Papapostolou H, Dimitrellou D, Kopsahelis N, Katechaki E, et al. 2009 Whey valorisation: A complete and novel technology development for dairy industry starter culture production. Bioresour Technol 100: 3734-3739.
34. Gonzalez Siso MI 1996 The biotechnological utilization of cheese whey: A review. Bioresour Technol 57: 1–11.
35. Kotoupas A, Rigas F, Chalaris M 2007 Computer-aided process design, economic evaluation and environmental impact assessment for treatment of cheese whey wastewater. Desalination 213: 238-252.
36. Ramos OL, Pereira RN, Rodrigues RM, Teixeira J, Malcata FX, et al. 2016 Whey and Whey Powders: Production and Uses. Encycl Food Heal 5: 498-505.
37. Mollea C, Marmo L, Bosco F. Valorisation of Cheese Whey, a By-Product from the Dairy Industry. Food Ind 2013: 549–88.
38. Bednarski W 1988 Possibilities for whey utilization. Przem Ferment I Owocowo-Warzywny 32:14-17.
39. Kosaric N, Asher Y 1982 Cheese whey and its utilization. Conserv Recycl 5: 23–32.
40. Ramchandran L, Vasiljevic T 2012 Whey Processing. Membr Process Dairy Beverage Appl 193-207.
41. Ramos OL, Pereira RN, Rodrigues RM, Teixeira J A, Malcata FX et al 2016 Whey and Whey Powders: Production and Uses. Encycl Food Heal 5: 498-505.
42. Smithers GW 2008 Whey and whey proteins-From “gutter-to-gold”. Int Dairy J 18:695-704.
43. Jelen P (2011) Whey processing: Utilization and Products. Elsevier: 731-737.
44. Cuartas-Uribe B, Vincent-Vela MC, Álvarez-Blanco S, Alcaina-Miranda MI, Soriano-Costa E 2007 Nanofiltration of sweet whey and prediction of lactose retention as a function of permeate flux using the Kedem-Spiegler and Donnan Steric Partioning models. Sep Purif Technol 56: 38-46.
45. Mawson AJ 1994 Bioconversions for whey utilization and waste abatement. Bioresour Technol 47: 195-203.
46. Booij CJ 1985 Use of lactose in the pharmaceutical and chemical industry. J Soc Dairy Technol 38: 105-109.
47. Zadow JG. Lactose: Properties and Uses. J Dairy Sci 1984;67: 2654-2679.
48. Paterson AHJ 2009 Production and uses of lactose. Adv. Dairy Chem 3: 105-120.
49. Panesar PS, Kennedy JF, Gandhi DN, Bunko K 2007 Bioutilisation of whey for lactic acid production. Food Chem 105: 1-14.
50. Panesar PS, Kennedy JF 2012 Biotechnological approaches for the value addition of whey. Crit Rev Biotechnol 32: 327-348.
51. Spălăţelu (Vicol) C 2012 Biotechnological valorisation of whey. Innov Rom Food Biotechnol 10: 1-8.
52. Pescuma M, de Valdez GF, Mozzi F 2015 Whey-derived valuable products obtained by microbial fermentation. Appl Microbiol Biotechnol 99: 6183-6196
53. Litchfield JH 2009 Lactic Acid, Microbially Produced . Encycl. Microbiol 362-372.
54. Wasewar KL 2005 Separation of Lactic Acid : RecentAdvances. Chem Biochem Eng Q 19: 159-172.
55. Roukas T, Kotzekidou P 1991 Production of lactic acid from deproteinized whey by coimmobilized Lactobacillus casei and Lactococcus lactis cells. Enzyme Microb Technol; 13:33-38.
56. Roukas T, Kotzekidou P 1998 Lactic acid production from deproteinized whey by mixed cultures of free and coimmobilized Lmtobacillus casei and Lmtococcus Zactis cells using fedbatch culture. Enzyme Microb Technol 22: 199-204.
57. Büyükkileci AO, Harsa S 2004 Batch production of L(+) lactic acid from whey by Lactobacillus casei (NRRL B-441). J Chem Technol Biotechnol 79: 1036-1040.
58. Ding S, Tan T 2006 l-lactic acid production by Lactobacillus casei fermentation using different fed-batch feeding strategies. Process Biochem 41: 1451-1454
59. Narayanan N 2004 L (+) lactic acid fermentation and its product polymerization. Electron J Biotechnol 7: 167-79.
60. Wee Y, Kim J, Ryu H 2006 Biotechnological Production of Lactic Acid and Its Recent Applications. Food Technol Biotechnol 44: 163-72.
61. Alvarez MM, Aguirre-Ezkauriatza EJ, Ramírez-Medrano A, Rodríguez-Sánchez Á, et al. (2010) Kinetic analysis and mathematical modeling of growth and lactic acid production of Lactobacillus casei var. rhamnosus in milk whey. J Dairy Sci 93: 5552-5560.
62. Stieber RW, Gerhardt P (1979) Continuous Process for Ammonium-Lactate Fermentation of Deproteinized Whey. J Dairy Sci 62: 1558-1566.
63. Panesar PS, Kennedy JF, Knill CJ, Kosseva M. Production of L(+) (2010) Lactic Acid using Lactobacillus casei from Whey. Brazilian Arch Biol Technol 53: 219-226.
64. Kosseva MR (2013) Use of immobilized biocatalyst for valorization of whey lactose. Food Ind. Wastes 137–156.
65. Bruno-Bárcena JM, Ragout a L, Córdoba PR, Siñeriz F. Continuous production of L(+)- (1999) lactic acid by Lactobacillus casei in two-stage systems. Appl Microbiol Biotechnol 51: 316–324.
66. Kosseva MR, Panesar PS, Kaur G, Kennedy JF (2009) Use of immobilised biocatalysts in the processing of cheese whey. Int J Biol Macromol 45: 437–447.
67. Aguirre-Ezkauriatza EJ, Aguilar-Yáñez JM, Ramírez-Medrano A, Alvarez MM (2010) Production of probiotic biomass (Lactobacillus casei) in goat milk whey: Comparison of batch, continuous and fed-batch cultures. Bioresour Technol 101: 2837-2844.
68. Fooks LJ, Fuller R, Gibson GR (1999) Prebiotics, probiotics and human gut microbiology. Int Dairy J 9: 53-61.
69. Chen LA, Sears CL (2014) Prebiotics, Probiotics, and Synbiotics. Mand. Douglas, Bennett’s Princ. Pract. Infect. Dis p.19–25.
70. Koushki M, Jafari M, Azizi M (2012) Comparison of ethanol production from cheese whey permeate by two yeast strains. J Food Sci Technol 49: 614–619.

71. Sansonetti S, Curcio S, Calabrò V, Iorio G (2009) Bio-ethanol production by fermentation of ricotta cheese whey as an effective alternative non-vegetable source. Biomass and Bioenergy 33: 1687–1692.
72. Jedrzejewska M, Kozak K (2011) Ethanol production from whey permeate in a continuous anaerobic bioreactor by Kluyveromyces marxianus. Environ Technol 32: 37–42.
73. Lane MM, Morrissey JP (2010) Kluyveromyces marxianus: A yeast emerging from its sister's shadow. Fungal Biol Rev 24: 17–26.
74. Dragone G, Mussatto SI, Almeida e Silva JB, Teixeira JA (2011) Optimal fermentation conditions for maximizing the ethanol production by Kluyveromyces fragilis from cheese whey powder. Biomass and Bioenergy 35: 1977–1982.
75. Gabardo S, Pereira GF, Rech R, Ayub MAZ (2015) The modeling of ethanol production by Kluyveromyces marxianus using whey as substrate in continuous A-Stat bioreactors. J Ind Microbiol Biotechnol 42: 1243-1253.
76. Gabardo S, Pereira GF, Klein MP, Rech R, Hertz PF, et al.(2016) Dynamics of yeast immobilized-cell fluidized-bed bioreactors systems in ethanol fermentation from lactose-hydrolyzed whey and whey permeate. Bioprocess Biosyst Eng 39: 141-150.
77. Jain DK, Tyagi RD, Kluepfel D, Agbebavi TJ (1991) Production of propionic acid from whey ultrafiltrate by immobilized cells of Propionibacterium shermanii in batch process. Process Biochem 26: 217-223.
78. Gupta A, Srivastava AK (2001) Continuous Propionic Acid Production from Cheese Whey Using In Situ Spin Filter. Biotechnol Bioprocess Eng 6: 1-5.
79. Alam S, Stevens D, Bajpai R (1988) Production of butyric acid by batch fermentation of cheese whey withClostridium beijerinckii. J Ind Microbiol 2: 359-364.
80. Arslan NP, Aydogan MN, Taskin M (2016) Citric acid production from partly deproteinized whey under non-sterile culture conditions using immobilized cells of lactose-positive and cold-adapted Yarrowia lipolytica B9. J Biotechnol 231: 232–239.
81. Raganati F, Olivieri G, Procentese A, Russo ME, Salatino P, Marzocchella A (2013) Butanol production by bioconversion of cheese whey in a continuous packed bed reactor. Bioresour Technol 138: 259-265.
82. Becerra M, Cerdán ME, González-Siso MI (2015) Biobutanol from cheese whey. Microb Cell Fact 14:27.
83. Nath K, Das D (2011) Modeling and optimization of fermentative hydrogen production. Bioresour Technol 102: 8569-8581.
84. Kargi F, Uzunca□r S (2013) Valorization of cheese whey by electrohydrolysis for hydrogen gas production and COD removal. Waste and Biomass Valorization 4: 517-528.
85. D??bowski M, Korzeniewska E, Filipkowska Z, Zieli??ski M, Kwiatkowski R (2014) Possibility of hydrogen production during cheese whey fermentation process by different strains of psychrophilic bacteria. Int J Hydrogen Energy 39: 1972-1978.
86. Ferreira Rosa PR, Santos SC, Silva EL (2014) Different ratios of carbon sources in the fermentation of cheese whey and glucose as substrates for hydrogen and ethanol production in continuous reactors. Int J Hydrogen Energy 39: 1288-1296.
87. Castro-Mayorga JL, Martínez-Abad A, Fabra MJ, Olivera C, Reis M, Lagarón JM (2014) Stabilization of antimicrobial silver nanoparticles by a polyhydroxyalkanoate obtained from mixed bacterial culture. Int J Biol Macromol 71: 103–110.
88. Colombo B, Sciarria TP, Reis M, Scaglia B, Adani F (2016) Polyhydroxyalkanoates (PHAs) production from fermented cheese whey by using a mixed microbial culture. Bioresour Technol 218: 692-699.
89. Gouveia AR, Freitas EB, Galinha CF, Carvalho G, Duque AF, et al. (2017) Dynamic change of pH in acidogenic fermentation of cheese whey towards polyhydroxyalkanoates production: Impact on performance and microbial population. N Biotechnol 37: 1080-116.
90. Akhtar P, Gray JI, Asghar A (1998) Synthesis of lipids by certain yeast strains grown on whey permeate. J Food Lipids 5: 283-297.
91. Castanha RF, Mariano AP, Morais LASD, Scramin S, Monteiro RTR (2014) Optimization of lipids production by Cryptococcus laurentii 11 using cheese whey with molasses. Brazilian J Microbiol 45: 379-387.
92. Espinosa-Gonzalez I, Parashar A, Bressler DC (2014) Heterotrophic growth and lipid accumulation of Chlorella protothecoides in whey permeate, a dairy by-product stream, for biofuel production. Bioresour Technol 155: 170-176.
93. Belem MAF, Gibbs BF, Lee BH (1997) Enzymatic production of ribonucleotides from autolysates of Kluyveromyces marxianus grown on whey. J Food Sci 62: 851-857.
94. Húngaro HM, Calil NO, Ferreira AS, Chandel AK, Silva SSD (2013) Fermentative production of ribonucleotides from whey by Kluyveromyces marxianus: Effect of temperature and pH. J Food Sci Technol 50: 958-964.
95. Sun ML, Liu SB, Qiao LP, Chen XL, Pang X, et al. (2014) A novel exopolysaccharide from deep-sea bacterium Zunongwangia profunda SM-A87: Low-cost fermentation, moisture retention, and antioxidant activities. Appl Microbiol Biotechnol 98: 7437-7445.
96. Zhou F, Wu Z, Chen C, Han J, Ai L, et al. (2014) Exopolysaccharides produced by Rhizobium radiobacter S10 in whey and their rheological properties. Food Hydrocoll 36: 362-368.
97. Chen Z, Shi J, Yang X, Nan B, Liu Y, et al. (2015) Chemical and physical characteristics and antioxidant activities of the exopolysaccharide produced by Tibetan kefir grains during milk fermentation. Int Dairy J 43: 15-21.
98. Farnworth ER (2005) Kefir - a complex probiotic. Food Sci Technol Bull: Funct Foods 2: 1-17.
99. Nielsen B, Gürakan GC, Ünlü G (2014) Kefir: A Multifaceted Fermented Dairy Product. Probiotics Antimicrob Proteins 6: 123-135.
100. Gradova NB, Khokhlacheva AA, Murzina ED, Myasoyedova VV (2015) Microbial components of kefir grains as exopolysaccharide kefiran producers. Appl Biochem Microbiol 51: 873-880.
101. Frengova GI, Simova ED, Beshkova DM, Simov ZI (2002) Exopolysaccharides produced by lactic acid bacteria of kefir grains. Z Naturforsch C 57: 805-810.
102. Maeda H, Zhu X, Omura K, Suzuki S, Kitamura S (2004) Effects of an exopolysaccharide (kefiran) on lipids, blood pressure, blood glucose, and constipation. Biofactors 22: 197-200.
103. Rodrigues KL, Carvalho JCT, Schneedorf JM (2005) Anti-inflammatory properties of kefir and its polysaccharide extract. Inflammopharmacology 13: 485-492.
104. Pop C, Apostu S, Salanță L, Rotar AM, Sindic M, et al. (2014) Influence of Different Growth Conditions on the Kefir Grains Production, used in the Kefiran Synthesis. Bull UASVM Food Sci Technol 71: 2344-2344.
105. Dailin DJ, Elsayed EA, Othman NZ, Malek R, Phin HS, et al. (2016) Bioprocess development for kefiran production by Lactobacillus kefiranofaciens in semi industrial scale bioreactor. Saudi J Biol Sci 23: 495-502.
106. Illanes A, Guerrero C, Vera C, Wilson L, Conejeros R, et al. (2016) Lactose-Derived Prebiotics: A Process Perspective. (1st edtn) Academic Press, Cambridge, USA.
107. Vera C, Guerrero C, Conejeros R, Illanes A (2012) Synthesis of galacto-oligosaccharides by β-galactosidase from Aspergillus oryzae using partially dissolved and supersaturated solution of lactose. Enzyme Microb Technol 50: 188-194.
108. Fischer C, Kleinschmidt T (2015) Synthesis of galactooligosaccharides using sweet and acid whey as a substrate. Int Dairy J 48: 15-22.
109. Kovacs Z, Benjamins E, Grau K, Rehman A, Ebrahimi M, et al. (2013) Recent developments in manufacturing oligosaccharides with prebiotic functions. Adv Biochem Eng Biotechnol 143: 257-295.
110. Rodriguez CB, Arrojo FL, Moriano MP, Ballesteros A, Plou F (2016) Continuous Packed Bed Reactor with Immobilized β-Galactosidase for Production of Galactooligosaccharides (GOS). Catalysts 6: 189.
111. Martínez CM, Copoví P, Olano A, Moreno FJ, Montilla A (2013) Synthesis of prebiotic carbohydrates derived from cheese whey permeate by a combined process of isomerisation and transgalactosylation. J Sci Food Agric 93: 1591-1597.
112. Torres DPM, Gonçalves MPF, Teixeira JA, Rodrigues LR (2010) Galacto-Oligosaccharides: Production, properties, applications, and significance as prebiotics. Compr Rev Food Sci Food Saf 9: 438-454.
113. Andorrà I, Berradre M, Rozès N, Mas A, Guillamón JM, et al (2010) Effect of pure and mixed cultures of the main wine yeast species on grape must fermentations. Euro Food Research Technol 231: 215-224.

114. Colinas BR (2013) Obtención Enzimática, Caracterización Y Propiedades Prebióticas De Oligosacáridos Empleados En Leches Infantiles, Universidad Autónoma de Madrid (UAM), Madrid, Spain.

115. Petrides D (2003) Bioseparations Science and Engineering, Oxford University Press, USA.

116. Peters MS, Timmerhaus KD, Ronald EW (2004) Plant design and economics for chemical engineers.(5th edtn), McGraw-Hill, USA.

117. Schultz N, Chang L, Hauck A, Reuss M, Syldatk C (2006) Microbial production of single-cell protein from deproteinized whey concentrates. Appl Microbiol Biotechnol 69: 515-520.

Author Affiliations

Top

[1]Department of Engineering in Agricultural and Environmental Sciences, Technical University of the North, Eucador

[2]Technical University of Machala, Machala, El Oro, Ecuador

[3]Postgraduate Unit of the National University of San Marcos-Peru

[4]Industrial Biotechnology and Bio products Research Group, Center for Nanoscience and Nanotecnology, CENCINAT, University of the Armed Forces (ESPE), Ecuador