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Whey to Vodka

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Abstract

Whey production can be an economic and environmental problem for small creameries and acid whey producers. The fermentation and distillation of whey not only eliminates the cost of disposing whey as waste while minimizing environmental impact but adds a revenue option through production of a value-added product. *Kluyveromyces marxianus* is typically utilized to ferment the pasteurized and pretreated whey. The fermented product contains approximately 3% ethanol v/v. Various options for distilling may be utilized such as a simple two-pot system or a more complex four-stage system to assure production of a neutral spirit. Quality of the distilled spirit is impacted by whey source, whey pretreatment, fermentation conditions, and the distilling process.

Keywords: *Kluyveromyces marxianus*, fermentation, distillation, spirits, ethanol, still, Carbery method

1. Introduction

Whey processing is a mature manufacturing sector. More than 75 years have passed since multiple effect evaporators and spray dryers were developed and applied to whey processing [1]. Nevertheless, the technology continues to evolve. The initial processes focused on removing water and concentrating all solids-non-fat into dry powders. Today, membranes, ion exchange resins, and chromatography are some of the new unit operations routinely applied in the processing of an increasingly diverse assortment of powders originating from whey.

This development has greatly benefitted larger cheese producers as these powders generally provide significant revenue potential. Unfortunately, smaller scale cheese processors are rarely able to benefit from these products. Whey powder facilities are expensive to construct and are therefore not an option for smaller cheese companies.

Large-scale cheese makers in the US typically only produce one type of cheese such as cheddar or mozzarella. This leads to production of large volumes of sweet whey streams with consistent composition that are well suited for current whey manufacturing facilities. In contrast, smaller specialty cheese producers tend to produce multiple different cheese types and must deal with different whey streams. While most hard renneted cheeses produce relatively similar whey streams, the lactic cheeses such as cottage or cream cheese along with Greek yogurt create acid whey. Acid whey primarily differs from sweet whey in mineral and acid content. Specifically, acid whey may have twice the calcium content and more than 10 times the lactic acid content as compared to sweet whey. The high levels of lactic acid interfere with the drying process as it contributes to forming sticky powder

agglomerates within dryers. Consequently, acid whey cannot be easily processed into whey powders.

Giving these limitations, small-scale cheese processors and acid whey producers have limited options for whey disposal. At best, they aim to dispose of whey without a cost. This could involve using whey as an animal feed source, land application, or disposal in farm lagoons. All of these options have potential negative consequences. Dragone et al. suggested that 47% of whey produced in Portugal (mostly from small scale producers) was disposed through land application or directly into streams [2]. The environmental consequences of this can be significant due to the high Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) of whey, which are 40–60 and 50–80 g/L respectively [3]. This leads to depletion of dissolved oxygen when disposed into lakes and streams. Whey does not appear to negatively impact the flavor of beef from cattle fed whey [4, 5], although some negative impacts such as acidosis and diminished carcass grade have been noted [6]. In addition, feeding whey back to the livestock at a farmstead creamery will likely increase the risk of phage development.

Nevertheless, self-disposal may be favorable compared to paying for disposal through municipal wastewater treatment systems or paying others to haul the whey away for disposal. Rates for disposal of waste through municipal water/waste treatment rates are based upon the mass of BOD being removed at the treatment facility thereby making it an expensive waste treatment option for whey. In fact, it may not even be an option as some municipalities refuse to treat whey. A recent (2015) unpublished survey of specialty cheesemakers in the US revealed that most of the very small artisan cheese makers manage to dispose of whey at no cost through feeding to own or local neighborhood animals. However, as soon as cheese production increases above 5000 kg/year, most are obliged to pay for disposal at rates up to $105/1000 kg of whey. This demonstrates that whey disposal can be a significant expense for medium scale cheese processors that are too small to produce whey powders and too large to dispose of whey through feeding or other small-scale disposal. As profit margins for small-scale cheese makers are tight [7], whey disposal costs can significantly impact business sustainability.

Due to these challenges, small-scale whey producers are continuously looking for whey disposal options. The fermentation and distillation of whey can be done to produce bioethanol or a potable spirit. The fermentation and distillation of whey to produce potable spirits may be a potential value-added option for small scale cheese makers. Not only does this allow for concentrating the initial whey stream, but it also enables the production of an additional high-priced product. For example, if a 750 ml bottle of vodka sells for $30 that would translate to approximately $1–1.5 per L of initial whey. This could potentially create as much revenue as the corresponding cheese.

2. Commercial whey spirits

The concept of producing whey-based spirits is not new. This process has been explored scientifically since the 1940s and the Carbery process was developed and commercialized in 1978 to produce potable ethanol from whey on an industrial scale [8, 9]. Analysis has been conducted illustrating that whey based spirits are composed of volatile compounds similar to other spirits and are safe for consumption [2]. Currently in New Zealand, potable ethanol is being produced using the Carbery process and is exported to Asian markets [9, 10]. There are multiple examples worldwide of commercially available whey-based spirits. All of these products highlight the dairy/whey connection; both on the label and in product description that emphasizes creamy flavors. They are all marketed as premium products and

Figure 1.
Commercially available whey-based spirits: Bertha's revenge gin, black cow vodka, Vermont white vodka, and sheep whey vodka.

sold at high prices. This demonstrates that consumers appreciate distilled spirits produced from dairy sources. Below is a summary of four commercial whey-based spirits (pictured in **Figure 1**).

- Bertha's Revenge and Slough Bertha are produced at Ballyvolane Guesthouse in Ireland (https://ballyvolanespirits.ie). The product is named after Bertha, a Droimeann cow from Sneem in Co. Kerry, who apparently lived to be 49 years old. Although this gin is labeled as an Irish milk gin, it is produced from fermented sweet whey. The alcoholic whey is distilled three times and flavored with local botanicals.

- Black cow vodka is produced in England (https://www.blackcow.co.uk). This vodka sells for a premium price. The product has significant worldwide distribution and in deference to regulations in various countries is sold in select countries as a spirit instead of vodka to recognize that it is not based on grains or potatoes.

- An American version is Vermont white vodka from Vermont Spirits (http://www.vermontspirits.com). Vermont Spirits converts multiple local agricultural products to spirits. Tasting notes for this product describes it as: "a traditional vodka with a bracing yet moderately light medicinal approach, then a finish that fades into a nice and lingering sweetness. Creamy, with just a hint of bittersweet chocolate."

- Sheep Whey Vodka is produced at a Tasmanian artisan creamery (http://grandvewe.com.au). This product is the only one of the four spirits that is produced at the creamery. In appreciation of the creativity of this product, it won Champion Vodka of Australia at the World Vodka Awards 2017 in London, along with the 2017 award for Australian Beverage of the year.

3. Controlling the whey source

An important conclusion from a recent study by Risner et al. is that aroma compounds within spirits differ significantly based on whey source [11]. Therefore, it is essential to understand and control the whey source prior to starting commercial fermentation and distillation of whey, composition of sweet whey depends on a wide variety of factors. Within cheese types, milk pretreatment and cheese processing parameters such as filtration, pasteurization, starter, rennet, and salting will all impact whey composition [12, 13]. Among different cheese types, the parameters listed above have even more impact with the largest differences

associated with lactic curd cheeses such as cottage cheese. In addition, external factors such as feed, season, and lactation influence whey composition [14]. This is particularly important for whey from goat and sheep milk cheeses as these animals are often on seasonal lactation schedules [15]. Small variations in compositions likely do not greatly affect fermentation and distillation; nevertheless, this can be a concern when striving to produce a consistent product.

4. Whey pretreatment

Although whey from different sources vary, there are tools available to pretreat the whey prior to fermentation and distillation. Traditionally, whey clarifiers are used to remove casein fines while whey separators are used to remove whey cream. This leaves behind non-fermentable substrates such as whey proteins, minerals, and acids, which do not contribute to the production of distilled beverages. Although whey proteins are soluble, they may precipitate when exposed to heat during pasteurization or during distillation, which could interfere with operation of the still. Therefore, some method of protein removal, such as ultrafiltration, would be beneficial prior to fermentation. Removal of other potentially interfering compounds such as minerals and acids could be achieved through nanofiltration. Nanofiltration has the additional advantage of concentrating lactose to increase the concentration of fermentable substrate within whey, which would essentially improve fermentation and distillation efficiencies. It is important to note that these unit operations are expensive and resource intensive and therefore not likely to be used in artisan dairy processing. Nevertheless, membrane units are utilized in some specialty cheese facilities and could therefore be a relevant option.

5. Whey to commercial spirit

The Carbery method is the industrial method used to convert whey/whey permeate to ethanol [8, 9]. The method is similar to other industrial ethanol production processes in that a microbial fermentation is performed to convert sugars within a substrate to ethanol and an extractive distillation occurs to concentrate and separate the ethanol from other volatile compounds. Once distillation has occurred the spirit can be treated as any other distilled spirit for subsequent processing (**Figure 2**).
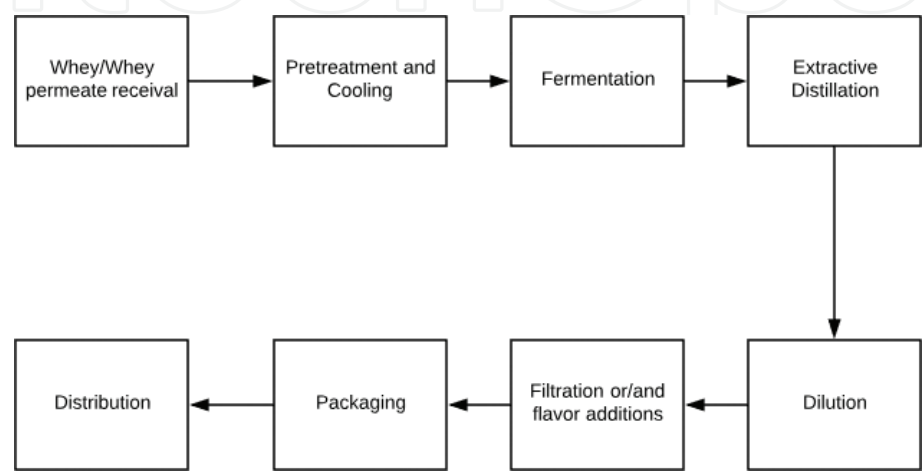

Figure 2.
Process overview of spirit production via the Carbery method.

There are several key differences in the Carbery method when compared to traditional spirit production. Whey/whey permeate is readily fermentable and a sugar conversion step such as mashing or cooking is not necessary. Whey/whey permeate should arrive at the facility well above the optimum fermentation temperature and must be cooled before inoculation. The main fermentable sugar within whey is lactose, which cannot be utilized by *Saccharomyces cerevisiae (S. cerevisiae)*, the yeast generally used for ethanol production. *Kluyveromyces marxianus (K. marxianus),* a lactose fermenting yeast is used to convert lactose to ethanol. The lactose levels within raw whey only allow for the production of a "beer" or "wash" with ethanol concentrations of 2–3% v/v. Whey permeate may be concentrated but ethanol production is limited by the sensitivity of *K. marxianus* to increased solute concentrations and ethanol. This low concentration of ethanol will increase the energy requirements during the distillation process. A beer still, extractive distillation unit and a rectifier are used during the extractive distillation process. A demethylizer is not employed during the extractive distillation process [8, 9] as very little methanol is formed during the fermentation process. The dilution, filtration, flavor additions, packaging, and distribution occur in a manner comparable to other spirits produced in a traditional manner.

6. Conversion of lactose to ethanol

Lactose [O-β-D-galactopyranosyl-(1→4)-D-glucopyranose] is a reducing sugar and disaccharide composed of β-1,4 glycosidically bonded galactose and glucose residues. Lactose is the primary carbohydrate constituent of whey and whey permeate [16, 17]. The conversion of lactose to ethanol is a two-step process. First, lactose must be hydrolyzed to galactose and glucose and then alcoholic fermentation occurs to produce ethanol.

6.1 Methods of lactose hydrolysis

The enzymatic hydrolysis of lactose is the most common method of lactose hydrolysis (**Figure 3**) and can be achieved in several ways. The common industrial conversion of lactose to ethanol uses an ethanol producing microbe, *K. marxianus* which enzymatically hydrolyzes lactose [8, 9]. Whey or whey permeate is cooled to the microbe's optimum fermentation temperature and then inoculated. Hydrolysis of lactose is achieved intracellularly via β-galactosidase and the organism subsequently metabolizes the constituents to produce ethanol [18]. It should be noted that the traditional brewing and distilling yeast used to produce ethanol, *S. cerevisiae* does not express the genes necessary to produce β-galactosidase, as an alternative a β-galactosidase producing yeast, *K. marxianus* is used. Genetic engineering of *S. cerevisiae* to produce β-galactosidase has been explored on an experimental scale

Figure 3.
Enzymatic hydrolysis of lactose.

for bioethanol production, but to the authors' knowledge this is not being used in beverage production [19–21].

A common method of lactose hydrolysis in dairy product production is the addition of lactase, an exogenous enzyme belonging to the β-galactosidases family [22–24]. The addition of this enzyme requires no additional processing equipment and lactase is widely available. Using lactase to hydrolyze lactose allows for the use of microbes, which do not produce β-galactosidase, to be used in the fermentation. This approach has been explored and documented on an experimental scale for bioethanol production [25, 26].

Other methods of hydrolysis of lactose include the use of immobilized enzyme systems, membrane reactor processes used to recover enzymes/cells and acid hydrolysis [24, 27]. Immobilized enzyme and membrane reactor systems could help reduce cost because both are enzyme conservation processes, but they require additional processing technology and are not widely implemented commercially. Acid hydrolysis requires the use of ultrafiltration because the whey permeate stream must be free of protein. The process involves the acidification and short heat treatment ranging from approximately 100–150°C. This treatment causes a brown discoloration in serum which requires color removal and purification steps [24, 27]. The color removal process would not be necessary during ethanol production. While these technologies and processes are currently not used in the commercial conversion of whey to ethanol, some have been explored to increase production efficiency [26, 28–30].

6.2 Fermentation after lactose hydrolysis

Alcoholic fermentation is a form of anaerobic energy production commonly used by plants, yeast and other microbes [31]. This metabolic pathway has been exploited by humans for food and beverage production for several millennia. During industrial production of ethanol from whey, an ethanol-fermenting strain of *K. marxianus* is used to convert lactose into ethanol. This strain of *K. marxianus* is used because it can intracellularly hydrolyze lactose and efficiently produce ethanol.

Alcoholic fermentation has two distinct phases. The first phase is glycolysis which converts glucose to pyruvate. The glycolytic pathway is common to nearly all
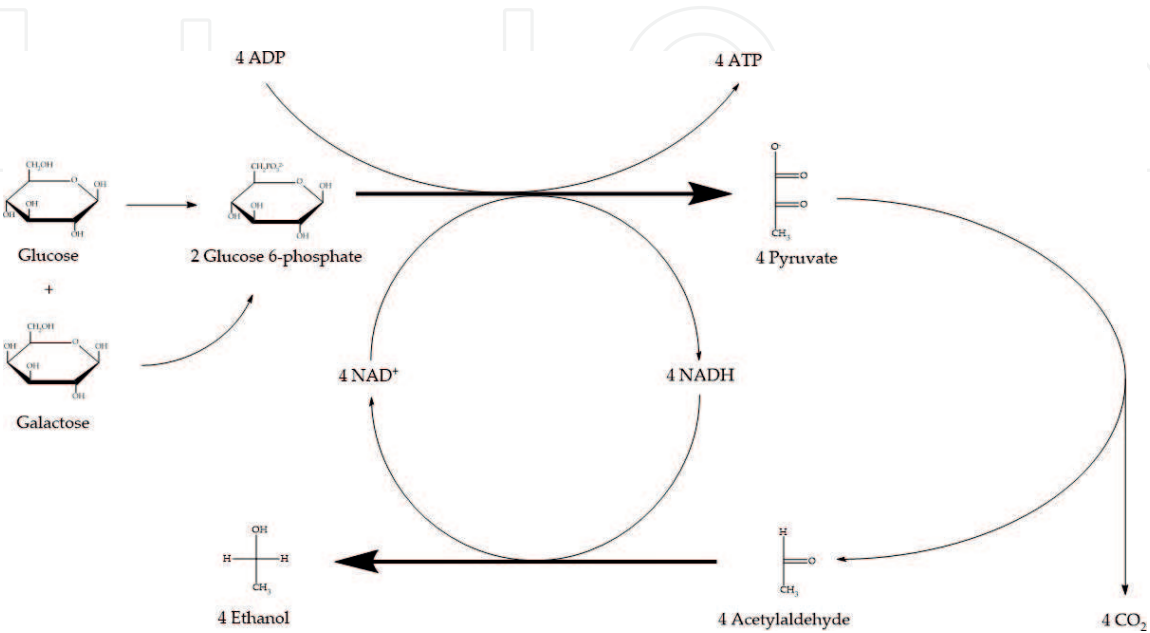
Figure 4.
Alcoholic fermentation after lactose hydrolysis.

cells and generates adenosine triphosphate (ATP) which is used for intracellular energy transfer. Galactose is enzymatically converted to glucose 6-phosphate, an intermediate product of glycolysis (**Figure 4**). The conversion of galactose to glucose 6-phosphate is a four step process; however, the cellular energetic cost is the same as the phosphorylation of glucose. The outcome of this glycolysis process is net production of 4 ATP, the conversion of glucose and galactose to 4 pyruvate molecules and the reduction of NAD^+ to NADH.

The second phase of alcoholic fermentation converts pyruvate into ethanol to regenerate NAD^+ used during glycolysis. Pyruvate is decarboxylated enzymatically which results in the production of CO_2 and the formation of acetaldehyde. The reduction of acetaldehyde to ethanol is catalyzed by alcohol dehydrogenase and NAD^+ is replenished in the process [31]. Ethanol is then passively diffused from the cell into the fermentation substrate.

7. Fermentation organisms

There are few yeast species which assimilate lactose to produce ethanol [32]. *K. marxianus* is the microorganism widely used in industrial lactose to ethanol conversion. Other microorganisms used in industrial food and beverage manufacture have been examined at an experimental scale for their suitability for lactose to bioethanol conversion. These organisms include *K. lactis, S. cerevisiae* and *Escherichia coli.* Use of genetically engineered organisms for alcoholic beverage manufacture is currently not a common commercial practice. This is likely due to perceived consumer concerns about the consumption of genetically modified organisms.

7.1 *K. marxianus* and considerations for lactose to ethanol conversion

K. marxianus ability to convert lactose to ethanol is widely reported in scientific studies concerning bioethanol production. *K. marxianus* is the fermentative organism used for large scale manufacture of potable spirits and bioethanol produced from whey/whey permeate [8, 9]. Scientific studies often reference *K. fragilis* as a lactose-fermenter; however, it is currently synonymous with *K. marxianus* [33]. Several studies have investigated the use of *Candida pseudotropicalis* as the fermentative organism for lactose to ethanol conversion [10]. *C. pseudotropicalis,* also referred to as *Candida kefir*, is the anamorph (asexual reproductive stage) of *K. marxianus* [33]. The species *K. marxianus* have a high degree of genetic variation and each strain's ability to produce ethanol can vary widely [34–36]. This is likely due to the species being present in a wide range of habitats [35]. *K. marxianus* is widely considered to be a Crabtree-negative organism, meaning the organism will preferentially respire instead of ferment when oxygen and glucose are abundant [37]. *K. marxianus* carries the genes necessary for fermentation and strains have been reported as Crabtree-positive (preferentially ferments in presence of oxygen and an abundance of glucose) [37, 38]. The ethanol tolerance of *K. marxianus* is lower than *S. cerevisiae* and can limit ethanol production [39]. Inhibition of ethanol production can occur at ethanol concentrations as low as 45–52 g/l or approximately 5.5–6.5% v/v [40]. Supplementation or concentration lactose within whey or whey permeate can cause substrate inhibition and limit ethanol production. This trait appears to be strain specific with reports varying of ethanol production inhibition at lactose concentration of 108–200 g/l [41, 42]. This wide variation in reported ranges highlights the importance of purchasing the proper fermentative strain of *K. marxianus* to meet each lactose to ethanol producer's requirements.

K. marxianus is generally recognized as safe (designated GRAS), which is advantageous for potable spirit producers because the yeast biomass can be further processed for livestock or human consumption. It has been reported that the fermentation process can reduce the biological oxygen demand of whey or whey permeate by 75% [43] and aerobic cultivation has reduced BOD by 90–95% [35].

7.2 Environmental considerations and fermentation parameters for *K. marxianus*

Several adjustable factors can influence the rate and quality of fermentation by *K. marxianus*. These factors include the presence of oxygen, nutrient supplementation, substrate, pH and fermentation temperature. In general, hypoxic and anoxic environments favor *K. marxianus's* fermentative metabolism, and ethanol yields are greater than in an aerobic environment [10, 43]. Aerobic conditions favor the building of cell density and are commercially applied for cell propagation in vessels called "Donas" [9]. The doubling time of *K. marxianus* is approximately 70 min, and it has one of the fastest growth rates of any eukaryote [37].

Nutrients are not added to the whey/whey permeate during commercial fermentations [9]. Additional supplementation of nitrogen and phosphorus to whey/whey permeate was shown not to affect ethanol production during fermentation [44]. It has been illustrated experimentally that supplementation of concentrated whey (200 g/l lactose) with bacto-peptone, ergosterol and linoleic acid reduced fermentation time from over 90 to less 60 h [10]. This is a substantial decrease in fermentation time, however large-scale commercial lactose to ethanol fermentations range from 12 to 24 h [8, 9].

K. marxianus is a thermotolerant yeast with reported maximum growth temperatures ranging from 47 to 52°C [10, 35]. Ethanol production has been reported at temperatures as high as 45°C [45] and, other studies indicate that the optimum fermentation temperature is lower. Studies indicate the optimal fermentation temperature range to be 30–40°C [36, 39, 41, 42, 46–48]. This wide range of reported temperatures can likely be attributed to the genetic diversity of *K. marxianus* strains and differences in experimental design. A pH of approximately 5 is widely reported as the optimum fermentation pH value [36, 39, 42, 46–48]. Agitation of fermenting whey/whey permeate occurs in industrial lactose to ethanol conversion and has been incorporated experimentally [8, 9, 47, 49]. To the authors' knowledge, the effects of the rate of agitation on fermentation efficiency of *K. marxianus* have not been examined.

Large scale lactose to ethanol production facilities will adjust fermentation time, temperature, tank pressure, and agitation rate to meet production goals [8, 9]. *K. marxianus* strain UFV-3 may have potential for potable ethanol production. *K. marxianus* strain UFV-3 was able to produce ethanol at yields 90% of the theoretical maximum with fermentation temperatures between 33.3 and 38.5°C, pH 4.7–5.7 and lactose concentrations between 50 and 108 g/l [42].

7.3 Other fermentation organisms

K. lactis is used to produce lactase and recombinant bovine chymosin on an industrial scale. It is the sister organism to *K. marxianus* that is more widely studied. *K. lactis* synthesizes β-galactosidase much like *K. marxianus* and most strains of *K. lactis* are considered Crabtree-negative [50]. A small number of isolate have been used by researchers working with *K. lactis* and it is ubiquitous to fewer environments than *K. marxianus* [10, 32]. This has led to less genetic variation than within in the species than *K. marxianus*. Some strains of *K. lactis* exhibit Crabtree-positive

metabolic characteristics [51, 52] and have been genetically engineered for lactose to bioethanol conversion [53]; however, they have not been adopted on a commercial level for lactose to ethanol conversion.

S. cerevisiae is the microorganism widely used in alcoholic beverage and bioethanol production. *S. cerevisiae* is used for traditional potable spirit production for several reasons including its fermentative capacity and ethanol tolerance, being considered Crabtree-positive (preferentially ferments in presence of oxygen and an abundance of glucose), and it's GRAS designation [10]. *S. cerevisiae* is ill-suited for the conversion of lactose to potable ethanol because wild *S. cerevisiae* does not express the genes necessary to produce β-galactosidase. This requires the lactose within whey/whey permeate to be pre-hydrolyzed or *S. cerevisiae* to be genetically engineered to produce β-galactosidase. While pre-hydrolysis of lactose has been explored on an experimental scale for bioethanol production, it would require an additional input (enzymes) and/or additional processing equipment. *S. cerevisiae* preferentially uptakes glucose after lactose hydrolysis and the presence of glucose causes the catabolic repression of enzymes necessary to uptake galactose [54]. The enzymes necessary to uptake galactose will only be synthesized after the glucose has been depleted. This repression causes an increase in fermentation time due to a diauxic lag [10, 55]. While *S. cerevisiae* has been genetically engineered to synthesize β-galactosidase and to reduce catabolic repression, genetically engineered yeast are not commonly used for beverage production [19, 21, 56].

E. coli has been genetically altered to produce ethanol since 1987 [57]. In 2010, *E. coli* was genetically modified to express the *Vitreoscilla* hemoglobin for direct fermentation of sugar to ethanol [58]. This technology has been experimentally developed for the efficient fermentation of whey and other organic by-products. Recently, microbial immobilization has been experimentally applied to *E. coli* expressing *Vitreoscilla* hemoglobin and has shown an increase in lactose to bioethanol production efficiency without producing the microbial biomass associated with the traditional fermentation process [59, 60].

The use of genetically modified organisms for the conversion of whey to potable spirit has the potential to increase production efficiency and reduce operating costs. The use of these organisms will require consumer acceptance of potable spirits produced from this technology.

8. Industrial whey fermentation process and technology for potable spirits

The fermentation process and technology used for the Carbery process are identical for potable spirits and bioethanol production [8, 9]. The Carbery process (**Figure 5**) is used for the industrial conversion of whey to potable spirits. Differences in the process occur during the distillation and during post-distillation processing. Whey/whey permeate is received at the facility and must be cooled to the specified fermentation temperature. Once cooled, the whey is pumped into fermentation tanks and inoculated with *K. marxianus*. The common inoculation rate for commercial spirit production is $1–5 \times 10^7$ cells/ml [61]. *K. marxianus* is grown in yeast propagation vessels referred to as "Donas". These yeast propagation vessels are aerobic and pumped with filtered air to promote yeast growth. This allows yeast to be maintained in growth phase which increases their ability to produce alcohol and reduces lag time when inoculated in whey [61]. The fermentation tanks are cylindroconical vessels jacketed with ethylene glycol or other coolant for temperature control. The quantity and size of the fermentation tanks vary based upon the production facility capacity. Compressed air is used for agitation during
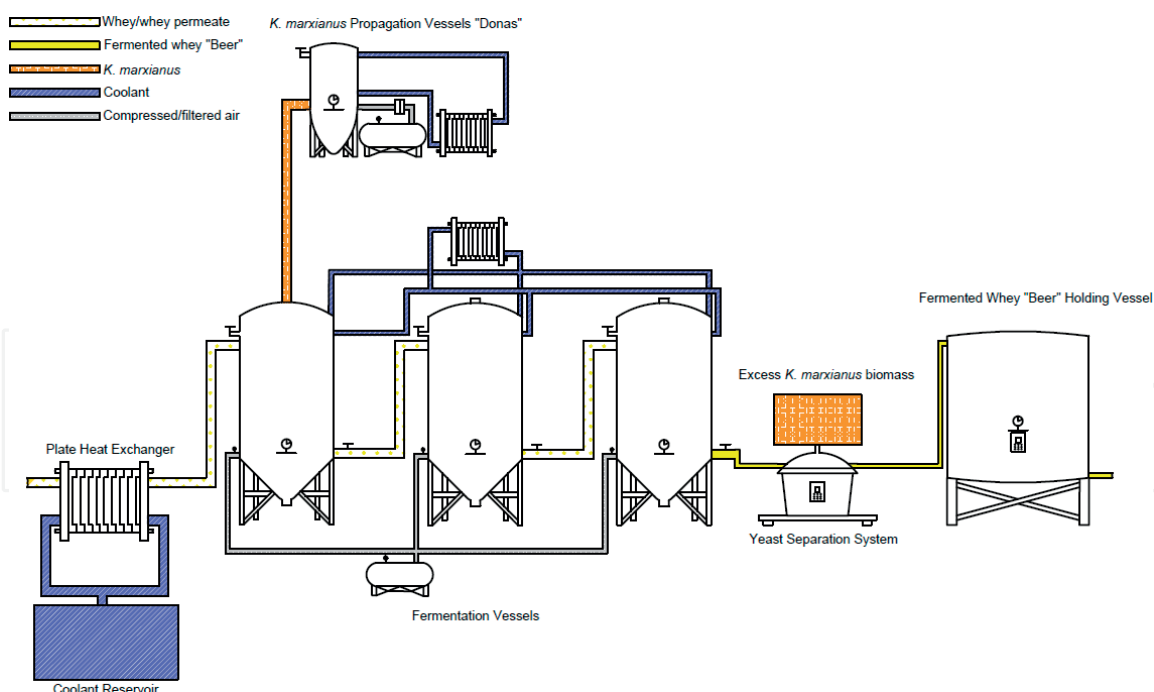
Figure 5.
Example of industrial (Carbery process) whey to potable ethanol fermentation process flow.

fermentation. The fermenting whey is pumped from vessel to vessel with monitoring of the specific gravity occurring throughout the process. The specific gravity of whey starts at approximately 1.022 g/cm^3 and drops to 1.008 g/cm^3 during fermentation. This drop in specific gravity is due the lactose within whey being converted to ethanol and CO_2. The specific gravity measurement is used to determine the process flow rate. Fermentation time ranges between 12 and 24 h [8, 9]. Once the designated specific gravity has been reached, the fermented whey is separated from the yeast via gravity (yeast falling out of solution) and/or through separation technology, such as centrifugation. The yeast can potentially be recycled for later batches or further processed for human or animal consumption. The fermented whey, now called a "beer" is held in a holding tank until distillation [8, 9].

9. Distillation

Once the whey sugars, primarily lactose and its monosaccharide constituents galactose and glucose, have been converted into ethanol, there is a need to concentrate the alcohol up to a strength that is appropriate for a spirituous product. Broadly speaking the ethanol yield from a whey fermentation will be typically 2–5% v/v, depending on the fermentation procedures and any preconcentration applied. The fermented feed though can contain significant levels of other whey constituents such as calcium salts and proteins. Depending on the process design, the whey may be pretreated to remove proteins and salts.

The requirements of the distillation operation are straight-forward, at least in principle. The fermented whey is to a first approximation a dilute solution of ethanol in water, and this ethanol needs to be concentrated by around an order of magnitude to generate the basis of an alcoholic spirit. However, other volatile components present, either from the parent whey or produced during fermentation as secondary metabolites, also termed congeners. Whilst these compounds are present in relatively low concentrations they can contribute to the flavor of the distilled spirit and the distiller needs to make a decision as to how much of these flavors should be retained in the resulting spirit.

In any case, the distillation process consists of three distinct activities: heating, to create vapor from the still feed, condensation, to convert vapor into the liquid spirit, and collection of the spirit. Each of these activities can be achieved using equipment of widely varying complexity and broadly speaking the higher the purity of the alcohol the more complex the equipment needs to be. For the distillation of fermented whey the common primary aim is to create "neutral alcohol" (i.e. alcohol that has no extraneous color or flavor) and so both the concentration of ethanol and removal of flavor-active components is usually required. To achieve this the ratio of surface area to volume in the still is a key design consideration. Generally, the introduction of more surface area tends to enhance the separation of ethanol and congeners, resulting in a cleaner, more neutral spirit. With the rapid development of the craft spirits industry, especially since the turn of the century, there has been a plethora of new still designs and fabricators available to the nascent distiller. To remove any congeners present is usually achieved by multiple distillations, the introduction of "plates" into a still or both.

Whilst the distilled spirit is the primary product from distillation, it is a relatively minor proportion of the still output. If the alcohol is around 3% v/v and the output is, say, 70% v/v, then the spirit fraction is only about 5% of the total feed volume, with the remaining 95% as "waste". However the removal of BOD (mainly present in whey as lactose) and the distillation of ethanol from the fermented whey, means that the BOD is substantially reduced, which in turn reduces effluent costs. If protein is removed prior to distillation and utilized elsewhere, then the resulting still waste stream is amenable to further treatment, for instance by anaerobic digestion. In any case, the distillation operation results in a significant waste stream in itself that must be considered in any process design.

The scale of the fermentation and distillation facilities is straight-forward to estimate. For a cheese plant that produces 5000 kg cheese per year, around 45,000 l of whey will be produced. On a weekly basis this is around 100 kg of cheese and 900 l of whey. If the lactose content is 5% w/v and the sugars are completely fermented (for instance using yeasts such as *K. marxianus*), the ethanol yield will be up to around 3% v/v. Allowing 5 days for a fermentation to complete, two fermenters of 1000 l will be required, and a still of 300–1000 l capacity. The exact capacity depends on how often the distilling operation is performed per week.

10. Still configuration

The recent growth of the craft spirits industry has spawned a wide range of still configurations, many of which focus on flexibility for different feeds. Such stills are referred to as hybrids. As mentioned above, ethanol is only part of the composition of the distilled spirit. A range of other compounds, especially a plethora of esters, short-chain fatty acids and methyl ketones are common secondary metabolites of whey fermentations. Their presence affects the final sensory performance of the spirit and therefore should be under control, either by fermentation management or by judicious distillation.

In principle, most "contaminating" secondary metabolites can be removed by employing four distillation approaches in sequence: stripping, rectification, hydro-extractive distillation and another rectification step. A distiller may not want to remove all the additional flavor-active components. Using a simple pot still, the fermented whey will distil to yield a product of around 15–20% v/v ethanol, depending on the initial ethanol concentration. This ethanol concentration can be increased up to around 70% v/v with a second pot still. This approach will yield a spirit that will retain significant levels of flavor compounds and so will be most

"whey-like". If a "cleaner" spirit is required more complexity is required in the distillation set-up.

At the other extreme to the two-pot system is the four-stage system indicated above. Stripping is followed by rectification, a process that typically employs a column of plates to enhance the separation of ethanol from the stripped feed. This should yield an output of close to 96% v/v, close to the maximum concentration of ethanol possible at atmospheric conditions from an aqueous ethanol system (the "azeotropic limit"). But this ostensibly clean spirit still retains flavor from the initial stripping feed and needs further processing to clean up the final spirit. To do this, water is perversely added back to the rectifier column output. This has the effect of increasing the volatility of the secondary metabolites, so that they are more easily separated from the distilling ethanol. The output of this column is still relatively water-rich so an additional rectification stage is the final part of the distillation process to elevate the ethanol concentration toward the azeotropic limit of around 96% v/v.

As mentioned above, there is an option of applying a demethylizer as a final column stage. This is an essential operation for pectin-rich distillation feeds such as those from stone fruits and potatoes. The pectin content of whey is negligible so this is unnecessary. One point to note concerning the use of a demethylizer is that it is most effective at low water concentrations (in contrast to hydro-extractive distillation) and so it is best employed after the second rectification step.

For a plant that only distils whey fermentations, the four-column process has most to commend it, as it will yield spirit that is relatively clean or "neutral". From a craft perspective this is a relatively complex distilling operation (with associated fabrication costs), so novel still configurations are becoming increasingly common. From a customer perspective there are three points to keep in mind when seeking distillation equipment:

- What quality of the final spirit is required in terms of ethanol concentration and levels of secondary metabolites?

- What is the expected range of initial feed ethanol concentration?

- What is the solids content of the original fermented whey feed?

The two former points help to define the distillation stages and the columns that may or may not be required (columns add significant cost to still fabrication). The latter is an important consideration when considering heat source. Direct heating such as electrical elements can be problematic if heating causes precipitation (e.g. of proteins) as they can congeal on to the heating surfaces and can cause heat transfer and burn-on issues for the spirit. The latter in particular can give rise to burnt-on flavors that are difficult to remove from the spirit despite repeated distillations.

11. Use of spirit

A spirit can be used in a range of final distilled spirits. Most commonly, these are vodka, gin and liqueur/cordial products. The specifications for spirit used for vodka production are usually the most exacting. Usually the final product has to be essentially neutral, so that the concentrations of secondary metabolites should be minimal. Typically, spirit for vodka has specifications for

total terpenoids, acetic acid, ethyl acetate and methanol. Spirit used for gin must also be neutral, but the use of botanicals to flavor the resulting spirit can help to mask any minor flavor deviations. Liqueurs and cordials based on neutral alcohol are often relatively strong in flavor. In principle a spirit that is less neutralized can be used with relative comfort.

One other aspect to bear in mind is that the addition of sugar, usually as syrups, can help to smooth out any "edges" to the mouthfeel of the spirit. Most liqueurs require significant levels of sugar addition during production, whilst for gin and vodka, only the London dry gin style has proscriptive sugar levels. Returning to the design of the still layout, the decisions there can be steered by the expected uses that the spirit will be put to, with vodka requiring the most tightly defined quality criteria. In any case, though the spirits produced for whatever duty should be of consistent quality.

One other option is to use the spirit for non-potable uses such as fuel. Generally, though the value of a non-potable alcohol product is substantially less than potable alternatives so there is less financial imperative for producing, say, fuel alcohol.

12. Reactive distillation

A relatively recent development in distillation development is the concept of reactive distillation, pioneered by Berglund at Michigan State University. Here the concept is to encourage reactivity between spirit components to alter the sensory attributes of the spirit. This has significant potential value for whey distillates as one demonstrated option is to induce fatty acids to react with ethanol to create esters, mediated by a solid-state acid catalyst. From a whey distillate perspective, this can in principle help to reduce the levels of short-chain fatty acids in spirit (with typical flavor descriptors such as cheesy, rancid) and convert them into fruit-flavored esters. Whilst this has yet to be demonstrated specifically for whey this approach offers a tantalizing option for enhancing the neutrality of whey-derived spirits.

13. Product quality

Spirit quality can be influenced by several factors including source of the whey, fermentation parameters, still configuration and post- distillation product treatment. Congeners, minor volatile constituents of a spirit influence it's the organoleptic qualities. The perception of congeners is considered a flaw in vodka. Congeners are present in raw whey and are formed as secondary metabolites during the fermentation process. Congeners within whey can be carried over during the distillation process and are similar to congeners in other spirits [2].

The source of whey and fermentation parameters can influence the composition of congeners in fermented whey. The composition of the volatile aroma compounds within milk and other dairy products can vary depending upon the source of the milk [62]. The milk producer's diet and geographic location can be attributed to the presence volatile compounds such as terpenes and terpenoids [63, 64]. The cheese production process can also influence volatile compound composition of whey, particularly the application of heat and exposure to microorganisms. Exposure to heat can create thermal artifacts and influence chemical reaction rates within whey. The exposure of milk or whey to microbes can influence the volatile compounds

present in whey. The metabolites produced by microbes can include alcohols aldehydes, esters and ketones, all which can influence organoleptic quality. The microbial populations can differ per facility and geographic location [62]. Each cheese production facility can potentially produce wheys with different volatile compound compositions. The source of whey can influence the composition of volatile compounds present in a spirit [11].

Fermentation conditions can influence the production of secondary metabolites of *K. marxianus* [65, 66]. Traditional industrial ethanol production facilities take a *laissez faire* approach to fermentation conditions related to congeners production. These facilities' chief concern is maximizing ethanol production. A similar approach is taken at industrial whey to ethanol production facilities. The extractive distillation process is used to separate ethanol from congeners and produce a neutral spirit. It should be noted that congeners with a similar volatility as ethanol may be more difficult to separate. Diacetyl is difficult to remove via extractive distillation and can impart rancid butter or butterscotch aromas to a spirit [67]. Spirit quality is influenced by the number of plates used to separate the ethanol from the other volatile compounds. A greater number of plates allow for greater separation volatiles reducing the presence of congeners within the final spirit. The use of copper plates or other components which have contact with the spirit during the distillation process can influence the organoleptic qualities of the final product. Copper contact during distillation reduces sulfur aromas in spirits and can reduce concentration of sulfur containing compounds in the final spirit [68]. Post- distillation of filtration of the spirit can reduce the presence of congeners in the final product. Filtration with activated carbon can reduce the congeners in spirits and which can have a perceivable impact on the organoleptic qualities of the spirit [69].

If the spirit is to be sold as a vodka it should have a clean taste with no perceivable aroma. These requirements may not be as stringent if the product is to be sold as flavored spirit or mixed with other ingredients to produce a beverage such as Irish cream. Flavorings may mask presence of congeners or congeners with positive organoleptic qualities may enhance the final product.

For cheese makers with no prior knowledge of distillation, this entire process may appear intimidating. Fortunately, assistance is available for people entering into the distillation business [70].

14. Environmental implications of whey spirit production

The production of potable spirits from whey has the potential to reduce environmental impacts of cheese and spirit production [71]. The fermentation process reduces the environmental impact of whey. The conversion of lactose to CO_2 and ethanol can reduce the BOD of whey by 75% [43] and aerobic cultivation can reduce BOD levels up to 95% [35]. The volume reduction during distillation and reduction of BOD during fermentation indicate that processing spent wash would be less economically and environmentally impactful than raw whey. *K. marxianus* is classified as GRAS and can be used as feed for livestock. It has also illustrated that production of a spirit from whey destined to be land spread instead of a similar grain-based spirit can reduce net CO_2-equivalent emissions [71]. This 2018 study also indicated that the production of a whey-based spirit required less water than a grain-based spirit [71]. These factors indicate that the production of a spirit from whey may be beneficial to the whey producer, distiller and the environment.

15. Conclusion

Whey production can be an economic and environmental problem for small creameries and acid whey producers. The production of potable ethanol from whey is currently occurring on an industrial scale and it may be a strategy worth pursing for smaller producers.

Conflict of interest

The authors do not have any conflict of interest regarding materials covered within this chapter.

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References

[1] Tunick M. Whey processing, functionality and health benefits. In: Onwulata C, Huth P, editors. Whey Processing, Functionality and Health Benefits. 1st ed. Hoboken, NJ: Wiley-Blackwell; 2008. p. 400

[2] Dragone G, Mussatto SI, Oliveira JM, Teixeira JA. Characterisation of volatile compounds in an alcoholic beverage produced by whey fermentation. Food Chemistry. 2009;**112**(4):929-935

[3] Panesar PS, Kennedy JF, Gandhi DN, Bunko K. Bioutilisation of whey for lactic acid production. Food Chemistry. 2007;**105**(1):1-14. Available from: https://www.sciencedirect.com/science/article/pii/S0308814607002816

[4] Dufey PA, Messadene J, Silacci P. Ingestion of whey on alpine pastures by beef cattle and quality of the meat. Agrar Forschung Schweiz. 2016;**7**(1):30-39

[5] Freudenreich P. Carcass value of cows. Effects of feeding sweet whey. Fleischwirtschaft. 1984;**64**(8):958-961

[6] Lynch GP, McDonough FE. USDA research on whey and whey products as feed for cattle. Journal of Agricultural and Food Chemistry. 1979;**27**(4):695-698. Available from: http://pubs.acs.org/doi/abs/10.1021/jf60224a061

[7] Durham CA, Bouma A, Meunier-Goddik L. A decision-making tool to determine economic feasibility and break-even prices for artisan cheese operations. Journal of Dairy Science. 2015;**98**(12):8319-8332. Available from: https://www.sciencedirect.com/science/article/pii/S0022030215007390

[8] Ling C. Whey to Ethanol: A Biofuel Role for Dairy Cooperatives? Washington, D.C.; 2008. Available from: https://www.rd.usda.gov/files/RR214.pdf

[9] Hamilton R, Wansbrough H. The Manufacture of Ethanol from Whey. Available from: https://nzic.org.nz/app/uploads/2017/10/3H.pdf

[10] Guimarães PMR, Teixeira JA, Domingues L. Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey. Biotechnology Advances. 2010;**28**(3):375-384. Available from: https://www.sciencedirect.com/science/article/pii/S0734975010000224?via%3Dihub

[11] Risner D, Tomasino E, Hughes P, Meunier-Goddik L. Volatile aroma composition of distillates produced from fermented sweet and acid whey. Journal of Dairy Science [Internet]. 1 Nov 2018;**0**(0). Available from: https://www.journalofdairyscience.org/article/S0022-0302(18)31009-9/fulltext

[12] Blaschek KM, Wendorff WL, Rankin SA. Survey of salty and sweet whey composition from various cheese plants in Wisconsin. Journal of Dairy Science. 2007;**90**(4):2029-2034. Available from: https://www.sciencedirect.com/science/article/pii/S0022030207716910

[13] Outinen M, Heino A, Uusi-Rauva J. Pre-treatment methods of Edam cheese milk. Effect on the whey composition. LWT-Food Science and Technology. 2010;**43**(4):647-654. Available from: https://www.sciencedirect.com/science/article/pii/S0023643809003223

[14] Johansen AG, Vegarud GE, Skeie S. Seasonal and regional variation in the composition of whey from Norwegian Cheddar-type and Dutch-type cheeses. International Dairy Journal. 2002;**12**(7):621-629. Available from: https://www.sciencedirect.com/science/article/pii/S0958694602000547

[15] Casper JL, Wendorff WL, Thomas DL. Seasonal changes in protein composition of whey from commercial manufacture of caprine and ovine specialty cheeses. Journal of Dairy Science. 1998;**81**(12):3117-3122. Available from: https://www.sciencedirect.com/science/article/pii/S002203029875876X

[16] Belitz H-D, Grosch W, Schieberle P. Carbohydrates. In: Food Chemistry. 4th ed. Berlin, Heidelberg: Springer Berlin Heidelberg; 2009. pp. 248-339. Available from: http://link.springer.com/10.1007/978-3-540-69934-7_5

[17] Belitz H-D, Grosch W, Schieberle P. Milk and dairy products. In: Food Chemistry. 4th ed. Berlin, Heidelberg: Springer Berlin Heidelberg; 2009. pp. 498-545. Available from: http://link.springer.com/10.1007/978-3-540-69934-7_11

[18] Bansal S, Oberoi HS, Dhillon GS, Patil RT. Production of β-galactosidase by *Kluyveromyces marxianus* MTCC 1388 using whey and effect of four different methods of enzyme extraction on β-galactosidase activity. Indian Journal of Microbiology. 2008;**48**(3):337-341. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23100731

[19] Domingues L, Guimarães PMR, Oliveira C. Metabolic engineering of *Saccharomyces cerevisiae* for lactose/whey fermentation. Bioengineered Bugs. 2010;**1**(3):164-171. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21326922

[20] Silva AC, Guimarães PMR, Teixeira JA, Domingues L. Fermentation of deproteinized cheese whey powder solutions to ethanol by engineered *Saccharomyces cerevisiae*: Effect of supplementation with corn steep liquor and repeated-batch operation with biomass recycling by flocculation. Journal of Industrial Microbiology and

Biotechnology. 2010;**37**(9):973-982. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20535525

[21] Tahoun MK, El-Nemr TM, Shata OH. A recombinant *Saccharomyces cerevisiae* strain for efficient conversion of lactose in salted and unsalted cheese whey into ethanol. Nahrung/Food. 2002;**46**(5):321-326. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12428446

[22] Schmidt C, Mende S, Jaros D, Rohm H. Fermented milk products: Effects of lactose hydrolysis and fermentation conditions on the rheological properties. Dairy Science and Technology. 2016;**96**(2):199-211. Available from: http://link.springer.com/10.1007/s13594-015-0259-9

[23] Dutra Rosolen M, Gennari A, Volpato G, Volken de Souza CF. Lactose hydrolysis in milk and dairy whey using microbial *β*-galactosidases. Enzyme Research. 2015;**2015**:1-7. Available from: http://www.hindawi.com/journals/er/2015/806240/

[24] Harju M, Kallioinen H, Tossavainen O. Lactose hydrolysis and other conversions in dairy products: Technological aspects. International Dairy Journal. 2012;**22**(2):104-109. Available from: https://www.sciencedirect.com/science/article/pii/S0958694611002251

[25] Das M, Raychaudhuri A. Supply chain of bioethanol production from whey: A review. Procedia Environmental Sciences. 2016;**35**:833-846. Available from: https://www.sciencedirect.com/science/article/pii/S187802961630189X

[26] Roukas T, Lazarides HN. Ethanol production from deproteinized whey by β-galactosidase coimmobilized cells of *Saccharomyces cerevisiae*. Journal of Industrial Microbiology. 1991;**7**(1):

15-18. Available from: http://link.springer.com/10.1007/BF01575597

[27] Tetra Pak. Whey processing. In: Tetra Pak, editor. Dairy Processing Handbook. Rockford, IL: Tetra Pak; 2018. Available from: http://dairyprocessinghandbook.com/chapter/whey-processing

[28] Coté A, Brown WA, Cameron D, van Walsum GP. Hydrolysis of lactose in whey permeate for subsequent fermentation to ethanol. Journal of Dairy Science. 2004;**87**(6):1608-1620. Available from: https://www.sciencedirect.com/science/article/pii/S0022030204733159

[29] Panesar PS, Kumari S, Panesar R. Potential applications of immobilized β-galactosidase in food processing industries. Enzyme Research. 2010;**2010**:473137. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21234407

[30] Kosseva MR, Panesar PS, Kaur G, Kennedy JF. Use of immobilised biocatalysts in the processing of cheese whey. International Journal of Biological Macromolecules. 2009;**45**(5):437-447. Available from: https://www.sciencedirect.com/science/article/pii/S0141813009002050

[31] Berg JM, Tymoczko JL, Stryer L. Glycolysis and glucogensis. In: Biochemistry. 5th ed. New York: W H Freeman; 2002. pp. 453-496

[32] Fukuhara H. *Kluyveromyces lactis*—A retrospective. FEMS Yeast Research. 2006;**6**(3):323-324. Available from: https://academic.oup.com/femsyr/article-lookup/doi/10.1111/j.1567-1364.2005.00012.x

[33] Lachance M-A. Kluyveromyces van der Walt [1971]. In: The Yeasts. London: Elsevier; 2011. pp. 471-481. Available from: https://www.sciencedirect.com/science/article/pii/B9780444521491000355?via%3Dihub

[34] Ortiz-Merino RA, Varela JA, Coughlan AY, Hoshida H, da Silveira WB, Wilde C, et al. Ploidy variation in *Kluyveromyces marxianus* separates dairy and non-dairy isolates. Frontiers in Genetics. 2018;**9**:94. Available from: http://journal.frontiersin.org/article/10.3389/fgene.2018.00094/full

[35] Fonseca GG, Heinzle E, Wittmann C, Gombert AK. The yeast *Kluyveromyces marxianus* and its biotechnological potential. Applied Microbiology and Biotechnology. 2008;**79**(3):339-354. Available from: http://link.springer.com/10.1007/s00253-008-1458-6

[36] Koushki M, Jafari M, Azizi M. Comparison of ethanol production from cheese whey permeate by two yeast strains. Journal of Food Science and Technology. 2012;**49**(5):614-619. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24082274

[37] Lane MM, Morrissey JP. *Kluyveromyces marxianus*: A yeast emerging from its sister's shadow. Fungal Biology Reviews. 2010;**24**(1–2):17-26. Available from: https://www.sciencedirect.com/science/article/pii/S1749461310000035

[38] Beniwal A, Saini P, Kokkiligadda A, Vij S. Physiological growth and galactose utilization by dairy yeast *Kluyveromyces marxianus* in mixed sugars and whey during fermentation. 3 Biotech. 2017;**7**(5):349. Available from: http://link.springer.com/10.1007/s13205-017-0985-1

[39] Zoppellari F, Bardi L. Production of bioethanol from effluents of the dairy industry by *Kluyveromyces marxianus*. New Biotechnology. 2013;**30**(6):607-613. Available from: https://www.sciencedirect.com/science/article/pii/S1871678412008746?via%3Dihub

[40] Grubb CF, Mawson AJ. Effects of elevated solute concentrations on the fermentation of lactose by *Kluyveromyces marxianus* Y-113. Biotechnology Letters. 1993;**15**(6): 621-626. Available from: http://link. springer.com/10.1007/BF00138552

[41] Dragone G, Mussatto SI, Almeida e Silva JB, Teixeira JA. Optimal fermentation conditions for maximizing the ethanol production by *Kluyveromyces fragilis* from cheese whey powder. Biomass and Bioenergy. 2011;**35**(5):1977-1982. Available from: https://www-sciencedirect-com. ezproxy.proxy.library.oregonstate. edu/science/article/pii/ S0961953411000602

[42] Diniz RHS, Rodrigues MQRB, Fietto LG, Passos FML, Silveira WB. Optimizing and validating the production of ethanol from cheese whey permeate by *Kluyveromyces marxianus* UFV-3. Biocatalysis and Agricultural Biotechnology. 2014;**3**(2):111-117. Available from: https://www. sciencedirect.com/science/article/pii/ S1878818113001035

[43] MIG S. The biotechnological utilization of cheese whey: A review. Bioresource Technology. 1996;**57**(1): 1-11. Available from: https://www. sciencedirect.com/science/article/pii/ 0960852496000363

[44] Kargi F, Ozmıhcı S. Utilization of cheese whey powder [CWP] for ethanol fermentations: Effects of operating parameters. Enzyme and Microbial Technology. 2006;**38**(5):711-718. Available from: https://www. sciencedirect.com/science/article/pii/ S0141022905004916

[45] Kourkoutas Y, Dimitropoulou S, Kanellaki M, Marchant R, Nigam P, Banat I, et al. High-temperature alcoholic fermentation of whey using *Kluyveromyces marxianus* IMB3 yeast

immobilized on delignified cellulosic material. Bioresource Technology. 2002;**82**(2):177-181. Available from: https://www.sciencedirect.com/ science/article/pii/ S0960852401001596?via%3Dihub

[46] Ferreira PG, da Silveira FA, dos Santos RCV, Genier HLA, Diniz RHS, Ribeiro JI, et al. Optimizing ethanol production by thermotolerant *Kluyveromyces marxianus* CCT 7735 in a mixture of sugarcane bagasse and ricotta whey. Food Science and Biotechnology. 2015;**24**(4):1421-1427. Available from: http://link.springer. com/10.1007/s10068-015-0182-0

[47] Ozmihci S, Kargi F. Comparison of yeast strains for batch ethanol fermentation of cheese whey powder [CWP] solution. Letters in Applied Microbiology. 2007;**44**(6):602-606. Available from: http://doi.wiley.com/ 10.1111/j.1472-765X.2007.02132.x

[48] Hadiyanto AD, Aini AP, Pinundi DS. Optimization of ethanol production from whey through fed-batch fermentation using *Kluyveromyces marxianus*. Energy Procedia. 2014;**47**: 108-112. Available from: https://www. sciencedirect.com/science/article/pii/ S1876610214002197

[49] Ozmihci S, Kargi F. Kinetics of batch ethanol fermentation of cheese-whey powder [CWP] solution as function of substrate and yeast concentrations. Bioresource Technology. 2007;**98**(16):2978-2984. Available from: https://www-sciencedirect-com.ezproxy.proxy. library.oregonstate.edu/science/article/ pii/S0960852406005232

[50] Rodicio R, Heinisch JJ. Yeast on the milky way: Genetics, physiology and biotechnology of *Kluyveromyces lactis*. Yeast. 2013;**30**(5):165-177. Available from: http://www.ncbi.nlm.nih.gov/ pubmed/23576126

[51] González Siso MI, Ramil E, Cerdán ME, Freire-Picos MA. Respirofermentative metabolism in *Kluyveromyces lactis*: Ethanol production and the Crabtree effect. Enzyme and Microbial Technology. 1996;**18**(8): 585-591. Available from: https://www. sciencedirect.com/science/article/pii/ 0141022995001514

[52] El-Batal AI, Farahat LM, El-Rehim HA. Ethanol production by *Kluyveromyces lactis* immobilized cells in copolymer carriers produced by radiation polymerization. Acta Microbiologica Polonica. 2000;**49**(2): 157-166. Available from: http://www. ncbi.nlm.nih.gov/pubmed/11093678

[53] González-Siso MI, Touriño A, Vizoso Á, Pereira-Rodríguez Á, Rodríguez-Belmonte E, Becerra M, et al. Improved bioethanol production in an engineered *Kluyveromyces lactis* strain shifted from respiratory to fermentative metabolism by deletion of NDI1. Microbial Biotechnology. 2015;**8**(2): 319-330. Available from: http://www. ncbi.nlm.nih.gov/pubmed/25186243

[54] Gancedo JM. Yeast carbon catabolite repression. Microbiology and Molecular Biology Reviews. 1998;**62**(2):334-361. Available from: http://www.ncbi.nlm. nih.gov/pubmed/9618445

[55] Mehaia MA, Cheryan M. Ethanol from hydrolyzed whey permeate using *Saccharomyces cerevisiae* in a membrane recycle bioreactor. Bioprocess Engineering. 1990;**5**(2):57-61. Available from: http://link.springer.com/10.1007/ BF00589146

[56] Ostergaard S, Olsson L, Nielsen J. Metabolic engineering of *Saccharomyces cerevisiae*. Microbiology and Molecular Biology Reviews. 2000;**64**(1):34-50. Available from: http://www.ncbi.nlm. nih.gov/pubmed/10704473

[57] Kambam PKR, Henson MA. Engineering bacterial processes for cellulosic ethanol production. Biofuels. 2010;**1**(5):729-743. Available from: https://www.tandfonline.com/doi/full/ 10.4155/bfs.10.46

[58] Sanny T, Arnaldos M, Kunkel SA, Pagilla KR, Stark BC. Engineering of ethanolic *E. coli* with the *Vitreoscilla* hemoglobin gene enhances ethanol production from both glucose and xylose. Applied Microbiology and Biotechnology. 2010;**88**(5):1103-1112. Available from: http://link.springer. com/10.1007/s00253-010-2817-7

[59] Sar T, Seker G, Erman AG, Stark BC, Yesilcimen Akbas M. Repeated batch fermentation of immobilized *E. coli* expressing *Vitreoscilla* hemoglobin for long-term use. Bioengineered. 2017; **8**(5):651-660. Available from: https:// www.tandfonline.com/doi/full/ 10.1080/21655979.2017.1303024

[60] Sar T, Stark BC, Yesilcimen Akbas M. Effective ethanol production from whey powder through immobilized *E. coli* expressing *Vitreoscilla* hemoglobin. Bioengineered. 2017;**8**(2):171-181. Available from: https://www. tandfonline.com/doi/full/10.1080/ 21655979.2016.1218581

[61] Kelsall DR, Lyons TP. Practical management of yeast: Conversion of sugars to ethanol. In: Jacques KA, Lyons TP, Kelstall DR, editors. Alcohol Textbook. 4th ed. Nottingham: Nottingham University Press; 2003. pp. 121-133

[62] Turbes GS. Impact of Terroir on Cheddar Cheese Flavor and the Influence of Farm-to-Farm Variability, Commingling, and Pasteurization. Oregon State University; 2014. Available from: https://ir.library. oregonstate.edu/concern/graduate_ thesis_or_dissertations/7p88ck174? locale=en

[63] Lejonklev J, Løkke MM, Larsen MK, Mortensen G, Petersen MA, Weisbjerg

MR. Transfer of terpenes from essential oils into cow milk. Journal of Dairy Science. Champaign, IL; 2013;**96**(7): 4235-4241. Available from: https://www-sciencedirect-com. ezproxy.proxy.library.oregonstate.edu/ science/article/pii/S0022030213003123

[64] Borge GIA, Sandberg E, Øyaas J, Abrahamsen RK. Variation of terpenes in milk and cultured cream from Norwegian alpine rangeland-fed and in-door fed cows. Food Chemistry. 2016; **199**:195-202. Available from: https://www-sciencedirect-com. ezproxy.proxy.library.oregonstate.edu/ science/article/pii/S0308814615302430

[65] İşleten Hoşoğlu M. Study of increasing the production of volatile flavor compounds by the yeast *Kluyveromyces marxianus* through optimization of carbon and nitrogen sources. Food and Health. 2018;**4**(2): 112-123. Available from: www. scientificwebjournals.com

[66] Wittmann C, Hans M, Bluemke W. Metabolic physiology of aroma-producing *Kluyveromyces marxianus*. Yeast. 2002;**19**(15):1351-1363. Available from: http://doi.wiley.com/10.1002/ yea.920

[67] Walker G, Hill A. *Saccharomyces cerevisiae* in the production of whisk[e] y. Beverages. 2016;**2**(38):1-15. Available from: file:///C:/Users/derri/Downloads/ beverages-02-00038[3].pdf

[68] Harrison B, Fagnen O, Jack F, Brosnan J. The impact of copper in different parts of malt whisky pot stills on new make spirit composition and aroma. Journal of the Institute of Brewing. 2011;**117**(1):106-112. Available from: http://doi.wiley.com/10.1002/ j.2050-0416.2011.tb00450.x

[69] Siříšťová L, Šárka P, Riddellová K, Hajšlová J, Melzoch K. Changes in quality parameters of vodka filtered through activated charcoal. Czech Journal of Food Sciences. 2012;**30**:474-482. Available from: https://www-cabdirect-org.ezproxy.proxy.library. oregonstate.edu/cabdirect/ FullTextPDF/2012/20123285582.pdf

[70] Risner D, Hughes P, Meunier-Goddik L, Ramirez S. Starting a Distilled Spirit Business: A Guide to Completing the Alcohol and Tobacco Tax and Trade Bureau Application and the Oregon Liquor Control Commission Licensing Process [Internet]. 1st ed. Corvallis: Oregon State University Extension Service; 2018. p. 29. Available from: http://sos.oregon.gov/business/Pages/ default.aspx

[71] Risner D, Shayevitz A, Haapala K, Meunier-Goddik L, Hughes P. Fermentation and distillation of cheese whey: Carbon dioxide-equivalent emissions and water use in the production of whey spirits and white whiskey. Journal of Dairy Science. 2018; **101**(4):2963-2973