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PRODUCCIÓN DE LÍPIDOS DEL SUERO DE LECHE MEDIANTE SISTEMAS MICROBIANOS

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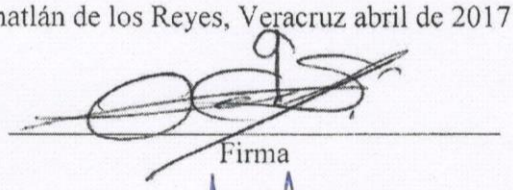

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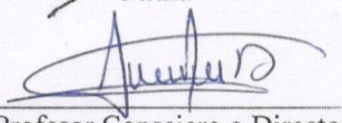

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PRODUCCIÓN DE LÍPIDOS DEL SUERO DE LECHE MEDIANTE SISTEMAS MICROBIANOS

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Colegio de Postgraduados, 2017

RESUMEN

El principal subproducto generado por el procesamiento de la leche en queso es el lactosuero. Se producen a nivel mundial 160 millones de toneladas al año. Más de 50 % del lactosuero se desecha sin tratamiento al drenaje, suelos o cuerpos de agua, lo que causa daños al medio ambiente por la cantidad de oxígeno que necesita para degradar altas concentraciones de materia orgánica. En este trabajo de investigación se realizó la producción de lípidos de la levadura oleaginosa *Cryptococcus laurentii*, en lactosuero de la producción de queso de leche de vaca y de cabra de dos queserías de Perote, Veracruz. La producción máxima de biomasa fue 18 gL^{-1} y 20 gL^{-1}, para el lactosuero bovino y caprino, respectivamente. Los factores tiempo y temperatura fueron los que más influyeron ($p = 0.000$ y $p = 0.010$). El mayor rendimiento de lípidos se obtuvo a partir del lactosuero dulce de bovino (4.13 %). Los principales ácidos grasos obtenidos de la fracción lipídica fueron oléico, palmítico, linoleico y esteárico.

Palabras clave: lactosuero, lípidos microbianos, *Cryptococcus laurentii*.

LIPID PRODUCTION FROM CHEESE WHEY BY MICROBIAL SYSTEM

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Colegio de Postgraduados, 2017

ABSTRACT

The main byproduct generated by the processing of milk in cheese is whey. Worldwide 160 million tonnes are produced annually. More than 50 % of the whey is discarded without treatment to drainage, soil or bodies of water, which causes damage to the environment by the amount of oxygen it needs to degrade high concentrations of organic matter. In this research, the production of lipids by oleaginous yeast *Cryptococcus laurentii* was carried out, in whey from the production of cow and goat cheese from two Perote, Veracruz cheese factories. The maximum biomass production was 18 gL^{-1} and 20 gL^{-1}, for bovine and goat whey, respectively. The time and temperature factors were the most influential ($p = 0.000$ and $p = 0.010$). The highest lipid yield was obtained from sweet bovine whey (4.13 %). The main fatty acids obtained from the lipid fraction were oleic, palmitic, linoleic and stearic.

Keywords: Cheese whey, microbial lipids, *Cryptococcus laurentii*.

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INTRODUCCIÓN GENERAL

Actualmente la industria láctea genera gran variedad de productos derivados de la leche, entre los que destacan yogur, helado, mantequilla y queso (Muñoz *et al*., 2014). De manera específica, la industria de elaboración de queso genera un residuo líquido llamado "suero de leche", "lactosuero" o "suero de queso" (Yadav, 2014). Este subproducto es altamente contaminante por sus características fisicoquímicas: altas concentraciones de proteínas, nitrógeno total, fósforo, azúcares disueltos y otros nutrientes (Tsolcha *et al*., 2015).

El lactosuero se caracteriza por sus altos valores de demanda química de oxígeno (DQO; 60-80 gL^{-1}) y demanda biológica de oxígeno (DBO; 35-45 gL^{-1}), producto de la concentración de materia orgánica (50-65 gL^{-1}) (Pais *et al*., 2014; Nath *et al*., 2015; Pundir y Dastidar, 2015). También es considerado generalmente un producto de desecho, y se elimina por el método más económico, se usa como alimento para animales (principalmente cerdos), se vierte sobre terrenos o en efluentes naturales, entre otros. La producción mundial de lactosuero es de $145x10^6$ toneladas por año, de las cuales $6x10^6$ toneladas corresponden a la lactosa (Das *et al*., 2015a).

La nueva cultura de valorización de subproductos agroindustriales y la innovación tecnológica, permiten aprovechar el lactosuero en compuestos de interés industrial (Koller *et al*., 2013), como biocombustibles (Bertin *et al*., 2013; Seo *et al*., 2015; Tomaszewska y Białonczyk, 2016), enzimas (Bansal *et al*., 2008), concentrados proteicos (Kalyankar *et al*., 2016), polímeros (Colombo *et al*., 2016), bacteriocinas (Garsa *et al*., 2014), entre otros.

La investigación sobre la producción de aceites vegetales se ha enfocado en la obtención de aceites producidos por microorganismos oleaginosos (Chang *et al*., 2013;). Los lípidos

microbianos se consideran fuentes prometedoras de aceites benéficos a la salud humana, que pueden utilizarse como aditivos alimentarios o para la producción de biodiesel (Meester, 2009). Estos aceites presentan ventajas sobre los aceites de origen vegetal: (1) no ponen en riesgo la seguridad alimentaria ya que no compiten con la producción de aceites comestibles; (2) la producción no está limitada a regiones geográficas ni a condiciones climáticas; (3) se pueden producir en condiciones controladas; y (4) los periodos de producción son más cortos comparados a los de fuentes vegetales o animales (Herrero y Alvarez, 2015).

El lactosuero es una opción para la obtención de lípidos microbianos, dado que es un subproducto abundante y subutilizado en el estado de Veracruz y en México, y así se podría contribuir a disminuir el efecto contaminante al medio ambiente. El objetivo de este trabajo de investigación fue desarrollar un método para la obtención de lípidos vía fermentativa, usando como biocatalizador una cepa microbiana de *Lypomyces kononenkoae* o *Cryptococcus laurentii*, a partir del lactosuero ácido y dulce de bovino y caprino, obtenido de dos queserías artesanales del centro de Veracruz.

Este documento se estructura en cinco secciones; en la primera se presenta la introducción general, el problema de investigación, los objetivos general y específicos, y la hipótesis; la segunda analiza la producción de lactosuero en el estado de Veracruz; la tercera muestra el estado del arte de los usos del lactosuero a nivel mundial; la cuarta desarrolla el trabajo experimental; y por ultimo, la conclusión general.

Problema de investigación

La mayor parte de los productores de queso de leche de cabra y vaca de la región de influencia de la zona central del estado de Veracruz, eliminan el suero de leche que se obtiene como subproducto en sus procesos de elaboración, provocando con esto daños al medio ambiente y convirtiendo un recurso potencial generador de ingresos en un desecho. Existen estudios en los que se propone utilizar el lactosuero como materia prima para otros procesos, tales como la producción de ácido cítrico, ácido láctico, enzimas y bebidas alcohólicas. Los resultados hasta el momento no han permitido llevar estas tecnologías a escala comercial en el país. Esta problemática se acentúa en sistemas de producción de baja escala debido a la carencia de tecnologías adaptables a este nivel y la falta de integración de redes de trabajo entre productores, investigadores, agroindustria e instituciones, a causa posiblemente por la distancia geográfica entre los actores, que podría solventarse con estudios locales conjuntos. Derivado de los anterior, en este trabajo de investigación se propone un proceso de producción de lípidos a partir del lactosuero generado en dos queserías artesanales de la región centro del estado de Veracruz, mediante fermentación con la levadura oleaginosa *Cryptococcus laurentii*.

Objetivos

Objetivo General

Desarrollar un proceso para la producción de lípidos a partir de suero de leche mediante sistemas microbianos.

Objetivos específicos

1. Caracterizar física y químicamente el lactosuero ácido producido en la región centro del estado de Veracruz.
2. Realizar una revisión del estado del arte sobre el aprovechamiento del lactosuero.
3. Evaluar el pretratamiento por hidrólisis química en el lactosuero.
4. Evaluar la producción de lípidos mediante estudios cinéticos de la cepa *Cryptococcus laurentii*.

Hipótesis

Es posible desarrollar un proceso sustentable para la producción de lípidos mediante fermentación, usando una levadura oleaginosa, a partir de lactosuero bovino y caprino proveniente de la pequeña agroindustria quesera de la región centro del estado de Veracruz.

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[1]CAPITULO I. POTENCIAL DE APROVECHAMIENTO DEL LACTOSUERO EN MÉXICO

Resumen

El lactosuero es el subproducto de la transformación de leche en queso, representa de 85 a 90 %. Su producción en el estado de Veracruz es de 351 662 toneladas, la mayor parte se vierte sin procesar al drenaje, terrenos aledaños y cuerpos de agua, lo que provoca contaminación ambiental debido a la alta demanda bioquímica y química de oxígeno que se requiere para degradar las altas concentraciones de materia orgánica que presenta, dado que aun contiene el 50 % de los nutrientes de la leche. El lactosuero representa un potencial de nuevos procesos de transformación para su aprovechamiento, su uso como sustrato para obtener productos con valor agregado: biocombustibles, enzimas, lípidos, ácidos orgánicos, se analizan como alternativas en el estado de Veracruz.

Abstract

As a by-product of the transformation of milk into cheese, a residue (85 % of the total milk used) is generated and called cheese whey. The current production of this waste in the State of Veracruz is 351 662 thousand liters and most is disposed of in water bodies, which causes damage to the environment due to the high biochemical and chemical demand for oxygen needed to degrade the high concentrations of organic matter present. This situation has generated the search for new processes for the transformation of this waste and several alternatives have been proposed for its use, one of these alternatives contemplates its use as a substrate for obtaining products with added value. This article mentions different alternatives for the use of whey produced in the State of Veracruz.

Palabras clave: derivados del lactosuero, valor agregado a subproductos lácteos.

[1] Agroproductividad. *Enviado.*

1. Introducción

El lactosuero es un subproducto poco aprovechado en México, incluso se le considera un problema ambiental potencial. El lactosuero es el residuo líquido generado en el proceso de elaboración de queso (Prudencio *et al*., 2014), este residuo contiene cerca del 50 % de los nutrientes presentes en la leche (Vázquez *et al*., 2009). Se estima que por 1 kg de queso producido se generan de 9 a 10 litros de suero (Padin y Díaz, 2009). La producción mundial de suero es de 160 millones de toneladas por año y está distribuido principalmente entre la Unión Europea con 1 790 872 toneladas y los Estados Unidos con 465 000 toneladas (FAOSTAT, 2015).

La industria láctea produce dos tipos de suero, dulce y ácido, el primero es obtenido por coagulación enzimática, el segundo se obtiene por coagulación por acidificación a través de cultivos lácticos o ácido orgánicos. El 50 % del suero generado se desecha en cuerpos de agua o en suelos (Aktas *et al*., 2006), lo que provoca un severo problema ambiental debido a la materia orgánica que contiene, principalmente lactosa de 40 a 60 % (Prazeres *et al*., 2012), por lo que la demanda química de oxígeno (DQO), de 60 a 80 g L^{-1} y la demanda bioquímica de oxígeno (DBO) de 30 a 50 g L^{-1} (Spalatelu, 2012) para llevar a cabo su degradación es elevada.

La descarga continua de suero sin tratamiento previo a los cuerpos de agua causa un rápido consumo de oxígeno, lo que produce eutrofización, formación de jabón, salinización, generación de malos olores y acidificación, entre otros elementos (Prazeres *et al*., 2013). Para analizar la producción y uso del suero, es necesario separarlo en dos grupos, el mercado de queso de las grandes agroindustrias y el amplio sector a pequeña escala que elaboran quesos tradicionales y comercializan en mercados locales y regionales, el objetivo de este análisis

es presentar un panorama del potencial de aprovechamiento del lactosuero generado por empresas rurales del estado de Veracruz y proponer alternativas de aprovechamiento.

2. Producción de leche en México

La leche es un alimento completo e indispensable para la nutrición humana, especialmente para los niños, por lo que su producción es de importancia para la seguridad alimentaria de México (Caballero, 2010). Con una producción de 11.8 millones de toneladas de leche, México ocupa el noveno lugar mundial, esto es: dos de cada diez litros de leche en el mundo son producidos en México (SAGARPA, 2015). El sector lechero mexicano desde la última década del siglo pasado, ha venido integrándose progresivamente a la economía mundial globalizada, con la consecuente apertura de sus mercados al comercio exterior (Abrego, 2011). La producción total de leche en el país ha aumentado durante la última década, hasta 2015 la producción fue de $1.15x10^7$ millones de litros (Figura 1a), de la que el 98.5 % ($1.14x10^7$ millones de litros; Figura 1b) es leche de bovino y solo el 1.5 % ($1.59x10^5$ miles de litros; Figura 1c) restante es leche de cabra (SIAP, 2016).

De la producción total de leche en el país, el estado de Veracruz contribuye con 695 762 miles de litros (Figura 2a), ocupa el sexto lugar a nivel nacional en producción de leche y aporta el 6.2 % del total nacional. Este Estado ocupa el doceavo lugar en producción de leche de cabra con 1,983 miles de litros (Figura 2b), aportando el 1.4 % de la producción total del país (SIAP; 2016).

Para fortalecer el acopio de leche en cuanto a infraestructura se refiere, en el año 2005 la SAGARPA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación) apoyó la construcción de 16 nuevos centros de acopio, no obstante la industrialización de leche líquida es a través de empresas como Nestlé, EVAMEX y LICONSA, las cuales se

localizan en diferentes municipios del Estado (Coatepec, Veracruz, Xalapa y Acayucan), en donde la materia prima es obtenida directamente con los productores (CTEE, 2009).

La producción de leche de bovino en el estado de Veracruz se concentra principalmente en diez municipios (Figura 3a), de los cuales Minatitlán es el más importante con una producción de 46 733.80 toneladas. La producción está concentrada en municipios ubicados en zonas tropicales, contrario a lo que ocurre con la producción de leche de cabra, en donde los principales municipios productores están ubicados en la parte de las altas montañas, en donde el clima predominante es semiárido, siendo el municipio de Perote el que mayor cantidad de leche aporta (Figura 3b), con una producción total de 210.57 toneladas (OEIDRUS, 2016).

3. Producción de queso en México

En México los productos lácteos como son los quesos y los yogures, así como las leches industrializadas: pasteurizada, ultra-pasteurizada y en polvo, ocupan los primeros lugares de comercialización con una tendencia hacia el abastecimiento de las zonas urbanas, ya que estas poseen vías de comunicación accesibles y concentran grupos con niveles de ingreso más altos, en contraste con las zonas rurales, donde el consumo de lácteos se limita principalmente a leche bronca y productos artesanales (Figueroa-Rodríguez *et al*., 2012).

La producción total de queso en el país es de 332 251 toneladas, distribuidas en 9 diferentes tipos, siendo los principales el queso fresco, el panela y el doble crema (SAGARPA, 2015; Figura 4).

La leche de cabra tradicionalmente se utiliza en la producción de dulces (cajeta principalmente); sin embargo, durante los últimos años ha tenido un incremento la producción de queso, principalmente por parte de pequeños productores que representan

alrededor del 40 % del sector lechero de cabra (Cuchillo *et al*., 2010). En México, la demanda de productos lácteos provenientes de las cabras mantiene una tendencia de crecimiento, lo que ha fomentado la integración de la cadena producción-comercialización (Escareño-Sánchez *et al*., 2011). Según la FAOSTAT (2015), la producción de queso a partir de leche de cabra en el país durante el 2013 fue de 15 698 toneladas.

En el estado de Veracruz los productos lácteos como el queso, están distribuidos en tres sectores principales, los producidos por los mismos productores, los producidos por intermediarios (la elaboración de este producto en los dos sectores antes mencionados se realiza de manera artesanal, utilizando equipos inadecuados, que en la mayor parte de las veces no cumple con las normas de calidad) y los producidos por diferentes empresas que figuran dentro de este ramo en el Estado (Quesos de Ozuluama, Jamapa, Rancho Chico, y La Joya) por mencionar algunos. No obstante, en el inventario estatal de agroindustrias del sector pecuario, se reportan un total de 124 queserías, las cuales representan el 54.4 % de este sector (CTEE, 2009).

4. Producción de suero de queso en México

Hasta el momento, no existen datos en las dependencias reguladoras internacionales (FAOSTAT) o nacionales (SIAP y SAGARPA), en relación al aprovechamiento de suero de queso para la producción de algún producto con valor agregado. Contrario a esto, desde inicios del siglo XX en el país se han desarrollado diferentes investigaciones referentes al proceso y aprovechamiento del suero de queso, los principales trabajos se refieren a la producción de bebidas saborizadas, ácidos orgánicos o como suplemento alimenticio.

5. Producción de suero de queso en el estado de Veracruz

De acuerdo a Montero-Lagunés *et al*. (2009), en México las queserías artesanales procesan de 2 000 a 10 000 litros de leche; en Veracruz el 56 % de la producción de leche se usa para

producir quesos (389 626 miles de litros de leche de bovino y 1 110 miles de litros de leche de cabra), si se toma en cuenta que por cada kilo de queso producido se utilizan 9-10 litros de leche y que por cada kilogramo de leche se producen de 8 a 9 litros de suero (Remón *et al*., 2016), en el año de 2015, la producción suero de queso en el Estado fue de 350 663 miles de litros de suero de queso a partir de la producción de queso de leche de bovino y de 999 mil litros de suero a partir de la elaboración de queso de leche de cabra, del cual el 45 % es desechado a los mantos acuíferos o al suelo, lo anterior representa una pérdida económica y provoca serios problemas de contaminación (eutrofización, acidificación o alcalinización del suelo), esto se debe a que los pequeños productores no cuentan con la tecnología necesaria para el aprovechamiento de este subproducto, ya que por su alto contenido de humedad y su rápida descomposición se dificulta su manejo y almacenamiento.

6. Usos potenciales del suero de queso

6.1 Producción de biomasa microbiana

Un aspecto muy demandado es la producción de proteína debido a que es insuficiente para la alimentación de la población. Una alternativa para solucionar esta deficiencia es la producción de biomasa (rica en proteína) a través de procesos de fermentación. Estos procesos pueden usar como sustrato suero de queso con diferentes tipos de microorganismos. La proteína unicelular, se puede utilizar para diferentes propósitos, por ejemplo como suplemento proteico en alimentos balanceados para el ganado o como suplemento en alimentos para consumo humano (Morr and Ha, 1993; Aguirre-Ezkauriatza *et al*., 2009; Wolz *et al*., 2016).

6.2 Obtención de lactosa

El compuesto de mayor importancia en el suero de queso es la lactosa (4.4 a 5.2 %). Este carbohidrato se puede utilizar en diversas aplicaciones, tales como: suplemento en fórmulas

lácteas para bebes, en la industria de los alimentos o en la producción de glucosa y galactosa por hidrólisis. La lactosa es importante en la industria de los alimentos y la industria farmacéutica debido a que este disacárido, presenta un bajo índice glicémico y calórico en comparación con otros azúcares. Este compuesto se puede obtener por medio de diferentes operaciones, dentro de las que destacan la concentración por evaporación, la cristalización y la filtración (Demirhan *et al*., 2007; Xinmin *et al*., 2008).

6.3 Extracción y producción de metabolitos secundarios

El suero de queso tiene gran potencial como sustrato para la obtención de compuestos benéficos para la salud, como los aminoácidos, los cuales están presentes en las proteínas de suero de queso, debido a que contienen altas concentraciones de aminoácidos de cadena ramificada como leucina, valina e isoleucina, los cuales tienen funciones como reguladores del metabolismo celular y son considerados importantes para el control de peso corporal, acción que ofrece la posibilidad de extender beneficios sobre la salud de los consumidores (Wu *et al*., 2009; Goulart *et al*., 2014).

Otros metabolitos obtenidos son las bacteriocinas, que son compuestos que actúan contra microorganismos patógenos presentes en los alimentos y que se producen mediante procesos fermentativos usando diferentes bacterias ácido lácticas (Anastasiadou *et al*., 2008; Altuntas *et al*., 2010).

A partir del suero de queso también se pueden producir exopolisacáridos, los cuales brindan diferente cualidades y funciones en los alimentos, ya que intervienen directamente en sus propiedades reológicas como agentes emulsificantes o gelificantes, adicionalmente, tienen efectos benéficos en la producción de alimentos gracias a la relación que existe entre estos compuestos y la protección que brindan contra ciertos compuestos tóxicos (Badel *et al*., 2011; Zhou *et al*., 2014; Haj-Mustafa *et al*., 2015).

6.4 Producción de ácidos orgánicos por fermentación

Un uso industrial importante que se le puede dar al suero de queso es la producción de diferentes ácidos orgánicos como el cítrico y el láctico, los cuales se obtienen a partir de la fermentación de lactosa mediante bacterias lácticas. Diversas industrias como la alimentaria, de bebidas, la farmacéutica y la cosmética los ocupan dentro de sus procesos de transformación como aditivos, debido a que actúan como conservadores, acidificantes, estabilizadores y potenciadores del sabor (Prasad *et al*., 2014; Cortés-Sánchez *et al*., 2015; Arslan *et al*., 2016).

6.5 Uso de microorganismos para la producción de aceites

Diferentes especies de microorganismos son capaces de acumular más del 20 % de su peso celular en lípidos. Algunos de estos microorganismos producen una cantidad mayor de grasa que la de algunos cultivos oleaginosos vegetales y a diferencia de estos no requiere de espacios grandes, se pueden producir en tiempos más cortos y no se ven afectados por las condiciones climáticas. La producción de lípidos mediante diferentes microorganismos y el uso del suero de queso como sustrato, es una alternativa prometedora para la obtención de ácidos grasos poliinsaturados, los cuales pueden ser utilizados como aditivos alimentarios o como materia prima para la producción de biodiesel (Yuzbasheva *et al*., 2014; Castanha *et al*., 2014; Tsouko *et al*., 2016).

6.6 Biocombustibles

La producción de bioetanol se considera una alternativa energética para disminuir el impacto ambiental negativo, provocado por el uso de combustibles fósiles. La conversión de lactosa proveniente de suero de queso crudo o suero de queso permeado a etanol, es una opción que puede competir con el uso de otros sustratos utilizados actualmente (caña de azúcar y almidón de maíz) o con la biomasa lignocelulósica (tecnología de segunda generación). Al ser un

residuo agroalimentario, el suero de queso tiene ventajas sobre materias primas comúnmente utilizadas para la producción de etanol, las cuales se consideran de primera necesidad, debido a que no compromete la seguridad alimentaria. Por otra parte, el etanol potable obtenido a partir de suero de queso, puede encontrar mercados adecuados, como la industria de procesos químicos, la industria automotriz, las industrias de cosméticos, de alimentos, de bebidas, y en la industria farmacéutica (Demirbas *et al*., 2010; Parashar *et al*., 2015).

La producción de biogás e hidrógeno se realiza a través de procesos químicos muy costosos, por lo que el proceso de producción biológico de ambos gases es una opción viable para resolver esa limitante, que al ser una fuente renovable, se tiene un impacto positivo sobre el medio ambiente, ya que la producción neta de gases de efecto invernadero que se generan durante su combustión es menor en comparación con la originada por el hidrógeno obtenido de materias primas fósiles. La principal variable a considerar en la producción biológica de hidrógeno o biogás es el costo de la materia prima, contenido de carbohidratos, biodegradabilidad y su disponibilidad en el mercado, entre las materias primas más utilizadas para la producción de biogás están las aguas residuales provenientes de la agroindustria, el suero de queso y estiércol líquido de bovino (Dareioti y Kornaros, 2015; Zhong *et al*., 2015).

7. Conclusión

El lactosuero es una fuente potencial para la obtención de productos con alto valor agregado por su alto contenido de nutrientes y con su aprovechamiento se contribuye a la disminución de la contaminación ambiental. Se han propuesto alternativas tecnológicas de procesamiento y aprovechamiento, pero se requiere de más investigación para la obtención de mayores rendimientos que hagan factible el proceso industrial, lo cual es un reto para la industria láctea. Estas tecnologías y productos pueden ser una ruta de desarrollo sustentable para la preservación del ambiente en un futuro cercano.

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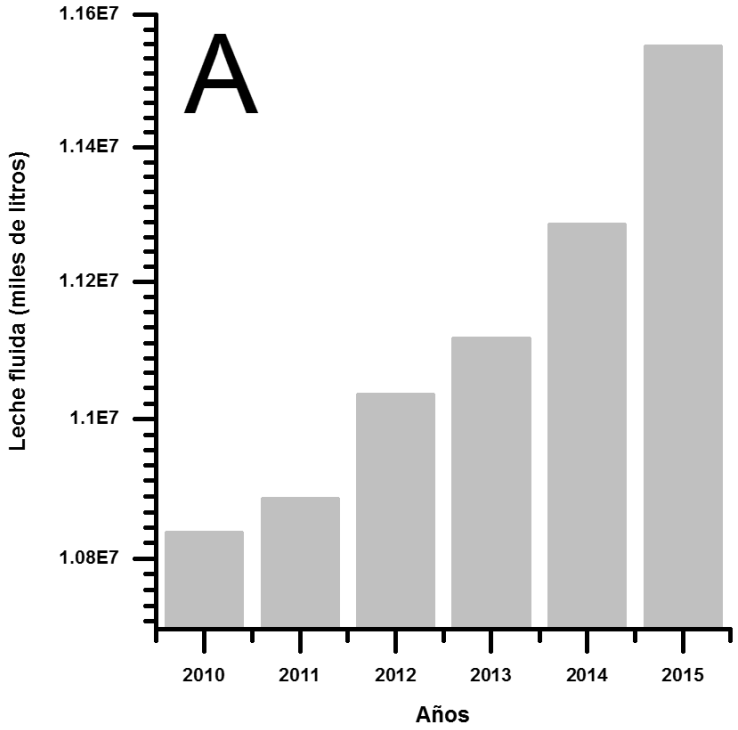
Figuras


A
1.16E7
1.14E7
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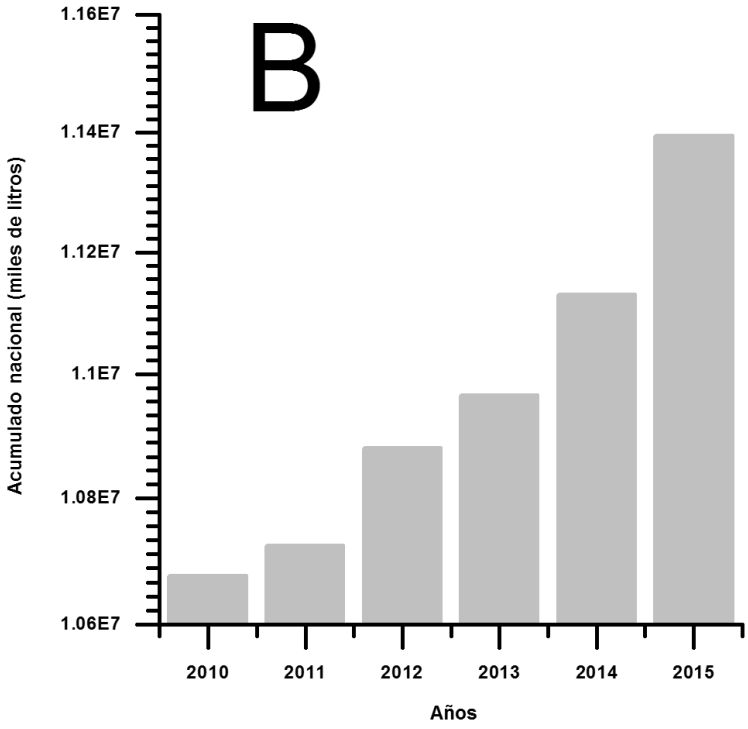
Leche fluida (miles de litros)
2010
2011
2012
2013
2014
2015
Años


B
1.16E7
1.14E7
1.12E7
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Acumulado nacional (miles de litros)
2010
2011
2012
2013
2014
2015
Años

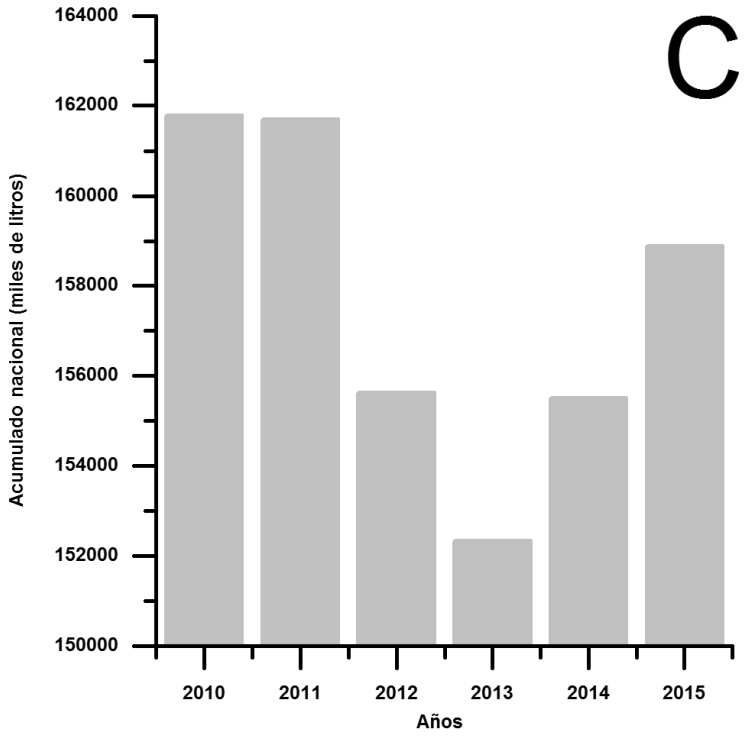

Figura 1. Producción total de leche en México (a), de bovino (b) y caprino (c) en los últimos seis años.

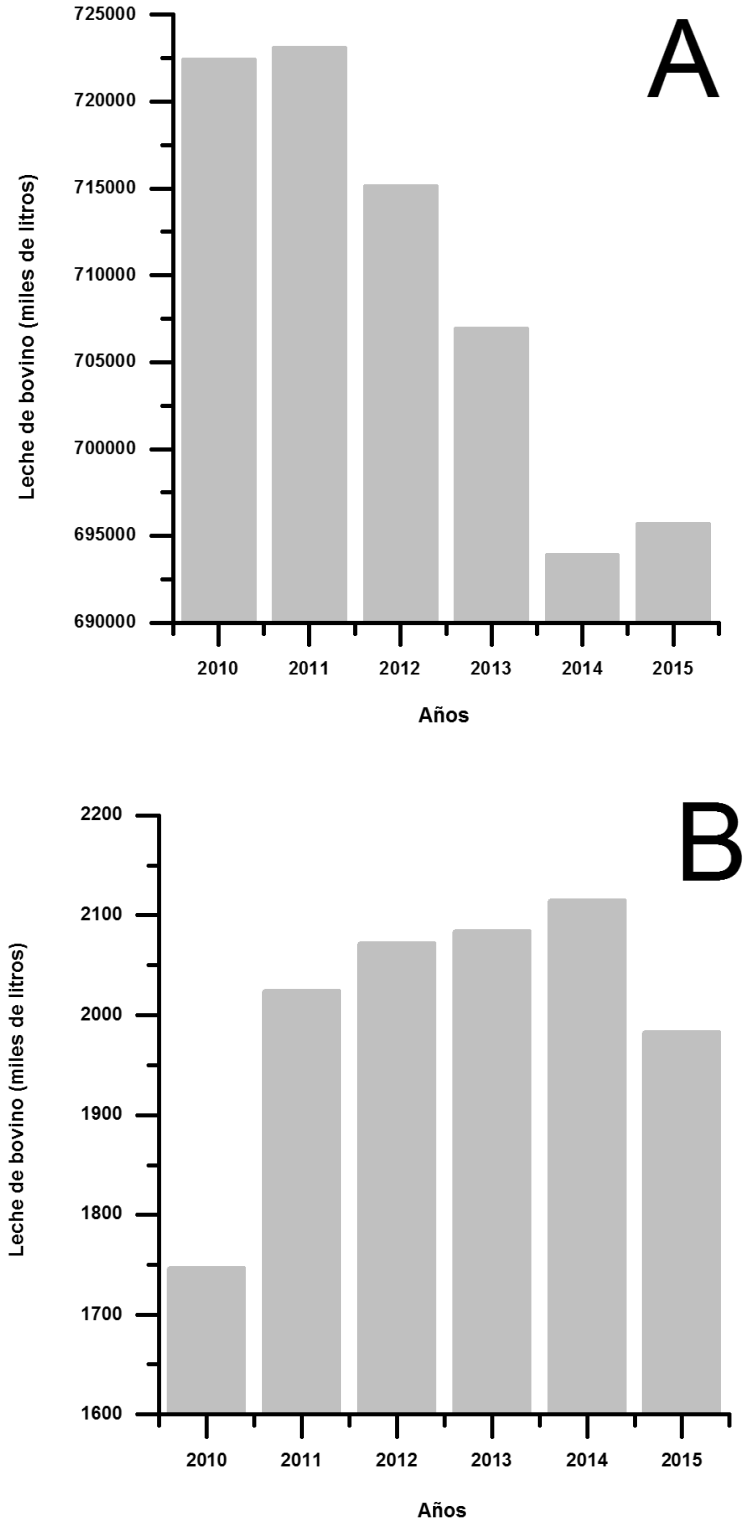

Figura 2. Producción de leche de bovino (a) y caprino (b) en el estado de Veracruz durante el periodo 2010 a 2015.

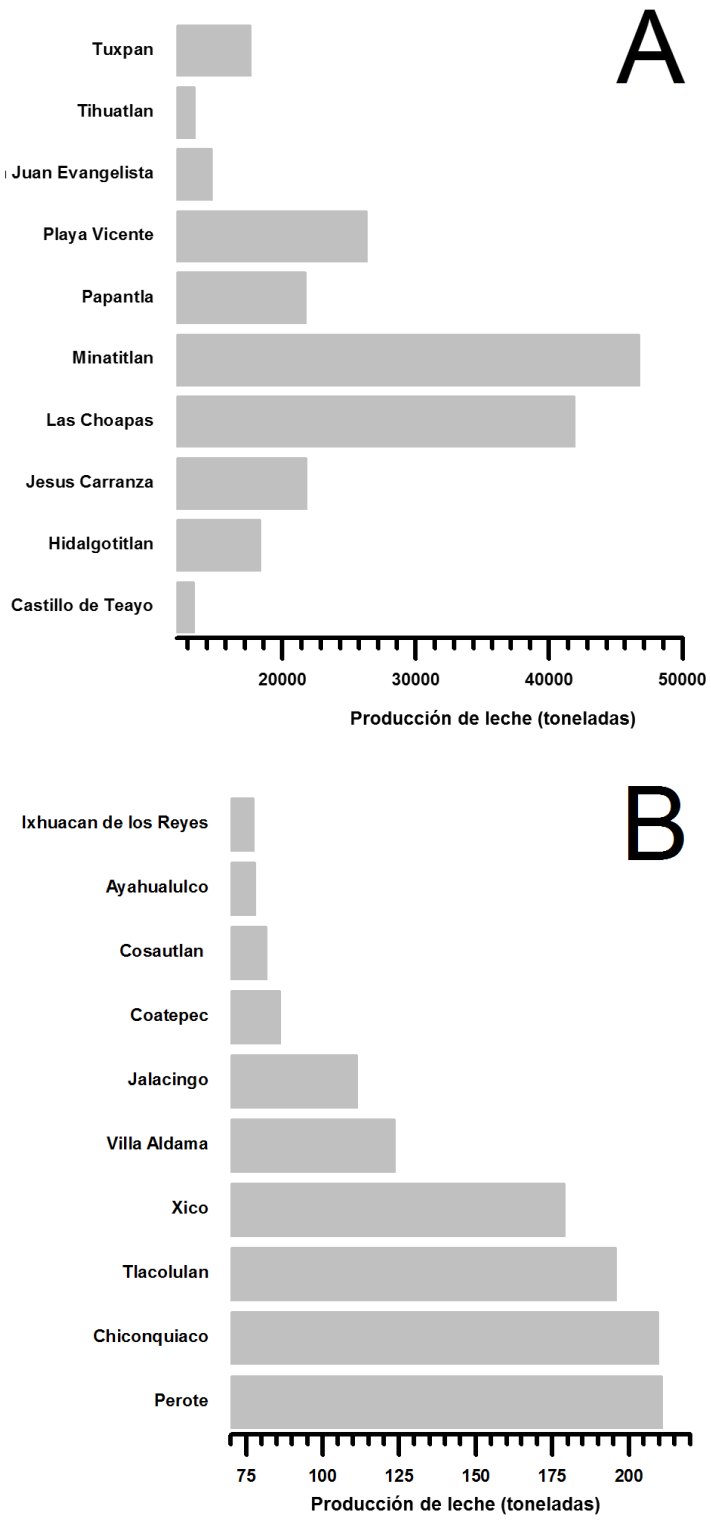

Figura 3. Principales municipios productores de leche de bovino (a) y de caprino (b) en el estado de Veracruz.

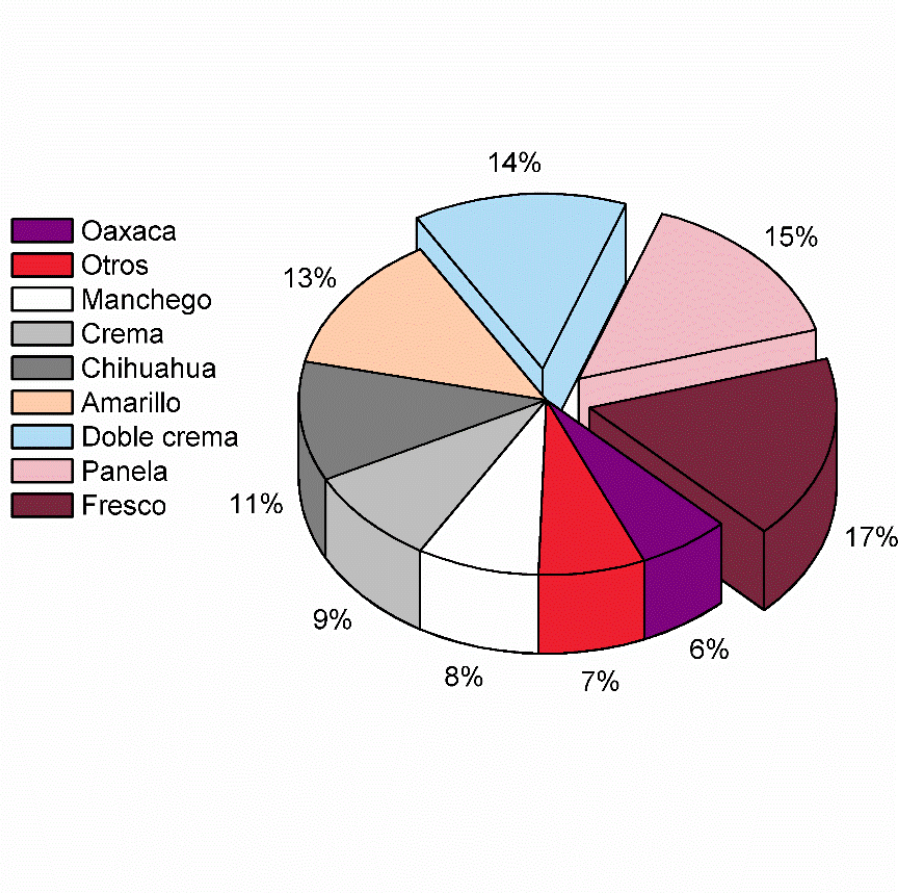

Figura 4. Producción de queso de todos tipos en México.

[2]CAPITULO II. PRODUCTION, USES AND APPLICATIONS OF CHEESE WHEY: A REVIEW

Resumen

Como subproducto de la transformación de la leche en queso, se genera un residuo (85% de la leche total utilizada) que se denomina suero de queso. La producción mundial de estos residuos por año es de 160 millones de toneladas y hasta la fecha, la mayor parte se descarta en los cuerpos de agua causando daños al medio ambiente debido a la alta demanda bioquímica y química de oxígeno que se necesita para degradar la presencia de altas concentraciones de materia orgánica. Esta situación ha generado la búsqueda de nuevos procesos para la transformación de estos residuos y se han propuesto varias alternativas para su uso, una de estas alternativas contempla su uso como sustrato para la obtención de productos con valor agregado. Los ejemplos clásicos son el etanol y la proteína unicelular; sin embargo, a través de procesos biotecnológicos se pueden obtener muchos bioproductos, entre los principales se encuentran lactosa, diferentes tipos de proteína (concentrada y aislada), ácidos orgánicos (acético, propiónico, láctico, cítrico) enzimas (β-galactosidasa, lipasa, poligalacturonasa), polímeros (polihidroxialcanoatos, polihidroxibutiratos), lípidos (ácidos grasos poliinsaturados), metabolitos secundarios (exopolisacáridos, prebióticos), biocombustibles (biogás, bioetanol, biodiesel). El objetivo fue determinar el estado del arte de las tecnologías utilizadas en la obtención de diferentes productos por la fermentación de suero de queso, sus ventajas en el proceso y beneficios que tienen para la salud, la economía y la preservación del medio ambiente.

[2] Environmental Technology Reviews. *Enviado.*

Abstract

Cheese whey is a by-product of the transformation of milk into cheese; (85 % of the total milk used). Worldwide production of this waste per year is 160 million tons and currently, most of it is discarded in water bodies causing damage to the environment due to the high biochemical and chemical demand of oxygen needed to degrade the presence of high concentrations of organic matter. This situation has generated the search for new processes for the transformation of this waste and several alternatives have been proposed for its use. One of these alternatives contemplates using it as a substrate for obtaining products with added value, such as ethanol and unicellular protein. Moreover, a great variety of bioproducts are obtained utilizing biotechnological processes, among the main ones are lactose, different types of protein (concentrated and isolated), organic acids (acetic, propionic, lactic, citric), enzymes (β-galactosidase, lipase, polygalacturonase), polymers (polyhydroxyalkanoates, polyhydroxybutyrates), lipids (polyunsaturated fatty acids), secondary metabolites (exopolysaccharides, prebiotics), biofuels (biogas, bioethanol, biodiesel) and compounds (vitamins, butanol, amino acids). This review is focused on the different processes used to obtain diverse products from cheese whey fermentation, the profits and benefits such products have for health, and the economy and preservation of the environment.

Key words: cheese whey, production, contamination, recovery.

Content

1. Introduction

High levels of pollution have led governments from all around the world to demand industries to focus on clean production of goods and services. This situation has forced companies to create solutions to reduce their pollutant loads (Panesar *et al*., 2007; Koutinas *et al*., 2009; Carvalho *et al*., 2013), by improving its processes or using by-products. The main waste generated by dairy industry is whey. The worldwide production of this waste per year is 160 million tons, with an annual growth of 1-2% (Dragone *et al*., 2009; Guimaraes *et al*., 2010; Ramírez Navas, 2012; Das *et al.,* 2015). In some cases, this whey is used directly for cattle feed; nevertheless, most of this product is poured into bodies of water or directly into the soil. Such event causes damage to the environment, since it contains a high concentration of dissolved organic substances (Pintado *et al.*, 2001; Mukhopadhyay *et al.,* 2003) that cause a high biochemical and chemical oxygen demand (35-45 mg/L and 80 mg/L respectively; Ergüder *et al*., 2001; Ozmihci and Kargi, 2007; Dragone *et al*., 2009; Das *et al.,* 2015). The variations in COD and BOD are directly determined by the type of milk and the lactose content (Mukhopadhyay *et al.*, 2003; Das *et al.,* 2015). Due to these characteristics, the new culture of valorization of agroindustrial wastes and the growing combination of new methodologies, could be used in other processes to obtain compounds of industrial interest (Koutinas *et al*., 2009; Bansal *et al*., 2008; Bertin *et al*., 2013; Dragone *et al*., 2011; Hernández-Ledesma *et al*., 2011), such as biogas (Bertin *et al*., 2013), enzymes (Bansal *et al*., 2008), ethanol (Dragone *et al*., 2011), and protein concentrates (Hernández-Ledesma *et al*., 2011). Another alternative for exploitation is the cultivation of oil-producing

microorganisms for lipids production that may substitute those obtained from the overexploited agricultural and animal sources (Ykema *et al*., 1988; González-Siso, 1996; Daniel *et al*., 1999; Meng *et al*. 2009). The lipids produced by this pathway can be used for human consumption (Panesar *et al*., 2007) and as raw material for the production of biodiesel (Meng *et al*., 2009; Moser, 2009; Faife-Pérez *et al*., 2012). The objective of this review is to present a perspective on the uses and applications of cheese whey, a subproduct of the dairy industry, with a biotechnological approach.

2. Cheese whey

Today only half of the whey produced worldwide receives some type of treatment for its recovery. This situation is mainly originated since small and medium producers are not able to acquire some technology to provide added value to this residue (Tavares y Malcata, 2016). One of the main products recovered from cheese whey is lactose, which can be used as an ingredient in the production of various other products (infant formula, bread, sweets, meats, etc.). However, when this sugar is fermented together with other essential nutrients for the growth of microorganisms, it is possible to obtain a wide variety of by-products (Soriano-Pérez *et al*., 2012; Hadiyanto *et al*., 2014; Valentino *et al*., 2015; Amado *et al*., 2016; Remon *et al*., 2016). The amount of cheese whey that is generated annually in the world is about 160 million tons (Dragone *et al.* 2009), this is due to each kilo of processed cheese generates 9 kilos of whey (Ozmihci y Kargi, 2007). This phenomenom has become a major environmental, health and economic problem, since a large number of small and medium producers, as well as milk processing plants, do not own a system for the appropriate treatment of this waste. In order to find a solution to this problem, several researches have been carried out on technologies for the use of whey and the generation of different products for specific sectors (food, pharmacy, health, cosmetics).

2.1 Types of cheese whey

The composition and type of whey depend on the processing technique used in the cheese making process. In the dairy industry, there are two types of whey: acid and sweet, the first type is obtained mainly by direct use of some organic acid as a coagulant in cheese processing or casein recovery. On the other hand, the sweet whey is mainly obtained by direct coagulation with animal or microbial enzymes (Anand *et al.,* 2013). The main difference between both wheys is the lactose and mineral content, the amount of protein and the degree of acidity (Guimaraes *et al*., 2010).

2.1.1 Acid whey

When lactic cultures are used for the production of cheeses acid whey is obtained as waste. The characteristic whey color is yellow, sometimes green, and a bluish tone is rarely perceived (Yadav *et al.,* 2015). One of the main characteristics of acid whey is that, due to its low pH value (4-5), it causes insolubility of some proteins present in the whey (Aydiner *et al.,* 2014; Kar *et al.,* 2015). The processes of elaboration of specific cheeses that involve prolonged processes of fermentation generate acid whey with high bacterial and enzymatic load (Chime *et al.,* 2009).

2.1.2 Sweet whey

The most frequently found cheese whey in the dairy industry comes from coagulation of casein by rennet (enzymatic chymosin complex, Carvalho *et al*., 2013). Casein coagulation enzymatically occurs at pH values of 6.5. The whey obtained during this treatment is called sweet whey (Kosseva *et al.,* 2009; Aydiner *et al.,* 2014; Kar *et al.,* 2015).

2.2. Cheese whey composition

The physical and chemical characteristics of whey are directly dependent on the process used to remove casein and the type of milk used (Pescuma *et al.,* 2015). The difference between

the two types of whey (Table 1) is defined by its lactose content, its acidity, the amount of protein, among others (Anand *et al*., 2013).

3. Cheese whey as a contaminant

In the second half of the 20th century, community action groups, environmental agencies, and processors equally recognized and highlighted the environmental damage caused by the elimination of untreated cheese whey. Due to only half of the world's whey production is processed and transformed into different products, this problem has become a priority in order to treat and take advantage of it (Yorgun *et al.*, 2008; Becerra *et al.,* 2015). Since governments in various jurisdictions around the world acted, with the exception of some developing countries, it is currently illegal to dispose of untreated whey in water bodies (Smithers *et al,* 2015). The contamination caused by this residue is originated by the high biochemical oxygen demand (BOD), which ranges are from 30 to 50 mgL^{-1}, and by the chemical oxygen demand (COD) that fluctuates between 60 and 80 mgL^{-1}. Such demand is mainly produced by the lactose content, approximately 70 % of the total solids that the whey contains (Das *et al*., 2015; Nath *et al*., 2015).

4. Pre-treatments for cheese whey

4.1 Deproteinization

The first processing for the recovery of the liquid whey generally consists of the separation of residual particles of the curd, as well as the recovery of fat (centrifugation or clarification, Grinstead *et al*., 2000).

4.1.1 Characteristics of whey proteins

Whey proteins are a by-product of cheese making process and when they are disposed, they represent a potential damage to the environment (Chelulei y Kulozik, 2015). Whey protein

is composed of at least nine types of protein fractions, from those five are the most important: β-lactoglobulins, α-lactalbumin, glycomacropeptides, immunoglobulins and protease-peptone (Krissansen, 2013; Wijayanti *et al*., 2014; Table 3). Due to the recent technological advances in the recovery and purification of these molecules, they have been classified into concentrated protein (WPC) or isolated protein (WPI). They are widely used in various segments of the food industry (dairy, bakery, and others, Barba and Beolchini, 2000, Dies-Municio *et al*., 2012, Wolz *et al*., 2016), as well as in the medical and pharmaceutical industry (Morr and Ha, 1993). The main whey protein is β-lactoglobulin. It is a rich source of cysteine (Eissa *et al*., 2014). This protein fraction is utilized as an active ingredient in the pharmaceutical industry (Chelulei and Kulozik, 2015), since it offers health benefits, such as prevention of type II diabetes mellitus (T2DM), as well as for the treatment of some disorders related to metabolism (Gillespie *et al*., 2015). In general, the protein fractions obtained from the whey may be used by humans to obtain essential amino acids (Wijayanti *et al*., 2014). However, from 35 to 55 % of WPC is used as an ingredient in animal feed and more than 70 % of WPI is used as an active or nutritional ingredient (Morr and Ha, 1993).

4.1.2 Precipitation

Several studies show that whey protein is involved in increased satiety (Paddon-Jones *et al*., 2008). However, its low thermal stability limits its application. Thermal stability can be defined as the ability of proteins to survive thermal treatment without detrimental changes in their chemical structure. In its natural state, whey protein is soluble in the pH range of 2 to 9. This encourages protein-water interactions over hydrophobic protein-protein interactions. These solution conditions (pH and ionic strength), have a substantial influence on thermal stability and aggregation of proteins (Ryan *et al*., 2012). The electrostatic interactions

between protein molecules and aggregates play an important role in the determination of their functional properties. Electrostatic interactions are particularly sensitive to their ionic environment and can be manipulated by the addition of minerals. When salt concentration increases, a more extensive shield negatively charged appears in whey protein aggregates, favoring faster gelation and a more rigid gel structure (Bryant and McClements, 2000).

4.1.3 Membrane technologies

In the last three decades membrane technologies (diafiltration, microfiltration, ultrafiltration, nanofiltration and osmosis) have become a very important tool in the food processing industry (El y Mietton-Peuchot, 2015). They have been used particularly in the dairy industry for the recovery of components of specific interest (lactose and proteins) due to their acceptable operating cost (Cuartas-Uribe *et al*., 2009; de Souza *et al*., 2010). The most common whey added value consists of the recovery of lactose and globular proteins, which have different applications within the food industry (Becerra *et al*., 2015). In this context, membrane technology plays an important role in the valorization of the main by-products recovered from cheese whey (Chandrapala *et al*., 2016a).

a) Reverse osmosis

Reverse osmosis is a membrane system (100-0.1 μm) in which a solution is purified by spontaneous osmosis (Aydiner *et al*., 2013). The main uses of reverse osmosis is the separation of the water present in the whey and the treatment of leachates. It is also used in the quantification of chemical oxygen demand (COD), for the elimination of ammoniacal nitrogen (Turan *et al*., 2000), for the purification of lactose and the demineralization of concentrated whey (Aydiner *et al*., 2014).

b) Microfiltration

Microfiltration (MF) is a method that provides opportunities for the use of residual whey in the milk industry and the products obtained from it. It is mainly used in the removal of bacteria, in the selective fractionation of fat and the elimination of casein particles (Ye *et al*., 2011; Das *et al*., 2015b). Additionaly, the use of low-pressure microfiltration can completely replace the centrifugation process as pretreatment of the whey (Ye *et al*., 2011). The cut size of the membranes used in this methodology oscillates between 0.1-10 μm, a sufficient size to retain all kinds of bacteria, turbidity, macromolecules, colloids, among others.

c) Ultrafiltration

The use of ultrafiltration began in the 70's, with the aim to separate and concentrate proteins from the whey. The ultrafiltration separation is based on molecular sizes that depend on the membrane retention characteristics themselves (Patel y Murthy, 2012). Ultrafiltration (UF) is also used in the dairy industry for the separation of protein concentrates. This methodology is mainly focused on a size-exclusion strategy based on pressure and through a separation membrane (Hodúr *et al*., 2009; Nath *et al*., 2015a). In order to carry out lactose separation, lactic acid recovery and demineralization of whey, nanofiltration (NF) has been used efficiently since this methodology is based on the principle of fractionation and continuous concentration (Chandrapala *et al*., 2016b). Ultrafiltration removes 0.001-0.1 μm particles. The membranes used in ultrafiltration may retain viruses, macroproteins and antibiotics.

d) Electrodialysis

Another alternative method for the recovery of lactic acid and for whey demineralization is electrodialysis. It is a methodology based on the transference of a constant electrical charge through membranes (Chen *et al*., 2016). Electrodialysis is an electrochemical separation process where, under the influence of an electric field, ions are able to move through selective

membranes made from synthetic polymers (styrene-divinylbenzene) and containing ion exchange groups (sulfonic, carboxylic, arsenic or phosphoric acids) when are exposed to potentials of 1-2 V. Among its main advantages, electrodialysis offers a low energy consumption and a high efficience under controlled conditions with a relatively ease of use (Chacón-Villalobos, 2006).

4.2 Recovery of lactose

4.2.1. Lactose

In the dairy industry, the most important carbohydrate is lactose (4.4-5.2%). It is present in milk, whey and some dairy products (Bury *et al*., 2001; Zisu *et al*., 2014). The recovery of this disaccharide is carried out by different processes, for instance concentration by evaporation, crystallization, electrodialysis and filtration (Xinmin *et al*., 2008). This compound may be present in various isomeric chemical forms (amorphous lactose such as α-lactose, β-lactose or as a mixture of both; Patel y Murthy, 2012). The use of this carbohydrate is diverse, due to its utility as a supplement in milk formulas for babies (de Souza *et al*., 2010), in the food industry (Patel y Murthy, 2012), or in the production of glucose and galactose by hydrolysis (Demirhan y Özbek, 2009; Erich *et al*., 2015). The food and pharmaceutical industries are interested in this disaccharide, due to its low glycemic and caloric index, compared to other sugars (Demirhan *et al*., 2007).

4.2.2. Hydrolysis of lactose

The hydrolysis of the lactose present in the whey contributes to solving several problems (health, technological and environmental) caused by the poor disposition and the lack of treatment of the residues from the elaboration of cheese (Panesar *et al*., 2006). The hydrolysis of the lactose can be carried out by acid treatment or by enzymatic treatment.

a) Chemical hydrolysis

Chemical hydrolysis is a process that is performed using high acid concentrations and the application of high temperatures. The products obtained (glucose and galactose) are not produced in equimolar amounts to the initial lactose concentration (Demirhan y Ozbek, 2009). Chemical hydrolysis also causes several problems (color and formation of unwanted compounds as well as denaturation of proteins; Sener *et al*., 2006). The use of acid in the hydrolysis of lactose is viable only for protein-free whey, such as permeated whey obtained from ultrafiltration (UF). Adjustment of the pH can be done by direct addition of acid or an ion exchange resin. Typically, the pH is adjusted to 1.2 and the temperature to 150 °C for a short period of time (Harju *et al*., 2012).

b) Enzymatic hydrolysis

In the dairy industry, the enzymatic hydrolysis of lactose is highly important since it offers different opportunities of biotechnological utilization (Nath *et al*., 2014; Vasileva *et al*., 2016). Lactase (β-D-galactosidase) is used as a biocatalyst. This enzyme is widely distributed in nature and can be isolated from different sources: plants (almonds, peaches, apricots, apples), animal organs, yeasts, bacteria and fungi (Ansari y Husain, 2012). Nevertheless, the most used enzyme is obtained from *Kluyveromyces* sp. or *Aspergillus* sp. (Ansari y Satar, 2012; Erich *et al*., 2015). Currently, lactase is one of the most important enzymes in the food industry (Klein *et al*., 2013). The lactose hydrolysis process is simple and does not require the use of special equipment within dairy factories (Muniraj *et al*., 2015). When using the enzyme in suspension (for single use) for the hydrolysis of lactose, several factors that affect the hydrolysis of lactose must be taken into account, including substrate concentration, operating pH, maximum temperature, the permissible contact time, the enzymatic activity and the cost (Sener *et al*., 2006). This type of hydrolysis is commonly used in the dairy

industry to elaborate lactose-free products. This process allows solving the problem that affects consumers who are intolerant to this carbohydrate (Nath *et al*., 2014; Erich *et al*., 2015).

c) Membrane reactors

Lactose hydrolysis in membrane reactors is a process that is usually carried out to obtain protein-free fluids, such as permeated milk or whey. The enzyme is recovered from the reaction mixture with a second ultrafiltration kit and the permeate containing hydrolyzed lactose (Miller y Brand, 1980). This process has not been commercially attractive due to its complexity to perform (Zadow, 1992). Table 2 shows the main bioreactors used in lactose hydrolysis.

d) Immobilized systems

Immobilized enzyme systems own a great potential for large-scale application in hydrolysis of milk and raw or permeated whey (Klein *et al*., 2013). Immobilized systems often use fungal lactase, due to fungal enzymes are very stable and the organisms used are on the *GRAS* list. This means that they can be used for food (Harju *et al*., 2012). In practice, the shelf life of an immobilized system can be several thousand hours, which significantly reduces costs compared to that of soluble enzymes. This process depends on a large amount of support used for enzyme immobilization (Albayrak *et al*., 2002; Vasileva *et al*., 2012).

5. Main products obtained from whey

5.1 Secondary metabolites obtained from cheese whey

Cheese whey has a great potential as a substrate for obtaining beneficial compounds for health. It is a residue with an interesting added value; therefore it has a high opportunity for generating new products and biotechnological processes (Brandelli *et al*., 2015).

5.1.1 Amino acids

Although the amino acid levels present in cheese whey proteins are lower than meat proteins, the methionine and cysteine content is higher (Nath *et al*., 2015b). Cheese whey proteins contain high concentrations of branched-chain amino acids (leucine, valine and isoleucine; Goulart *et al*., 2014). These amino acids are regulators of the cellular metabolism and are considered important for the control of corporal weight. These characteristics offer the possibility to extend benefits on the health of the consumers (Ha y Zemel, 2003; Katsanos *et al*., 2008; Wu, 2009).

5.1.2 Prebiotics

Currently, population is more aware of the relationship among food, nutrition and good health. Consequently, there has been a considerably increseament on the researching on the identification of food and its components, as well as knowledge of the benefits they provide to the consumers (Agrawal, 2005). Many of the investigations performed are related to bioactive peptides (antioxidant, antihypertensive and antimicrobial activities, antihypertensive), which are mostly derived from the hydrolysis of bovine caseins (Brandelli *et al*., 2015). In the last decade, the study of compounds of biological nature with biological activity (bioactive peptides) has aroused a great interest in the dairy industry (de Jesús *et al*., 2015; Anand *et al*., 2013). Some important variables in the production of bioactive peptides are the source of the precursor protein element, the type of enzyme involved, and the conditions how the peptides are obtained (Mulero-Cánovas *et al*., 2011; Chelulei y Kulozik, 2015). The release of the bioactive peptides may occur during digestion in the stomach or during commercial fermentation processes carried out by the metabolic activity of different types of microorganisms (Arruda *et al*., 2012). These compounds present biological

properties of great importance in different processes of the food industry: antimicrobial activity (Osman *et al*., 2016), antioxidant activity (Corrêa *et al*., 2011) and increased shelf life (Anand *et al*., 2013). It is also relevant for the treatment of different disorders that affect human health such as hormonal induction (Krissansen, 2007; González-Chaves *et al*., 2009), regulation of the immune system (Amjres *et al*., 2010; Fisher y Kleinschmidt, 2015) and the prevention of chronic diseases (Haque y Chand, 2008). The main sources of these compounds are milk and the main residue obtained of their transformation is cheese whey (Baró *et al*., 2001; Alvarado-Carrasco and Guerra, 2010).

5.1.3 Bacteriocins production

Some bacteriocins produced by lactic acid bacteria (LAB, Table 4) have activity against pathogenic microorganisms present in food (Ananou *et al*., 2008; Altuntas *et al*., 2010). The range of bacteria that produce bacteriocins is very wide. However, they are produced by lactic acid bacteria (BAL), that is particularly interesting for the food industry since these bacteria have the status of "generally recognized as safe" (GRAS; Anastasiadou *et al*., 2008).

In general, bacteriocins are a valuable tool in different types of industries (dairy, meat, fishery, medicine, pharmaceutical, Abee *et al*., 1995). The main use of bacteriocins is as preservative (Anastasiadou *et al*., 2008b; Abbasiliasi *et al*., 2012). Bacteriocins are divided into three classes: antibiotics, small hydrophobic thermostable peptides and large heat-labile proteins (Klaenhammer, 1993).

The production of bacteriocins is performed on complex substrates (MRS, Elliker, brain and heart infusion, tryptone glucose extract, trypticase soy broth) that promote high growth and yields (Albano *et al*., 2007; Bali *et al*., 2016). However, these cultures are expensive and the presence of large amounts of proteins interfere in the purification step (Somkuti y Gilbreth,

2007). An alternative substrate is cheese whey that may serve as a growth medium for bacteriocins production (Mota y Brandelli, 2003). Due to the previously mentioned, the biotechnological production of bacteriocins from this dairy industry waste could be considered as a viable alternative (Kumar *et al*. 2012).

5.1.4 Exopolysaccharides production

Exopolysaccharides are compounds of molecular weight between 10-1000 kDa (Wang *et al*., 2010). They may be homopolymers (composed of residues of glucans and fructans) or heteropolymers (consisting of several oligosaccharides: galactose, xylose, mannose, among others; de Vuyst y Degeest, 1999). Exopolysaccharides can be added to food products or produced directly during the fermentation of dairy products (Haj-Mustafa *et al*., 2015). These compounds have different functions in food. They directly intervene in the rheological properties as emulsifying or gelling agents (Zhou *et al*., 2014). Additionally, they have beneficial effects on human health. In recent years, the interest in the production of exopolysaccharides has increased thanks to the relationship of these compounds with the protection of microbial cells against toxic compounds (Badel *et al*., 2011). These benefits have increased the consumer demand of dairy products (new and existing) containing this type of compounds (Welman, 2015). The ability to produce EPS is widespread among acid-lactic yeasts (L. casei, L. acidophilus, L. brevis, L. curvatus, L. delbrueckii spp. Bulgaricus, L. helveticus, L. rhamonosus, and L. plantarum), although they can also be produced by microalgae (Cerning *et al*., 1994; Briczinski and Roberts, 2002; Dayananda *et al*., 2007; Li *et al*., 2014; Lavari *et al*., 2015). Bacteria also have good yields for the production of EPS (Gram-negative bacteria; Vaningelgem *et al*., 2004; Whitney and Howell, 2013). An

excellent substrate for the production of exopolysaccharides is cheese whey since it has a high lactose content.

5.2 Biopolymers production

During the last thirty years, the investigation on the production of biodegradable polymers has been intensified due to the increaseament of petroleum-based polymers comsuption (150 million tons/year) and the serious pollution problems caused to the environment by its slow degradation (Castilho *et al*., 2009). Different studies have focused on the production of biopolymers (polyhydroxyalkanoates, PHA) since they are excellent substitutes for plastics obtained from petroleum derivatives (de Jesus *et al*., 2015; Elain *et al*., 2016). These polymers are mainly produced by a microbial fermentation process (Table 5), helping to reduce production costs (Valentino *et al*., 2015). They are intracellularly stored by different microorganisms under restricted nutritional conditions (Shang *et al*., 2003; Braunegg *et al*., 2004; Berlanga *et al*., 2006).

For the production of these biopolymers, residues obtained from the transformation of milk are utilized (Bosco y Chiampo, 2010). In the production of these compounds, it has been found that the costs of the substrate (carbon source) constitute up to 50 % of the general cost of the production. Due to this factor, abundant and low-cost raw materials have been sougth such as cheese whey that is a viable feedstock for the production of microbial polymers (Obruca *et al*., 2011).

5.3 Organic acids production

Various industries (food, beverages, pharmacy, cosmetics) currently utilize a great variety of organic acids within their processes of transformation. They are used as additives, acidifiers, stabilizers, flavor enhancers or preservatives (de Jesus *et al*., 2015). Some examples of additives are citric and lactic acids (López *et al*., 2006; Arslan *et al*., 2016), which are used

as food additives, in the synthesis of biodegradable polymers and in the biomedical textile industry (Cui *et al*., 2012a; Soriano-Pérez *et al*., 2012; Prasad *et al*., 2014). Another example is succinic acid, used in the chemical synthesis of surfactants, detergents and green solvents (Wan *et al*., 2008). Moreover, the propionic acid is utilized as an important intermediate in herbicides and pharmaceuticals (Morales *et al*., 2006). On the other hand the lactobionic acid is mainly used in biomedicine and in the development of platforms of nanoparticles (Alonso *et al*., 2015). Finally, the hyaluronic acid is used in the clinical and pharmaceutical industry (Amado *et al*., 2016). The production of organic acids by fermentation using residues of the dairy industry (cheese whey) as substrate and the use of different microorganisms could be a sustainable response to the constant demand of these compounds in different industries.

5.4 Oil production

There are certain microorganisms with the ability to accumulate a high amount of lipids, commonly called oleaginous microorganisms. They are able to store more than 20 % of their cellular weight (Huang *et al*., 2013; Magdouli *et al*., 2014; Tsouko *et al*., 2016). Within these microorganisms are mainly yeasts, fungi, algae and some bacteria (Wältermann *et al*., 2000). Many microorganisms produce a greater amount of oil than some vegetable oleaginous crops (Beligon *et al*., 2016) and, unlike them, they do not require big spaces, they can be produced in shorter times and they are not affected by the climatic conditions (Li *et al*., 2008; Yuzbasheva *et al*., 2014). One of the main problems of microbial lipid production is the raw material (Muniraj *et al*., 2015). Dairy wastes have been proposed as a solution, since they can be used as a substrate for the production of fatty acids by different species of microorganisms (Zhu *et al*., 2008, Castanha *et al*., 2014; Ahmad *et al*., 2015; Table 6). Under favorable conditions these microorganisms may accumulate from 200 to 700 g of fresh lipid

per kilogram of carbohydrate. Most of these lipids are triglycerides (TAGs; Alvarez and Steinbüchel, 2002; Zhang *et al*., 2011).

The biotechnological production of lipids by fermentation takes place under restricted conditions of nitrogen source and with an excess of carbon source (Ahmad *et al*., 2015). The accumulation of lipids in oleaginous microorganisms is due to the presence of ATP-Citrate lyase that acts as a catalyst in the formation of acetyl-CoA, a raw material used in the fatty acid biosynthesis pathway (Ratledge, 2004; Xu *et al*., 2015). The fatty acids produced mainly are polyunsaturated fatty acids (PUFAs, palmitoleic, stearic, oleic, linoleic, among others, Castanha *et al*., 2014), which can not be synthesized by mammals and, therefore, they must be consumed in food (Beligon *et al*., 2016).

5.5 Enzymes production

Enzymes have played a transcendental role in the history of mankind since they have been used in different biologic systems for a wide variety of purposes (Headon and Walsh, 1994). Its main function is to act as a catalyst with high specificity and a high capacity to regulate its catalytic power (Ramos and Malcata, 2011; Prado-Barragán *et al*., 2016; Ventura-Sobrevilla *et al*., 2015). Currently, enzymes are produced from a wide variety of raw materials (Debeuckelaere, 2015).

Cheese whey and its derivatives (permeates) have been widely used for the production of β-galactosidase, a very important enzyme in the enzymatic hydrolysis of lactose (Tari *et al*., 2010). For this purpose a wide variety of "natural" and "engineering" microorganisms are used, (Streptococcus thermophilus, Bacillus stearothermophilus, Kluyveromyces lactis, Kluyveromyces fragilis, Guehomyces Pullulans, Aspergillus tubingensis, Escherichia coli; Bacillus B-2; Rao y Dutta, 1977; Sonawat *et al*., 1981; Rech *et al*., 1999; Bansal *et al*., 2008; Xu *et al*., 2012; Raol *et al*., 2015; Kamran *et al*., 2016; Tomizawa *et al*., 2016). Cheese whey

has also been used for the production of other enzymes, such as proteases (Dias *et al*., 2008; El-Shora and Metwally, 2008), amylases (Bajpai *et al*., 1991, 1992), carboxypeptidases Lee, 1998), lipases (Tommaso *et al*., 2011) and polygalacturonases (Murad and Foda, 1992; Harsa *et al*., 1993).

5.6 Biofuels production

Recently, the international community has focused on the renewable energy generation (Dareioti and Kornaros 2015). Fuels obtained from biological sources are called biofuels. There are several types of them such as solid biomass (wood and agricultural residues, Demirbaş, 2004, 2006, 2010), liquid fuels (bioethanol and biodiesel) and biogas (methane and hydrogen; Staopoulou *et al*., 2011). One of the main advantages of using biofuels is their environmental safety. The greenhouse gasses they produce as a by-product of their combustion are lower and sometimes null compared to those produced by fossil fuels (Ghali *et al*., 1989, Muniraj *et al*, 2015).

5.6.1 Bioethanol

The conversion of lactose from cheese whey to ethanol is economically competitive with the currently used substrates (sugarcane and corn starch) or with lignocellulosic biomass (second generation technology) (Kádár *et al*., 2011, Sarris and Papanikolaou, 2016). However, being a waste product represents an advantage over the raw materials of fermentation related to essential products such as corn, due to it does not endanger food safety. On the other hand, the production of drinkable ethanol from cheese whey may find suitable markets, for example in food, beverage, pharmaceutical, cosmetic industries (Guimarães *et al*., 2010; Parashar *et al*., 2015) and recently in the automotive industry as a raw material, solvent or fuel.

Furthermore, 95 % of ethanol produced worldwide is via fermentation (Sarris and Papanikolaou, 2016).

The fermentation of cheese whey to ethanol using yeasts has been reported since the 40´s decade (Table 7). Consequently, it is very important to choose a strain with adequate physiological characteristics to achieve an efficient transformation of lactose to ethanol from whey (Zafar and Owais, 2006).

The fermentation process can be performed in different modes. The most used forms are batch, fed-batch and continuous batch (Christensen *et al*., 2011; Hadiyanto *et al*., 2014).

5.6.2 Biodiesel from microbial lipids

The production of biodiesel derived from oilseeds, animal fat or residual cooking oils is an alternative to petroleum-derived fuel (Wang *et al*., 2015; Wasilenko *et al*., 2015). Nonetheless, this production difficultly satisfy the existing demand for fuel in the transportation industry (Wild *et al*., 2008). As a consequence, the search for production processes has intensified, especially in those that can be operated continuously and without limitations (quantities of arable land and climate conditions) (Beopoulos *et al*., 2011; Wasilenko *et al*., 2015).

In this context, lipids obtained from microbial systems seem to be an ideal source for the production of renewable biodiesel to contribute to satisfy the global fuel demand (Liu and Zhao, 2007, Vicente *et al*., 2009, Thiru *et al*., 2011). Recent advances demonstrate that some microbial species such as yeasts, fungi, and microalgae can be used as potential sources in the production of lipids for biodiesel. They can synthesize and store large amounts of fatty acids in their biomass (Yang *et al*., 2015). Therefore, microbial lipids are an alternative raw material with great potential for biodiesel production (Chang *et al*., 2015).

5.6.3 Biogas

Nowadays, commercial hydrogen production is carried out through very expensive chemical processes (Karadag *et al*., 2014). As a result, a high amount of energy is consumed. Biological hydrogen processes are a viable option to solve this issue (Azbar *et al*., 2009; Hublin *et al*., 2012). The cost of raw material, carbohydrate content, biodegradability and market availability are considered to determine the use of waste material in the biological production of hydrogen or methane (Kapdan and Kargi, 2006). The production of biogas from renewable sources has a positive impact on the environment. The production of greenhouse gasses generated during its combustion is extremely low compared to those produced by hydrogen from fossil fuels (Hublin *et al*., 2012, Moreno *et al*., 2015, Zhong *et al*., 2015). Among the most used raw materials for biogas production are wastewater from agro-industry, cheese whey and liquid bovine manure (Ghaly, 1989; Hublin and Zelic, 2012; Dareioti and Kornaros 2015). The production of hydrogen from biological processes can be divided into three types, photo-fermentation (cyanobacteria algae) photosynthetic (chemosynthetic-fermentative bacteria), and fermentation in obscurity with anaerobic bacteria (Ueno *et al*., 1996; Antonopoulou *et al*., 2008; Powell *et al*., 2013; Lin *et al*., 2014). Hydrogen production from dark fermentation is more advantageous than photo-fermentation due to the high H_2 production and the potential to use different residues as a substrate (Stamatelatou *et al*., 2011; Frascari *et al*., 2013; Gadhe *et al*., 2013).

6. Conclusion

Cheese whey is a by-product generated by the dairy industry. It is highly polluting if directly disposed in water sources, soils and wastewater processing plants. Due to its high nutrient content, whey is a potential source for obtaining products with high-added value. The harnessing of this resource contributes to the reduction of pollution and reduces the damage

caused by humans. Although different technological alternatives have been developed for its processing, more research is required to obtain higher yields that can make the industrial scaling of processes feasible. Finally, these technologies and products may be a sustainable development pathway for the preservation of the environment in the near future. The objective of this review was to summarize all the products that can be obtained by fermentation of cheese whey, its utilities and the benefits that these own for health, for economy and for the preservation of the environment. However, technological developments for the use and disposal of cheese whey remain as an important point of discussion for the dairy industry.

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7. References

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Table 1. Chemical composition of two different types of whey.

	Bovine		Goat	
Components	**Sweet whey g/L**	**Acid whey g/L**	**Sweet whey g/L**	**Acid whey g/L**
Total solids	64.3	63-70	70	62.91
Lactose	63.0	44-46	50	39.18
Protein	6.8	6-8	6.3	0.66
Fat	1.8	0.4	8.4	0.40
Ash	6.97	8.0	5.7	8.3
pH	6.2	3.8	6.34	--
DQO	--	--	--	--

Compiled from Boudjema *et al*., 2015; Yadav *et al*., 2015; Sanmartìn *et al*., 2012; Moulin and Galzy, 1984.

Table 2. Parameters identifying the main protein fractions isolated from cheese whey.

Protein fraction	Igs	BSA	Lf	β-LG	α-LA	Glycomacro-peptides	Protease-Peptone	LP	Reference
Molecular weight (kDa)	150-1000	66.0	76.5	18.3	14.0	6.8	4-22	78.0	Yadav *et al.* 2015
	150-1000	66	78	18.4	14.1	<7	--	89	Lech *et al.*, 2015
	150-1000	69	78	18.36	14.1	--	--	--	Patil *et al.*, 2014
	--	66	78	18.3	14.2	--	--	78	Nath *et al.*, 2015a
Concentration in whey (g / L)	0.4-1.0	0.4	--	2-4	0.6-1.7	--	--	--	Morr y Ha, 1993
	0.65	0.4	0.1	2.7	1.2	Varios	--	0.02	Lech *et al.*, 2015
	0.65	0.4	--	2.7	1.2	--	--	--	Cowan y Ritchie, 2007
pH (pl)	5.5-8.3	4.7-4.9	9.0	5.2	4.5-4.8	Varios	--	9.5	Lech *et al.*, 2015
	5.5-8.3	5.13	9.5-10.0	5.35-5.49	4.2	4.3-4.6	--	9.5	Yadav *et al.* 2015
	5.5-8.3	4.7-4.9	9.0	5.2	4.5-4.8	--	--	--	Patil *et al.*, 2014
	--	4.9-5.1	8.0	5.2-5.4	4.2	--	--	9.6	Nath *et al.*, 2015a

Immunoglobulins (Igs), Bovine Serum Albumin (BSA), Lactoferrine (Lf), β-Lactoglobulin (β-LG), α-Lactoalbumin (α-LA), Lactoperoxidase (LP)

Table 3. Bioreactors used in the hydrolysis of lactose.

Sustrate	Reactor	Enzime used	Product	Reference
Commercial lactose solution	Polymeric and inorganic membranes	β- galactosidase	Oligosaccharides	Czermak *et al.*, 2004
Permeated whey	Ultrafiltration with shaking	β- galactosidase	Oligosaccharides	Foda y Leyva, 2000
Commercial lactose solution	Cellulose membrane	β- galactosidase	Oligosaccharides	Chockchaisawasde *et al.*, 2004
Purified cheese whey	Polypropylene membrane	β- galactosidase	Oligosaccharides	Vasileva *et al.*, 2016
Commercial lactose solution	Polysulfone fiber membrane	β- galactosidase	Oligosaccharides	Gonawan *et al.*, 2016
Commercial lactose solution	Ceramic membrane	β- galactosidase	Oligosaccharides	Córdova *et al.*, 2016

Table 4. Main bacteria producing bacteriocins

Bacteriocin	Sustrate	Microorganism	Reference
Nisina A y Z	Permeated whey	*Lactococcus lactis*	Amiali *et al.*, 1998; Arauz *et al.*, 2009
Antipediococcus termófila 110	Permeated whey	*Streptococcus thermophilus*	Somkuti y Gilbreth, 2007
Pediocina	Supplemented whey	*Pediococcus acidilactici*	Guerra y Pastrana, 2001; Pérez-Guerra *et al.*, 2005
Mesenterocina 5	Supplemented whey	*Leuconostoc mesenteroides*	Daba *et al.*, 1993
ST194BZ	Hydrolyzed whey	*Lactobacillus plantarum*	Rodríguez-Pazo *et al.*, 2013
Divercina	Hydrolyzed whey	*Carnobacterium divergens*	Grajek *et al.*, 1996
Liquenina	Whey powder	*Bacillus licheniformis*	Cladera-Olivera *et al.*, 2004
Enterocina	Raw whey	*Enterococcus faecium*	Schirru *et al.*, 2014
Linenscina	Raw whey	*Brevibacterium linens*	Mota y Brandelli, 2003

Table 5. Major microorganisms producing biopolymers in cheese whey.

Sustrate	Microorganism	Reference
Raw whey	*B. megaterium*	Ramkumar-Pandian *et al.*, 2010; Obruca *et al.*, 2011
Hydrolyzed whey	*H. mediterrani*	Pais *et al.*, 2015
Raw whey and propionic acid	*Ralstonia eutropha*	Marangoni *et al.*, 2002
Bovine whey powder	*Escherichia coli*	Wong y Lee, 1998; Ahn *et al.*, 2001; Park *et al.*, 2002
Permeated whey and hydrolyzed whey	*Hydrogenophaga pseudoflava*	Povolo y Casella, 2003; Koller *et al.*, 2008
Acid whey	*Methylobacterium* sp	Yellore y Desai, 1998; Nath *et al.*, 2008;

Table 6. Main lipid-producing microorganisms from cheese whey.

Microorganism	**Sustrate**	**Reference**
Yarrowia lipolytica	Deproteinized whey	Taskin *et al.*, 2015
Candida sp.	Raw whey	Moon *et al.*, 1978; Yönten y Aktas, 2014
Rhodosporidium toruloides	Permeated whey	Akhtar *et al.*, 1998
Cryptococcus sp.	Permeated whey	Otto *et al.*, 1999; Castanha *et al.*, 2014
Zygomycetes (*Mortierella isabellina, Thamnidium* elegans y *Mucor* sp.)	Raw whey	Vamvakaki *et al.*, 2010
Hongos xerofilos (*Aspergillus* sp., *Emericella* sp., *Eurotium* sp. Y *Fennellia*)	Raw whey	El-Kady *et al.*, 1995
Apiotrichum curvatum	Hydrolyzed whey	Davies *et al.*, 1990
Lypomyces starkeyi	Permeated whey	Akhtar *et al.*, 1998

Table 7. Main strains used in ethanol production from cheese whey.

Sustrate	Strain	Reference
Deproteinized whey and raw whey	*K. marxianus*	Sansonetti *et al.,* 2009; Christensen *et al.,* 2011
Permeated whey	*S. cerevisiae*	Parashar *et al.,* 2015
Whey supplemented	*S. cerevisiae*	Rodrigues *et al.,* 2016
Raw whey and whey powder supplemented with molasses	*E.coli*	Akbas *et al.,* 2014
Whey powder	*K. marxianus* and *S.cerevisiae*	Guo *et al.,* 2010
Permeated whey	*E. coli*	Liu *et al.,* 2016
Raw, permeated and hydrolyzed whey	*S. cerevisiae*	Tomaszewska y Białonczyk, 2016

[3]CAPITULO III. LIPID PRODUCTION FROM BOVINE AND GOAT CHEESE WHEY

Resumen

El principal residuo generado por el procesamiento de la leche en queso se llama suero de queso. La producción mundial de este residuo es de 160 millones de toneladas al año. Lo anterior causa daños al medio ambiente debido a la cantidad de oxígeno que necesita para degradar la presencia de altas concentraciones de materia orgánica. Esta investigación presenta la producción de lípidos de una levadura oleaginosa, a partir de suero de queso bovino y de cabra de dos microempresas del centro del estado de Veracruz, México. Para la caracterización fisicoquímica se evaluó la materia seca, grasa, proteína, cenizas, lactosa, pH, ácido láctico y DQO. La hidrólisis enzimática de la lactosa se realizó en un modo discontinuo para estudiar el efecto de los siguientes parámetros: temperatura, pH, tiempo de reacción y concentración enzimática. Para la hidrólisis química se evaluó: tiempo de reacción, temperatura y concentración de ácido. Para la producción de biomasa y lípidos, se evaluó: temperatura, pH y tiempo de fermentación. Los valores máximos de producción de biomasa para suero bovino y de cabra fueron de 18 gL^{-1} y 20 gL^{-1}, respectivamente, donde los factores de tiempo y temperatura fueron los más influyentes ($p = 0.000$ y $p = 0.010$ respectivamente). El mayor rendimiento de lípidos se obtuvo a partir de suero de queso dulce bovino (4.13 %). Los principales ácidos grasos obtenidos de la fracción lipídica fueron el oleico, palmítico, linoleico y esteárico.

Palabras clave: ácidos grasos, fermentación del lactosuero, *Cryptococcus*.

[3] Bioresource Technology. *Enviado.*

Abstract

The main waste generated from the transformation of milk into cheese is called cheese whey. World production of this waste per year is 160 million tons. This process causes damage to the environment due to amount of oxygen required to degrade the presence of high concentrations of organic matter. This research focuses on the lipid production by oleaginous yeast from bovine and goat cheese whey of two micro craft-cheese in the central region of Veracruz, Mexico as a mechanism from exploitation. For the physicochemical characterization, the dry matter, fat, protein, ash, lactose, pH, lactic acid and COD were evaluated. Enzymatic hydrolysis of lactose was performed in a batch mode to study the effect of the following parameters: temperature, pH, reaction time and enzyme concentration. For the chemical hydrolysis, the reaction time, temperature and acid concentration were evaluated. For the biomass and lipid production, the temperature, pH and fermentation time were evaluated. For physicochemical characterization the dry matter, fat, protein, ash, lactose, pH, lactic acid, DQO were evaluated. Enzymatic hydrolysis of lactose was performed in a batch mode to study the effect of following parameters: temperature, pH, reaction time and enzyme concentration. For the chemical hydrolysis, was evaluated: reaction time, temperature and acid concentration. For the biomass and lipid production, was evaluated: temperature, pH and fermentation time. The maximum values in biomass production for bovine and goat whey were of 18 gL^{-1} and 20 gL^{-1} respectively, where the time and temperature factors were the most influential ($p=0.000$ and $p=0.010$ respectively). On the other hand, the higher lipids yield was obtained from bovine sweet whey (4.13 %). Moreover, the main fatty acids obtained from the lipid fraction were oleic, palmitic, linoleic and stearic acids.

Keywords: cheese whey, lipids, production, fermentation, *Cryptococcus*

1. Introduction

The cheese whey is the waste obtained after the precipitation and removal of casein from milk (Carvalho *et al.,* 2013). The worldwide annual production of cheese whey is of 160 million tons (Das *et al.,* 2015). Its composition varies according to the type of milk and cheese, and the manufacturing process (Pescuma *et al*., 2015). Due to these characteristics, cheese whey has been explored as growth medium for different fermentation processes such as bioethanol (Tomaszewska y Białonczyk, 2016) and high-valued products, including poly-3-hydroxy alkanoate (PHA; Pais *et al*., 2015), exopolysaccharides (Haj-Mustafa *et al*., 2015), organic acids (Amado *et al*., 2016) and lipids (Taskin *et al*., 2015).

In this sense, the microbial lipids generated from different substrate have emerged as promising alternative as food additive or feedstock for biodiesel production. Currently, the production of yeast oil is more expensive than the production of vegetable oils. Nevertheless, it is considered that single-cell oil fermentations may be economically feasible only when the oil produced own an added value. Accordingly, it is necessary to utilized various processes focused on engineering to obtain a higher lipid production and, therefore, make this process more economically feasible (Thiru *et al.,* 2011).

Concerning the oleaginous microorganisms, some species have been reported to be able to produce lipids (Donot *et al*., 2014) since they are able to accumulate more than 20 % of its cell weight (Tsouko *et al*., 2016). These microorganisms are mainly yeasts, fungi, algae and a few bacteria (Wältermann *et al*., 2000). The same as the plants, microorganisms use a carbon source to produce lipids; some strains produce a greater amount of fat than some oilseed crops (Beligon *et al*., 2016).

Strains of *Cryptococcus* are already recognized as good producers of lipids, especially *C. curvatus* that has been widely studied in fermentations using different substrates (Leiva-

Candia *et al.,* 2014; Tanimura *et al.,* 2014). On the other hand, *C. laurentii* has been little used for lipid production, and so far its main application is as a biocontrol agent against post-harvest pathogens (Blum *et al*., 2004). With the aim of searching new processes to decrease the production costs of lipids by combining oleaginous microorganisms with low-cost feedstock (cheese whey), the present study was based on lipid production, utilizing two types of cheese whey (sweet and acid) from two different species (bovine and goat) with *Cryptococcus laurentii*.

2. Materials and Methods

2.1 Biological material

Two types of cheese whey were obtained in batches from two different industries located in the central region of Veracruz, Mexico. A sweet whey was obtained from the production of fresh cheese, where protein precipitation was performed using renin. On the other hand, an acid whey was obtained from lactic paste (goat) and Oaxaca cheese (bovine) using lactic cultures and organic acids (respectively).

2.2 Chemical composition

The chemical composition of the two types of cheese whey was determined using oven drying at 105 °C until constant weight for the dry matter (A.O.A.C., 2005), incineration at 550 °C for 5 h for the ash (A.O.A.C., 2005), the Lowry method for the protein (Lowry, *et al.,* 1951), and the Gerber method for the fat (NOM-F-155-SCFI-2003). The pH was directly determined using a Hanna potentiometer (NMX-F-317-S-1978), whereas the chemical oxygen demand (CQO) was determined using a HACH® kit. Finally, the lactose quantification was measured with a Lactoscan MCC Milkotronic® equipment. All determinations were made in triplicate.

2.3 Pretreatment of the cheese whey

The cheese whey was subjected to a heat treatment with the following conditions: two temperatures (120 °C and 100 °C), two different times (30-90 minutes) and three different acid concentrations (0.01, 0.1 and 1 M).

2.4 Microorganism, culture maintenance, and inoculum preparation

The strains *Cryptococcus laurentii* CDBB-L-652 used in this study were provided by the National Collection of Microbial Strain and Cultures Cellular of CINVESTAV Mexico. The laboratory stocks of the culture were grown in a commercial solid PDA medium (BD Bioxon, Mexico) and were maintained at 4 °C until inoculation of a new culture. To prepare the inoculum, three loops of the culture were transferred to an Erlenmeyer flask (250 mL) containing 100 mL of YM (malt extract) culture medium and was put to 28 °C in a rotary shaker incubator at 180 rpm for 48 h.

2.5 Lipid production

The Erlenmeyer flasks (125 mL) containing 50 mL of fermentation medium (hydrolyzed whey) were inoculated with $1x10^7$ cel/mL of seed culture and put under stirring at 180 rpm in a rotatory shaker incubator Lab Companion SI-600. The effects of fermentation time (100, 200 and 300 hours), pH (5.0, 5.7 and 6.5), and temperature (20 and 28 °C) on the biomass and lipids production were evaluated.

2.6 Lipid extraction

For the lipid extraction, 50 mL of the final broth were centrifuged and the wet cells were dried at 60 °C for 24 h. The cell-wall rupture was carried out according to Thakur *et al.*, (1998), where the dry biomass was subjected to acid hydrolysis (boiling with 1 mol/L HCl for 1 h). Subsequently, it was centrifuged (10 min at 12470 X*g*) and the supernatant was

discarded. For the second stage of the lipid extraction the method reported by Castanha *et al.* (2014) was followed. The biomass was mixed with 4 mL of distilled water, 10 mL of methanol (J.T. Baker) and 5 mL of chloroform (J.T. Baker). The mixture was stirred in a rotary shaker for 2 h at 220 rpm and further diluted with 5 mL of chloroform and 5 mL of 1.5 % sodium sulfate (J.T. Baker). Finally, the separation of the two layers was obtained by centrifugation for 2 min at 173.29 X*g*. The upper aqueous layer containing methanol, water, and non-lipid compounds was discarded. The lower layer (chloroform) was filtered using Whatman filter paper grade 1 with 1 g of anhydrous sodium sulfate and it was collected in pre-weighed glass vials. The lipid content was expressed as lipid grams per liter of fermentation broth.

2.7 Fatty acid composition

Fatty-acid methyl esters (FAMEs) were obtained by transesterification from extracted lipid according to Seo *et al.,* (2014). Methanol was used as a reactant and sulfuric acid as a catalyst at 100 °C for 20 min. After the reaction, the samples were cooled to room temperature and then centrifuged at 4000 rpm for 10 min. In the organic phase, FAMEs were analyzed with an Agilent 7890B gas chromatograph, with a flame ionization detector, equipped with an Agilent 122-2362 DB-23 column with 60 m x 250 μm and 0.25 μm film thickness. The injector and the detector temperature was the same (270 °C). The oven was programmed as follows: 140 °C for 0 min; then 6.5 °C min^{-1} to 170 °C for 0 minutes; next 2.75 °C min^{-1} to 200 °C for 14 minutes; and finally, 3°C min^{-1} to 230 °C min^{-1} where the temperature was held for 6 minutes. The sample solution (1 μL) was injected, and the split was opened after 2 minutes. Each fatty acid was identified and quantified by comparing the retention times and peak areas with the Supelco 37 Component FAME Mix (Sigma-Aldrich, USA).

2.8 Statistical analysis

In order to study the effects of the different factors, a surface response design was used to evaluate the different interactions on the pretreatment of cheese whey and a full factorial statistical design of three factors (fermentation time, pH, and temperature) was utilized for biomass and lipids production. The best treatment was analyzed with a complete randomized design (CRD) and variance analysis (ANOVA) with a Fisher´s test. For analysis of data was used a MINITAB® software.

3. Results and Discussion

3.1 Chemical composition

The chemical composition of the cheese whey is variable as it depends on some factors (Table 1), according to Ramirez-Navas (2011) the mainly are: type of milk, type of processed cheese, the form of casein removal (acid or enzymatic), feedstock used (enzyme complexes, lactic cultures or organic acids).

3.1.1 Bovine cheese whey

The dry matter content is higher than that reported by Boujdema *et al.,* (2015) for sweet whey and similar to reported by Kosseva *et al.,* (2009) for acid whey. The pH for sweet whey is similar to reported by Boujdema *et al.,* (2015), the fat amount (0.2 %) it was higher than reported by Boujdema *et al*. (2015), the fat value for the acid whey (0.7 %) was more high respect to reported by Yadav *et al.,* (2015), this difference is mainly due to use of technology during cheesemaking. The protein content was low compared to that reported by Kosseva *et al*. (2009) and Chen *et al*. (2016), for sweet and acid whey, respectively. The values obtained for the ash content in sweet and acid whey (1.18 and 0.87 respectively), is higher compared to report by Boujdema *et al*. (2015) at sweet whey and to Yadav *et al*. (2015) for acid whey.

According to what was expected (Yadav *et al*., 2015), the acidity is higher in whey from cheese processing in that lactic cultures or citric acid were used to promote protein precipitation, such as "Oaxaca Cheese", due to the solubility of the acids in the liquid fraction (whey). The difference in chemical oxygen demand dependent on the amount of lactose present in the whey (56.76 gL^{-1} and 74.58 gL^{-1}, respectively), the values found are within the ranges reported for Ozmihci and Kargi (2007).

3.1.2 Goat cheese whey

The pH value for sweet whey was similar to that reported by Casper *et al.*, (1998). The amount of fat (0.3 %) was lower than that reported by Sanmartín *et al*. (2012). This difference is mainly due to the use of technology during cheese making (automatic temperature control, the particle size of the curd, the process of separation of the curd, among others) (Moreno-Indias *et al.*, 2009). The dry matter content was higher than the reported by Sanmartín *et al*. (2012) for sweet whey, and similar to that reported by Casper *et al*. (1998) for acid whey. The protein content was relatively lower than that reported by Pintado *et al.* (2001) regarding the acid whey. The protein content was influenced by the animal feed, the lactation period, the breed, the season of the year, among others (Hilario *et al.*, 2010). In this work, milk from Saanen and Alpine goats was used. It was reported that this type of milk owns a low protein content (Tziboula-Clarke, 2003). The content of ash in sweet and acid whey (0.90 y 0.79 % respectively) was higher than that reported by Sanmartín *et al.* (2012) for sweet whey and by Casper *et al.* (1998) for acid whey. The mineral content was largely influenced by the solubilization of the calcium salts ($CaCl_2$), added during the process of cheese making (Johansen *et al*., 2002; Jelen, 2003).

3.2 Pretreatment cheese whey

The chemical pretreatment using dilute acid solutions has been used in different natural sources as a common way for the hydrolysis of different polysaccharides. This pretreatment may be used as a substrate in the production of lipids through microorganisms without repercussions in their vitality (Yu *et al.*, 2011; Liang *et al.*, 2012). The result of the chemical hydrolysis did not show a significant difference for double interaction (P=0.123) and the time factor (P=0.245). Regarding the acid concentration, although the yield was slightly higher at 1M concentration in relation to 0.1 M, the hydrolysates obtained presented a dark coloration and lumps formation that made the handling of the samples difficult during the fermentation. On the other hand, the pH of these hydrolysates was very low (pH = 1) that required high concentrations of NaOH to neutralize, with the consequent formation of saline precipitates. With these results, the best hydrolysis conditions for obtaining fermentable sugars were 30 minutes, 0.1 M hydrochloric acid and 120 °C temperature.

3.3 Biomass and lipid production

Cryptococcus sp. is an important type of oleaginous yeast in the production of lipids from different lignocellulosic raw materials and some residues (active sludge supernatant, hydrolyzed wheat straw, bagasse hydrolyzate from sweet sorghum) (Deeba *et al*., 2016).

In this research, the analysis performed in each of the interactions of the variables (triple, double and main effects) in biomass production showed that there exist a significant effect in the time and temperature factors over microbial growth (p = 0.000 and p = 0.010

respectively). In this sense, when the time and temperature increased, the highest growth values were obtained (Table 2).

Figure 1 shows the highest values for the two types of cheese whey, where 18.2 gL^{-1} was obtained with 300 hours of fermentation, a pH of 6.5 and a temperature of 20 °C in bovine acid whey; and 19.3 gL^{-1} to 200 hours of fermentation, a pH of 5 and temperature of 20 °C in goat acid whey.

Table 3 shows the lipid production using *C. laurentii* in two types of cheese whey. In the production of lipids in the bovine cheese whey, the fermentation time (300 h) was the factor with the highest significative effect (P=0.023); therefore, in this time the highest content was obtained. For the lipid production in goat cheese whey, the temperature was the factor with the highest effect (p=0.000). The highest content was obtained with a temperature of 20 °C.

Figure 2 shows highest values for the two types of cheese whey, where 4.13 % was obtained with 300 hours of fermentation, pH 6.5 and 20 °C in bovine acid whey and 1.93 gL^{-1} to 200 hours of fermentation, pH 5 and 20 °C was obtained for goat acid whey.

In general, oleaginous yeasts accumulate a substantial amount of lipids during the stationary growth phase, as for the pH factor, the best lipid production for these whey´s was observed in the lowest value (pH 5), contrary to what was reported by Castanha *et al*. (2014) and Seo *et al*. (2014) where the best yield was obtained with a pH high values (6.5 and 12 respectively).

Table 4 shows the yields obtained in some of the studies that have been carried out to obtain biomass and lipids from *Cryptococcus* sp. In different raw materials, which are considered low cost and renewable. In this sense the strain that was used in this study (*C. laurentii*) using only reducing sugars as the sole carbon source after the hydrolysis treatment produced a similar lipid content compared to other recently reported low-cost substrates.

Some studies report inhibition of the growth of *Cryptococcus* in raw cheese whey, this is mainly because the whey in its natural form contains a wide range of native microorganisms (mainly lactic acid bacteria), which produce compounds (lactic acid and bacteriocins) that inhibit the growth of coexisting microorganisms (Noike *et al*., 2002). The strain used in this study (C. laurentii) responded positively, showing an increase in cellular biomass production and lipid content in relation to other studies in that cheese whey has been used as a substrate. From the economic point of view, the cheese whey can greatly reduce the costs of raw material, since it does not have a sale price or an established market; this makes it a profitable raw material and suitable for obtaining products with added value and also contribute to the prevention-remediation of environmental pollution caused by this agro-industrial waste.

3.4 Fatty acid profile

The fractionation of the lipids indicated that the fatty acid distribution was similar between the samples where was used bovine cheese whey how substrate (Table 5), where the fatty acids consisted mainly of chain from 16 to 24 carbon atoms, this composition is comparable with those some vegetable oils (palm, soy and sunflower oils). The three major fatty acids were palmitic acid, stearic acid, and oleic acid, this phenomenon was consistent with previous observations where similar fatty acids compositional profiles were found for lipids produced by species from *Cryptococcus* genre.

The absence of palmitoleic acid (C16:1) in both types of whey of the two species was observed in the fatty acids that make up the lipid fraction. The content of oleic acid (C18:1) obtained from fermentations at 28 °C in bovine whey is similar to that reported by Seo *et al*. (2014); Castanha *et al.* (2014) and Carota *et al.* (2017). However, an increase in the proportion of this acid was observed in the fermentations carried out at 20 °C, this indicate

that temperature has a direct effect on lipids composition. The presence of two fatty acids that were not reported in previous works (Lignoceric and Nervonic acids) were observed.

Type of carbohydrates present in cheese whey may be a key factor in the composition of lipids; some studies report that yeasts of the genus *Cryptococcus* that were grown in lactose-rich media had a lower content of oleic acid compared to yeasts grown in media with glucose as the only source of carbon (Seo *et al.*, 2014). In that sense, the composition of the lipids obtained by the use of oil-microorganisms, specifically yeasts, can be directed towards the production of specific fatty acids (neutral lipids, glycolipids, sphingolipids or phospholipids), modifying the composition of the substrate (cheese whey). The most common way do this, is by adding different types of mineral salts (NH_4Cl, KH_2PO_4, Na_2HPO_4, $MgSO_4 \cdot 7H_2O$), this effect is observed directly in the metabolic pathway of microorganisms during lipogenesis and storage of lipids (Garay *et al.*, 2014). In this work, the fatty acids obtained are those that microorganism produces natively, due to the cheese whey that was used as substrate were not supplemented.

Table 6 shows the profile of fatty acids obtained from the fermentations with goat whey (sweet and acid), where, as in bovine whey, oleic acid was the most abundant and where the proportion was higher with respect to another whey. However, there was a decrease in the proportion of lignoceric acid (C24:0) and nervonic acid (C24:1) in lipids produced at 28 °C compared to bovine whey. On the other hand, nervonic acid was present in goat whey in both fermentation temperatures (28 and 20 °C), respect to bovine whey, where it was only presented in fermentations carried out at 28 °C.

These results are important since it is the first work that reports the composition of the lipids produced from the fermentation of two types of whey (acid and sweet) from the production of goat cheese. In general, the lipid fractions and fatty acid compositional profiles indicated

that microbial lipid produced from bovine and goat cheese whey how substrate could be explored as sustainable feedstock for food additives or biodiesel production.

4. Conclusion

Cheese whey is a by-product generated by the dairy industry. It is very polluting if it is disposed of directly in water sources, floors and sewage treatment plants. Due to its high nutrient content, whey is a potential source for products with high added value. The use of this resource contributes to the reduction of pollution and reduces the damage caused by humans. Although different technological alternatives have been developed for its processing, more research is needed to obtain higher yields that can make the industrial scale of the processes viable. Finally, these technologies and products can be a path of sustainable development for the preservation of the environment in the near future. The production of microbial biomass was higher in goat acid whey, this result is attributed to the fact that this substrate contains higher amounts of lactose. While lipid production was higher in bovine acid whey, this result is mainly due to the fact that these whey contain less protein, which is considered an inducer or inhibitor of accumulation. The main fatty acids obtained from the fermentation of *C. laurentii* cheese whey were oleic, linoleic, palmitic and stearic, which can be used as a food additive or as a raw material for the production of biodiesel. However, the technological developments for the use and disposal of whey remain a major point of discussion for the dairy industry.

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Table 1. Chemical composition (%) of goat and cow cheese whey (sweet and acid).

Parameter	Sweet	Acid	Goat (sweet)	Goat (acid)
Dry matter	7.44±1.27	6.80±0.20	8.11±0.01	7.04±0.06
Fat	0.23±0.58	0.7±0.00	0.3±0.00	0.4±0.00
Protein	0.022±0.05	0.023±0.00	0.027±0.00	0.027±0.01
Ash	1.18±0.03	0.87±0.03	0.90±0.01	0.79±0.21
Lactose	4.2±0.07	3.52±0.14	5.3±0.02	4.7±0.01
pH	6.60±0.02	4.42±0.18	5.11±0.03	4.05±0.13
Lactic acid	0.50±0.00	5.03±1.24	2.34±0.10	5.55±0.06
DQO (g/L)	56.76±0.00	74.58±0.02	63.86±0.00	58.77±0.00

Mean values ± standard deviation (n=3)

Table 2. Biomass production using *C. laurentii* from cheese whey.

		Biomass production					
		Sweet cheese whey (bovine)			Acid cheese whey (bovine)		
	Time/ pH	100	200	300	100	200	300
20°C	5	9.50±0.04	12.50±0.04	15.00±0.07	10.00±0.00	**16.50±0.04**	13.00±0.07
	5.7	8.00±0.00	12.80±0.04	14.10±0.06	13.50±0.04	14.50±0.04	9.80±0.01
	6.5	6.30±0.02	14.20±0.06	**18.20±0.01**	13.50±0.04	8.20±0.01	13.50±0.04
28°C	5	**13.00±0.07**	8.10±0.01	9.00 ±0.07	**8.10±0.01**	6.10±0.01	6.00±0.00
	5.7	4.20±0.01	9.00±0.07	9.50±0.11	6.20±0.01	6.30±0.02	4.10±0.01
	6.5	7.00±0.07	4.10±0.01	9.00±0.07	4.20±0.01	6.20±0.01	4.00±0.00
		Sweet cheese whey (goat)			**Acid cheese whey (goat)**		
20°C	5	4.10±0.01	**11.70±0.02**	10.20±0.01	6.30±0.02	**19.30±0.05**	15.70±0.02
	5.7	4.20±0.01	11.50±0.04	8.10±0.01	9.90±0.01	16.50±0.04	14.30±0.02
	6.5	6.00±0.00	10.40±0.03	9.80±0.01	7.70±0.02	15.80±0.01	13.80±0.01
28°C	5	2.30±0.01	2.10±0.01	2.10±0.01	2.30±0.02	3.90±0.01	**6.10±0.01**
	5.7	2.00±0.00	2.30±0.02	**4.20±0.01**	2.20±0.01	4.10±0.01	3.80±0.03
	6.5	2.80±0.01	2.80±0.03	2.10±0.01	2.10±0.01	4.20±0.01	3.90±0.02

Table 3. Lipid production using *C. laurentii* from cheese whey.

		Lipid production					
		Sweet cheese whey (bovine)			Acid cheese whey (bovine)		
	Time/ pH	100	200	300	100	200	300
20°C	5	0.52±0.42	1.56±0.19	1.61±0.74	2.16±0.95	2.86±0.07	3.97±0.78
	5.7	0.65±0.39	1.18±0.27	1.84±0.35	1.95±0.16	3.18±0.75	3.24±0.42
	6.5	0.52±0.21	1.20±1.39	**3.19±0.49**	0.32±0.16	3.54±0.61	**4.13±0.10**
28°C	5	0.22±0.00	1.71±1.06	1.48±0.14	0.20±0.04	**1.15±0.16**	0.96±0.05
	5.7	0.21±0.35	**1.78±0.14**	1.66±0.07	0.33±0.28	0.95±0.21	0.94±0.05
	6.5	0.57±0.64	1.03±0.35	1.74±0.28	0.94±0.28	0.85±0.42	0.23±0.01
		Sweet cheese whey (goat)			**Acid cheese whey (goat)**		
20°C	5	1.06±0.14	**1.78±0.21**	1.46±0.21	1.78±0.28	**1.93±0.64**	1.89±0.41
	5.7	1.57±0.35	1.71±0.64	1.62±0.28	1.83±0.42	1.91±0.28	1.86±0.07
	6.5	1.00±0.57	1.64±0.35	1.72±0.21	1.82±0.35	1.83±0.57	1.78±0.42
28°C	5	1.01±0.85	**1.04±0.35**	0.53±0.49	1.04±2.83	1.56±1.41	1.22±0.14
	5.7	0.99±0.35	0.76±0.14	0.81±0.71	1.02±1.41	0.97±0.35	0.57±0.42
	6.5	0.88±0.11	0.66±0.21	0.78±1.41	1.28±1.41	**1.57±0.42**	1.15±0.71

Table 4. Lipid production rates from *Cryptococcus* in various substrates

Oilyeast	Carbon source	Dry biomass (g/L)	Yield* (g/L)	Lipid content (%)	Reference
C. aerius	Municipal solid waste	14.1	4.31	30.6	Ghanavati *et al*. (2015)
C. curvatus	Cheese whey	7.2	4.68	65	Seo *et al*., 2014
C. humicola	Corn stover	15.5	5.58	36	Sitepu *et al*. (2014)
C. curvatus	Sweet sorghum	10.83	7.93	73.26	Liang *et al*. (2012)
C. curvatus	Wheat straw	17.2	5.76	33.5	Yu *et al*. (2011)
C. laurentii	Bovine acid cheese whey	13.50	4.13	30.56	**This study**

* Calculate on based yield=lipid content*dry biomass/100

Table 5. Lipid compositions from differents species of *Cryptococcus*

Fatty acid	Seo *et al*., 2014 (*C. curvatus*; Cheese whey)	Castanha *et al*., 2014 (*C. laurentii*; Cheese whey with molasses)	Carota *et al*., 2017 (*C. laurentii*; Ricotta cheese whey)	This study (*C. laurentii*; Bovine sweet whey 28°C)	This study (*C. laurentii*; Bovine acid whey 28°C)	This study (*C. laurentii*; Bovine sweet whey 20°C)	This study (*C. laurentii*; Bovine acid whey 20°C)
C 16:0 (Palmitic)	23.3	21.0	18.53	15.82	20.53	16.79	18.68
C 16:1 (Palmitoleic)	0.71	--	0.39	--	--	--	--
C 18:0 (Stearic)	10.4	28.8	5.45	11.34	12.40	6.10	7.86
C 18:1 (Oleic)	45.3	36.0	47.16	45.88	32.36	55.64	52.84
C 18:2 (Linoleic)	6.40	5.0	23.47	4.81	14.93	16.55	15.28
C24:0 (Lignoceric)	--	--	--	6.32	1.38	2.29	1.93
C24:1 (Nervonic)	--	--	--	9.99	1.55	--	--

Table 6. Lipid compositions of *Cryptococcus laurentii* using goat cheese whey

Fatty acid	Goat sweet whey (28°C)	Goat acid whey (28°C)	Goat sweet whey (20°C)	Goat acid whey (20°C)
C 16:0 (Palmitic)	20.10	18.11	17.39	17.64
C 16:1 (Palmitoleic)	--	--	--	--
C 18:0 (Stearic)	13.87	10.71	7.53	17.47
C 18:1 (Oleic)	31.53	47.14	50.91	49.44
C 18:2 (Linoleic)	5.11	13.22	18.62	8.42
C24:0 (Lignoceric)	3.60	0.08	1.48	2.54
C24:1 (Nervonic)	0.39	0.34	0.16	0.21

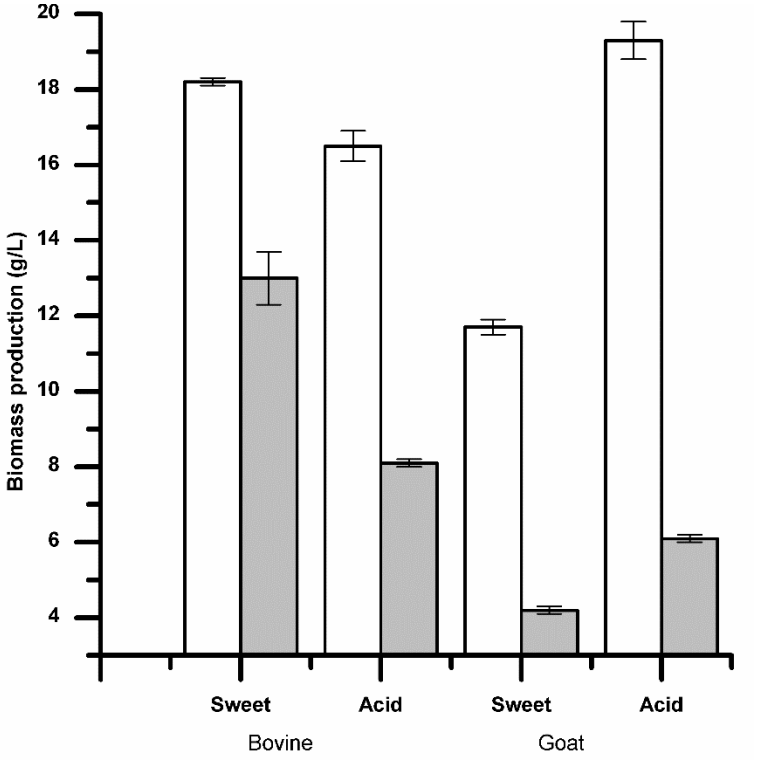

Figure 1. Biomass production from two different cheese whey: white (20°C) and gray (28°C).

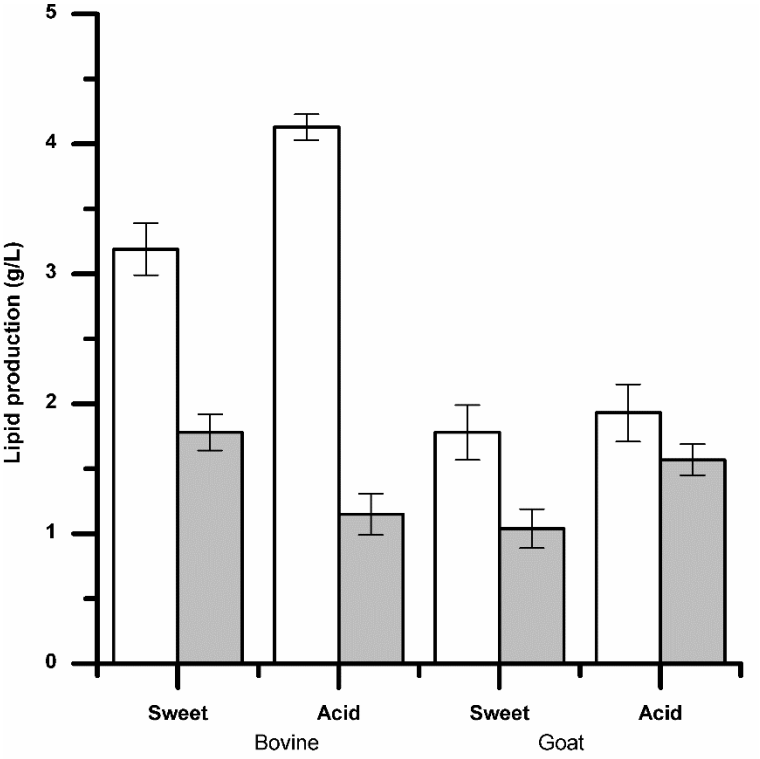

Figure 2. Lipid production from two different cheese whey: white (20 °C) and gray (28 °C).

CONCLUSIÓN GENERAL

El suero de queso es un subproducto generado por la industria láctea. Es muy contaminante si se elimina directamente en fuentes de agua, suelos y plantas de tratamiento de aguas residuales. Debido a su alto contenido de nutrientes, el suero de leche es una fuente potencial para obtener productos con alto valor agregado. El aprovechamiento de este recurso contribuye a la reducción de la contaminación y reduce el daño causado por los seres humanos. Aunque se han desarrollado diferentes alternativas tecnológicas para su procesamiento, se requieren más investigaciones para obtener mayores rendimientos que puedan hacer viable la escala industrial de los procesos. Por último, estas tecnologías y productos pueden ser un camino de desarrollo sostenible para la preservación del medio ambiente en un futuro próximo. La producción de biomasa microbiana fue mayor en sueros ácidos de queso de cabra, este resultado se atribuye a que este sustrato contiene mayores cantidades de lactosa. Mientras que, la producción de lípidos fue superior en suero ácido bovino, este resultado se debe principalmente a que estos sueros contienen menor cantidad de proteína, la cual es considerada como inductor o inhibidor de la acumulación. Los principales ácidos grasos obtenidos a partir de la fermentación de suero de queso con *C. laurentii* fueron oleico, linoleico, palmítico y esteárico, los cuales pueden ser utilizados como aditivo alimentario o como materia prima para la elaboración de biodiesel. No obstante los desarrollos tecnológicos para el uso y disposición del suero de queso siguen siendo un punto de discusión importante para la industria láctea.