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Valorization Studies on Ice-Cream Wastewater and Whey Permeate

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VALORIZATION STUDIES ON ICE-CREAM WASTEWATER AND WHEY PERMEATE

BY

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DISSERTATION ACCEPTANCE PAGE

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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This dissertation is dedicated to my amazing mother who sacrificed her entire life for my success and encouraged me to pursue my dreams and finish Ph.D. studies.

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CONTENTS

ABBREVIATIONS

A_s	Absorbance of the sample
A_b	Absorbance of the blank
A_{st}	Absorbance of the serine standard (100 mg L^{-1})
A_t	Absorbance of the mixture at the steady state
A_o	Absorbance of the mixture at the beginning
AWP	Acid whey permeate
B	Constant for whey protein (0.4)
DH	Degree of hydrolysis (%)
DH_t	Degree of hydrolysis at a specific time (%)
DH_o	Initial value of degree of hydrolysis (%)
DH_f	*F*inal value of degree of hydrolysis (%)
DPPH	2,2,diphenyl-1-picrylhydrazyl
E	Average absolute percentage of residuals (%)
E_a	Apparent activation energy (k J mol^{-1})
EC_{50}	Concentration of protein needed to reduce 50% of the *DPPH* activity
h	Hydrolyzed bonds
h_{tot}	Number of total peptide bonds per protein equivalent
IC_{50}	Concentration of protein needed to inhibit 50% of ACE activity
k_i	Hydrolysis rate constants (min^{-1}) for equations (2-3)
k_T	Reaction rate at a reference temperature (min^{-1})
LA	Lactose
LBA	Lactobionic acid

LAU	Lactulose
P	Protein content of the wastewater (%)
p	Pressure (bar)
R	Universal gas constant (8.314 J mol^{-1} K^{-1})
R^2	Coefficient of determination
R^2_{Adj}	Adjusted coefficient of determination
S	Serine protein miliequivalent (0.9516 meqv L^{-1})
SWP	Sweet whey permeate
$t_{cooling}$	Cooling time (min)
$t_{heating}$	Heating time (min)
$t_{reaction}$	Hydrolysis time (min)
T	Reaction temperature (K)
T_r	Reference reaction temperature (K)
V	Volume of wastewater (L)
TS	Total solids (%)
TVS	Total volatile solids (%)
α	Scale parameter
α_T	Scale parameter at a reference temperature
β	Shape parameter

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ABSTRACT

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Ever growing demand for dairy products in many countries has led to advancements in the manufacture of such products. Nowadays, revolutionary trends for high-protein dairy foods and drinks products have brought about new challenges from processing requirements and manufacturing aspects. Using different membrane processing has enabled dairy manufacturers to produce different fraction of milk proteins such as micellar caseins, ultra-filtered (UF) milk proteins, whey proteins concentrate (WPC), and micellar casein concentrate (MCC) to meet the nutritional needs with variety of milk product options. However, the installation of new processing lines and establishing further manufacturing steps requires more consumption of water and raises in the generation of waste streams and byproducts. Dairy wastewater is defined as a biomass with high biological oxygen demand (BOD), chemical oxygen demand (COD), nutrients, organic and inorganic materials. Therefore, dairy wastewater requires multifaceted treatment prior disposal into the municipal sewage system to avoid environmental jeopardies. In this research, the valorization of two main biomass streams from dairy manufacturing have been discussed – ice cream wastewater and whey permeate.

During ice-cream manufacturing, the amount of generated wastewater is estimated between 1 and 2 gallons per pound of product. Recently, subcritical water hydrolysis has

been recognized as a promising method for conversion of ice-cream wastewater into valuable chemicals such as surfactants, foaming agents, emulsifiers, and animal feed. Hydrolysis ice-cream wastewater under subcritical treatment (230˚C, 40 bar, and 240 min) reduced its organic materials significantly (87-97 and 25-70% of the BOD and COD, respectively). Moreover, the subcritical process hydrolyzed 38 to 56% of the protein fraction, and the resulting hydrolysates showed antioxidant activity (14-19% of the DPPH) and antihypertension (ACE) activity (60-97%). Additionally, the hydrolysates contained a high ratio of glutamic acid (20-26%) and proline (10-15%), which confirmed the relevant functional properties.

Whey is the main byproduct obtained from the manufacture of cheese, yogurt, and milk protein concentrates. After separation of whey proteins through membrane processing, the remained stream is known as whey permeate, and it contains a considerable amount of lactose (75–80% on dry basis). As a feedstock, lactose has the potential to undergo different reactions leading to the formation of value-added compounds. The simultaneous production of lactulose (LAU), lactobionic acid (LBA), and organic acids from sweet and acid whey permeate (SWP and AWP) via catalytic synthesis (5% Ru/C) was studied in a continuous stirred-tank reactor. At selected conditions (60˚C, 60 bar, and 600 rpm), a maximum conversion of lactose (37 and 34%) was obtained after 90 min for SWP and AWP, respectively. The highest yield calculated with respect to the initial concentration of lactose for LAU was 22.98 ± 0.81 and 15.29 ± 0.81% after only 30 min for SWP, and AWP, respectively. For LBA, a maximum yield was found in SWP (5.23%) after 210 min, while about 2.2% was found in AWP. Six major organic acids (gluconic,

pyruvic, lactic, formic, acetic, and citric acid) were quantified during the one-pot synthesis of lactose. The synthesis of a pool of molecules through a one-pot approach represents an alternative approach for the utilization of streams of lactose. Upon further separation, organic acids can be used as building blocks of numerous applications in the manufacture of herbicides, bioplastics, and biofertilizers.

CHAPTER 1

INTRODUCTION

The modern lifestyle or industrialization has brought about many pros and cons to the life of people globally. The main influence of modern lifestyle is on health issues related to diet patterns. As consumers are getting more concerned about negative effects of fast foods, as well as increasing the number of diabetic and overweight people, they are looking for more healthy and functional food choices. On the other side, the health and nutritional benefits of dairy food products have become more evident. Accordingly, consumers are looking for functional and high-protein products in the spectrum of dairy. In recent decades, demand for dairy products showing an ascending movement and dairy industry need to acquire advances in veterinary science, farming methods, operation units, and processing to increase the production yield of milk and milk products (Kushwaha, Srivastava, & Mall, 2011). This momentum has changed enormously the infrastructure of dairy industry to expand category of dairy products in response to consumers' nutritional needs. Improvement in dairy processing by optimizing the production parameters, as well as implementing advanced techniques such as membrane processing have made a promising revolution in dairy manufacturing. Also, using valorization methods to utilize dairy byproducts and to produce value-added materials have helped dairy producers to secure their sustainability All these strategies would possibly reduce the cost of operation and provide consumers with wide spectrum of dairy food options and to maintain food security by increasing the milk production yield. Also, implementing the best processing practices and using membrane systems such as reverse osmosis (RO) and ultrafiltration

(UF) as alternative methods for evaporation and extraction whey proteins could save more energy and water resources from economic and environmental aspects, and develop more functional and nutritional dairy products to meet consumers' demands.

With growth of dairy industries in number, capacity, and diversity of milk products, subsequently the volume of generated wastewater and byproducts have been enhanced. It is worthy to point out that from the food sector, dairy industry presents high production of waste in both liquid and solid state per unit of manufacturing. It is estimated that annually about 4 to 11 million tons of dairy effluents is discharged into the water bodies and polluting environment, as well as endangering biodiversity (Ahmad et al., 2019).

The major sources of effluent and wastewaters in dairy industries are from cleaning-in-place practice and washing operation units based on hygienic procedure. It is also reported that approximately 2% of the total milk is going to the drainage system during processing (Munavalli & Saler, 2009). Three major types of dairy effluents are whey, dairy slurries, and wastewater-originated from processing, cleaning, and sanitary practices. Due to high diversity of dairy products, processing, equipment, handling, packaging, transportation, and storage, the quality and quantity of dairy effluents differ extensively (Britz, Van Schalkwyk, & Hung, 2006). High content of nutrients (fat, proteins, carbohydrates, and minerals), biological and chemical oxygen demands (BOD and COD), and organic and inorganic materials make dairy effluent as one of the most pollutant biomass for environment. Additionally, they encompass substantial levels of detergents and sterilizing substances which influence on the quality of soil, water, and air (Ahmad et

al., 2019). Furthermore, the fat components make a greasy layer on the surface of water bodies and acts a depletion of dissolved oxygen for aquatic creatures and jeopardizes their survival (Rosa et al., 2009).

Ice-cream manufacturing and cheese processing are two important sectors of dairy industry from the nature of their generated effluents.

The complex composition of ice-cream wastewater due to variation in ingredients (milk proteins, fats, carbohydrates) and other additives, as well as presence of detergents have created many impetuses for its treatment (Borja & Banks, 1995). Identifying an appropriate and efficient methods for treatment of ice-cream wastewater could play a crucial role in sustainability of dairy industry. Biological and physicochemical methods are as conventional techniques used for ice-cream wastewater treatment. Biological methods include activated sludge process, aerated lagoons, sequencing batch reactor (SBR), anaerobic sludge blanket reactor, and anaerobic filters. Most applicable physicochemical methods are coagulation and flocculation processes, and membrane techniques such as nanofiltration (NF) and reverse osmosis (RO) (Demirel, Yenigun, & Onay, 2005). The low cost of reagents and higher efficiency in reduction of COD are mentioned as advantages of biological methods. Water and residual proteins are two predominant fractions in ice-cream wastewater which make it as a potential stream for production of amino acids through hydrolysis reactions. Subcritical water or water at elevated temperature and pressure (above boiling point; 100˚C and 0.01 MPa, and below critical conditions; 374˚C and 22 MPa) shows outstanding properties for hydrolysis and extraction purposes. So, subcritical

hydrolysis has been proposed as an alternative to chemical and enzymatic methods. Within the region of boiling and critical point, water exhibit high ion product which triggers ionic reactions mainly hydrolysis that is considerably improved. Relevant features of water at subcritical conditions which behaves as a reaction medium for hydrothermal biomass transformation are reviewed somewhere else (Möller, Nilges, Harnisch, & Schröder, 2011). Subcrtitical water has been recognized to be an effective reaction environment for hydrolysis of organic materails and converting them valuable susbtances. The protein content of ice-cream wastewater under subcrtical condition go through hydrolysis reaction and produce peptide fractions and realising amiono acids, which can be further utilized as feedstock.

Whey is the main byproduct obtained from the manufacture of cheese, yogurt, and milk protein concentrates (Nath et al., 2016). It is the yellowish liquid separated from curds, and it is mainly made of water (~94%), lactose (~5%), proteins (~1%), minerals (~1%), and milk fat (~0.5%) (Prazeres, Carvalho, & Rivas, 2012). As a byproduct, whey is concentrated and further fractionated to produce a wide array of products and ingredients with food and pharmaceutical applications. Examples of ingredients derived from whey are milk serum protein concentrate, whey powder, whey protein concentrate, whey protein hydrolysate, and whey protein isolate (Smithers, 2008, 2015). The byproduct derived from the production of protein ingredients is known as whey permeate, and it contains considerable amount of lactose (75-80% on dry basis). After separating from milk or whey, lactose in different purity specification can be used as an ingredient in feed, food and pharmaceutical applications. Lactose can also serve as a precursor of lactose-derived

bioactive substances, which, like lactose itself, have applications in food and pharmaceuticals (Schaafsma, 2008). Different types of lactose derivatives with broad applications in food and pharmaceutical fields can be produced in laboratory or industrial scale by using numerous methods, including hydrolysis, oxidation, hydrogenation or reduction, isomerization, and epimerization (Gänzle, Haase, & Jelen, 2008). The synthesis of a pool of molecules through a one-pot approach represents an alternative approach for the utilization of lactose streams (Cheng, Metzger, & Martínez-Monteagudo, 2020). The current strategies and later scientific improvement in valorization methods should speed up the growth of platforms to expand the utilization of lactose from lactose permeate and to convert it to lactose derivatives as important ingredients to formulate more functional food products and pharmaceutical preparation, as well as raw substance for a big market share in nonfood acquisition.

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CHAPTER 2

LITERATURE REVIEW

2.1. Sustainability spectrum in dairy industry

Improvement in dairy processing is possible through optimizing the production parameters and using valorization methods to utilize dairy waste stream and byproducts for production of value-added ingredients. These practices would possibly reduce the cost of operation and processing, as well as providing consumers with wide spectrum of dairy food options and maintain food security by increasing the milk production yield. Also, implementing the best processing practices and using membrane technologies such as reverse osmosis (RO) and ultrafiltration (UF) as alternative methods for evaporation and extraction whey proteins could save more energy and water resources from economic and environmental aspects, and develop more functional and nutritional dairy products to meet consumers' demands.

To better understand sustainability in dairy industry, it is worthy to define its terminology. In 1997, Paul B. Thompson has defined sustainability as a norm and said: "The word sustainability always includes an aspect that considers social values" (Thompson, 1997). According to current US legal definition (US Code Title 7, Section 3103), sustainability is defined as "an integrated system of plant and animal production practices having a site-specific application that will over the long-term: satisfy human food and fiber needs, enhance environmental quality and the natural resource base upon which the agriculture economy depends, make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and

controls, sustain the economic viability of farm operations, and enhance the quality of life for farmers and society as a whole." Sustainability covers three main elements of environment, economic, and social. So, the type of processing, equipment, and characteristics of products contribute significantly to maintain the sustainability of dairy industry.

To consider dairy industry as a sustainable platform, the role of processing and equipment is very vital from environment and economic aspects. For instance, utilizing advanced facilities in milking and equipment for in-farm cooling and storage systems, as well as using membrane system for pre-concentrating milk and reducing the cost of transportation have helped farmers economically. Additionally, recent developments in membrane processing has made a big revolution in dairy industry. Using different membrane processing has enabled dairy manufacturers to produce different fraction of milk proteins such as micellar caseins, ultra-filtered (UF) milk proteins, and whey proteins concentrate (WPC) to meet the nutritional needs with variety of dairy foods options (Cheryan, 1998; Huffman & Harper, 1999; Pouliot & Sumner, 2008). However, installation of new processing lines and establishing further manufacturing steps requires more consumption of water and raises in the generation of waste streams and byproducts. Accordingly, dairy industry needs to identify proper treatment techniques and valorization methods to provide its sustainability.

2.2. Dairy wastewater

A confluence of social and economic factors has made the dairy industry consider waste streams as essential actors in the transition to sustainable manufacture. Dairy

industry generates large quantities of wastewater due to their strict hygienic standards (Demirel, Yenigun, & Onay, 2005). Dairy wastewater is defined as a biomass with high biological oxygen demand (BOD), chemical oxygen demand (COD), nutrients, organic and inorganic materials (Britz, Van Schalkwyk, & Hung, 2006). Therefore, dairy wastewater requires multifaceted treatment prior disposal into the municipal sewage system to avoid environmental jeopardies.

General characteristics of dairy wastewater

The most volume of generated dairy wastewater is through processing phases including cleaning and washing the floors, vehicles, trollies, vehicles, and during the cleaning-in-place (CIP) practices for manufacturing units such as tanks and piping. The CIP system in dairy manufacturing includes three phases including: 1) pre-rinse phase- removing any residuals of raw materials or final products, 2) hot-caustic wash phase- cleaning operation units surfaces, and 3) cold finish rinse phase- removing any trace residuals of caustic solution.

The properties of dairy effluents such as quantity, physiochemical composition, and biological load are influenced by type of product, manufacturing program and method, processing steps, layout of processing facilities in the plant, the level of water management, and the volume of water usage. Accordingly, there are three main classifications of dairy wastewater:

1) Processing water- the water utilized for heating and cooling. This type of effluent is free of pollutant and chemicals agents and requires the least treatment for reusing or used as rain runoff water.

2) Cleaning wastewaters- which are generated through cleaning of processing units that are in contact with milk and dairy products, leakage of milk products, cheese and whey production, CIP protocols, and water derived from failures of processing equipment. This type of waste stream contains residuals from milk, cheese, whey, cream, and water from separator, dilution water from yogurt manufacturing, and fruit particles.

3) Sanitary wastewater- that is regularly transferred to the sewage system (Britz et al., 2006).

The dairy effluents emanate via cleaning system encompass different types of sanitizing agents and detergents. So, the pH level of the dairy stream varies noticeably depending on the CIP and cleaning process. Caustic soda, nitric acid, phosphoric acid, and sodium hypochloride are among the most utilized for CIP process which influence on pH of wastewater (Danalewich, Papagiannis, Belyea, Tumbleson, & Raskin, 1998). The CIP chemicals contribute notably on the levels of biochemical and chemical oxygen demands (BOD and COD) which considerably impact on toxicity level of waste stream.

The features of dairy wastewater vary among factories according to the type of products and manufacturing process. This determines the appropriate treatment method according to its nutrients, organic and inorganic materials. Underneath, the various

conventional treatment methods, and recent developments in conversion of dairy effluents have been discussed.

2.3. Dairy wastewater treatment methods

According to nature of generated wastewater, various treatments methods or integration of numerous procedures including grease traps, oil-water separators, clarifiers, biological, and chemical treatments could be employed. Some authors documented a comprehensive review about different treatment methods and utilization of dairy waste streams (Ahmad et al., 2019). The main dairy effluent treatments can be categorized in three groups including biological, physicochemical, and wetland (natural process) methods. Some of the most promising methods for dairy waste stream are discussed followingly.

2.3.1. Biological treatment

Treatment of dairy wastewater through biological methods are highly recommended due to their efficiency in removing organic compounds from dairy (Carvalho, Prazeres, & Rivas, 2013). The most useful biological treatments are trickling filters, aerated lagoons, activated sludge, up flow anaerobic sludge blanket (UASB), anaerobic filters, sequential batch reactor (SBR), etc. (Yonar, Sivrioğlu, & Özengin, 2018). Some of drawbacks of biological methods are formation of sludge as a result of aerobic degradation of organic materials and nutrients, , the high cost of process for treatment of sewage sludge, and environmental concerns due to adsorption of organic compounds and toxic metals by the formed sludge (Dąbrowski, Żyłka, & Malinowski, 2017). However, it

is reported that using appropriate microorganisms with capability of hydrolyzing complicated organic compounds and adsorbing heavy metals could solve the mentioned disadvantage of biological methods (Britz et al., 2006).

According to oxygen intake, biological treatment can be categorized into two different groups of aerobic and anaerobic techniques.

For aerobic process, microbial degradation and oxidation of waste substances require oxygen which could be done by activated sludge, trickling filters, aerated lagoons, and or integration of them (Kushwaha, et al., 2011). Except proteins and fats, all other substances in dairy waste is biodegradable. Combination of enzymatic and anerobic treatments has resulted in satisfactory reduction of organic materials (Vidal, Carvalho, Mendez, & Lema, 2000).

Sequential batch reactor (SBR) has been recognized as one of the most efficient aerobic methods for treatment of dairy wastewater. About 90% reduction of COD value was reported for treatment of wastewater form an industrial milk factory by using a bench scale aerobic SBR process (Mohseni & Bazari, 2004). Recently, simultaneous application of aerobic granular activated sludge SBR or called GAS-SBR has shown high efficiency of aerobic treatment with improved settling of residuals for treatment of dairy wastewater (Wichern, Lübken, & Horn, 2008). In a study by Schwarzenbeck, et al., 2005, an aerobic granular activated sludge SBR was applied for treatment an industrial dairy wastewater that resulted in 90% reduction of total COD, 80% of total nitrogen, and 67% total phosphorus.

During the last decades, anaerobic processes have found promising applications in treatment of dairy waste streams (Omil, Garrido, Arrojo, & Méndez, 2003). Also, several drawbacks of aerobic treatment such as high energy consumption have motivated researcher for other cost-effective process. Certain characteristics of dairy effluent like high organic content and COD value, as well as high temperature, has proposed it as a superb matrix for anaerobic treatment. Additionally, no need for aeration, minimum amount of surplus sludge formation, and low required space are pinpointed as benefits of anaerobic treatments methods over aerobic process (Wheatley, 1990).

Up flow anaerobic sludge blanket or called UASB is one the most applicable anaerobic treatment for dairy effluents. Fundamentally, the UASB reactor is composed of sludge blanket, influent-distribution system, gas-solid separator, and attached to an effluent-withdrawal port (Borja & Banks, 1994; Gavala, Kopsinis, Skiadas, Stamatelatou, & Lyberatos, 1999). In a study, 97.5% of COD removal and 98% lactose conversion were obtained using a conventional UASB reactor for treatment of dairy wastewater (Najafpour, Hashemiyeh, Asadi, & Ghasemi, 2008). In an investigation, the possibility of using UASB reactors was revealed for treating dairy wastewater to the magnitude of 96% COD reduction (Ramasamy, Gajalakshmi, Sanjeevi, Jithesh, & Abbasi, 2004).

Anaerobic filter (AF) reactor is proposed for treatment of dairy effluents with low amounts of suspended solids. The basic function of AF reactor is entrapment of suspended solids and giving enough retention time for biomaterials. Consequently, the retention time for suspended solids and hydraulic retention time (HRT) impact noticeably on efficiency of AF reactor. It needs to consider the porosity of supporting media which considerably influences on the efficiency of the AF reactor. Using the supporting media with large

surface area increases the attachment of biomaterials and enhanced porosity results in low required volume of reactor, and subsequently diminish the clotting of filter (Kushwaha, Srivastava, & Mall, 2011). Omil et al., 2003, investigated the efficiency of AF reactor for treatment of industrial complex dairy wastewater and they achieved more than 90% of COD reduction. In a pilot trial for treatment of dairy waste using an upflow AF reactor, an average COD removal of 70% was obtained (Monroy, Johnson, Wheatley, Hawkes, & Caine, 1994).

2.3.2. Physicochemical treatment

Physicochemical methods including coagulation/flocculation, adsorption techniques, and membrane process have been used for removal of suspended, colloidal, and dissolved compounds. Coagulation and flocculation are common processes for purification purposes in industrial waste streams. Utilizing chemical coagulants improve removal of insoluble particles and dissolved organic materials following by sedimentation, floatation, and filtration steps (Kushwaha et al., 2011). Numerous coagulation agents such as $FeCISO_4$, H_2SO_4, and carboxy methyl cellulose (CMS) have been used for treatment of dairy streams. Using lactic acid by addition of CMC has shown promising trend in chemical treatment of dairy wastewater. Although, addition of $FeCISO_4$ has removed 2-3% more COD than H_2SO_4 plus CMS, and 4-6% more COD than lactic acid plus CMC (Rusten, Lundar, Eide, & Ødegaard, 1993).

Membrane processing such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO), and electrodialysis have found promising applications in treatment of dairy streams. In a comprehensive study by Vourch, Balannec,

Chaufer, & Dorange, 2008, treatment of dairy wastewater through RO resulted in 90 to 95% water recover (Vourch, Balannec, Chaufer, & Dorange, 2008). After treating dairy wastewater using single RO or NF + RO methods, the recovered water showed satisfactory properties such as total organic carbon (TOC) and conductivity for reusing for heating, cooling, and cleaning purposes.

Some inexpensive adsorbents in conjunction with powdered activated (PAC) have been applied extensively to treat dairy wastewater (Rao & Bhole, 2002). Usage of PAC has been more efficient in reducing the level of totals dissolved solids (TDS) than other adsorbents such as bagasse, straw and saw dust, fly ash, and coconut coir. The efficiency of using chitosan and other inorganic coagulants at various pH levels as pretreatment of dairy wastewater followed by membrane processing such as UF and RO have been evaluated. Using chitosan and PAC at pH of 4.0 followed by RO showed high efficiency in removal of color and odor from dairy wastewater and consequently yielded better quality of recycled water. Also, chitosan at concentration of 10-50 mg L^{-1} resulted in 57% removal of COD (Sarkar, Chakrabarti, Vijaykumar, & Kale, 2006).

2.3.3. Wetland treatment

Over half a century, the treatment of wastewater in developed countries is conducted by conventional biological processes which require consolidated infrastructure, costly operation units, continuous maintenance, high energy consumption, production of generation of harmful carbon dioxide and unpleasant odors. For some developing countries, installment of these processing methods for treatment of wastewater in not possible from economic aspects which resulting in dumping of waste stream in water bodies. So, wetland treatment is defined as a sustainable wastewater treatment with similar

functionality of the conventional methods in an economic manner, eco-friendly and energy efficient fashion (Stefanakis, Akratos, & Tsihrintzis, 2014). Wetlands have been applied effectively for treatment of dairy wastewater in some countries such as Italy, Canada, Ireland, and Argentina (Ahmad et al., 2019). Although, some drawbacks of wetland treatment like requirement for large surface area, possible danger for surface and ground water, and accumulation of insects and harmful volatile compounds have been reported (Kolev Slavov, 2017).

2.4. Recent advancements in dairy wastewater utilization

Recently, biotechnological processes like fuel cell, aerobic and anaerobic microbial fermentation, and anaerobic digestion have opened new windows for bioconversion of dairy streams. In this way, integration of biotechnological and physicochemical methods can be employed for manufacturing value-added substances. Recently, hydrolysis technology like subcritical water has been employed successfully to convert biomass waste (fish waste, chicken waste, hair, and feather) to value-added substances such as amino acids (Cheng et al., 2008). Also, valuable carbohydrates such as monosaccharides and oligosaccharides have been hydrolyzed from bakery stream and food wastes through subcritical water treatment (Mohd Thani et al., 2020).

2.5. Valorization of ice-cream wastewater

During the manufacturing of ice-cream, the amount of wastewater generated is estimated to be between 1 and 2 gallons per pound of product. Ice-cream wastewater is a complex colloidal mixture of suspended solids (flavoring compounds), soluble material (sugar, lactose, minerals, and other carbohydrates), dispersed particles of different size

(proteins and emulsified fat), and residues of cleaning agents (Demirel et al., 2013; Hu, Thayanithy, & Forster, 2002).

Currently, biological and physicochemical methods are used for ice-cream wastewater treatment. Biological methods encompass activated sludge process, aerated lagoons, sequencing batch reactor (SBR), anaerobic sludge blanket reactor, and anaerobic filters. Most applicable physicochemical methods are coagulation and flocculation processes, membrane techniques such as nanofiltration and reverse osmosis (Demirel et al., 2005). Advantage of biological methods is the low cost of reagents and higher efficiency in reduction of COD (Möller, Nilges, Harnisch, & Schröder, 2011). While the use of anaerobic digestion as a mean to reduce the levels of organic load prevails in the treatment of wastewater for food and dairy wastewater (Demirel et al., 2005). Although, anaerobic treatment is not effective for removing fat from dairy waste due to its high content and inhibitory activity (Vidal et al., 2000). Some studies have reported the enzymatic hydrolysis as a pre-treatment followed by anerobic digestion to reduce organic load. In a study conducted on babassu cake using *Penicillium restrictum* lipase and combined with UASB reactor, a high removal of COV value around 90% was achieved (Cammarota, Teixeira, & Freire, 2001).

It should be noticed that all these approaches have been focusing on reducing the organic load, and valorization strategies for ice-cream wastewater have not been acquired yet.

The complex composition of ice-cream wastewater due to variation in ingredients and formulation, as well as presence of detergents has created many impetuses for researchers. Therefore, implementing identifying appropriate and efficient methods for treatment of ice-cream wastewater could play a crucial role in sustainability of dairy industry.

A promising method for conversion of ice-cream wastewater involves the breakdown of the compounds forming the colloidal mixture in the presence of pressurized heated water, which results in compounds of a lower molecular size (peptides and free fatty acids) that can be separated and further converted into valuable chemicals such as surfactants, foaming agents, emulsifiers, and animal feed. More importantly, peptides derivate from milk proteins are known for providing additional health benefits beyond their essential nutrition (Kilara & Panyam, 2003).

In recent decades, subcritical water has gained attention of researchers due to its unique characteristics as a reaction medium. This technology is also known as subcritical water hydrolysis, water above the boiling and below the critical point (Möller et al., 2011). The rationality of using pressurized water lies on physicochemical properties induced by the combination of pressure and temperature. Thermodynamic properties of water under pressure are well documented and available through National Institute of Standards and Technology database.

In summary, water within the subcritical state possesses relative low dielectric permittivity and high ion strength, which favors reactions such as nucleophilic substitution,

eliminations, and hydrolysis (Brunner, 2009). Hydrolysis as a result of subcritical water offers unique advantages in valorizing wastewater streams. For instance, the water content of the waste stream acts as a reaction agent and solvent, meaning that streams of wastewater can be directly treated without any pretreatment. The hydrolysis occurring in subcritical water is often accompanied by secondary reactions (oxidation, hemolysis, and additions). Subcritical hydrolysis showed to be an effective process for the hydrolysis of ice-cream wastewater. Controlled subcritical hydrolysis through the hydrolysis time and temperature showed to be significant variables to obtain a high degree of hydrolysis from ice-cream wastewater.

2.6. Whey permeate valorization

One of the most prominent methods to maximize the value of milk and economically supporting farmers and dairy producers, are creating value from dairy by-products. Whey is a coproduct of cheese and Greek yogurt manufacturing which contains around 6% totals Per one Kg production of cheese, almost 7-9 Kg of whey is generated. According to USDS report in 2018, the annual dry whey production from cheese and casein manufacturing amounted to 71.3 million pounds in United States (USDA, 2019).

Lactose as the major component of whey solid which accounts about 80% of the solid materials and presents high biological and chemical oxygen demands (BOD and COD). Due to its high organic content, there are some strict environmental regulations for dairy manufacturers to find valorization methods to utilize lactose from whey streams. Consequently, creating value from dairy byproducts and particularly whey permeate and its lactose content is very vital to have a sustainable dairy industry from economic and environmental aspects. Currently, lactose crystallization is not a profitable industry and

due to its low solubility and less sweetness value, as well as malabsorption by a certain population its utilization has been limited in food preparations and pharmaceuticals. Recently, there is a considerable industrial interest to further utilize lactose as a feedstock to produce lactose-based ingredients. Accordingly, developing cost-effective methods to convert lactose to its valuable derivatives will undoubtedly create a more sustainable platform for dairy processing. In this way, several pathways have been applied to benefit from lactose. Chemical, enzymatic, and microbial methods have been used to synthesize lactose derivatives, such as lactulose, lactobionic acid, lactosucrose, lactic acid, galacto-oligosaccharide, tagatose, and lactitol.

Newly, development in renewable resources has gained noticeable attention in industrial chemistry. In this regards, sugar alcohols or polyols derived from hydrogenation of sugar molecules are so resourceful molecules with various applications such as low-caloric sweeteners. Carbohydrates account for 75% of available biodegradable materials and one of the main resources in the area of green chemistry. Accordingly, since the last two decades ago carbohydrates have been proposed as biofeedstocks for chemical industry (Lichtenthaler & Peters, 2004). Although, beside all naturally occurring carbohydrates as a main source of renewable biomass, their derivative products should be developed as alternative raw biomaterials for maintaining a flexible evolution trend into a biomass conversion industry. From naturally occurring polysaccharides such as hemicellulose to produce simpler monosaccharides and disaccharides that act as initial substances for production of value-added derivatives and more importantly sugar alcohols. These molecules are formed through the reduction of carbonyl group in sugar structure during

chemical reaction with aid of chemical agents such as sodium borohydride or through catalytic hydrogenation using homogeneous or heterogeneous catalyst system with molecular hydrogen.

2.7. Lactose uses[1]

Over the last 30 years, obesity has changed from being an outstanding occurrence to a condition affecting a significant proportion of the population (Haslam, 2016). In the United States of America, two-thirds of the adult population is obese or overweight, and approximately 17% of children and adolescent are obese (Farran, Ellis, & Barron, 2013). Americans spend about $200 billion annually in medical expenses to address diseases closely related to obesity (AANP Forum, 2016). The alarming prevalence of obesity has been attributed to a variety of socio-economic factors including eating environment, sedentary lifestyle, genetics, and dietary patterns (Story, Kaphingst, Robinson-O'Brien, & Glanz, 2008). Concerns about sugar intake in human health dates from the 1970s where the onset of the obesity epidemic was first observed (Kavey, 2010). Epidemiological studies have closely associated sugar intake with several chronic diseases through the development of obesity (Kavey, 2010; Reedy & Krebs-Smith, 2010). Health professionals recommend reducing the caloric intake and increasing physical activity as a means to achieve sustainable weight loss (Patton, Hubrich, & O'Brien-Nabors, 2016). Alternatively, increasing the number of healthy food choices can be an effective strategy for promoting weight loss (Wing & Jeffery, 2001). There is a national urgency to reformulate energy-dense food products to make them healthier. Industrially processed food products are

[1] A version of this content has been published in Trends in Food Science and Technology, Volume 83, January 2019, Pages 181-191.

formulated with a variety of processing ingredients and aids to provide desirable texture, flavor, and shelf-life (Wahlgren, Bergenståhl, Nilsson, & Rayner, 2015). Though taste and convenience remain the top reasons for selecting a particular product, perceived health benefits are increasingly important for the consumer to choose a product over another. While the use of sucrose and sweeting syrups as a source of sweetness prevails in the manufacture of foodstuffs, many sugar replacers have been developed offering new opportunities for product development (Aidoo, Depypere, Afoakwa, & Dewettinck, 2013; Grembecka, 2015). Sugar replacers are classified as nutritive and non-nutritive sweeteners. Examples of nutritive sweeteners are sugar alcohols, including sorbitol, xylitol, isomalt, lactitol, and mannitol, while aspartame, saccharin, sucralose, stevia, and acesulfame-K are examples of non-nutritive sweeteners. The role of nutritive sweeteners in food formulations has been reviewed by Grembecka (2015). In today's supermarket, it is common to find food products formulated with nutritive sweeteners including drinks, chocolate, desserts, puddings, and others.

Sugar alcohols or polyols are traditionally synthesized from corn syrup or cellulose biomass through a set of chemical reactions including oxidation, hydrolysis, and hydrogenation. Among sugar alcohols, lactitol is a versatile compound having diverse applications in the field of food, dairy, and pharmaceutical. In this chapter, key functional properties of lactitol and applications thereof are discussed.

2.7.1. Historical progress

Table 1 provides a historical review of selected milestones for the lactitol industry. Lactitol was first reported almost 100 years ago by Senderens (1920), who hydrogenated lactose in the presence of active nickel. Soon after, it became clear that Senderens' process was not reliable since extensive hydrolysis of lactitol was observed.

Table 1. Selected scientific and commercial milestones in lactitol production and applications.

Year	Milestone	Reference
1920	First reported catalytic hydrogentation of lactose	Senderens (1920)
1925	Invention of a catalytic material	Raney (1925)
1937	Experiments on some properties of lactitol	Karrer & Büchi (1937)
1938	Crystalline lactitol	Wolfrom et al. (1938)
1952	Experiments on lactitol dihydrate	Wolfrom et al. (1952)
1953	Continuous hydrogenation of sugars	Kasehagen (1953)
1963	Application of lactitol in infant food	Tervalon (1966)
1966	Polyethers made of lactitol	Stockburger et al. (1966)
1976	Lactitol as a sweetener	Hayashibara (1976)
1977	Experiments on taste and sweetness	Lee (1977)
1979	Fermentation of sugar alcohols	Havenaar et al. (1978)
1980	Incorporation in toothpaste	Suganuma et al. (1980)
1982	Metabolic studies	Van der Wiel-Wetzels (1982)
1983	Lactitol for the treatment of liver disease	Booij (1983)
1983	Toxicological evaluation	JECFA (1983)
1987	Sugarless confectionary formulations	Bunick et al. (1987)
1987	Chewing gum formulations	Yang (1988)
1987	Animal feed	Harju et al. (1988)
1988	Low calorie chocolate	Kong-Chan (1988)
1992	Experiments on lactitol crystalline structure	Kivikoski et al. (1992)
1993	GRAS status	US FDA (1993)
1996	Food labeling and health claims of lactitol	US FDA (1996)
2008	Hair styling products	Huynh et al. (2008)
2009	Cleaning compositions	Wilson (2009)

Further research in catalyst led to the invention of the sponge nickel, a catalytic preparation that facilitated the synthesis of sugar alcohols (Raney, 1925). Years later, the concept of lactitol as a low caloric ingredient was introduced by Karrer and Büchi (1937), who demonstrated that lactitol was minimally hydrolyzed by enzymes. Wolfrom, Burke, Brown, and Rose (1938) obtained anhydrous lactitol in crystalline form by purification and further crystallization of the hydrogenated slurry. In 1952, research in crystallization showed a second anhydrous crystalline form of lactitol, dihydrate, having different melting point than the monohydrate form (Wolfrom, Hann, & Hudson, 1952). Soon after, Kasehagen (1953) invented a process for the continuous production of sugar alcohols (sorbitol, mannitol, and lactitol) by direct hydrogenation of reducing sugars in the presence of solid catalyst. In the years thereafter, lactitol stimulated research in the field, nutrition, material science, and biotechnology. Fortification of infant food (Tervalon, 1966), synthesis of lactitol-based polyethers (Stockburger, Wright, & Brandner, 1966), sweetening agent (Hayashibara, 1976), animal feed (Harju, Setala, Heikonen, & Linko, 1988), and fermentation (Havenaar, Huis in ’t Veld, B., & de Stoppelaar, 1978) are remarkable examples of industrial applications of lactitol.

The sweetness intensity of lactitol was demonstrated by Lee (1977), who developed a sweetness scale (relative sweetness intensity) using sucrose as a reference. For the first time, a numerical value was used to quantify the sweetness of lactitol and other sugar alcohols, leading to a hundreds of patents in subsequent years including confectionary formulations (Bunick, Hutchinson, & Cifrese, 1987), chewing gum (Yang, 1987), and low-calorie chocolate (Kong-Chan, 1988). The applicability of lactitol in the

field of hygiene and medicine was demonstrated in the 1980's, where lactitol was successfully incorporated into toothpaste, mouthwashes and aseptic products for improving taste. Metabolic concerns related to lactitol consumption were first addressed by Wiel-Wetzels (1981). In years thereafter, lactitol enters in the field of medicine. For instance, it was used for the treatment of liver disease (Booij, 1983). In 1983, an expert committee of food additive approved lactitol as a safe product after years of safety studies in experimental animals (World Health Organization, 1983).

In the early 1990's, Kivikoski, Pitkänen, Valkonen, and Heikkilä (1992) elucidated the different crystalline forms of lactitol and characterized their respective melting points. In 1993, the Food and Drug Administration (FDA) granted the status of Generally Recognized As Safe (GRAS) by FDA (Food and Drug Administration, 1993). Soon after, the FDA regulated the labeling of lactitol and its health claims (Food and Drug Administration, 1996). More recently, lactitol was used in hair styling products due to its stability and hygroscopicity (Huynh, Hohenstein, & Santos, 2008). Wilson (2009) developed cleaning formulations using lactitol based surfactants. In nowadays, lactitol and other sugar alcohols represent a significant global market with various applications, and its production is projected to reach 1.9 million metric tons by 2022.

2.7.2. Lactitol production

Lactitol is not found in nature and it is industrially produced through catalytic hydrogenation of lactose. Catalytic hydrogenation refers to a set of chemical reactions in which hydrogen is added to a reactive functional group. In the case of lactose, the hydrogen

is added to the carbonyl group of the glucose molecule. Overall, the reaction temperature is an important variable influencing the yield of catalytic hydrogenation of lactose. The reaction temperature ranged from 110 to 150°C, while the pressure of hydrogen gas varied between 20 and 70 bars. The transition metals used as a catalyst are Ni, Pd, or Ru at a concentration from 1.5 to 10% supported in either carbon or alumina. These metals favor the dissociation of H_2 molecules, which produces reactive hydrogen molecules. Such unstable species react with the carbonyl group of lactose when both are adsorbed at the surface of the supported catalyst. The primary reaction product is lactitol with reaction yields in the range of >90%. A significant amount of lactulitol is also found, 1.7-1.9%. Lactitol may be hydrolyzed and further hydrogenated leading to the formation of sorbitol and galactitol (yield of 4.6-4.8%). Once the reaction is completed, the catalyst is separated by filtration, and the lactitol slurry is purified by ion-exchange resins. The purified slurry is evaporated to obtain lactitol syrup, which subsequently is crystallized, centrifuged and dried.

2.7.3. Properties

Chemical and crystalline forms

Earlier observations by Wolfrom et al. (1938) and (1952) showed the existence of two forms of anhydrous lactitol having different melting points. Indeed, lactitol in solid state can exist in different crystalline forms. XRD and IR-spectra revealed three hydrate forms (mono-, di-, and tri-hydrate), two anhydrate (A and B), and one amorphous form (Kanters, Schouten, & van Bommel, 1990; Valkonen, Perkkalainen, Pitkänen, & Rautiainen, 1994; Yajima, Okahira, & Hoshino, 1997). **Table 2** summarizes the X-ray

diffraction peaks and endothermic peaks of four solids forms of lactitol. The most common form of lactitol is monohydrate, which is obtained through slow crystallization of the lactitol slurry. Lactitol is a monoclinic polyol with one intra- and eight inter-molecular hydrogen bonds in its chemical structure (Kivikoski et al., 1992). All hydroxyl H-atoms form hydrogen bonds, which give rise to an eight membered ring, chair conformation of the galactopyranosyl ring. Yajima et al. (1997) reported a melting point of around 98°C for lactitol monohydrate. Similarly, Halttunen, Rajakylä, Nurmi, Perkkalainen, and Pitkänen (2001) evaluated the melting point of lactitol monohydrate using DSC. The melting point was found to be within the range of 93 to 100°C, depending on the grinding and drying of the lactitol monohydrate.

Table 2. Summary of X-ray diffraction peaks and endothermic peaks of the different crystalline forms of lactitol. Experimental data reported by Yajima et al. (1997).

Crystalline form	X-ray diffraction peaks	Endothermic peak
Monohydrate	8.9, 17.3, and 17.8°	98.9°C
Dihydrate	7.7, 15.8, and 16.2°	78.3°C
Anhydrate A	9.1, 9.2, 12.0, 18.7, and 22.5°	124.1°C
Anhydrate B	12.9°	151.5°C
Experimental data reported by Yajima et al. (1997).		

The crystalline form of lactitol dihydrate is tetragonal with three intra- and twelve inter-molecular hydrogen bonds in its chemical structure (Kivikoski, Valkonen, & Nurmi, 1992). Similar to lactitol monohydrate, all hydroxyl H-atoms form hydrogen bonds resulting in a chair configuration of the galactopyranosyl ring. Halttunen, Nurmi, Perkkalainen, Pitkanen, and Raisanen (1997) evaluated the effect of water content on the crystal structure of lactitol monohydrate. It was found that over 23% of the water content

can be removed from the crystal structure by slow drying without significant structural changes. Contrary, during rapid drying of crystalline lactitol monohydrate, a third form of anhydrous can be formed, namely trihydrate lactitol. The crystalline structure of lactitol trihydrate is orthorhombic with one intra- and thirteen inter-molecular hydrogen bonds (Kivikoski et al., 1992). Upon heating, the trihydrate form undergoes phase transformation into anhydrate A (85°C) and anhydrate B (105°C). The melting point of the two polymorphic forms is within the range of 120-124 and 150-151°C, respectively (**Table 2**). Yajima et al. (1997) investigated the transformation of solid forms of lactitol using XRD, IR spectra, and DSC. **Figure 1** illustrates the interconversion path of five solid forms.

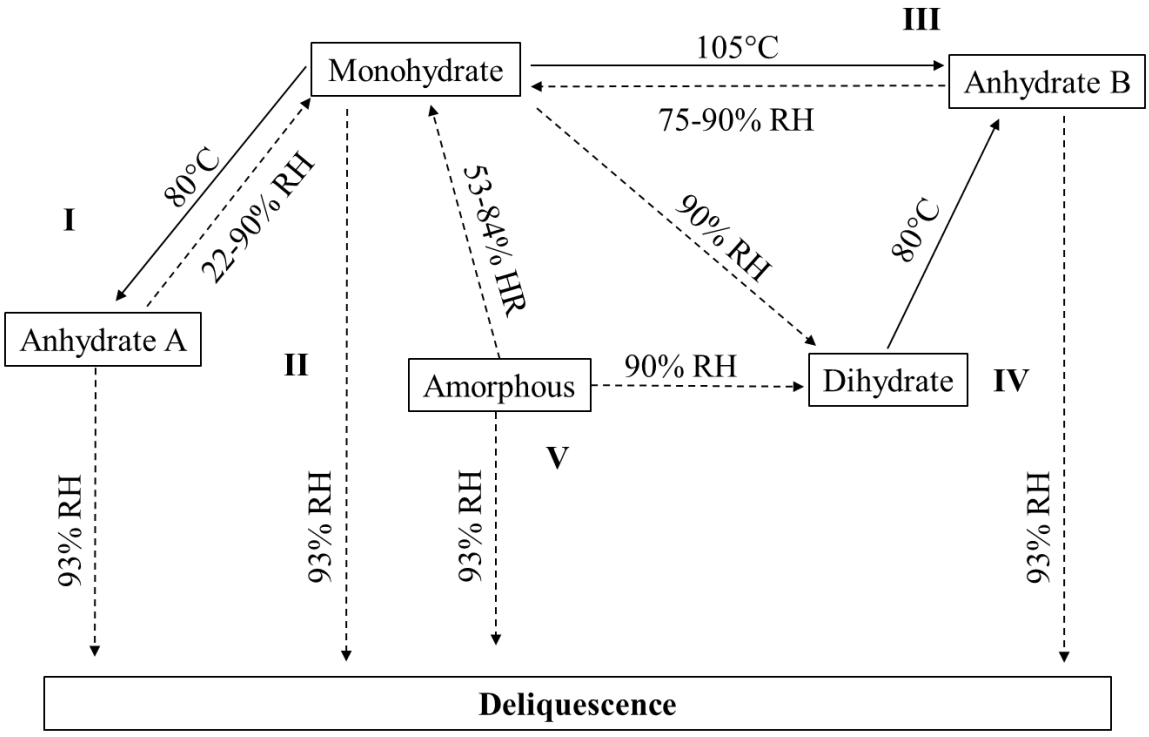

Figure 1. Schematic representation of the interconversion of the solid forms of lactitol. The continuous line represents interconversion induced by heating, while the discontinuous line represents interconversion induced by changes in the relative humidity (RH). Adapted from Yajima et al. (1997).

Lactitol monohydrate can be converted into anhydrate A upon heating at 80°C, which subsequently converts into deliquescence state at a relative humidity of 93%, path I. Anhydrate A may convert back into monohydrate within the range of relative humidity of 22-90%. Additionally, lactitol monohydrate may convert directly to deliquescence state at a relative humidity of 93%, path II. Anhydrate B is formed by heating monohydrate at temperatures around 105°C, which further be converted into deliquescence state at a high relative humidity of 93%. Anydrate B converts back to monohydrate at a relative humidity range of 75-90%. The conversion of anhydrate B is illustrated in path III. Lactitol dihydrate is formed either from monohydrate or amorphous at a relative humidity of 90%. Interestingly, dihydrate does not convert directly into deliquescence state instead; it is converted into anhydrate B, path IV. The amorphous form of lactitol may convert into deliquescence state, dihydrate, and monohydrate when the relative humidity is around 93, 90 and 53-84%, respectively (path V). Halttunen, Hurtta, Pitkänen, and Nurmi (2005) studied the phase transitions of lactitol through sorption and desorption using humidity chambers prepared with aqueous solutions of salt. Lactitol monohydrate was more stable towards moisture than dihydrate and trihydrate. Thermogravimetric analysis (weigh loss upon heating) revealed that the monohydrate form does not evaporate water easily, while dehydrate partly eliminates water changing into an amorphous form. On the other hand, lactitol trihydrate easily loses part of its crystal water and converts into lactitol dehydrate.

Solubility

Methods for commercial preparation of lactitol has been a topic of interest in years past. Lactitol is available as a crystalline powder, and its properties and potential application strongly depend of the given crystalline form. After hydrogenation, the catalyst is removed through ion-exchange followed by evaporation under vacuum, crystallization under prescribed protocol, centrifugation and drying. During the crystallization step, a specific crystal form can be favored other another. General guidelines of crystallization of carbohydrates are applicable for lactitol. However, it is worth to mention that little is known in relation to nucleation of lactitol. Nevertheless, the work described by Nurmi and Kaira (2002) is the best guide available in the literature for the crystallization of lactitol. **Figure 2** shows the solubility curves of the different crystalline forms of lactitol.

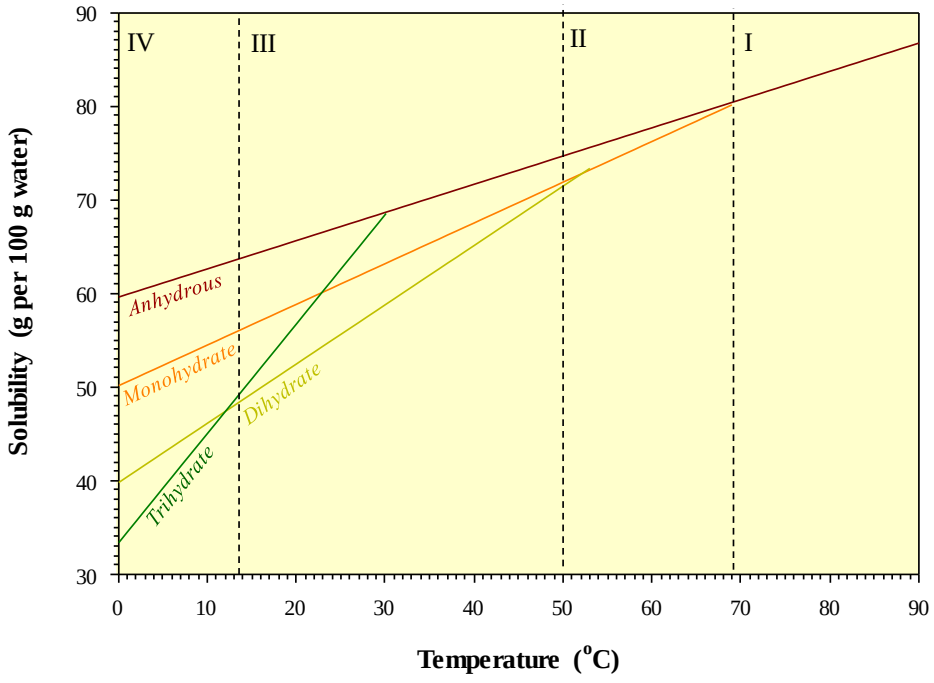

Figure 2. Solubility curves of lactitol anhydrous, monohydrate, dihydrate, and trihydrate. The solubility lines were obtained according to the regression equations reported by Nurmi & Kaira (2002).

From a practical point of view, the solubility curve of lactitol was arbitrarily divided into regions I to IV. Region IV represents the crystallization conditions yielding anhydrous lactitol. Nurmi and Kaira (2002) obtained lactitol in anhydrous form by crystallizing a 91% solution of lactitol. The mass was crystallized by cooling the solution from 95 to 75°C over 10 h. Similarly, Heikkila, Nurmi, and Pepper (1998) crystallized lactitol anhydrous by cooling (90 to 80°C) of a concentrated lactitol solution (90%). Region II illustrates the working conditions for obtaining lactitol monohydrate. This has been exemplified by Heikkilä et al. (2002), who performed a four-step crystallization to obtain lactitol monohydrate. The protocol consisted of cooling from 70 to 40°C in 16 h a seeded lactitol solution (82%). Wijnman, Van Velthuijsen, and Van Den Berg (1998) obtained lactitol monohydrate through seeding an aqueous solution of lactitol (80%) and crystallized it from 75 to 50°C in 18 h. The remaining mother liquid from the lactitol monohydrate was seeded and further cooled down to 18-15°C in order to produce lactitol dihydrate (region II in **Figure 2**). Wijnman et al. (1998) followed a similar protocol and reported a 60% yield of lactitol dihyrate. Lactitol trihydrate (region I) is obtained by further crystallization of the mother liquid at temperatures lower than 10°C (Nurmi & Kaira, 2002).

Caloric value

Evidence of reduced calorie value of lactitol dates back to 1930's, where the enzymatic hydrolysis of lactitol was found to be significantly slower than that of lactose (Karrer & Buchi, 1937). This observation pointed out the possibility of reduced calorie effect of lactitol. Indeed, Hayashibara (1976) injected a 20% solution of lactitol in rabbits

and measured the concentration of lactitol left in the intestines after several hours. Interestingly, the concentration of lactitol was unchanged while 85% of the glucose (control treatment) was lost due to digestion. Van Es, De Groot, and Vogt (1986) analyzed the metabolized energy derived from lactitol and sucrose and found that the energy contribution to the body was 60% less than for sucrose. European labeling considers a blanket caloric value of lactitol as 2.4 kcal g^{-1} (Radeloff & Beck, 2013), while the Food and Drug Administration (FDA) allows a value of 2.0 kcal g^{-1}, which correspond to a reduction of 48-40% with respect to sucrose.

Sweetness

Lactitol has been described as a crystalline powder having a mild clean sweet taste (Zacharis & Stowell, 2011). Relative sweetness is measured in relation to a reference value of 1, which correspond to the sucrose sweetness at a given concentration (Radeloff & Beck, 2013). The relative sweetness of lactitol varies from 0.3 to 0.42, increasing with concentration from 10-20% (wt./wt.). For practical reasons, the sweetness of lactitol is often considered to be around 30-35% compared to that of sucrose. Consequently, replacing sucrose with lactitol in equal sweetness value would require a substantial amount of lactitol. Thus, lactitol is combined with high-intensity sweeteners to effectively reduce the sucrose concentration.

Health claims

Although sugar alcohols are not essential nutrients, its consumption has been clinically linked to numerous healty claims. A comprehensive review of the health claims

of sugar alcohols can be found elsewhere (Livesey, 2003; Nath et al., 2017). In general, sugar alcohols are a poor source of energy for oral bacteria as opposed to other sugars (sucrose and starches), resulting in less acid production. This has been exemplified by van der Hoeven (1986) who studied the cariogenicity of lactitol in program-fed rats and observed that replacing sucrose with lactiotl significantly reduced caries increment. This observation was supported by the fermentation rates of oral bacteria, where the acid production from lactitol occurred at much lower rate than the acid production from sucrose. Clinical evidence showed that substitution of sucrose with sugar alcohols in chewing gum and candies reduce the incidence of caries. van Loveren (2004) suggested that caries-preventive effects of gums and candies formulated with sugar alcohols are due to a stimulation of the salivary flow, which provides buffer capacity to wash away soluble. However, there is no evidence for a minimal dose required to effectively reduced caries. Nevertheless, van Loveren (2004) concluded that chewing of sugar-free chewing gum 3 or more times daily may reduce caries incidence irrespective of the type of sugar alcohol added. Lactitol is one of the most frequently prescribed laxative agents to treat chronic constipation (Prasad & Abraham, 2017). As a laxative agent, lactitol is minimally absorbed in the small intestine, and when it reaches the large intestine, it creates an osmotic gradient that increases the water retention in the stool, enhancing its passage. Miller, Tennilä, and Ouwehand (2014) performed a meta-analysis on the efficacy and tolerance of lactitol for adult constipation. It was found that lactitol supplementation not only was well tolerated but also significantly improve symptoms of constipation.

2.7.4. Applications

Cryoprotectant and dryoprotectant

Table 3 summarizes investigation where lactitol was used as a cryoprectant. Lactitol is a polyol with the ability to prevent physical and chemical degradation of protein preparations during processing (frozen and drying) and subsequent storage.

Table 3. Summary of studies on the use of lactitol as cryopretectant and dryopretectant.

Product	Process	Lactitol form	Concentration	Remarks	Reference
Rainbow trout (*Oncorhyncus mykiss*) muscle	Frozen-stored	Not reported	14 mg mL^{-1}	- Lactitol helped preserving the structural stability of myosin of fish muscle	Herrera and Mackie 2004
Threadfin bream (*Nemipterus spp.*) surimi	Frozen-stored up to 6-month	Not reported	6%	- Surimi blends added with lactitol withstanded protein denaturation during 6-month of frozen storage	Nopianti et al. (2012)
Duck surimi	Freeze-thaw cycles during 4-month of frozen storage	Not reported	6% (w/w)	- Duck surimi added with lactitol mantained protein solubility after 5 cycles of freeze-thawing	Ramadhan et al. (2012)
Ling cod surimi	Frozen-stored up to 4-month	Monohydrate	4-12%	- Blends of polyols including lacitiol yielded acceptable gel formation after frozen storage.	Sultanbawa and Li-Chan (1998)
Ling cod (*Ophiodon elongatus*) surimi	Frozen-stored and 8 freeze-thaw cycles	Not reported	1-8%	- Optimal blends of cryoprotectants prevented loss of gelling capacity during frozen storage	Sultanbawa and Li-Chan (2001)
Threadfin bream (*Nemipterus Japonicus*) surimi powder	Freeze-drying	Not reported	5%	- Lactitol prevented protein denaturation during freeze-drying	Santana et al. (2017)
Buffer solution (pH=7.0) of L-lactic dehydrogenase (0.05 mg mL^{-1}) Buffer solution (pH=7.0) of bovine albumin serum (10 mg mL^{-1})	Freeze-drying and subsequent storage (50°C for 7 d)	Monohydrate	100 mg mL^{-1}	- The presence of lactitol retained the activity of L-lactic dehydrogenase - The structure of bovine albumin serum was preserved by the addition of lactitol	Kadoya et al. (2010)

An investigation on the effectiveness of lactitol as a cryoprotectant agent for myofibrillar proteins of fish muscle (rainbow trout) showed that addition of lactitol preserved the structural stability of myosin (Herrera & Mackie, 2004). Additionally, lactitol reduced the kinetic of hydrophobic residues over the protein surface. Similarly, Nopianti, Huda, Noryati, Fazilah, and Easa (2012) evaluated the effect of adding lactitol on protein denaturation of threadfin bream surimi during 6 months of frozen storage. It was found that a formulation containing 6% of lactitol yielded comparable protective effect with that obtained for polydextrose and sorbitol. Ramadhan, Huda, and Ahmad (2012) evaluated the cryoprotective effect of lactitol on duck surimi after five cycles of freeze-thaw during 4-month of frozen storage. These authors reported that 6% (w/w) of lactitol yielded higher protein solubility and gel firmness when compared with the control treatment. More importantly, lactitol yielded comparable protective parameters with those obtained for other cryoprotectants such as polydextrose, trehalose, and palatinit. The cryoprotectant ability of lactitol has also been evaluated in combination with other compounds. Sultanbawa & Li-Chan (1998) used blends of cryoprotectant (sorbitol, lactitol, polyphosphate, and Litesse™) to prevent protein damage during frozen storage of ling cod surimi. A blend of 4% made of sucrose, sorbitol, Litesse™, and lactitol (ratio 1:1:1:1) was found to stabilize surimi during frozen at -18°C and storage for 4 months. Similarly, Sultanbawa and Li-Chan (2001) evaluated individual and blends of cryoprotectants during frozen-stored surimi from ling cod (*Ophiodon elongatus*). The optimized blends effectively prevented loss of gelling capacity after 8 freeze-thaw cycles.

As a cryoprotectant agent, lactitol forms glassy matrix that immobilizes the protein system and preventing unfolding. It is also though that lactitol forms hydrogen bonds with the protein structure, which helps preserving the activity of enzymes. Interestingly, the postulated mechanisms are also valid for drying of protein preparations. This has been exemplified by Santana, Zilda, and Huda (2017), who evaluated the effect of adding lactitol (5%) during the freeze-drying of threadfin bream surimi. It was found that lactitol not only prevented protein denaturation during freeze-drying but also performed comparable with other dryoprotectant agents (sorbitol and maltodextrin) in terms of physiochemical parameters (whiteness, gel formation, and foaming). Kadoya et al. (2010) used lactitol monohydrate as a cryoprotective agent during the freeze-drying of buffer solutions of L-lactic dehydrogenase and bovine serum albumin. Microscopic observation under vacuum revealed that lactitol forms hydrogen bonds that substitute water molecules, which maintains the activity of L-lactic dehydrogenase and preserves the structure of bovine albumin serum during freeze-drying and subsequent storage. Preserving the activity and structure of proteins is an essential characteristic for pharmaceutical applications that helps minimizing product immunogenicity. Lactitol has been used to preserved archeological artifacts. An investigation on the stability of waterlogged archaeological wood showed that the impregnation of lactitol before freeze-drying resulted in better hygroscopic properties compared with polyethylene glycol impregnation (Majka, Babiński, & Olek, 2017). Similarly, Babiński (2015) evaluated changes in dimensions and moisture content of waterlogged archeological oak treated with lactitol. It was found that the application of lactitol significantly reduced the shrinkage of the wood. Lactitol can partially replace water

molecules and fill the cell walls and lumen, reducing contraction and deformation upon drying.

Surfactant and hydrogel

Lactitol is chemically more stable than related disaccharides, lactose and sucrose. The stability of lactitol arises from its chemical structure, where the absence of a carbonyl group provides stability within a broad range of pH (3-9). Another important characteristic derived from its chemical structure is that lactitol is not a reducing sugar, meaning that does not participate in Maillard reactions. These properties of lactitol have opened a window for non-conventional applications, including the synthesis of surfactants, emulsifiers, and hydrogels. **Table 4** exemplifies studies on the use of lactitol for the synthesis of surfactants, emulsifiers, and hydrogels. Van Velthuijsen (1979) synthesized a non-ionic lactitol based emulsifier by direct esterification with palmitic acid under alkaline conditions. Mono- and higher lactitol fatty esters showed detergent activity through removing oil and stains from kitchen towels. Dupuy et al. (1998) evaluated the micellization in water of lactitol-based surfactants. These authors found that lactiol surfactants were weakly dispersed at low concentration. Furthermore, the formed micelles were schematized by oblate ellipsoids due to their stearic hindrance. Similarly, Drummond and Wells (1998) synthesized mono-esters of lactitol of different chain length, octyl (C8), dodecyl (C12) and hexadecyl (C16). These surfactants were evaluated for their interfacial tension in contact with hexadecane and triolin. Above the critical micelle concentration, increasing chain surfactant slightly lower the interfacial tension than their shorter chain homologous.

Table 4. Summary of studies on the use of lactitol for the synthesis of surfactants, emulsifiers, and hydrogels.

Product	Process	Lactitol form	Remarks	Reference
Lactitol palmitate	Esterification	Monohydrate	- Lactitol palmitate showed promising detergent activity	Van Velthuijsen (1979)
(*N*-dodecylamino) lactitol	Esterification	Not reported	- Micellization was characterized by oblate ellipsoidal	Dupuy et al. (1998)
Octyl, dodecyl, and hexadecyl mono esters of lactitol	Transesterification with vinyl esters	Not reported	- Lactitol based surfactants showed good surface and interfacial activity	Drummond and Wells (1998)
Rigid polyurethane foam	Propoxylation of lactitol	Dihydrate	- Polyurethane foam prepared from lactitol showed comparable characteristics with commercial polyols	Wilson et al. (1996)
Rigid polyurethane foam	Propoxylation of lactitol	Hydrogenated sweet whey permeate	- The thermal stability of the lactitol based foams were similar to that of commercial foams	Hu et al. (1997)
Polyether polyol hydrogel	Propoxylation of lactitol	Dihydrate	- Lactitol based hydrogels exhibited desirable swelling properties and thermal stability	Lin et al. (1998)
Polyether polyol hydrogel	Propoxylation of lactitol and further cross-link	Monohydrate	- Drug release rate of lactitol hydrogel were controlled by the cross-linking ratio	Han et al. (2000a)
Cross-linked hydrogel	Cross-link	Monohydrate	- Lactitol hydrogels showed desirable characteristics in terms of swelling ratio, viscosity, and release rate	Han et al. (2000b)
Polyether polyol hydrogel	Acylation	Dihydrate	- Upon heating (up to 40°C), 90% of protein release within the first hour	Chacon et al. (2000)
Cholesterol-diethenyl decandiote-lactitol (Drug carrier)	Lipase-catalyzed esterification	Not reported	- Lactitol was used as a target group during the synthesis of the drug carrier	Luo et al. (2014)

Additionally, lactitol surfactants showed the ability to form stable foam over 30 min. Such properties are important indicators suggesting that lactitol based surfactants have the potential to be used as emulsifiers. Although the surfactants derived from lactitol has shown promising properties, no commercial application have been developed.

Edible and renewable disaccharides has been used as building blocks for the production of polymers and hydrogels. This has been illustrated by Wilson, Hu, Kurth, Hsieh, and Krochta (1996), who synthesized and characterized polyether polyols by propoxylation of lactitol under alkaline conditions. The physical characteristics of lactitol polyether polyols showed similar viscosity and hygroscopicity as their analogous sucrose-based polyether polyols of the same hydroxyl number. Interestingly, shorter reaction times were required than the sucrose-based polyols. More importantly, the decomposition of lactitol, as well as the formation of browning compounds, were negligible. Wilson et al. (1996) used the lactiol polyether polyols as building blocks to prepare rigid polyurethane foams. Such rigid polyurethane foams showed comparable physical properties than that of commercial foams. Hu et al. (1997) synthesized polyurethane foams by propoxylation of hydrogenated sweet whey permeate. The resulting foams were evaluated showed low densities, high closed cell contents, strong mechanical properties, and high thermal stability. Similarly, Lin, Hu, Hsieh, Kurth, and Krochta (1998) modulated the propoxylation of lactitol to yield polyether polyols having up to nine polypropylene oxide branches. Ring opening polymerization of propylene oxide initiated by the hydroxyl group of lactitol was proposed as a reaction mechanism. The resulting lactitol polyether polyols were further used to generate hydrogel by reacting acylated polyethylene glycol bis

carboxymethyl ether. The lactitol hydrogels showed the ability to absorb water up to 1000% of their dry weight. Remarkably, these hydrogels expelled free water at a temperature above 30°C.

As a platform chemical, lactitol has also been used to engineer delivery systems for bioactive compounds. Han, Krochta, Kurth, and Hsieh (2000) synthesized poly (ether polyol) hydrogel using lactitol as a platform chemical. These authors evaluated the drug release ability of such hydrogel using acetylsalicylic acid over a pH range of 4-9, and it was found that the release rate can be controlled by the degree of crosslinking of the hydrogel. Han, Krochta, Hsieh, and Kurth (2000) incorporated model protein into the lactitol based cross-linked hydrogel and evaluated the release of them into the surrounding fluid. These authors found that the release rate of β-lactoglobulin, bovine serum albumin, and γ-globulin was constant over 2 h at a temperature range of 37-45°C. Such a constant release rate approaching the human body temperature is of high relevance for drug delivery in clinical applications. Chacon, Hsieh, Kurth, and Krochta (2000) synthesized lactitol hydrogels with swelling capacity up to 81-fold. The swelling capacity was controlled by the length of polypropylene oxide branches and the extend of crosslinking. The space between the polypropylene oxide branches within the lactitol governs the free volume available to bind water and subsequently swell. The relative high swelling behavior of lactitol hydrogels makes them promising candidates for controlled release systems. Indeed, Chacon et al. (2000) incorporated lipase into the lactitol hydrogel structure and evaluated its release as a function of temperature. It was found that over 90% of the enzyme was released during the first hour when the temperature raised between 25 to 40˚C. Another

investigation used lactitol as a target group during the synthesis of a novel drug delivery (Luo et al., 2016). The resulting carrier was incorporated in docetaxel liposomes to improve the cure rate of liver disease.

Bakery

Table 5 illustrates investigation on the use of lactitol in bakery products. Reducing or replacing sugar in bakery formulations is not a trivial task since sugar plays a major role in the rheological properties of the batter/dough and therefore on the final quality of the product. This has been illustrated by Psimouli and Oreopoulou (2012), who used lactitol as a sugar replacer in cake formulations. It was found that replacing sugar with lactitol in equal amount resulted in a batter of comparable flow index and temperature of starch gelatinization. More importantly, the sensory analysis showed no significant difference between the batter formulated with lactitol and the one formulated with sugar. Frye and Setser (1992) used lactitol as a sweetener to optimize cake formulations with approximately 45% calorie reduction having comparable attributes with a standard yellow layer cake. Zoulias, Oreopoulou, and Kounalaki (2002) investigated the effect of lactitol and other polyols as a sucrose replacement on the texture profile of cookie dough. The dough formulated with lactitol and maltitol yielded medium values of hardness and consistency, and relatively high values of adhesiveness and cohesiveness. Such texture attributes are similar to those obtained from cookie dough formulated with sucrose. Similarly, Similarly, Zoulias et al. (2002) formulated fat-free and sugar-free cookies without significant changes in hardness and brittleness using lactitol combined with sorbitol and maltitol.

Table 5. Summary of studies on the use of lactitol for reducing sugar in bakery formulations.

Product	Lactitol form	Remarks	Reference
Batter cake	Not reported	- Lactitol were able to substitute functional properties of sucrose	Psimouli and Oreopoulou (2012)
Low-fat cookies	Not reported	- Lactitol yielded favorably texture properties (soft and less brittle) of low-fat cookies	Zoulias et al. (2000)
Low-fat and low-sugar cookies	Not reported	- Formulations with lactitol resulted in higher moisture and water activity than the control	Zoulias et al. (2002)
Reduced-calorie cake	Not reported	- Formulated cakes with lactitol yielded comparable textural attributes with commercial samples	Frye and Setser (1992)

Chocolate and confectionary

The development of sugar-free chocolate is a significant challenge since all the sugar needs to be replaced, which changes the rheological and melting properties of the final product. General guidelines for manufacturing sugar-free chocolates can be found elsewhere (Aidoo et al., 2013). **Table 6** summarizes studies on the use of lactitol for formulating chocolate and confectionary products. Mentink and Serpelloni (1994) developed a formulation for low-calorie chocolate containing an equimolar blend of maltitol, lactitol, and isomaltulose. The resulting chocolate showed technical and organoleptic properties comparable to those of traditional sucrose-containing chocolate. Synergistic effects have been observed when combining sugar alcohols with other sweeteners. de Melo, Bolini, and Efraim (2009) formulated a sugar-free chocolate with an acceptable sensory profile by combining high-intensity sweeteners with blends of polydextrose/lactitol (60/40% w/w).

Table 6. Summary of studies on the use of lactitol for reducing sugar in chocolate and confectionary formulations.

Product	Lactitol form	Remarks	Reference
Low-calorie chocolate	Monohydrate	- An equimolar blend (maltitol, lactitol, and isomaltulose) yielded desirable technical and organoleptic properties	Mentink and Serpelloni (1994)
Milk chocolate	Not reported	- Combinations high-intensity sweeteners with polydextrose and lactitol yielded acceptable sensory profile	de Melo et al. (2009)
Chocolate	Not reported	- Combinations of fructose-lactitol yielded chocolate formulations with increased hardness	Belščak-Cvitanović et al. (2015)
Sweeting syrup	Monohydrate and lactitol from hydrogentation	- Syrup mixed (sorbitol, maltitol, mannitol, xylitol, lactitol, and isomalt) was developed for coating	Serpelloni and Ribadeau-Dumas (1995)
Sweeting syrup	Not reported	- 40% of the sugar was replaced with lactitol in a standard confectionary syrup	Onwulata et al., 2000
Sweeting syrup	Monohydrate and lactitol from hydrogentation	- Lactitol based syrup suitable for soft confectionary product was developed	Blankers et al. 2002

Similarly, Belščak-Cvitanović et al. (2015) compared the use of sugar alcohols, syrups, and natural sweeteners as a sucrose alternative in the production of reduced sugar chocolates. Combination of lactitol and fructose yielded a reduction of 20% in the caloric value than sucrose formulated chocolate. The relatively large particles of bulk lactitol resulted in a two-fold increase in the hardness. Another relevant application of sugar alcohol as sugar replacers is during the manufacture of hard-boiled sweets. Lactitol, sorbitol and other polyols are hygroscopic in nature resulting in sticky texture. This technological challenge has limited the applicability of sugar alcohols for manufacturing hard-boiled sweets and coating applications. Serpelloni and Ribadeau-Dumas (1995) improved the process of hard coating using a syrup of polyols (sorbitol, malitol, mannitol, xylitol, lactitol, and isomalt) and pulverized powder of polyols. The process reduces the number of coating cycles, while providing the desirable organoleptic characteristics. Another investigation on the effect of substituting sugar in confectionary syrups showed up to 40% of the sugar content was replaced with lactitol without significant changes in the moisture content and density of the syrup (Onwulat, Konstance, & Holsinger, 2000). On the other hand, the addition of lactiol resulted in a two-fold increase in the viscosity of the syrup. Blankers, Evers, Putker, and Terlouw (2002) formulated a syrup sweeting suitable for soft confectionary applications. The syrup is made of lactitol and polydextrose and it is combined with the lactitol slurry derived from lactose hydrogenation. Bunick et al. (1987) formulated a sugarless nougat-type confection with desirable mouthfeel by replacing hard-boiled corn syrup with hydrogenated starch and mixtures of sugar alcohols (xylitol, mannitol, sorbitol, galactitol, and lactitol).

Desserts

Attempts to formulate dessert products with reduced sugar using lactitol have been investigating. Gurditta et al. (2015) evaluated the effect of replacing sugar in Chhana-murki, a dairy dessert from India, by using blends of isomalt and lactitol. These authors found that a formulation containing up to 15% (w/w) of polyol blend yielded desirable color, appearance, sweetness, and overall acceptance. Santos and Silva (2012) evaluated the physical and chemical properties of reduced sugar ice-cream using lactitol and Splenda as a sugar replacement. A 50% reduction in the caloric content was obtained by a combination of lactitol and Splenda. However, the apparent viscosity and the ability to incorporate air were adversely affected.

Chewing gum

Lactitol in combination with other sugar alcohols are used to formulate sugar-free chewing gum. The hygroscopicity of lactitol is relatively low, which facilities its incorporation into the gum base. This has been illustrated by Huzinec, Kearns, and Schindeldecker (1999) who incorporated flavor and sweetness compounds including lactitol into a carrier made of microcrystalline cellulose. Such blend extended the release of flavor and sweetness in chewing gums. McGrew et al. (2005) combined active compounds with bulk sweeteners (mannitol, xylitol, maltitol, lactitol, and hydrogenated starch hydrolysates) to develop a controlled release of an active agent embedded within a chewing gum base. Similarly, Yatka, Richey, and Meyers (1995) formulated a generic chewing gum base containing oligofructose and sugar alcohols (sorbitol, maltitol, xylitol, lactitol, and mannitol) as bulking agents. This generic formulation was blended with

glycerol and evaporated to produce a low-moisture and sugar-free chewing gum. The combination of oligofructose and sugar alcohols improved texture, moisture adsorption, and shelf-life compared with conventional sugar-based formulation. Reed, Hook, and Schnell (1994) formulated a hard-coated chewing gum using a coating layer made of lactitol, maltitol, and sorbitol. The wet syrup was used to cover the gum base, and further dried with air until reaching a final moisture content of 8%. The coated mixture improved the stability during prolonged storage. Similarly, Michael A Reed et al. (1997) formulated pellets of chewing gum successively coated with syrups of lactitol, maltitol, and isomaltulose. Such coating process improved the quality and stability of hard coated pellets. Schobel and Yang (1989) encapsulated sweeting agents (lactitol, mannose, fructose, and aspartame) with carboxymehtylcellulose. The encapsulated sweeting agents were further incorporated into the gum base, which provides prolonged release of sweetness.

Biosensor development

Lactitol is used as additive for the development of biosensors due to its ability to stabilize enzymes during production and storage. Karamitros and Labrou (2017) immobilized isoenzyme glutathione transferase in the presence of lactitol. It was found that 5% of lactitol led to prolonged stability of the enzyme activity. Similarly, Gibson and Woodward (1993) used combinations of diethylaminoethyl-dextran hydrochloride (DEAE-Dextran) and lactitol to stabilize enzymes in dry state. It was found that the combination of DEAE-Dextran (10%) and lactitol (5%) preserved 95% of the enzymatic activity after 16 d. Zhybak et al. (2016) used lactitol as stabilizer during the immobilization

of creatinine deaminase and urease and reported significant improvement in the stability of the biosensor. More importantly, the selectivity of the biosensor was not impacted by the addition of lactitol. The ability of lactitol to stabilize enzymes has attracted the attention of researchers for prolonging the stability of immobilized enzymes, and therefore development of improved biosensors.

Stabilizer agent

As discussed earlier, the absence of a carbonyl group within the lactitol structure provides stability within a broad range of conditions. This characteristic has been explored by Klewicki (2007) who evaluated the hydrolysis of juices during pasteurization and found that only 3% of the oligosaccharides were hydrolyzed in the presence of lactitol. Similarly, the addition of lactitol showed a protective effect on the activity of α-amylase during heating (Samborska, Guiavarc'h, Van Loey, & Hendrickx, 2006). Ma, Lee, and Kwong (2002) evaluated the transesterification of lactitol and methylparaben, an antimicrobial, to enhance its solubility and incorporation within the excipient. The transesterification of lactitol minimized the formation of byproducts compared with the transesterification of sugars.

2.7.5. Opportunities and future challenges

Nutritive sweeteners or sugar alcohols are increasingly used in many food and pharmaceutical applications due to their functional properties and health benefits. An overview of the industrial application of sugar alcohols as sweeteners is reviewed by Grembecka (2015).

Although the functional properties of lactitol provided by Zacharis and Stowell (2011), a systematic and comprehensive evaluation of lactitol physical properties has not been reported. Research strategies for expanding the applicability of lactitol are needed including, solubility at different conditions, rheological behavior, heat stability, thermogravimetric analysis, stability toward heat and pH, particle size, bulk and particle density, and crystallization kinetics.

CONCLUSION

Dairy industry has been identified as one of the key segments of food manufacturing that uses high amount of water and generates massive volume of waste stream. The characteristics of wastewater vary from industry to industry and processing methods. Due to high organic content, dairy wastewater requires to be treated thoroughly before putting into the sewage. Moreover, the complexity of wastewater from ice-cream processing and its high COD and BOD values makes its treatment challenging. Among biological and physicochemical treatment methods, subcritical hydrolysis has shown high ranking potential in transformation of ice-cream wastewater biomass to value-added materials. Accordingly, applying appropriate treatment method is very vital for sustainability of dairy industry.

Byproducts from dairy processing can be converted to valuable substances and feedstocks. Whey as foremost byproduct of cheese, Greek yogurt, and casein manufacturing contains considerable amount of lactose after separating whey protein. Lactose is mainly responsible for high BOD and COD values of whey permeate and finding valorization methods to convert lactose to functional lactose derivatives shows a promising

trend in byproduct management. Unlike other lactose derivates (lactulose, lactobionic acid, and sorbitol), lactitol is only produced through catalytic hydrogenation. Lactitol is a versatile ingredient that can be employed by product developers not only to deliver sweetness at low caloric value but also to assist food formulation as a bulking agent, humectant, cryoprotectant, and prebiotic source. Lactitol has shown potential to be used for the synthesis of surfactants, emulsifiers, polymers, and hydrogels. Research efforts are necessary to understand the chemistry behind the applications of lactitol as well as a comprehensive evaluation of the final product properties.

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CHAPTER 3

SUBCRITICAL HYDROLYSIS OF ICE-CREAM WASTEWATER: MODELING AND HYDROLYSATE PROPERTIES[2]

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2018

3.1. ABSTRACT

The hydrolysis kinetics of ice-cream wastewater was studied under subcritical conditions (130-230°C and 20-60 bar) in a continuous stirred-tank reactor. The kinetic was monitored by measuring the degree of hydrolysis (DH, %) at different time intervals (up to 240 min). Samples of ice-cream wastewater were collected from the university dairy plant after a typical clean-in-place protocol. Overall, the reaction time and temperature significantly increased the DH, reaching a maximum value of 40.99±0.81, 34.44±0.47, 20.61±0.42, and 5.74±0.36% after 200-240 min at 130, 170, 200 and 230°C, respectively. The experimental data were modeled using the Weibull distribution model showing a satisfactory correlation between experimental data and predicted values (R2=0.981). The apparent activation energy for subcritical hydrolysis was 37.53±5.21 kJ mol-1. After 240 min of reaction, the hydrolyzates were recovered, and their antiradical ability was measured through free radical scavenging (2,2-diphenyl-1-picrylhydrazyl) method. Additionally, the angiotensin converting enzyme (ACE)-inhibitory ability was determined. The inhibition of a free radical was found to increase linearly with the DH (R2=0.991). The hydrolysate recovered at 230°C showed the highest ACE-inhibitory ability (98.0 ±

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1.6%). The study outcomes present an opportunity for utilizing subcritical hydrolysis to convert wastewater into valuable materials.

3.2. INTRODUCTION

A confluence of social and economic factors has made the dairy industry consider waste streams as essential actors in the transition to sustainable manufacture. Dairy industry generates large quantities of wastewater due to their strict hygienic standards (Demirel et al., 2005). During the manufacturing of ice-cream, the amount of wastewater generated is estimated to be between 1 and 2 gallons per pound of product. Ice-cream wastewater is a complex colloidal mixture of suspended solids (flavoring compounds), soluble material (sugar, lactose, minerals, and other carbohydrates), dispersed particles of different size (proteins and emulsified fat), and residues of cleaning agents (Hu et al., 2002; Demirel et al., 2013). Although the concentration of such compounds depends on the throughput, type of ice-cream, equipment used, and manufacturing and cleaning protocols, the relatively high content of organic material has proved to be problematic to handle for the receiving effluent treatment plant (Hu et al., 2002). While the use of anaerobic digestion as a mean to reduce the levels of organic load prevails in the treatment of wastewater for food and dairy wastewater (Demirel et al., 2005), numerous approaches have been developed offering opportunities for reducing the organic load. Examples of such approaches are membrane filtration (Andrade et al., 2014), ultrasound (Adulkar et al. 2014), ultraviolet-C light (Millan-Sango et al., 2017), electrochemical (Davarnejad and Nikseresht 2016), and pressurized fluids (Prado et al., 2016). It is worth to point out that all these approaches have been focusing on reducing the organic load, and valorization strategies for ice-cream wastewater have not yet been developed.

A promising method for conversion of ice-cream wastewater involves the breakdown of the compounds forming the colloidal mixture in the presence of pressurized heated water, which results in compounds of a lower molecular size (peptides and free fatty acids) that can be separated and further converted into valuable chemicals such as surfactants, foaming agents, emulsifiers, and animal feed. More importantly, peptides derivate from milk proteins are known for providing additional health benefits beyond their essential nutrition (Kilara and Panyam, 2003). Examples of the properties related to bioactive peptides are antioxidant ability, antimicrobial effect, anti-inflammatory potential, and antihypertension (Nongonierma and Fitzgerald, 2015). Strategies for producing bioactive peptides from milk proteins have become a topic of industrial and commercial interest.

Recently, subcritical water has gained attraction of researchers due to its unique characteristics as a reaction medium. This technology is also known as subcritical water hydrolysis, water above the boiling and below the critical point (Möller et al., 2011). Hydrolysis through subcritical water has demonstrated in the valorization of various agricultural wastes including streams from sweet blue lupin hull (Ciftci and Saldaña 2015), sugar cane bagasse (Lachos-Perez et al., 2016), animal proteinaceous biomass (Mekonnen et al., 2015), and coffee waste residues (Mayanga-Torres et al., 2017).

The rationality of using pressurized water lies on physicochemical properties induced by the combination of pressure and temperature. Thermodynamic properties of

water under pressure are well documented and available through National Institute of Standards and Technology database. In summary, water within the subcritical state possesses relative low dielectric permittivity and high ion strength, which favors reactions such as nucleophilic substitution, eliminations, and hydrolysis (Brunner 2009). Hydrolysis as a result of subcritical water offers unique advantages in valorizing wastewater streams. For instance, the water content of the waste stream acts as a reaction agent and solvent, meaning that streams of wastewater can be directly treated without any pretreatment. The hydrolysis occurring in subcritical water is often accompanied by secondary reactions (oxidation, hemolysis, and additions). These reactions are mainly driven by the temperature and might influence the final products of the hydrolysis treatment. The objectives of this research were: 1) to obtain experimental data and model the subcritical hydrolysis kinetic of ice-cream wastewater, and 2) to evaluate the antiradical and antihypertensive ability of the recovered hydrolysate.

3.3. MATERIAL AND METHODS

Physicochemical analyses of ice-cream wastewater

Samples of ice-cream wastewater were obtained from the Davis Dairy Plant at South Dakota State University (Brookings, SD). The samples were collected from the returned line after a typical clean-in-place program. The wastewater samples were analyzed for pH, total protein, fat content, total solids, and total volatile solids. The pH was measured in 10 mL of the sample using an Orion Versa Star Pro (Thermo Fisher Scientific, Waltham, MA). The protein content was determined by Kjeldahl method (AOAC, 1990). The fat content was measured gravimetrically according to the method of Mojonnier fat extraction (Bligh

and Dyer, 1959). Total solids (*TS*) and total volatile solids (*TVS*) were determined using the Association of Official Analytical Chemists (AOAC) methods. The average composition of the ice-cream wastewater is given in **Table 7**.

Table 7. Average composition of the ice-cream wastewater used for the subcritical hydrolysis.

Parameter	Value
pH	3.18 ± 0.04
Total solids (g 100 g^{-1})	0.89 ± 0.01
Total Volatile Solids (g 100 g^{-1})	0.04 ± 0.03
Fat (g 100 g^{-1})*	24.26 ± 0.67
Total protein (g/100 g^{-1})*	6.81 ± 0.81
* Calculated on dry basis	

Subcritical hydrolysis

The subcritical hydrolysis of ice-cream wastewater was performed in a continuous stirred-tank reactor from Berghof Products & Instruments (BR-300, Berghof Products & Instruments, Berghof, Germany). The reactor system consists of a high-pressure vessel made of stainless steel T316 with an internal volume of 500 mL, a cooling coil, a sampling port, a rupture disk, a pressure inlet valve, and a gas release valve. The reactor lid is adapted with a magnetic clutch connected to a stirrer drive with controlled speed (0-2000 rpm). The reactor system was heated with an electrical heating jacket (BR-500, Berghof Products & Instruments). A K-type thermocouple (stainless steel, transition junction with high temperature molded construction, Omega Engineering, Stamford, CT) located inside the vessel was used to record the temperature of the sample, while a pressure sensor (Berghof Products & Instruments, Germany) was used for recording the pressure inside the vessel.

Both parameters were recorded every 2 sec during the subcritical hydrolysis with the aid of a desktop computer equipped with a data logger software (BTC-1000, Berghof Products & Instruments). The reactor was loaded with 350 mL of recently collected ice-cream wastewater, and the reactor lid was set and clamped. The vessel was then heated at either 130, 170, 200, or 230°C. Afterward, the vessel was pressurized at either 20, 40, or 60 bar with nitrogen as a pressuring gas (N_2 purity 99.99%, Praxair, Sioux Falls, SD). Once the vessel reached the working temperature and pressure, the subcritical hydrolysis of ice-cream wastewater was studied within the range of 0-240 min, withdrawing samples for analysis every 40 min.

Degree of hydrolysis

The degree of hydrolysis (*DH*) expressed as a percentage of cleaved peptide bonds was measured according to the methodology developed by Nielsen et al. (2001) with some modifications. Four hundred μL of hydrolyzed ice-cream wastewater was added into wells of a 12-well cell culture plate (Corning Incorporated, NY) containing 3 mL of OPA reagent. The mixture was held for 2 min at room temperature before reading its absorbance at 340 nm using a spectrophotometer (VARIAN Cary 50 UV-Visible, Agilent Co., Santa Clara, CA). The OPA reagent was prepared by dissolving 160 mg o-phthaldialdehyde (98%, Alfa Aesar, Haverhill, MA) in 4 mL ethanol (95%, Fisher Scientific). The ethanol solution was mixed with 176 mg dithiothreitol (99%, Fisher Scientific) dissolved in 50 mL of distilled water. The new mixture was added to 100 mL of distilled water containing 7.62 g di-Na-tetraborate decahydrate (Fisher Scientific) and 200 mg UltraPure Na-dodecyl-sulfate (99.5%, Fisher Scientific). Then, the solution was diluted with distilled water to

reach a volume of 200 mL. Then, the mixture was stirred on a magnetic stirrer plate until the reagents were dissolved. The standard of serine was prepared by dissolving 50 mg L-serine (99%, Fisher Scientific) in 500 mL distilled water, which corresponds to 0.9516 meqv L^{-1}, while distilled water was used as a blank. The *DH* was calculated from the ratio of the number of hydrolyzed bonds (*h*) per the number of total peptide bonds per protein equivalent (h_{tot}), Equation (1):

$$DH(\%) = \frac{\left[\left(\frac{A_S - A_b}{A_{st} - A_b}\right)\cdot\left(\frac{S\cdot V}{P}\right) - B\right]}{h_{tot}} \cdot 100 \qquad (1)$$

where A_s, A_b, and A_{st} is the absorbance of the sample, blank, and serine standard (100 mg L^{-1}), respectively; *S* is the serine protein milliequivalent (0.9516 meqv L^{-1}); *V* is the volume of wastewater (0.35 L); *P* is the protein content of the wastewater (%); and *B* is a constant for whey protein (0.4). Absorbance readings of each sample, standard, and blank were performed in four replicates.

Modeling the subcritical hydrolysis

The different kinetic models used to represent the experimental data are presented in **Table 8**. The parameters of each model were calculated using Athena Visual Workbench, a powerful software package for modeling and parameter estimation (www.athenavisual.com). The predictive capability of the individual models was assessed by the coefficient of determination (R^2), the adjusted coefficient of determination (R^2_{Adj}), residual analysis, and average absolute percentage of residuals (*E*). Experimental runs were

conducted at least in four replicates, and all figures were made using Sigmaplot software V11 for Windows (SPSS Inc., Chicago, IL, USA).

Table 8. Kinetic equations used to model the subcritical hydrolysis of ice-cream wastewater.

Model	Mathematical expression	Equation number
First-order	$\frac{DH_t}{DH_o} = exp^{(-k_i \cdot t_{reaction})}$	2
Biphasic	$\frac{DH_t}{DH_o} = A_1 exp^{(-k_1 \cdot t_{reaction})} + A_2 \cdot exp^{(-k_2 \cdot t_{reaction})}$	3
Weibull	$\frac{DH_t - DH_o}{DH_f - DH_o} = 1 - exp^{\left(-\left(\frac{t_{reaction}}{a}\right)^n\right)}$	4
DH_t – degree of hydrolysis at a given time; DH_o – initial value of degree of hydrolysis; DH_f – the final value of the degree of hydrolysis; k_i – hydrolysis rate constants for equations (2-3); A_1 and A_2 – regression parameters of equation (3) $t_{reaction}$ – reaction time; α, β – scale and shape parameters, respectively.		

The influence of temperature on the rate constant was expressed by the Arrhenius equation (5):

$$k_i = k_T \cdot exp^{\left(\frac{-E_a}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_r}\right)\right)} \qquad (5)$$

where, k_i is the hydrolysis rate constant (min^{-1}); k_T is the reaction rate constant at a reference temperature (T_r); E_a is the apparent activation energy (kJ mol^{-1}); T is the temperature (K); R is the universal gas constant (8.314 J mol^{-1} K^{-1}). The average value of the experimental temperatures was used as T_r. In the case of the Weibull model, the influence of temperature

on the α and β parameter was described by the Arrhenius-type model and empirical linear equation, Equation (6) and (7), respectively:

$$\frac{1}{\alpha} = \frac{1}{\alpha_T} \cdot exp^{\left(\frac{-E_a}{R}\cdot\left(\frac{1}{T}-\frac{1}{T_r}\right)\right)} \quad (6)$$

$$\beta = m \cdot \left(\frac{1}{T} - \frac{1}{T_r}\right) + b \quad (7)$$

where $1/\alpha_T$ is the rate constant at a reference temperature (T_r); E_a is the apparent activation energy (kJ mol^{-1}); R is the universal gas constant (8.314 J mol^{-1} K^{-1}); *m* and *b* are the slope and intercept, respectively.

Antiradical ability

The ability of the hydrolysate to quench a stable free radical was spectrophotometrically estimated according to the methodology described by Brand-Williams et al. (1995) with some modifications. This method measures the reduction in the absorbance of free radical *DPPH* (2,2,diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Saint Louis, MO) when it reacts with an antiradical compound. Different volumes of the recovered hydrolysate were mixed with 3 mL of a methanol solution of DPPH (100 µM). The absorbance of the mixture was measured at 517 nm when the reaction reaches the steady state. In all cases, the steady state was reached after 2 min at room temperature. The percentage of remaining *DPPH* was determined by Equation (8):

$$DPPH\ (\%) = \frac{A_t}{A_o} \cdot 100 \qquad (8)$$

where: A_t and A_o are the absorbance of the mixture at the steady state and the absorbance of the mixture at the beginning, respectively.

ACE-inhibitory

The inhibitory activity of angiotensin I-converting enzyme (ACE) of the recovered hydrolysate was evaluated spectrophotometrically according to the methodology described by Nakamura et al. (1995) with some modifications. The assay mixture (200 μL) consisted of sodium borate buffer 0.1 M (pH 8.3) containing 0.3 M NaCl (Fisher Scientific, Fair Lawn, NJ) and 5 mM hippuryl-L-histidyl-L-leucine (BACHEM, Torrance, CA) was mixed with 20-60 μL of the recovered hydrolysate whose pH was adjusted to 8.3. The mixture was pre-incubated at 37°C for 3 min prior to the addition of 40 μL of the ACE solution (Sigma-Aldrich, Saint Louis, MO). The concentration of the ACE solution was 0.1 U per mL of distilled water. Upon the addition of the ACE solution, the mixture was held at 37°C for 90 min. Afterward, the reaction was stopped by adding 300 μL of 0.1N HCl. The liberated hyppuric acid was extracted with 1.7 mL ethyl acetate and then centrifuged gently (3000 rpm for 10 min). Afterward, 1 mL of the organic phase was transferred to a clean glass vial and evaporated under vacuum conditions (100°C, 15 min). Then, the residue (the recovered hyppuric acid) was dissolved in 1 mL distilled water and subsequently measured spectrophotometrically at 228 nm. The ACE-inhibitory ability was calculated as a percentage.

3.4. RESULTS AND DISCUSSION

Composition of ice-cream wastewater

Table 7 shows the mean values of selected physicochemical parameters of ice-cream wastewater. The pH value of wastewater collected after clean-in-place was 3.18 ± 0.04, while the fat and protein content were 24.26 ± 0.67 and 6.81 ± 0.81% on dry basis, respectively. These values are within the range of those reported by Demirel et al. (2013). The relative high content of protein greatly contributes to the total organic load and it can be potentially explored for value-added derivates.

Temperature-pressure history

Figure 3 shows a representative temperature and pressure history for the subcritical hydrolysis of ice-cream wastewater. The total or treatment time is the sum of the heating ($t_{heating}$), reaction ($t_{reaction}$), and cooling ($t_{cooling}$) time. The temperature of the sample increased from room temperature (25°C) to the target temperature by programing the electrical heating jacket. The average heating rate was 2.29 ± 0.13, 1.94 ± 0.11, 2.21 ± 0.12, and 2.09 ± 0.12 °C min^{-1} for 130, 170, 200, and 230°C, respectively. The pressurization (arrow (1) in **Figure 3**) took place within the first minute of heating. The start of the $t_{reaction}$ was considered when both the target temperature and pressure had been reached, pseudo-isothermal and pseudo-isobaric conditions (arrow (2) in **Figure 3**). The sample temperature during the $t_{reaction}$ slightly fluctuated, resulting in a final temperature of 129 ± 0.67, 169 ± 0.82, 199 ± 0.73, and 229 ± 1.11°C, while the fluctuation in pressure was minimal. At the end of the predetermined $t_{reaction}$ (arrow (3) in **Figure 3**), the reactor was cooled down at a rate of 3.92 ± 0.85°C min^{-1}, and subsequently, the reactor was

manually depressurized to atmospheric pressure, $t_{cooling}$ (arrow (4) in **Figure 3**). The temperature ($130 \leq T \leq 230$°C) and pressure ($15 \leq p \leq 60$ bar) range used in this investigation lie within the window of reaction conditions described earlier for subcritical water hydrolysis (Möller et al., 2011; Saldaña and Valdivieso-Ramirez, 2015). **Figure 3** provides a detailed characterization of the experimental conditions used for the subcritical hydrolysis of ice-cream wastewater. Such characterization will help to meaningfully interpret the experimental findings and facilitate comparison with the literature.

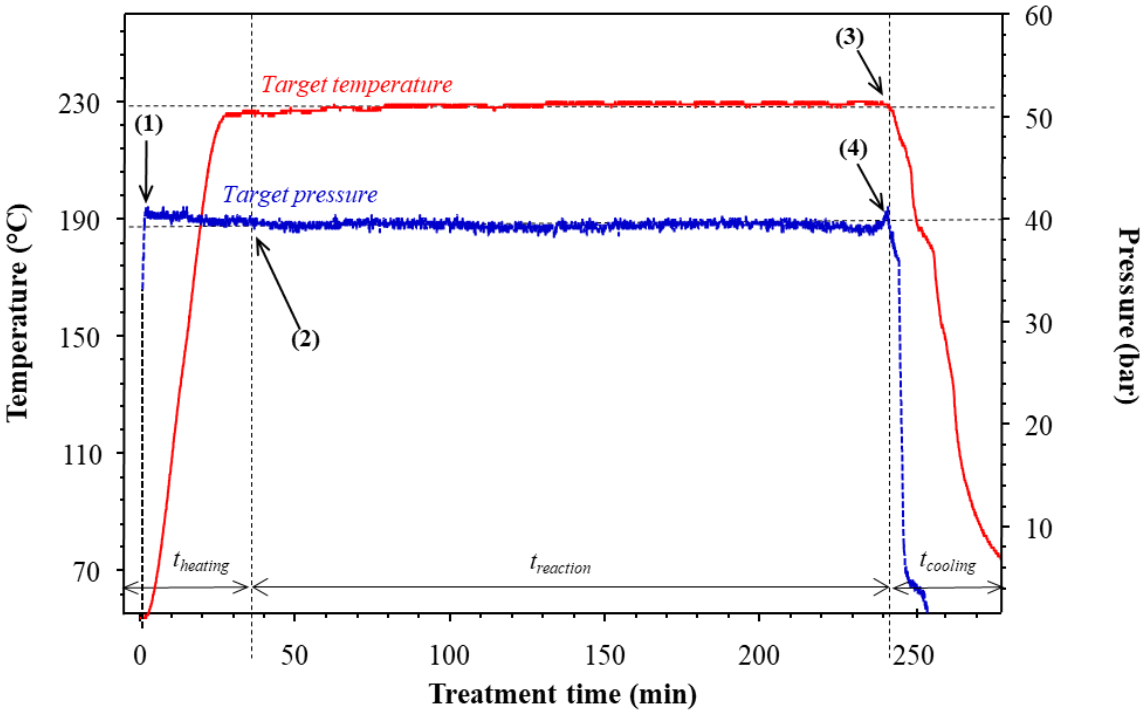

Figure 3. Representative temperature-pressure history of subcritical hydrolysis of ice-cream wastewater. Temperature = 200˚C, pressure = 40 bar, agitation = 600 rpm. $t_{heating}$ – heating time, $t_{reaction}$ – reaction time;$t_{cooling}$ – cooling time. Arrows (1), (2), (3), and (4) represent the start of pressurization, the beginning of the reaction time, the end of the reaction time, and the pressurization step, respectively.

Effect of pressure on the degree of hydrolysis

The influence of pressure was evaluated at a temperature of 169 ± 0.82°C and a pressure range of 15-60 bar (**Figure 4**). After 240 min of hydrolysis, the *DH* varied within a narrow range (~19-20%), regardless of the pressure used.

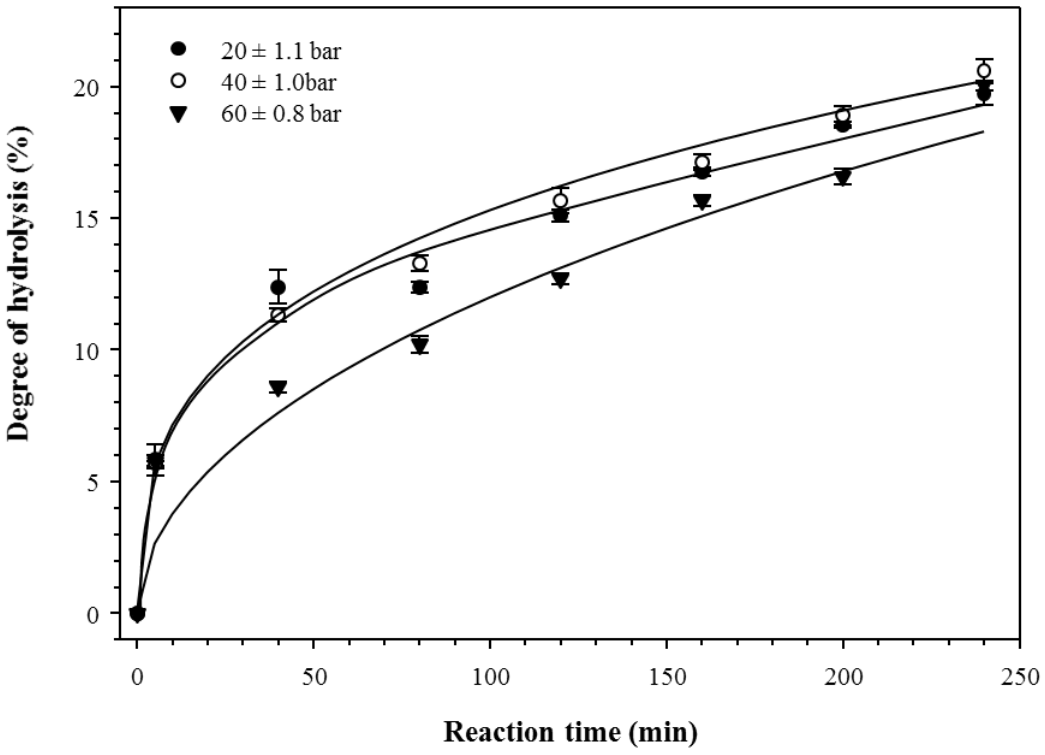

Figure 4. Influence of pressure on the degree of hydrolysis (DH) of ice-cream wastewater. Hydrolysis time = 240 min, temperature = 170˚C, agitation = 600 rpm. The continuous lines represent the initial reaction rates calculated with Equation (8). The error bars represent the standard deviation of four replicates.

The analysis of the initial reaction rates may provide more insights into the effect of pressure on the subcritical hydrolysis of ice-cream wastewater. Equation (9) was used to evaluate the initial reaction rates, which were plotted against pressure (**Figure 5**):

$$r_o = \frac{d[HD_t]}{dt}\Big|_{t=0} \qquad (9)$$

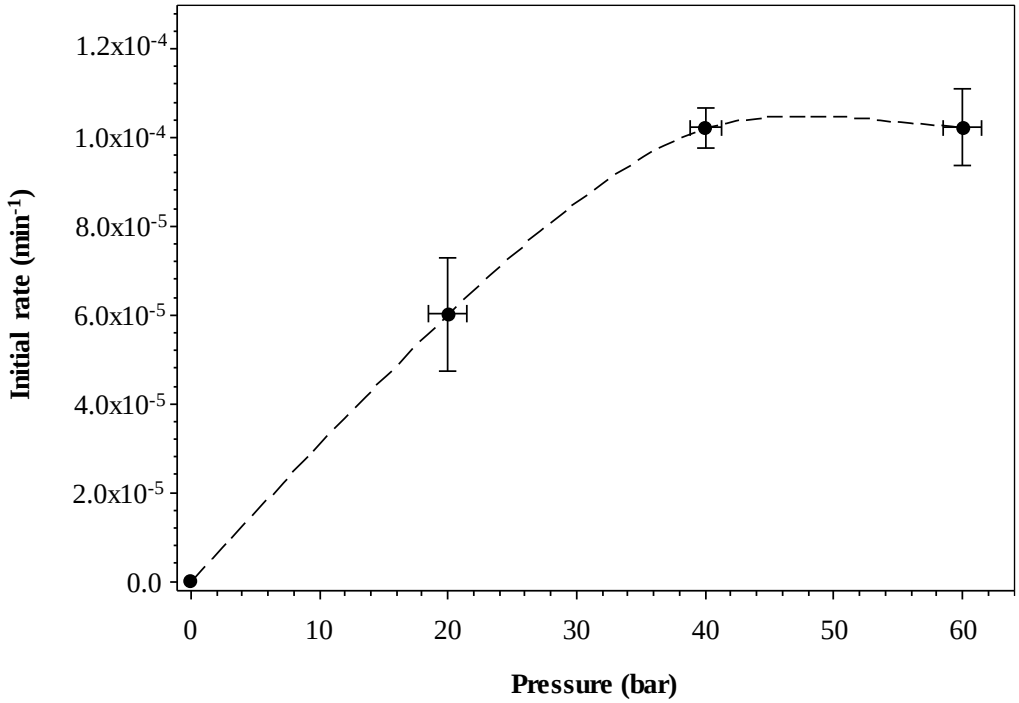

Figure 5. Effect of pressure on the initial reaction rates of subcritical hydrolysis of ice-cream wastewater. Hydrolysis time = 240 min, temperature = 170˚C, agitation = 600 rpm. The error bars from the initial reaction rates correspond to 95% confidence interval, while the error bars from the pressure values represents the fluctuation.

Increasing the pressure from 20 to 40 bar yields higher values of initial reaction rates, while further increment of the pressure (up to 60 bar) results in the same initial rates. Thus, further experiments were conducted at a fixed pressure of 40 bar. This observation is relevant from an economic point of view since the application of high pressure will bring high cost and difficult to operate. The subcritical hydrolysis of ice-cream wastewater was conducted using N_2 as a pressuring gas to prevent oxidation. An investigation on the effect of CO_2, air, and N_2 on the production of amino acids from fish waste showed that N_2 produced a higher yield for specific amino acid, leucine, and tyrosine (Cheng et al., 2008).

More importantly, N_2 not only prevents oxidation of the initial protein but also minimizes further degradation of individual hydrolysates.

Effect of temperature on the degree of hydrolysis

The effect of temperature on the subcritical hydrolysis of ice-cream wastewater is illustrated in **Figure 6**. Overall, the degree of hydrolysis increases gradually with the hydrolysis time. More importantly, increasing the reaction temperature caused notorious changes in the *DH*. At 130°C, the degree of hydrolysis increased linearly with the $t_{reaction}$, reaching values of 8.6 ± 0.33% after 240 min of hydrolysis. Increasing the temperature to 170°C results in 18.04 ± 0.31% of the degree of hydrolysis, and a further increase in the reaction temperature, 200 and 230°C, yielded a degree of hydrolysis of 34.44 ± 0.44 and 41.01 ± 0.71%, respectively. A similar influence of temperature has been reported in the hydrolysis of bovine spongiform encephalopathy (Mekonnen et al., 2015) and hog hair (Esteban et al., 2008). Within the domain of subcritical conditions, water molecules experience autodissociation ($2H_2O \leftrightarrows H_3O^+ + OH^-$) leading to an increase in the concentration of hydroxinium ions (H_3O^+) and hydroxide ions (OH^-) (Möller et al., 2011). In the presence of such ions, water exhibits properties of acid catalyst and therefore an enhanced hydrolysis capacity (Abdelmoez et al., 2007). Moreover, the autodissociation of water increases with increasing the temperature (Brunner, 2009). This physicochemical phenomenon explains the observed influence of temperature on the hydrolysis values .

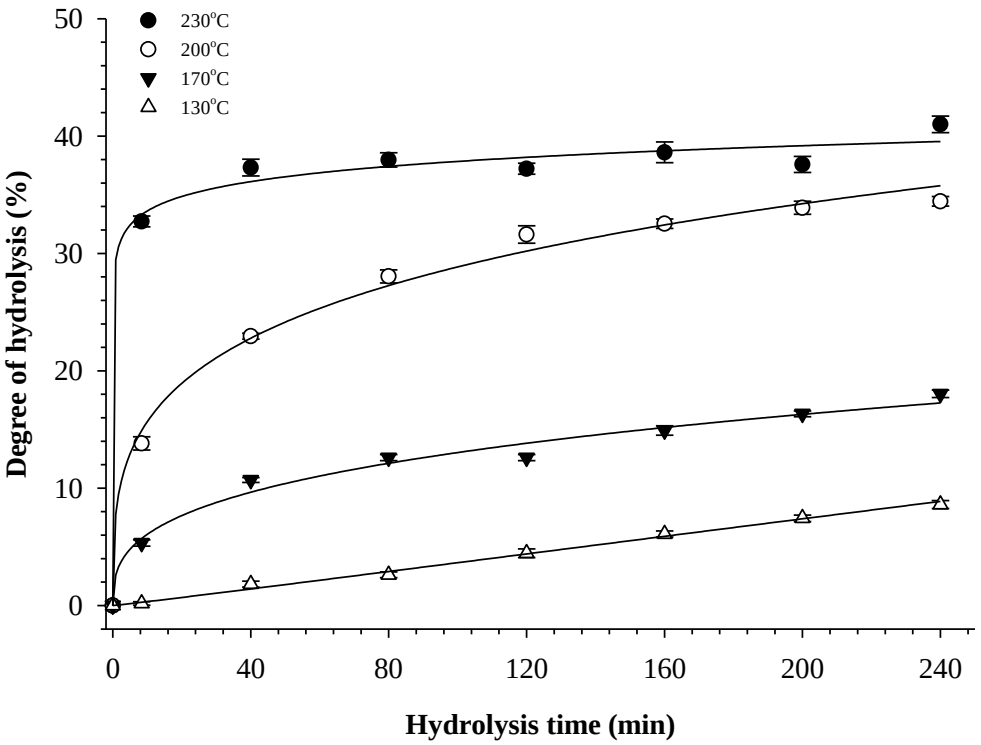

Figure 6. Kinetics of subcritical hydrolysis of ice-cream wastewater. Hydrolysis time = 240 min, pressure = 40 bar, agitation = 600 rpm. Curves represent data obtained with the Weibull model. The error bars represent the standard deviation of four replicates.

Although the maximum autodissociation of water has been reported at temperatures approaching the supercritical state (>350°C), the resulting hydrolysate may undergo rapid decomposition at temperatures more than 260°C (Kang et al., 2001; Cheng et al., 2008). The highest temperature used in the current investigation (230°C) is below the suggested degradation temperature, and it is reasonable to assume that the degradation of the formed hydrolysate is negligible. Espinoza et al. (2012) hydrolyzed whey protein isolate under subcritical conditions (150-320°C) and reported values of the degree of hydrolysis (12%) at 250°C after 50 min. These values are considerable lower to that reported in **Figure 4**. The difference observed in the degree of hydrolysis can be attributed to the pH of the medium. Although Espinoza et al. (2012) did not report the pH values, it can be assumed

that the pH of a whey protein isolate solution is 5.5-6.0, while the pH of the wastewater used in the current investigation was 3.18 ± 0.04. Under acidic conditions, the α-carboxylic end of the amino acid gains an H^+ ion from the medium, a reaction favors under subcritical conditions. On the other hand, pH values approaching the neutrality the α-amino end of the amino acid loses an H^+ ion to the medium already containing a relatively high concentration of H^+ ion (Abdelmoez et al., 2007).

Kinetic models

Table 9 shows the fitting performance of the different models used to represent the subcritical hydrolysis of ice-cream wastewater. The first-order model somewhat seems to be adequate to describe the experimental data (R^2>0.91 and E<19). However, the value of constant rate for both models was lower than its variability (confidence interval), meaning that the rate constant is not statistically different from zero and therefore should not be used for further modeling. Moreover, the experimental data clearly showed deviation from an idealized first-order behavior. This observation is not surprising since one of the assumptions of a first-order reaction is that all reactants molecules are in the same state neither affected by the matrix nor the reaction conditions (Martínez-Monteagudo and Saldaña 2015). On the other hand, the biphasic model is another way to express a first-order reaction and it accounts for the presence of fractions with different susceptibility toward hydrolysis, where the labile fraction is clearly identified from the stable fraction. However, our experimental data cannot confirm the existence of different fractions for the subcritical hydrolysis, which challenges the applicability of the biphasic model.

Table 9. Fitting performance of the different kinetic equations used for modeling the subcritical hydrolysis of ice-cream wastewater.

Parameters	First-order model	Biphasic model	Weibull model
R^2	0.914	0.964	0.976
R^2_{Adj}	0.912	0.963	0.975
E (%)	19.42	21.55	15.47
R^2 – Coefficient of determination; R^2_{Adj} – adjusted coefficient of determination; E –average absolute percentage of residuals.			

The Weibull model showed the best fitting performance throughout the entire experimental domain having the highest R^2 and R^2_{Adj}, and the lowest E values. The R^2_{Adj} and E are commonly used for evaluating the fitting performance of kinetics models. The R^2 values is a measure of how well the model can describe the experimental data, R^2_{Adj} indicates the influence of the number of parameters, and E compares the overall error in terms of percentage (Martínez-Monteagudo and Saldaña, 2014a). The residual analysis (standardized residual against predicted values) of Weibull model revealed random patterns (graph not shown), which validates the estimation of parameters and prediction of new observations. The application of the Weibull model for subcritical hydrolysis assumes that the reaction followed an exponential probabilistic distribution, regardless of the reaction mechanism.

Modeling the subcritical hydrolysis

The Weibull model in the form of Equation (4) was used to fit the experimental data, from which the influence of temperature on the scale and shape parameter is obtained (**Figure 7**).

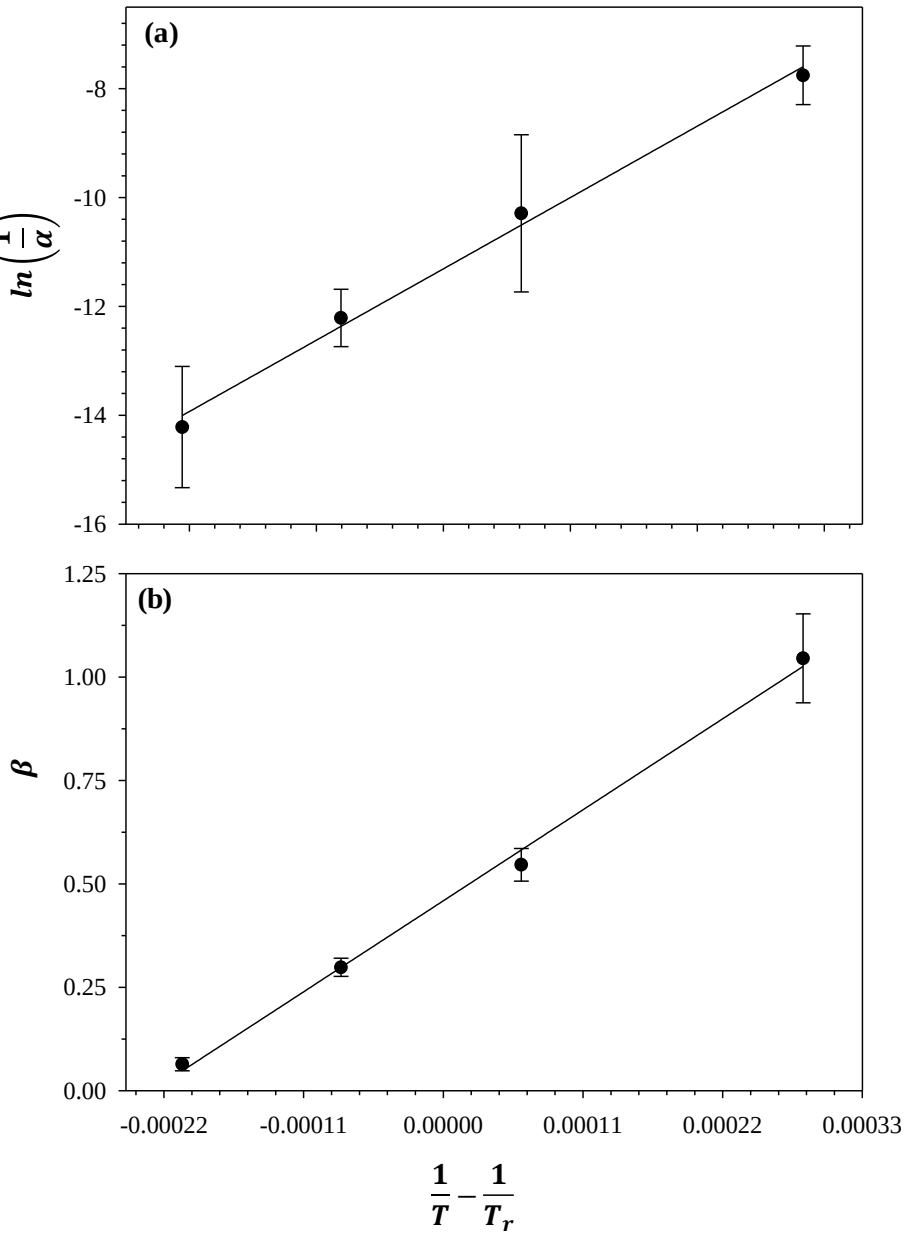

Figure 7. Influence of the temperature on the scale parameter (a) and shape parameter (b). The error bars correspond to 95% confidence interval obtained through non-linear regression analysis. The reference temperature was 182˚C.

A linear relationship between the reciprocal of the scale parameter and the inverse of the reaction temperature was observed (**Figure 7a**). Thus, the scale parameter is temperature-dependent according to Arrhenius law, which validates the use of Equation

(6). **Figure 7b** shows the influence of temperature on the shape parameter. The shape parameter was 1.04 ± 0.01, 0.54 ± 0.03, 0.29 ± 0.02, and 0.06 ± 0.01 for 130, 170, 200, and 230°C, respectively. Similar to the scale parameter, a linear relationship was observed between the shape parameter and the inverse of the reaction temperature in the form of Equation (7). It should be highlighted that Equation (7) is an empirical relation and no attempt was made to link to reaction mechanisms.

Once the influences of temperature were determined, Equation (6) and (7) were incorporated into the Weibull model (Equation (4)), yielding a global kinetic equation, Equation (9).

$$\frac{DH_t - DH_o}{DH_f - DH_o} = 1 - exp\left[-\left(\frac{t_{reaction}}{\alpha_T} \cdot exp\left(\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_r}\right)\right)\right)^{m\cdot\left(\frac{1}{T} - \frac{1}{T_r}\right)+b}\right] \qquad (9)$$

Table 10 shows the estimated kinetic parameters with the highest probability of being correct according to their 95% confidence interval. The scale parameter at a reference temperature is inversely proportional to the frequency or pre-exponential factor of the Arrhenius equation. Alternatively, it can be seen as the hydrolysis rate when no energetic barrier is present (Martinez-Monteagudo and Saldaña 2014b). The calculated E_a was 37.53 ± 5.21 kJ mol^{-1}, and it is often considered as the most relevant kinetic parameter. Cheng et al. (2008) reported slightly higher values of E_a (>46 kJ mol^{-1}) for the subcritical hydrolysis of individual amino acids. Such differences can be explained by the effect of the reaction

medium. Cheng et al. (2008) used isolated amino acids, while the current investigation used ice-cream wastewater, where the presence of other substances cannot be ignored.

Table 10. Fitting parameters (Equation (8)) for the subcritical hydrolysis of ice-cream wastewater.

Parameter	Value	CI95%
α_T	3.26×10^4	2.54×10^3
E_a	37.53	5.21
m	596.93	64.11
b	26.71	0.04
α_T – Scale parameter at a reference temperature; E_a – activation energy (kJ mol^{-1}); m and b – regression parameters; CI95% – 95% confidence interval.		

Time-temperature diagram

The estimated kinetic parameters (**Table 10**) were used to predict the degree of hydrolysis at different time ($0 \leq$ time $\leq$ 20,000 s) and temperature ($130 \leq$ temperature $\leq$ 230°C) combinations. **Figure 8** shows the time-temperature combinations needed to achieve a specific degree of hydrolysis. For instance, 40% of hydrolysis can be obtained by the combination of time-temperature corresponding to the contour line of 40. It should be mentioned that the degree of hydrolysis accounts for the accumulative percentage of cleaved peptides bonds, meaning that the amino acids profile of a specific degree of hydrolysis might be different depending on the temperature used. Knowing how different combinations of time and temperature impact the speed of subcritical hydrolysis of ice-cream wastewater enables better process design and optimization; in addition to the development of a kinetic databank.

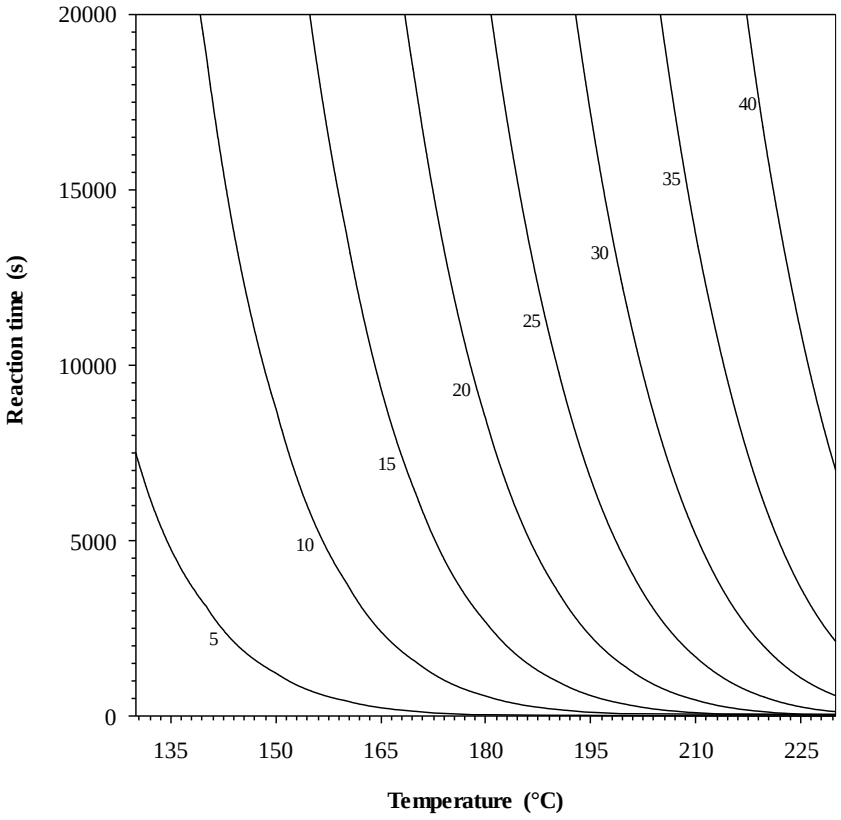

Figure 8. Temperature-time combinations needed to achieve a specific level of hydrolysis during the subcritical hydrolysis of ice-cream wastewater. Contour lines correspond to simulated data using Equation (8).

Hydrolysate properties

The hydrolysate fractions were recovered after 240 min of subcritical hydrolysis at different temperatures (130, 170, 200, and 230°C). The protein content of the recovered fractions was 10.55 ± 0.13, 19.07 ± 0.08, 28.84 ± 0.54, and 27.59 ± 0.41 % (dry basis) at 130, 170, 200, and 230°C, respectively. Interestingly, the recovered fractions presented antiradical activity measured by the *DPPH* test. **Figure 9** shows the percentage of remaining *DPPH* as a function of grams of hydrolysate per mmole of *DPPH*.

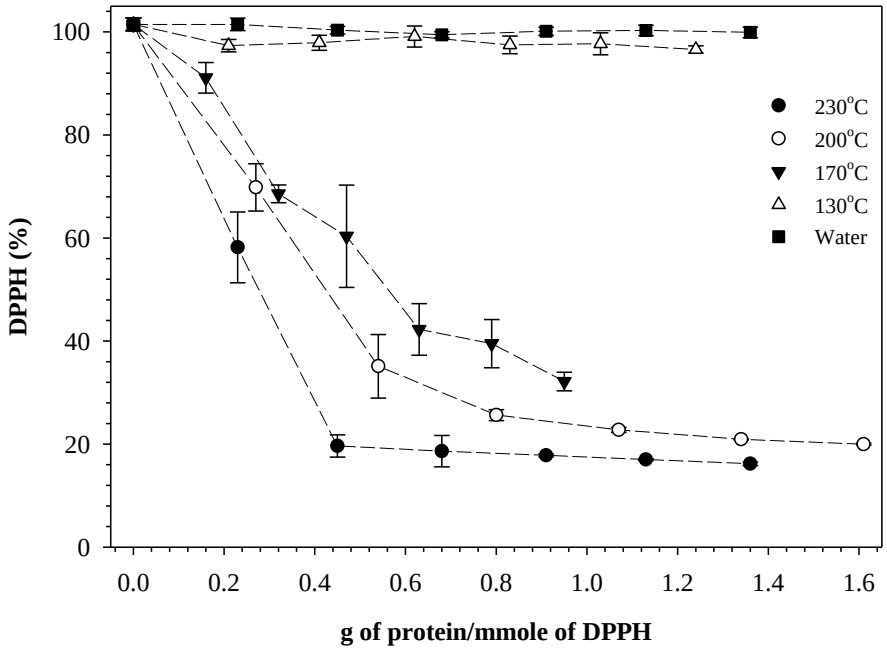

Figure 9. Antiradical ability of the hydrolysate recovered after subcritical hydrolysis of ice-cream wastewater. Hydrolysis time = 240 min, pressure = 40 bar, agitation = 600 rpm.

The recovered fraction from the hydrolysis conducted at 130˚C showed negligible antiradical activity. Indeed, no difference was detected when comparing to a reference sample (distilled water). On the other hand, the remaining *DPPH* decreased with increasing the amount of hydrolysate regardless of the reaction temperature. One way to interpret the *DPPH* test is by determining the concentration or ratio needed to reduce 50% of the *DPPH* activity, EC_{50} value. Low value of EC_{50} indicates higher antiradical ability (Brand-Williams et al., 1995). The EC_{50} was 0.56, 0.42, and 0.27 g of protein per mmole of *DPPH* for the fractions obtained after hydrolysis at 170, 200, and 230˚C, respectively. These results showed the potential use of hydrolysates as antiradical agents. Moreover, a linear relationship was found between EC_{50} and *DH* (R^2=0.991). However, such interpretation must be taken cautiously because the speed of the reaction (antiradical vs radical) depends

on the type and structural conformation of the recovered hydrolysate. Additional studies are necessary to further understand the reaction mechanism and establish links between hydrolysis conditions and biological properties.

ACE-inhibitory effect

The ACE-inhibitory activity of the recovered hydrolysates is given in **Figure 10**. Overall, the percentage of inhibition increased with the amount of hydrolysate. The maximum inhibition obtained was 78.41 ± 1.46, 68.86 ± 0.48, 92.01 ± 2.01, and 98.01 ± 1.60% using ~9.31, 5.88, 12.01, and 10.20 mg of hydrolysate per enzymatic unit at 130, 170, 200, and 230°C, respectively. A common way to express the ACE-inhibitory ability is by the IC_{50} value, amount of hydrolysate needed to inhibit 50% of the ACE activity. The IC_{50} values were 4.21, 3.29, 3.84, and 2.73 mg of hydrolysate per enzymatic unit for the fractions recovered at 130, 170, 200, and 230°C, respectively. Interestingly, no linear relationship was found between *DH* and IC_{50}, which contradicts previous research where the *DH* is positively correlated with the ACE-inhibitory ability of peptides (Gonzalez-Gonzalez et al., 2011). In their investigation, Gonzalez-Gonzalez et al. (2001) fractionated peptides derived from the fermentation of lactic acid bacteria, while the current investigation generated the hydrolysate under subcritical conditions. It is expected that subcritical hydrolysis will produce different types of hydrolysates than those obtained via fermentation. Subcritical hydrolysis offers the opportunity to produce hydrolysate biologically active and the combined action of different fractions may lead to a greater inhibition of ACE. Nevertheless, further research is certainly necessary to link the inhibitory ability of the recovered fraction with a specific group of peptides.

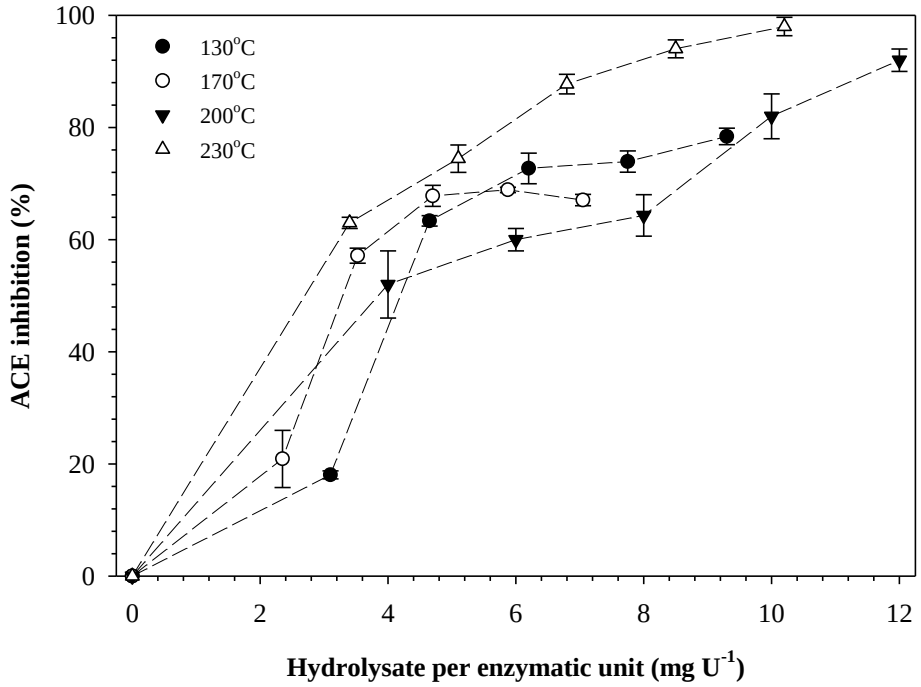

Figure 10. Inhibitory ability of angiotensin I-converting enzyme (ACE) of the hydrolysate recovered after subcritical hydrolysis of ice-cream wastewater. Hydrolysis time = 240 min, pressure = 40 bar, agitation = 600 rpm.

3.5. CONCLUSION

Subcritical hydrolysis showed to be an effective process for the hydrolysis of ice-cream wastewater. Controlled subcritical hydrolysis through the hydrolysis time and temperature showed to be significant variables to obtain a high degree of hydrolysis from ice-cream wastewater, reaching maximum values of ~41% at a temperature of 230°C and reaction time of 240 min. The experimental data were well described by the Weibull model. The influence of the temperature on the degree of hydrolysis was described by an Arrhenius-type equation. The recovered hydrolysate showed antiradical and antihypertensive ability. Future research will be focused on how to enhance the hydrolysis of peptides and effectively suppress the decomposition of amino acids. The study outcomes

present an opportunity for utilizing subcritical hydrolysis to convert wastewater into valuable materials.

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CHAPTER 4

HYDROTHERMAL CONVERSION OF ICE-CREAM WASTEWATER[3]

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2020

4.1. ABSTRACT

Ice-cream wastewater (WW) was subjected to a hydrothermal process (230°C, 40 bar, and 240 min) to reduce the organic load and to produce functional hydrolysates of protein as value-added components. Samples of WW from six different production days were collected after a typical clean-in-place protocol. Untreated WW contained high values of total protein (12-22% on dry basis), biological oxygen demand (BOD, 8-38 g L^{-1}), and chemical oxygen demand (COD, 7-30 g L^{-1}). The hydrothermal process reduced 87-97 and 25-70% of the BOD and COD, respectively. Moreover, the hydrothermal process hydrolyzed between 38 to 56% of the protein fraction, and the resulting hydrolysates showed antioxidant activity (14-19% of the DPPH) and antihypertension (ACE) activity (60-97%). Additionally, the hydrolysates showed a high ratio of glutamic acid (20-26%) and proline (10-15%), which confirmed the relevant functional properties of hydrolysates.

4.2. INTRODUCTION

The industrial manufacture of ice-cream ranks highest within the dairy industry in the usage of water per unit of product (Ackermann et al., 2007). Indeed, the benchmark for the usage of water established by the International Finance Corporation is between 4 to 5 L of water per liter of milk processed into ice-cream (World Bank 2007). In addition to the

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water used to manufacture ice-cream, there is a corresponding amount of wastewater (WW) generated due to accidental spills, product changeovers, and cleaning protocols (Kushwaha, Srivastava, & Mall, 2011). A typical ice-cream WW may contain about 10 and 24% of protein and fat on a dry basis, respectively (Enteshari & Martínez-Monteagudo, 2018). Such an amount of organic material significantly contributes to the biological oxygen demand (BOD) and chemical oxygen demand (COD) of ice-cream WW that are roughly 20 times higher than that of the domestic WW (Demirel, Yenigun, & Onay, 2005).

In general, the treatment of WW requires a combination of several operations, including grease traps, oil-water separators, sedimentation, biological, and chemical treatments. The presence of fats, oils, and grease (FOG) in the effluents is known to cause blockages in the sewer system, leading to overflow it and a reduction of its capacity (Khorsha, 2011). In ice-cream WW, the fat is emulsified and dispersed in the form droplets of relatively small size (1-10 μm), which negatively impacts the performance of grease traps and abatement devices. Another concern in ice-cream WW is the variations in the pH from day to day due to the presence of flavoring compounds, sugar, protein, emulsified fat, surfactants, and residual cleaning agents (Enteshari & Martínez-Monteagudo, 2018; Slavov, 2017). The treatment of ice-cream WW has become an environmental concern, and the implementation of new technologies for treating it has gained momentum worldwide. Aerobic, anaerobic, and physicochemical treatments are examples of treatments currently used for ice-cream WW. The different treatments used for the treatment of ice-cream WW have been reviewed elsewhere (Kushwaha et al., 2011). Aerobic and anaerobic treatments are aimed at reducing the organic matter by means of

degradation process (Karadag, Köroğlu, Ozkaya, & Cakmakci, 2015). However, the presence of protein and FOG inhibits the action of aerobic and anaerobic treatments, respectively. On the other hand, physicochemical treatments remove suspended solids and colloidal particles using treatments such as coagulation, adsorption, and membrane filtration. The combination of membrane filtration with chemical treatments has been used for the removal of FOG from dairy WW. Andrade, Mendes, Espindola, and Amaral (2014) treated WW from the production of Ultra-high-temperature milk (UHT), yogurt, and cheese with a membrane reactor followed by nanofiltration. With such an arrangement, about 99 and 93% of the COD and total solids were removed, respectively. Similarly, Suárez, Fidalgo, and Riera (2014) employed reverse-osmosis spiral-wound membrane to remove 98 and 97% of the conductivity and COD, respectively, from UHT WW. All these approaches have focused on the removal of solids, reduction of organic matter, and reusability of water.

The water and protein content in the ice-cream WW present opportunities to produce amino acids by hydrothermal conversion. Already, Enteshari and Martínez-Monteagudo (2018) hydrolyzed up to 41% of the proteins in ice-cream WW under subcritical conditions. Water within its boiling and critical point, exhibit high ion product, meaning that ionic reactions, including hydrolysis, are greatly enhanced. Relevant properties of water concerning hydrothermal conversion are discussed elsewhere (Möller , Nilges , Harnisch, & Schröder, 2011). The objective of this work is to evaluate the feasibility of the hydrothermal process to reduce the organic load in ice-cream WW and to produce functional hydrolysates as value-added compounds.

4.3. MATERIAL AND METHODS

Ice-cream wastewater and characterization

Samples of WW were collected from six different production days (six weeks) from the Davis Dairy Plant at South Dakota State University (Brookings, SD). The samples were collected from the returned line after a typical clean-in-place program. After collection, the samples were stored at 4°C prior to the analysis of pH, total protein, fat, moisture content, total solids, BOD, and COD. The pH was measured in 10 mL of the sample using an Orion Versa Star Pro (Thermo Fisher Scientific, Waltham, MA). The protein content was determined by the Kjeldahl method, while the fat content was measured using the Mojonnier extraction. Total solids (TS) were determined using the standard methods for the examination of WW (Rice, Baird, Eaton, & Clesceri, 2012). The WW was also analyzed for BOD and COD before and after the hydrothermal process, according to the methodology reported elsewhere (Rice et al., 2012). The BOD was obtained from the amount of oxygen consumed (g L-1) after 5-day incubation at 20°C using seeded samples with appropriate dilutions. The seeding sample was obtained from the municipal water treatment (Brookings, SD). In the case of COD, the amount of oxygen needed to oxidize the organic matter in samples of WW was spectrophotometrically measured. Potassium dichromate was used as an oxidizing agent, and sulfuric acid was used as a solvent. The oxidation was conducted at150˚C for 2 h using a DRB200 reactor (HACH Company, Loveland, CO). Then, samples were brought to room temperature before recording the absorbance at 600 nm using a DR/4000 U spectrophotometry (HACH Company, Loveland, CO). A calibration curve was constructed within the range of 2.5 to 10 g L^{-1} using a

standard solution (HACH 10236 TNTplus 825, mercury-free). Samples with values outside the range of the calibration curve were diluted accordingly.

Hydrothermal conversion

A continuous stirred-tank reactor (BR-300, Berghof Products & Instruments, Berghof, Germany) was used for the hydrothermal conversion of ice-cream WW (**Figure 11**). The handling of the samples, as well as the operational steps of the reactor, can be found elsewhere (Enteshari & Martínez-Monteagudo, 2018). Each experimental run consisted of 350 mL of WW heated at 230°C, pressurized at 40 bar, and held for 240 min. These conditions corresponded to the maximum hydrolysis (~40%) reported by Enteshari and Martínez-Monteagudo (2018). At the end of the hydrothermal process, the vessel was brought to room temperature using a circulating glycol bath. A representative description of the temperature-pressure history is given in the Chapter 3 (**Figure 3.**).

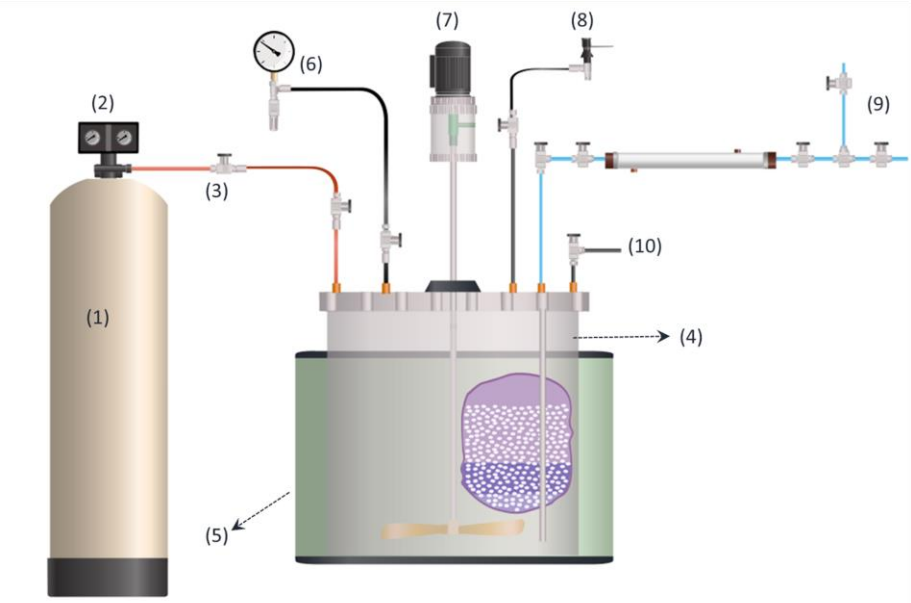

Figure 11. Schematic of the continuous stirred-tank reactor used for the thermochemical conversion of ice-cream wastewater: (1) nitrogen reservoir, (2) high pressure regulator, (3) needle valve, (4) high pressure reactor, (5) electrical heater, (6) rupture valve, (7) stirrer, (8) transition valve, (9) sampling port, (10) relief valve.

Degree of hydrolysis

The percentage of cleaved peptide bonds was measured as the degree of hydrolysis (DH), following the methodology reported by Nielsen, Petersen, and Dambmann (2001) with some modifications. Briefly, an aliquot (400 μL) of treated WW was mixed with the OPA reagent (3 mL). Then, the absorbance of the mixture was measured at 340 nm using a Varian spectrophotometer (Cary 50 UV–vis, Agilent Co., Santa Clara, CA). Details on the preparation of reagents and subsequent calculations can be found elsewhere (Enteshari & Martínez-Monteagudo, 2018).

Antiradical ability

The DPPH assay evaluated the ability of the hydrolysate to quench a stable free radical following the guidelines reported by Brand-Williams, Cuvelier, and Berset (1995). Three mL of a methanol solution of DPPH (100 μM) was mixed with different volumes of the recovered hydrolysate. The absorbance was recorded at 517 nm, and its reduction was expressed as a percentage of remaining DPPH.

ACE-inhibitory

The produced hydrolysates were tested for their ability to inhibit the activity of angiotensin I-converting enzyme (ACE) according to the method reported by Nakamura et al. (1995) with some modifications. Preparation of the reagents and their required volumes can be found elsewhere (Enteshari & Martínez-Monteagudo, 2018). The reduction in the absorbance was expressed as a percentage of ACE-inhibitory ability.

Amino acid profile of the produced hydrolysates

The amino acid profile (AA) before and after hydrothermal process was analyzed by a technical laboratory (Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia, MO, USA), according to AOAC Official Method (2006).

Statistical analysis

Experimental runs were conducted in triplicates, and all the figures were plotted using SigmaPlot software V11 for Windows (Systat Software, Inc, Chicago, IL, USA). Comparison of mean values was performed with one-way analysis of variance using Tukey's post hoc ($p < 0.05$).

4.4. RESULTS AND DISCUSSION

Characterization of ice-cream wastewater

Table 11 shows the composition of the ice-cream WW collected for six different production days. For simplicity, samples were named week 1 to 6. Overall, the variations in all the tested parameters did not show any particular trend since the values account for the production day that typically involves the manufacture of four different flavors. As discussed earlier, the variations in the pH (2.9 to 6.9, **Table 11**) is a common feature for ice-cream WW due to the wide portfolio of flavors, and solids added. The pH values reported in **Table 11** are within the range of previously reported values (Borja & Banks, 1994; Borja & Banks, 1995; Demirel et al., 2005; Hu, Thayanithy, & Forster, 2002).

Table 11. Characterization of the ice-cream wastewater used for the thermochemical conversion.

Parameters	Ice-cream wastewater					
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
pH	2.99 ± 0.02	3.18 ± 0.04	3.56 ± 0.03	2.18 ± 0.01	6.99 ± 0.03	4.77 ± 0.01
TS (g 100 g^{-1})	0.22 ± 0.01	0.27 ± 0.01	0.30 ± 0.02	0.32 ± 0.01	0.38 ± 0.01	0.30 ± 0.01
Total protein (g 100 g^{-1}, db)	12.48 ±1.17	15.43 ± 0.90	15.42 ± 0.12	18.12 ± 0.10	22.85 ± 0.27	21.25 ± 0.14
Total fat (g 100 g^{-1}, db)	26.37 ± 0.48	24.26 ± 0.67	33.78 ± 2.55	29.10 ± 0.82	31.34 ± 1.70	27.78 ± 0.21
COD (g L^{-1})	7.87 ± 0.78	11.61± 0.13	14.18 ± 0.62	15.13 ± 0.85	30.01 ± 0.16	18.81 ± 0.24
BOD (g L^{-1})	8.94 ± 0.08	11.59± 0.41	11.66 ± 0.20	38.21 ± 0.57	24.65 ± 3.97	22.62 ± 1.53
TS – total solids; COD – Chemical oxygen demand; BOD – Biochemical oxygen demand						

The protein content measured as total nitrogen content varied from 12 to 22 g 100 g^{-1} on a dry basis, while the fat content ranged from 26 to 33 g 100 g^{-1} on a dry basis. Enteshari and Martínez-Monteagudo (2018) reported similar values of protein and fat. The values of COD ranged from 7.87 to 30.01 g L^{-1}, which varied in accordance with the total nitrogen content. The literature reported values of COD from 1.1 to 10.4 g L^{-1} (Borja & Banks, 1994; Borja & Banks, 1995; Demirel et al., 2005; Hawkes, Donnelly, & Anderson, 1995). The protein content directly increases the COD of WW. On the other hand, the BOD varied from 8.9 to 38.2 g L^{-1}. Borja and Banks (1994) reported BOD of 2.45 g L^{-1} in ice-cream WW. Similarly, Moradi and Maleki (2013) reported BOD values of 1.5 g L^{-1} in synthetic ice-cream WW. The composition of ice-cream WW reported in **Table 11** exemplifies the complexity of ice-cream WW on a day to day production.

Degree of hydrolysis

Figure 12 shows the degree of hydrolysis after hydrothermal conversion of the different samples. The hydrothermal process yielded values of DH from 38 to 56%. A similar range of DH has been reported in ice-cream WW (Enteshari & Martínez-Monteagudo, 2018), and whey protein isolate (Espinoza & Morawicki, 2012). The employed conditions (230°C and 40 bar) induce dissociation of water molecules, leading to the formation of hydroxinium ions and hydroxide ions and enhancing the hydrolysis capacity of water (Möller et al., 2011). Interestingly, the protein content (**Table 11**) was not correlated with the DH of the ice-cream WW. For instance, the highest and lowest DH (56.67 ± 0.61 and 38.91 ± 0.83%, respectively) were obtained in those samples whose protein content was 12.48 ± 1.17 and 21.25 ± 0.14 g 100 g^{-1} on a dry basis, respectively.

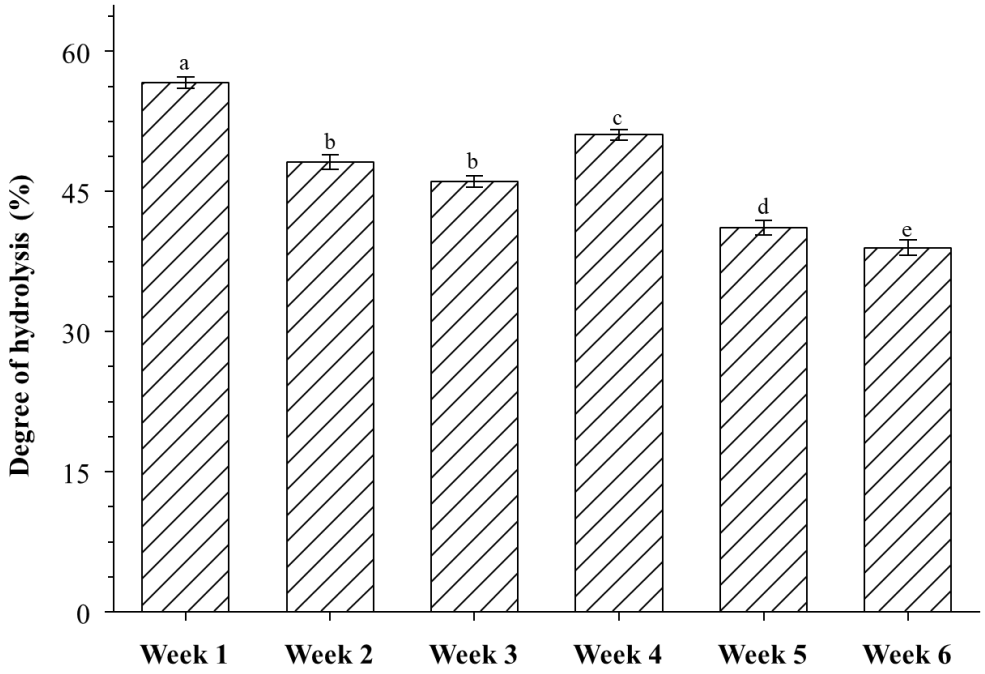

Figure 12. Degree of hydrolysis (DH) during the thermochemical conversion of ice-cream wastewater (230˚C, 60 bar, 600 rpm, 240 min). Mean ± standard deviation (n=3) within treatments with different letters are (a–e) significantly different ($p < 0.05$) according to Tukey test.

On the other hand, the values of DH (**Figure 12**) and the pH of the samples (**Table 11**) seem to be somewhat related. For instance, samples having the lowest pH (2.18 ± 0.01 and 2.99 ± 0.02) produced the highest values of DH (51.07 ± 0.55 and 56.67 ± 0.61%, respectively). The acidic medium may donate a hydrogen ion to the α- carboxylic end within the amino acids, favoring the cleavage of the peptide bond. On the other hand, those samples with the highest pH (4.77 ± 0.01 and 6.99 ± 0.03) yielded the lowest DH (38.96 ± 0.83 and 41.11 ± 0.78%, respectively). Under basic conditions, the α-amino end of the amino acid may loss a hydrogen ion to the medium (Abdelmoez, Nakahasi, & Yoshida, 2007), a condition that is less favorable for the formation of hydrolysis products.

Amino acid profile

Table 12 shows the total amino acid content of the different samples of ice-cream WW before and after the hydrothermal process. The amino acid content was expressed as a percentage with respect to the total protein since it changes from sample to sample. Glutamic acid, leucine, and aspartic acid were the most abundant amino acids before the treatment (16.83-19.83, 9.91-10.43, and 7.20-7.83%, respectively). After the treatment, glutamic acid, proline, leucine, and tyrosine were the most abundant amino acids whose concentration varied from 20.62-26.19, 10.31-15.38, 8.33-10.14, and 7.14-10.14%, respectively. Espinoza, Morawicki, and Hager (2012) reported a similar distribution of amino acid during the subcritical hydrolysis of whey protein isolate.

The study of the production of amino acids from proteins and their further degradation is a complex process since some amino acids are intermediate products from the decomposition of other amino acids (Abdelmoez et al., 2007). Sato, Quitain, Kang, Daimon, and Fujie (2004) studied the formation and further degradation of amino acids produced from fist waste. Kang et al. (2001) reported a maximum yield of amino acids within 60 min of reaction followed by decomposition.

A better understanding of the hydrothermal process can be obtained through kinetic studies, which is beyond the scope of this investigation. Nevertheless, a qualitative explanation of the formation and degradation of amino acids is provided. In general, the individual amino acids either decreased, increased, or unchanged after the hydrothermal process (230°C, 40 bar, and 240 min).

Table 12. Concentration of amino acids (%) in different samples of ice-cream wastewater before and after thermochemical process (230˚C, 60 bar, 600 rpm, 240 min).

	Week 1		Week 2		Week 3	
Amino acids	**Before**	**After**	**Before**	**After**	**Before**	**After**
Taurine	N.D.	2.90 ± 0.14	N.D.	N.D.	1.03 ± 0.05	2.06 ± 0.10
Hydroxyproline	N.D.	N.D.	N.D.	2.06 ± 0.10	N.D.	4.12 ± 0.21
Aspartic Acid	7.83 ± 0.39	2.90 ± 0.14	7.75 ± 0.39	2.06 ± 0.10	7.75 ± 0.39	2.06 ± 0.10
Threonine	3.91 ± 0.20	N.D.	4.00 ± 0.20	N.D.	3.88 ± 0.19	N.D.
Serine	5.22 ± 0.26	N.D.	4.75 ± 0.24	N.D.	4.65 ± 0.23	N.D.
Glutamic Acid	19.13 ± 0.96	26.09 ± 1.30	19.75 ± 0.99	24.74 ± 1.24	18.35 ± 0.92	20.62 ± 1.03
Proline	9.13 ± 0.46	11.59 ± 0.58	9.25 ± 0.46	12.37 ± 0.62	9.56 ± 0.48	10.31 ± 0.52
Lanthionine	0.43 ± 0.02	N.D.	0.25 ± 0.01	N.D.	1.03 ± 0.05	2.06 ± 0.10
Glycine	2.17 ± 0.11	2.90 ± 0.14	2.00 ± 0.10	2.06 ± 0.10	2.07 ± 0.10	2.06 ± 0.10
Alanine	3.48 ± 0.17	2.90 ± 0.14	3.25 ± 0.16	3.09 ± 0.15	3.36 ± 0.17	3.09 ± 0.15
Cysteine	0.87 ± 0.04	2.90 ± 0.14	0.75 ± 0.04	2.06 ± 0.10	1.29 ± 0.06	2.06 ± 0.10
Valine	6.52 ± 0.33	5.80 ± 0.29	6.50 ± 0.33	6.19 ± 0.31	6.46 ± 0.32	6.19 ± 0.31
Methionine	2.61 ± 0.13	2.90 ± 0.14	2.50 ± 0.13	4.12 ± 0.21	2.58 ± 0.13	4.12 ± 0.21
Isoleucine	6.09 ± 0.30	5.80 ± 0.29	6.00 ± 0.30	6.19 ± 0.31	5.94 ± 0.30	5.15 ± 0.26
Leucine	10.43 ± 0.52	10.14 ± 0.51	10.25 ± 0.51	9.28 ± 0.46	10.34 ± 0.52	9.28 ± 0.46
Tyrosine	2.61 ± 0.13	10.14 ± 0.51	3.75 ± 0.19	9.28 ± 0.46	2.07 ± 0.10	8.25 ± 0.41
Phenylalanine	5.65 ± 0.28	7.25 ± 0.36	5.25 ± 0.26	8.25 ± 0.41	5.43 ± 0.27	8.25 ± 0.41
Hydroxylysine	N.D.	4.35 ± 0.22	N.D.	5.15 ± 0.26	N.D.	5.15 ± 0.26
Ornithine	N.D.	N.D.	N.D.	1.03 ± 0.05	N.D.	1.03 ± 0.05
Lysine	8.26 ± 0.41	1.45 ± 0.07	8.25 ± 0.41	1.03 ± 0.05	8.01 ± 0.40	2.06 ± 0.10
Histidine	2.17 ± 0.11	N.D.	2.50 ± 0.13	1.03 ± 0.05	2.33 ± 0.12	1.03 ± 0.05
Arginine	3.48 ± 0.17	N.D.	3.25 ± 0.16	N.D.	3.88 ± 0.19	1.03 ± 0.05

Amino acids	Week 4		Week 5		Week 6	
	Before	**After**	**Before**	**After**	**Before**	**After**
Taurine	1.57 ± 0.08	3.08 ± 0.15	1.44 ± 0.07	1.19 ± 0.06	0.78 ± 0.04	N.D.
Hydroxyproline	N.D.	2.31 ± 0.12	N.D.	2.38 ± 0.12	N.D.	N.D.
Aspartic Acid	7.48 ± 0.37	1.54 ± 0.08	7.41 ± 0.37	1.19 ± 0.06	7.20 ± 0.36	1.67 ± 0.08
Threonine	4.00 ± 0.20	N.D.	4.09 ± 0.20	N.D.	4.25 ± 0.21	N.D.
Serine	4.52 ± 0.23	0.77 ± 0.04	3.98 ± 0.20	N.D.	4.51 ± 0.23	N.D.
Glutamic Acid	19.83 ± 0.99	23.85 ± 1.19	17.70 ± 0.88	26.19 ± 1.31	16.83 ± 0.84	25.83 ± 1.29
Proline	9.04 ± 0.45	15.38 ± 0.77	8.96 ± 0.45	13.10 ± 0.65	9.11 ± 0.46	12.50 ± 0.63
Lanthionine	N.D.	0.77 ± 0.04	0.55 ± 0.03	1.19 ± 0.06	0.78 ± 0.04	N.D.
Glycine	2.09 ± 0.10	2.31 ± 0.12	1.88 ± 0.09	1.79 ± 0.09	1.91 ± 0.10	1.67 ± 0.08
Alanine	3.30 ± 0.17	3.85 ± 0.19	3.21 ± 0.16	3.57 ± 0.18	3.21 ± 0.16	3.33 ± 0.17
Cysteine	1.74 ± 0.09	N.D.	0.77 ± 0.04	1.19 ± 0.06	0.69 ± 0.03	1.67 ± 0.08
Valine	6.43 ± 0.32	6.92 ± 0.35	6.53 ± 0.33	7.14 ± 0.36	6.85 ± 0.34	7.50 ± 0.38
Methionine	2.61 ± 0.13	N.D.	2.77 ± 0.14	5.95 ± 0.30	2.78 ± 0.14	5.00 ± 0.25
Isoleucine	5.74 ± 0.29	5.38 ± 0.27	5.75 ± 0.29	4.76 ± 0.24	5.90 ± 0.29	5.83 ± 0.29
Leucine	9.91 ± 0.50	9.23 ± 0.46	9.96 ± 0.50	8.33 ± 0.42	9.97 ± 0.50	10.00 ± 0.50
Tyrosine	2.78 ± 0.14	7.69 ± 0.38	4.98 ± 0.25	7.14 ± 0.36	4.86 ± 0.24	8.33 ± 0.42
Phenylalanine	5.04 ± 0.25	7.69 ± 0.38	5.09 ± 0.25	7.74 ± 0.39	5.20 ± 0.26	8.33 ± 0.42
Hydroxylysine	0.35 ± 0.02	6.15 ± 0.31	N.D.	4.17 ± 0.21	N.D.	5.00 ± 0.25
Ornithine	N.D.	0.77 ± 0.04	N.D.	0.60 ± 0.03	N.D.	0.83 ± 0.04
Lysine	8.17 ± 0.41	1.54 ± 0.08	8.41 ± 0.42	1.19 ± 0.06	8.50 ± 0.42	1.67 ± 0.08
Histidine	2.43 ± 0.12	0.77 ± 0.04	2.88 ± 0.14	0.60 ± 0.03	2.86 ± 0.14	0.83 ± 0.04
Arginine	2.96 ± 0.15	N.D.	3.65 ± 0.18	0.60 ± 0.03	3.82 ± 0.19	N.D.
N.D. – not detected						

Upon closer inspection of **Table 12**, those amino acids whose R group is positively charged (lysine, histidine, and arginine) showed a 3.8-8.0-, 2.1-4.8-, and 2.9-6.1-fold reduction after the treatment, respectively. Abdelmoez et al. (2007) reported a 2-fold reduction of the positively charged amino acids under subcritical conditions (225-290°C). Cheng et al. (2008) reported a reduction in the formation of arginine during the subcritical hydrolysis of chicken waste. Also, a significant reduction of 2.3-6.2-fold was observed in the concentration of aspartic acid. Kang and Chun (2004) hydrolyzed fish waste and reported a maximum yield of aspartic acid, followed by a sharp reduction due to the formation of organic acids. Contrary, negatively charged amino acids, such as glutamic acid, increased up to 1.54-fold, while proline, a hydrophilic polar amino acid, increased up to 1.37-fold. Finally, those amino acids having hydrophobic non-polar R group (valine, methionine, isoleucine, and leucine) unchanged after the hydrothermal process.

COD and BOD

Figure 13 shows the reduction of BOD and COD after the hydrothermal process for the different samples. For the BOD, the reduction values were relatively high (87-97%) and varied within a narrow range (**Figure 12**). In the case of COD, the reduction values were from 25 to 70% and did not show any relationship with the DH. The highest reduction (70.92 ± 0.91%) was obtained in the sample with lowest DH (38.96 ± 0.83%, **Figure 11**), while the lowest COD reduction (25.08 ± 1.68%) corresponded to the sample hydrolyzed to 41.11 ± 0.79%. Similar reduction values of COD have been reported in the literature, where a variety of treatments were used. Andrade et al. (2014) reduced up to 99% of the COD in dairy WW using a membrane reactor coupled with nanofiltration. Similarly,

Davarnejad and Nikseresht (2016) applied an electro-chemical treatment on dairy WW to reduce 98% of the COD. An investigation on the electro-coagulation of dairy WW showed a reduction of 39% of the COD (Hamdani, Mountadar, & Assobhei, 2005). Bensadok, El Hanafi, and Lapicque (2011) reduced up to 80% of the COD in dairy effluents using an electro-chemical treatment. Kushwaha, Srivastava, and Mall (2010) reduced 70% of the COD by a combination of electro-coagulation and electro-oxidation.

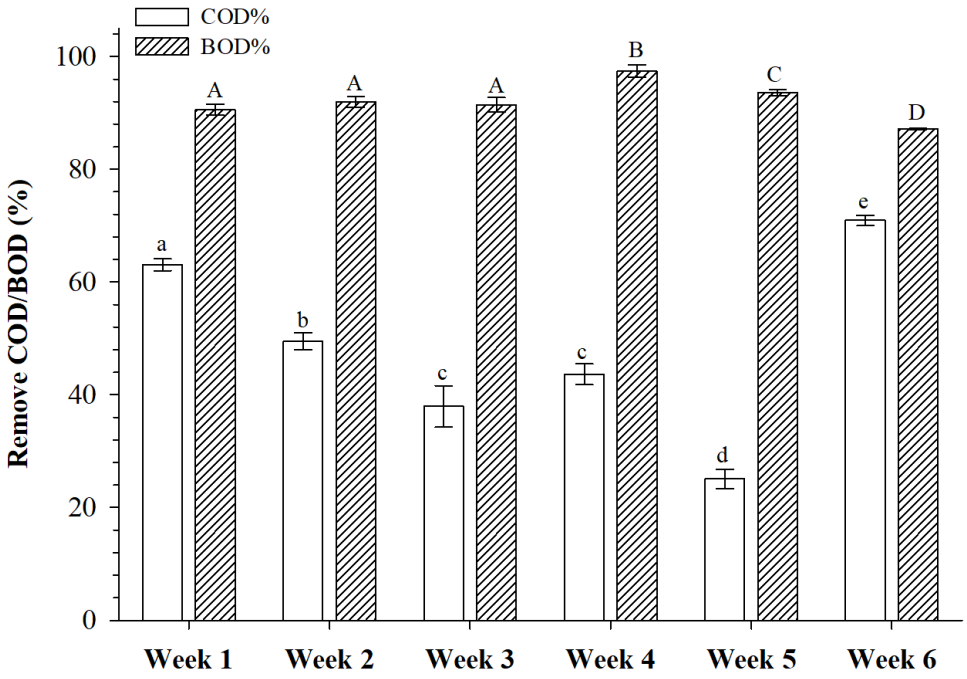

Figure 13. Reduction of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) before and after thermochemical process of different samples of ice-cream wastewater. Reaction conditions: 230°C, 40 bar, 240 min, and 600 rpm. Mean ± standard deviation (n=3) within COD column with different letters are (a–e) significantly different ($P < 0.05$) according to Tukey test. Mean ± standard deviation (n=3) within BOD column with different letters are (A–C) significantly different ($P < 0.05$) according to Tukey test.

Antiradical ability

Upon hydrolysis, proteins produce hydrolysates of different molecular sizes and a wide range of distribution of amino acids. Such hydrolysates may possess antiradical

activity, as previously reported elsewhere (Nongonierma & FitzGerald, 2015). A graphical representation of the antiradical activity of the produced hydrolysates is given in **Figure 14**, where the percentage of the remaining radical (DPPH) is plotted against a given volume of hydrolysates.

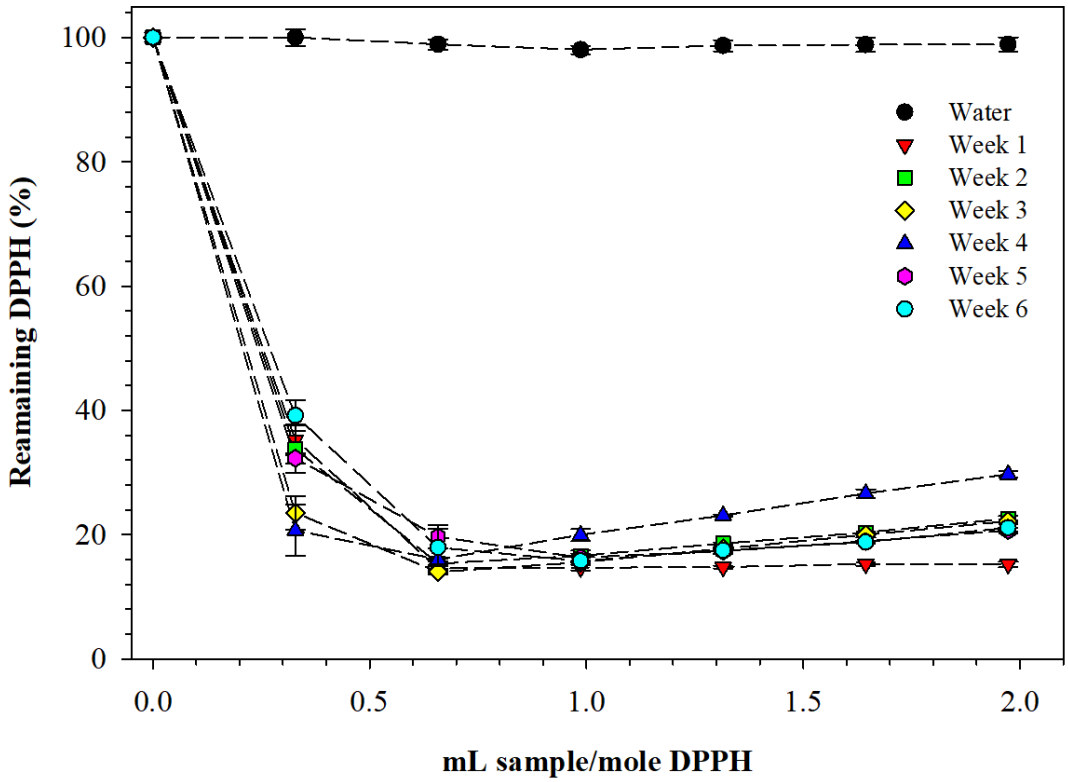

Figure 14. Antiradical ability of the produced hydrolysate after thermochemical treatment of different wastewater sample (230˚C, 60 bar, 600 rpm, 240 min).

For all the samples, a sharp decrease in the remaining DPPH was observed at a ratio of 0.32 mL of hydrolysates per mole of DPPH, reaching values between 20 and 38%. Increasing the ratio to 0.65 mL of hydrolysates per mole of DPPH yielded the lowest remaining DPPH (14-19%). Further increment of the ratio resulted in slightly increased with the remaining DPPH. Similar values of antiradical activity for hydrolysates derived from ice-cream WW can be found elsewhere (Enteshari & Martínez-Monteagudo, 2018).

ACE-inhibitory effect

Another relevant functional property of protein hydrolysate is the inhibitory ability of the angiotensin I-converting enzyme (ACE) (**Figure 15**). The highest values of ACE-inhibition (96.38 ± 1.17 and 92.60 ± 0.30) were obtained for the samples corresponding to a DH of 48.18 ± 0.77 and 46.07 ± 0.63%, respectively (week 2 and week 3). On the other hand, the lowest ACE-inhibition (59.34 ± 5.05%) corresponded to the samples with the highest DH (56.67 ± 0.62%). Although the produced hydrolysates showed relevant ACE-inhibitory ability, no correlation was found with DH or protein content.

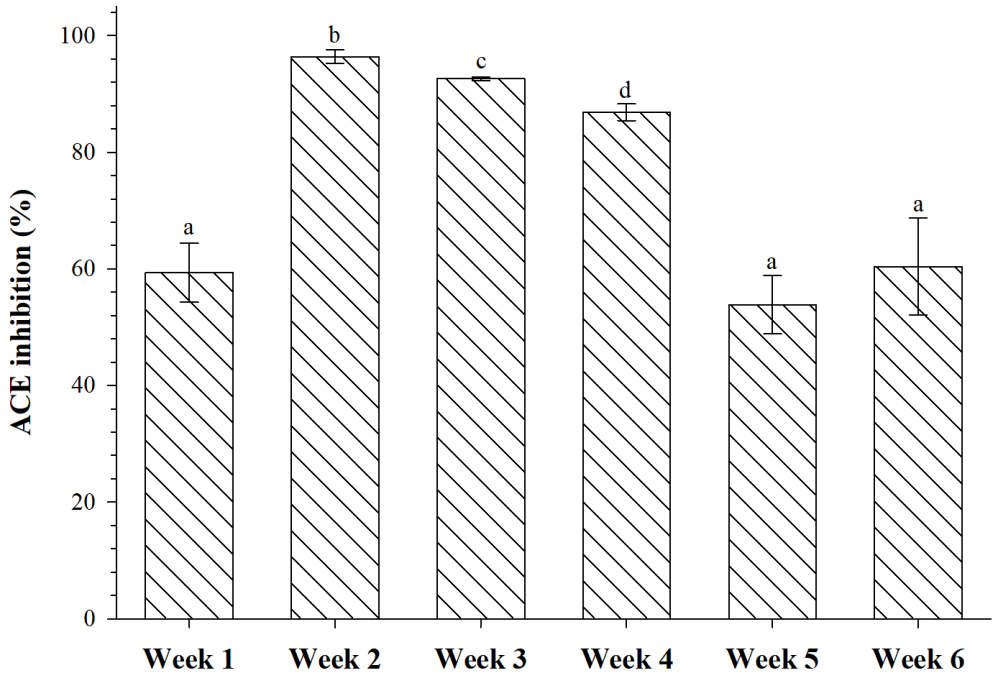

Figure 15. Inhibitory ability of angiotensin I-converting enzyme (ACE) of the produced hydrolysate after thermochemical process of different wastewater samples (230˚C, 60 bar, 600 rpm, 240 min). Mean ± standard deviation (n=3) within treatments with different letters are (a–d) significantly different ($P < 0.05$) according to Tukey test.

4.5. CONCLUSION

Wastewater derived from ice-cream manufacture showed significant differences from batch to batch in terms of protein, fat, and pH. The hydrothermal process showed promising results, yielding values of degree hydrolysis from 38 to 56%. The produced hydrolysates were high in glutamic acid, proline, and leucine. Aspartic acid was the least stable amino acid, showing reductions of 2.7-6.2-fold. After the hydrothermal process, 87-97% of the BOD was reduced, while the COD was reduced between 25 and 70%. Hydrolysates showed relevant antiradical and antihypertensive activity. The outcomes of this investigation demonstrated the potential use of subcritical hydrolysis in reducing the organic load of WW, and to convert into value-added compounds.

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CHAPTER 5

SUBCRITICAL HYDROLYSIS OF ICE-CREAM WASTEWATER FOR AMINO ACID PRODUCTION[4]

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2020

5.1. ABSTRACT

The generation of wastewater (WW) in ice-cream manufacturing results from cleanup operations. It is estimated that around 8-12 L of WW is generated per kg of product. The protein content in the ice-cream WW significantly contributed to its high content of organic load, which has proved to be problematic for the receiving effluent treatment plant. The goal of this study was to study the subcritical hydrolysis of ice-cream WW to produce a feedstock of amino acids for value-added applications. The influence of time (0-200 min), temperature (170-230˚C), and pH (2-9) was studied in a continuous stirred-tank reactor operated at 40 bar. The extent of hydrolysis was monitored by measuring the degree of hydrolysis (DH), recovered total amino acids and amino acid profile in the hydrolyzed fraction. The reaction time and temperature significantly increased the DH, reaching maximum values between 85-98% depending on the pH. The highest value of recovered amino acids in the hydrolyzed fraction was ~500 mg of amino acid per g protein, depending on the pH and gradually decreased with time and temperature (100-150 mg amino acid per g protein). The production of amino acids strongly depends on the hydrolysis conditions (time, temperature, and pH). Glutamic acid was the predominant amino acid in the hydrolyzed fraction (26-33%), followed by proline (11-

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14%), leucine (8-10%), and valine (6-8%). The recovery of amino acids such as aspartic acid, serine, cysteine, methionine, and histidine was less than 5%. The outcomes of this research indicate that subcritical hydrolysis is a controlled pathway to convert an otherwise waste material into valuable industrial feedstock.

5.2. INTRODUCTION

Dairy industry as one the most important agricultural processing industries in the United States with very speedy growth rate during last decades. Fluid milk and milk products have been identified as the most important sources of wastewater generated through dairy manufacturing. During milk reception and milk bottling, wastewater can be generated through cleaning-in-place (CIP) operations and washing bottles due to strict and hygienic regulation of good manufacturing practice in dairy industry. The quantities of wastewater are increasing in more proceed dairy products including condensed milk, powdered milk and milk protein fractions, fermented milk products, butter, cheese, concentrated whey and whey powder products. Approximately, 6-10 L of wastewater is generated per liter of processed milk in dairy manufacturing (Ahmad et al., 2019). Dairy wastewater is identified as one of the most pollutant industrial stream due to its high biological and chemical oxygen demands (BOD and COD), nutrients, and organic and inorganic materials (Kushwaha, Srivastava, & Mall, 2011). The approximate BOD value of milk is 100,000 mg L^{-1}. Additionally, more wastewater volume could be generated through processing of other dairy products such as cheese and butter with BOD levels of 1,500 to 3,000 mg L^{-1} (Liu & Liu, 2007).

Among dairy industries, ice-cream manufacturing utilizes massive volume of water per unit of product (Ackermann et al., 1999). In addition to the water used to manufacture ice-cream, considerable amount of wastewater (WW) is generated through incidental overflows, product switches, and cleaning-in-place protocols (Kushwaha, Srivastava, & Mall, 2011). Additionally, ice-cream WW is reported as one of the most pollutant dairy stream due to its high levels of BOD and COD (8-38, and 7-30 g L^{-1}, respectively) (Enteshari & Martínez-Monteagudo, 2020). So, discharging the WW from ice-cream processing without proper treatment to lower its organic load brings about severe environmental dilemmas (Montuelle, Goillard, & Le Hy, 1992). To keep safe environment, strict regulations and standards have imposed on dairy industries.

According to nature of generated WW, different treatments methods or integration of numerous procedures including grease traps, oil-water separators, clarifiers, biological, and chemical treatments could be employed. In 2019, Ahmed et al., documented a comprehensive review about different treatment methods and utilization of dairy waste streams (Ahmad et al., 2019). The complex composition of ice-cream WW due to variation in ingredients and formulation, as well as presence of detergents has created many impetuses for researchers. Therefore, utilizing an appropriate and efficient methods for treatment of ice-cream WW could play a crucial role in sustainability of dairy industry.

Currently, biological and physicochemical methods are used for ice-cream WW treatment. Biological methods encompass activated sludge process, aerated lagoons, sequencing batch reactor (SBR), anaerobic sludge blanket reactor, and anaerobic filters.

Most applicable physicochemical methods are coagulation and flocculation processes, membrane techniques such as nanofiltration and reverse osmosis (Demirel, Yenigun, & Onay, 2005). Advantage of biological methods is the low cost of reagents and higher efficiency in reduction of COD. Water and residual proteins are two important fractions in ice-cream WW which make it as a potential stream for production of amino acids through subcritical hydrolysis. In a previous study by Enteshari and Martínez-Monteagudo (2018) reported the degree of hydrolysis up to 41% of the proteins in ice-cream WW under subcritical conditions. Treatment of ice-cream WW from different production batches through subcritical method has shown considerable hydrolysis of proteins between 38 to 56% with profound antioxidant and antihypertensive activities (14-19% and 60-97%, respectively) (Enteshari & Martínez-Monteagudo, 2020). Within the region of boiling and critical point, water exhibit high ion product which triggers ionic reactions mainly hydrolysis that is considerably improved. Relevant features of water at subcritical conditions which behaves as a reaction medium for hydrothermal biomass transformation are reviewed somewhere else (Möller, Nilges, Harnisch, & Schröder, 2011). Subcrtitical water has been demonstrated to be an effective reaction environment for hydrolysis of organic materails. This study investigates the effect of vacuum evaporation followed by subcritical technique to hydrolyze the protein fraction of ice-cream WW under various pH and temperature conditions, and to produce valuable stream rich in amino acids.

5.3. MATERIAL AND METHODS

Ice-cream wastewater

Sample of WW was collected from individual batch of ice-cream production from the Davis Dairy Plant at South Dakota State University (Brookings, SD). The ice-cream WW sample obtained through returned line after a typical clean-in-place program. After collection, the WW sample was stored at 4˚C prior to the analysis of pH, total solids, total protein, fat, moisture content, BOD, and COD. The pH was measured in 10 mL of the sample using an Orion Versa Star Pro (Thermo Fisher Scientific, Waltham, MA). Total solid (TS) was determined using the standard methods for examination of WW (Rice, Baird, Eaton, & Clesceri, 2012). The protein content was measured by the Kjeldahl method, while the fat content was quantified using Mojonnier extraction.

The WW was also analyzed for BOD and COD before vacuum condensation, according to the methodology described elsewhere (Rice et al., 2012). The BOD was obtained from the amount of oxygen consumed (mg L^{-1}) after 5-day incubation at 20˚C using seeded sample with appropriate dilutions. The seeding sample was obtained from the municipal water treatment (Brookings, SD). To measure COD, the amount of oxygen needed to oxidize the organic matter in sample of WW was spectrophotometrically measured. Potassium dichromate was used as an oxidizing agent, and sulfuric acid was used as a solvent. The oxidation was conducted at 150˚C for 2 h using a DRB200 reactor (HACH Company, Loveland, CO). Then, samples were brought to room temperature before recording the absorbance at 600 nm using a DR/4000 U spectrophotometry (HACH

Company, Loveland, CO). A calibration curve was built within the range of 0 to 1000 mg L^{-1} using a standard solution (HACH 10236 TNTplus 825, mercury-free).

Pre-treatment - Vacuum evaporation and pH modification

To increase the total solid content, the sample of ice-cream WW was condensed using a rotary vacuum evaporator (Heidolph North America, Wood Dale, IL). The water removed from the WW under vacuum evaporation conditions of 70˚C at constant speed rate of 50 rpm. After reaching appropriate total solid content, the pH of concentrated WW was modified to levels of 2, 6, and 9 by addition of NaOH 0.1N while stirring continuously during pH measurement. The subcritical hydrolysis was conducted as described in previous chapters.

Degree of hydrolysis

The degree of hydrolysis (DH) was determined as percentage of cleaved peptide bonds was measured as the using the methodology documented by Nielsen, Petersen, and Dambmann (2001) with some minor alterations (Enteshari & Martínez-Monteagudo, 2018).

Amino acid profile

The amino acid profile (AA) of vacuum evaporated ice-cream WW treated at various pH (2,6, and 9) followed by subcritical hydrolysis was analyzed by a technical laboratory (Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia, MO, USA), according to AOAC Official Method (2006).

Statistical analysis

Experimental runs were conducted in triplicates, and all the figures were plotted using SigmaPlot software V11 for Windows (Systat Software, Inc, Chicago, IL, USA). Comparison of mean values was performed with one-way analysis of variance using Tukey's post hoc ($P < 0.05$).

5.4. RESULTS AND DISCUSSION

Characterization of wastewater

Table 13 shows the composition of non-evaporated and vacuum evaporated ice-cream WW collected from a batch of ice-cream production. The pH level of the non-evaporated and vacuum evaporated WW sample is very low and in the acidic range (pH~2) which is within the range of previously reported values (Enteshari & Martínez-Monteagudo, 2018; Enteshari & Martínez-Monteagudo, 2020).

Table 13. Physicochemical properties of ice-cream wastewater (non-evaporated and vacuum evaporated) used for subcritical hydrolysis.

Parameters	**Ice-cream wastewater**	
	Non-evaporated	**Vacuum evaporated**
pH	2.18 ± 0.01	2.06 ± 0.01
TS (g 100 g^{-1})	1.22 ± 0.01	3.15 ± 0.02
Total protein (g 100 g^{-1}, db)	9.13 ± 0.30	9.54 ± 1.57
Total fat (g 100 g^{-1}, db)	29.10 ± 0.82	N.D.
BOD (mg L^{-1})	38,200 ± 565.68	N.D.
COD (mg L^{-1})	15,132 ± 845.70	N.D.
TS – total solids; BOD – Biochemical oxygen demand; COD – Chemical oxygen demand.		

After vacuum evaporation, the level of total solids (TS) increased 2.5 times and reached to 3.15±0.02 g 100 g^{-1}. The protein contents measured as total nitrogen content for non-evaporated and vacuum evaporated WW sample were obtained 9.13 ± 0.30 and 9.54 ± 1.57 g 100 g^{-1} on a dry basis (db). Enteshari and Martínez-Monteagudo (2020) reported similar values of protein and fat for weekly collected ice-cream wastewater samples. Additionally, the biological load of ice-cream WW sample was determined by measuring the BOD and COD levels. For non-evaporated ice-cream WW, the obtained values of BOD and COD were 38,000 and 15,000 mg L^{-1}, respectively which are in the range of earlier studies (Borja & Banks, 1995; Scott & Smith, 1997).

Degree of hydrolysis

Figure 16 illustrates the degree of hydrolysis (DH) of vacuum evaporated ice-cream WW followed by subcritical hydrolysis treatment at various pH levels and temperature during 200 min. The hydrolysis degree of WW increased over the time and reached to maximum DH about 98% after 200 min subcritical process at 170˚C. Interestingly, in this study higher degree of hydrolysis was observed compared to earlier studies (Enteshari & Martínez-Monteagudo, 2018; Enteshari & Martínez-Monteagudo, 2020). As a result of condensation and obviously higher amounts of total solid and protein content, higher degree of hydrolysis was observed in vacuum evaporated WW. As presented in **Table 13**, the high protein content of evaporated WW proves the increase of DH under subcritical process. These results demonstrate the effectiveness of vacuum evaporation as a pre-treatment followed by subcritical hydrolysis to improve the hydrolysis degree of proteins in ice-cream WW. It is stated that concentration of reactants under

subcritical significantly influence of hydrolysis degree and it is more desirable from energy efficiency aspects (Möller et al., 2011). However, some authors have reported the negative effect of high concentration on hydrolysis process (Knezevic, van Swaaij, & Kersten, 2009). It was observed that increasing the concentration of glucose adversely influenced on relative decomposition of glucose solutions. It could be concluded that formation of soluble and insoluble polymers is greatly increased at higher concentration of substrate which inhibits hydrolysis process.

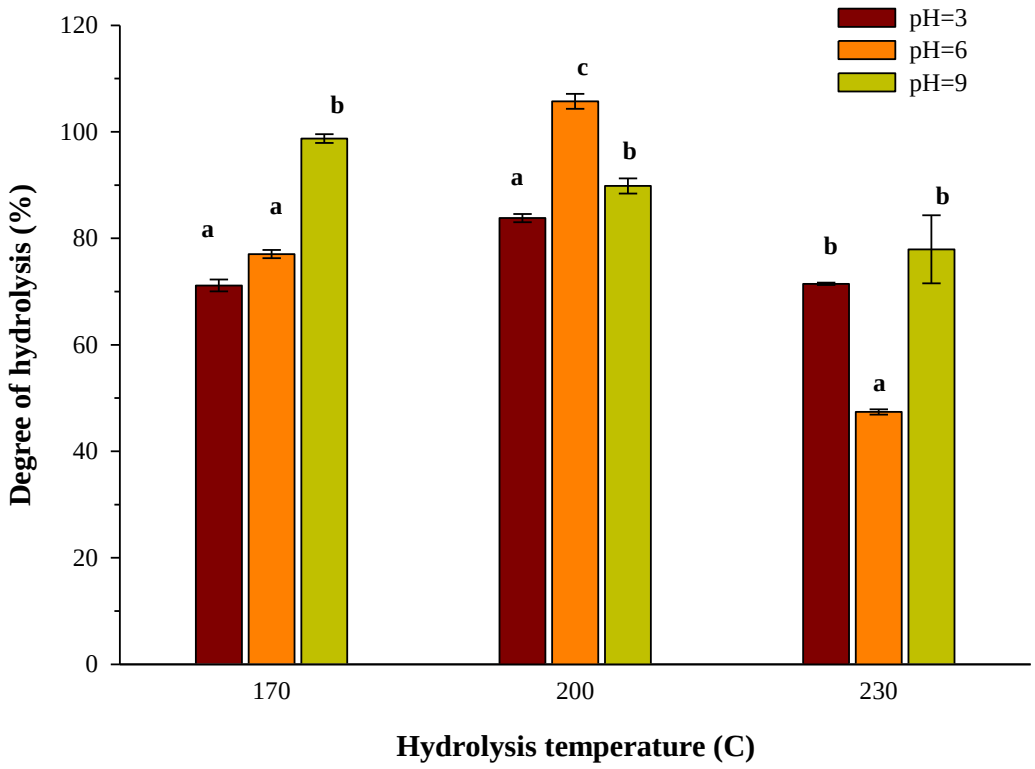


Figure 16. Degree of hydrolysis (DH) of vacuum evaporated ice-cream wastewater at different pH and temperature under subcritical hydrolysis treatment (40 bar, 600 rpm, 200 min). Mean ± standard deviation (n=4) within treatments with different letters are (a–c) significantly different ($P < 0.05$) according to Tukey test.

Remarkably, increasing the pH value from 2 to 9 induced more hydrolysis degree by subcritical treatment at lower hydrolysis temperature (170˚C). For instance, after 50 min subcritical hydrolysis of condensed WW its degree of hydrolysis was highest at alkaline medium than acidic (85 and 65% at pH of 9 and 2, respectively) (data is not presented). This result is mainly correlated with electrically charged R groups of some amino acids in peptide fractions and proteins which will be discussed later in this context. Also, the maximum degree of hydrolysis was achieved at 200˚C and pH 6 (105.71±1.41%). However, lower values of hydrolysis degree through subcritical process are documented in the literature for of whey protein isolate (WPI), DH 50% at 291˚C for 28 min (Espinoza & Morawicki, 2012), whey protein isolate (WPI), DH 11% at 305˚C for 17 min (Espinoza, Morawicki, & Hager, 2012), and ice-cream WW, DH 38 to 56% at 230˚C for 240 min (Enteshari & Martínez-Monteagudo, 2020).

The obtained higher degree of hydrolysis in present study could be related to acidic nature of ice-cream WW. It is believed that acidic medium may donate a hydrogen ion to the α- carboxylic end within the amino acids, encouraging the dissociation of peptide bond. Therefore, more concentration of protons in the reaction medium assist in hydrolysis degree induced by subcritical process (Möller et al., 2011). Also, milder subcritical hydrolysis conditions (lower temperature and longer time) could favor cleavage of peptides and gradually releasing amino acids without their further degradation to organic acids and other fractions. In contrast, under alkaline conditions, the α-amino end of the amino acid may lose a hydrogen ion to the medium a condition that is less favorable for the formation of hydrolysis products (Abdelmoez, Nakahasi, & Yoshida, 2007).

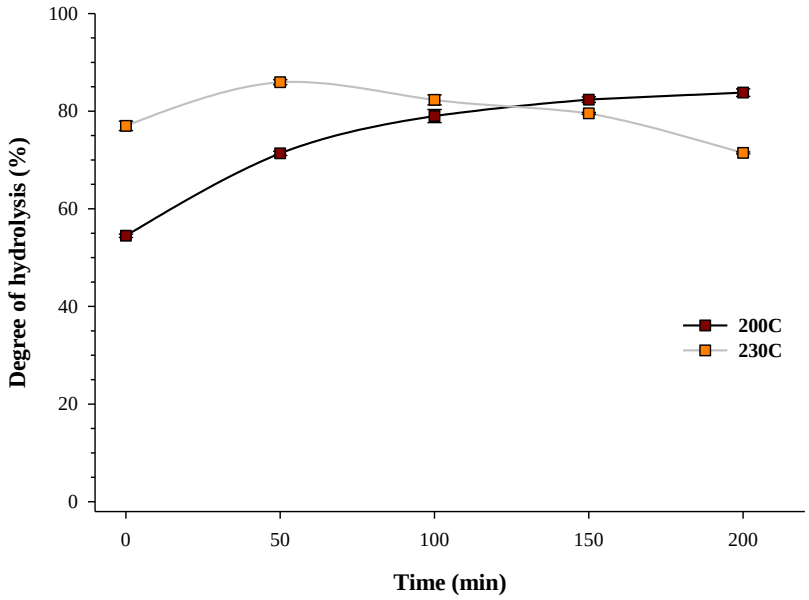
Figure 17 clearly shows that there is a positive effect of higher temperature on hydrolysis degree. After 50 min, at applied temperatures of 200 and 230˚C the hydrolysis degree reached to maximum values of 71.36 ± 0.42 and 85.95 ± 0.52%, respectively. With continuing the reaction time, the hydrolysis degree at 230˚C showed a decreasing trend, while an increasing pattern was observed at 200˚C and reached to value of 83.80 ± 0.88% after 200 min. This result can be explained by degradation of amino acids derived from cleavage of peptide bonds and formation of organic acids and other side products. This general finding was proved in hydrothermal conversion of glucose, fructose, and cellulose (Knezevic et al., 2009; Sasaki, Fang, Fukushima, Adschiri, & Arai, 2000; Watanabe et al., 2005). It is demonstrated that temperature plays a decisive role in effectiveness of subcritical water hydrolysis and determines degradation pathways, type of formed products, and the extent of hydrolysis degree (Möller et al., 2011). Hence, the formation of hydroxinium and hydroxide ions has been enhanced at applied subcritical conditions

(200˚C and 40 bar) because of higher dissociation of water molecules, which resulted in greater hydrolysis potency of water.

Figure 17. Degree of hydrolysis (DH) of vacuum evaporated ice-cream wastewater at 200 and 230˚C and variable times (0 to 200 min) under subcritical hydrolysis (pH 2, 40 bar, 600 rpm).

Amino acid profile

To determine efficiency of subcritical process on hydrolysis of proteins, the amino



acid composition in vacuum evaporated WW before and after subcritical hydrolysis was

Table 14. Concentration of amino acids (%) in vacuum evaporated ice-cream wastewater treated at different pH levels (2, 6, and 9).

Amino acids	**Untreated vacuum evaporated ice-cream WW**		
	pH 2	**pH 6**	**pH 9**
Taurine	N.D.	N.D.	1.85±0.09
Hydroxyproline	0.27±0.01	N.D.	N.D.
Aspartic Acid	7.97±0.40	7.59±0.40	7.90±0.39
Threonine	4.30±0.22	4.36±0.22	4.41±0.22
Serine	4.66±0.23	4.75±0.24	4.62±0.23
Glutamic Acid	19.18±0.96	19.38±0.97	18.56±0.93
Proline	9.23±0.46	9.21±0.46	8.92±0.45
Lanthionine	N.D.	N.D.	N.D.
Glycine	2.33±0.12	2.33±0.12	2.26±0.11
Alanine	3.76±0.19	3.78±0.19	3.69±0.18
Cysteine	3.23±0.16	3.00±0.15	2.67±0.13
Valine	6.27±0.31	6.30±0.31	6.15±0.31
Methionine	2.60±0.13	2.62±0.13	2.56±0.13
Isoleucine	5.47±0.27	5.52±0.28	5.44±0.27
Leucine	9.68±0.48	9.69±0.48	9.64±0.48
Tyrosine	0.63±0.03	0.58±0.03	0.62±0.03
Phenylalanine	5.11±0.26	5.14±0.26	5.23±0.26
Hydroxylysine	0.36±0.02	0.29±0.01	0.41±0.02
Ornithine	N.D.	N.D.	N.D.
Lysine	9.23±0.46	9.30±0.47	9.13±0.46
Histidine	3.23±0.17	3.29±0.16	3.38±0.17
Arginine	2.42±0.12	2.52±0.13	2.56±0.13
N.D. – not detected			

determined. The amino acid content was expressed as a percentage with respect to the total protein. As shown in **Table 14**, glutamic acid, leucine, lysine, and proline were the most abundant amino acids in the vacuum evaporated WW before subcritical treatment (18.56-19.38, 9.64-9.69, 9.13-9.30, and 8.92-9.23%, respectively).

After conducting the subcritical hydrolysis, glutamic acid, proline, leucine, and valine were the most abundant amino acids whose concentration varied from 20.62-26.19, 10.31-15.38, 8.33-10.14, and 7.14-10.14%, respectively (**Table 15**). Similar distribution of amino acid during subcritical hydrolysis of whey protein isolate is identified by other authors (Espinoza et al., 2012). Formation of glutamic acid and proline increased noticeably about 2-fold at more acidic medium (pH 2 to 6) and hydrolysis temperature of 200˚C. This result is compatible with the increasing pattern of hydrolysis degree which was observed for subcritical hydrolysis at 200˚C and pH of 2. Aspartic acid was found as the most labile amino acid, showing reductions of 1.6 to 6.4-fold. It is assumed that stability of amino acids is affected by the presence of other amino acids. Additonally, interactions among amino acids may induce their degradtion (Abdelmoez et al., 2007).

Additonally, the level of lysine decreased 4.3-fold after sucbritical hydrolysis at 230˚C under pH of 2. Similar result was reported by Abdelmoez et al. (2007). They reported a 2-fold reduction of the positively charged amino acids under subcritical conditions (225-290˚C). The study of amino acids production from proteins, kinetic of hydrolysis reactions, and their further degradation is discussed elsewhere (Andersson & Holm, 2000; Sato, Quitain, Kang, Daimon, & Fujie, 2004).

Table 15. Concentration of amino acids (%) in vacuum evaporated ice-cream wastewater treated at different pH levels (2, 6, and 9) and hydrolysis temperatures (170, 200, 230˚C) under subcritical conditions (40 bar, 600 rpm, 200 min).

Amino acids	**Subcritical hydrolysis temperature (˚C)**								
	170			**200**			**230**		
	pH 2	**pH 6**	**pH 9**	**pH 2**	**pH 6**	**pH 9**	**pH 2**	**pH 6**	**pH 9**
Taurine	N.D.	3.07±0.15	1.76±0.09	1.57±0.08	1.11±0.06	1.45±0.07	3.86±0.19	1.96±0.10	4.65±0.23
Hydroxyproline	N.D.	N.D.	N.D.	0.63±0.03	0.22±0.01	N.D.	10.73±0.54	3.27±0.16	4.65±0.23
Aspartic Acid	1.75±0.09	3.51±0.18	4.12±0.21	1.25±0.06	3.32±0.17	3.86±0.19	1.72±0.09	4.90±0.25	N.D.
Threonine	4.29±0.21	4.39±0.22	4.71±0.24	2.51±0.13	1.77±0.09	1.93±0.10	1.72±0.09	N.D.	N.D.
Serine	3.70±0.19	2.63±0.13	2.94±0.15	0.94±0.05	0.66±0.03	0.48±0.02	0.43±0.02	N.D.	N.D.
Glutamic Acid	27.68±1.38	25.44±1.27	24.71±1.24	32.60±1.63	32.96±1.65	26.09±1.30	16.31±0.82	26.47±1.32	26.74±1.34
Proline	10.33±0.52	10.53±0.53	10.88±0.54	13.79±0.69	11.28±0.56	10.63±0.53	14.59±0.73	10.78±0.54	12.21±0.61
Lanthionine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Glycine	2.73±0.14	3.07±0.15	3.24±0.16	2.82±0.14	2.88±0.14	2.90±0.14	3.00±0.15	2.61±0.13	2.91±0.15
Alanine	3.70±0.19	3.95±0.20	3.82±0.19	3.13±0.16	3.98±0.20	3.86±0.19	3.00±0.15	3.27±0.16	3.49±0.17
Cysteine	1.75±0.09	2.19±0.11	3.82±0.19	0.63±0.03	4.42±0.22	4.35±0.22	0.86±0.04	5.56±0.28	N.D.
Valine	7.02±0.35	6.58±0.33	6.76±0.34	6.90±0.34	6.86±0.34	6.76±0.34	6.87±0.34	6.86±0.34	8.14±0.41
Methionine	3.12±0.16	3.07±0.15	3.82±0.19	2.51±0.13	5.53±0.28	5.31±0.27	1.72±0.09	6.54±0.33	N.D.
Isoleucine	6.04±0.30	6.14±0.31	5.88±0.29	5.33±0.27	4.42±0.22	4.83±0.24	4.29±0.21	3.27±0.16	4.07±0.20
Leucine	9.55±0.48	10.09±0.50	10.29±0.51	8.15±0.41	7.96±0.40	9.66±0.48	7.73±0.39	7.84±0.39	10.47±0.52
Tyrosine	1.36±0.07	1.75±0.09	1.76±0.09	1.88±0.09	0.88±0.04	3.38±0.17	4.72±0.24	1.63±0.08	5.23±0.26
Phenylalanine	5.65±0.28	6.58±0.33	5.29±0.26	6.58±0.33	5.97±0.30	8.70±0.43	7.30±0.36	6.86±0.34	10.47±0.52
Hydroxylysine	0.58±0.03	1.32±0.07	0.29±0.01	2.82±0.14	1.11±0.06	N.D.	6.01±0.30	2.29±0.11	N.D.
Ornithine	0.58±0.03	0.44±0.02	0.59±0.03	0.63±0.03	0.66±0.03	0.48±0.02	0.43±0.02	0.65±0.03	0.58±0.03
Lysine	6.63±0.33	3.51±0.18	2.94±0.15	3.76±0.19	2.88±0.14	3.38±0.17	2.15±0.11	3.59±0.18	4.07±0.20
Histidine	2.73±0.14	1.75±0.09	2.35±0.12	1.57±0.08	1.11±0.06	1.93±0.10	1.29±0.06	0.65±0.03	1.16±0.06
Arginine	0.78±0.04	N.D.	N.D.	N.D.	N.D.	N.D.	1.29±0.06	0.98±0.05	1.16±0.06
N.D. – not detected									

As shown in **Figure 18**, glutamic acid, proline, leucine, and valine are the most prominent amino acids in recovered hydrolysate. Glutamic acid accounted for about 33% of total amino acids. Additionally, the formation of glutamic acid was highest at more acidic and near-natural pH which could be due to negative charge of R group in its structure. Perhaps, the high concentration of protons or presence of hydroxinium ions triggers more formation of glutamic acid. The recovery of other amino acids such as aspartic acid, serine, cysteine, methionine, and histidine was less than 5%.

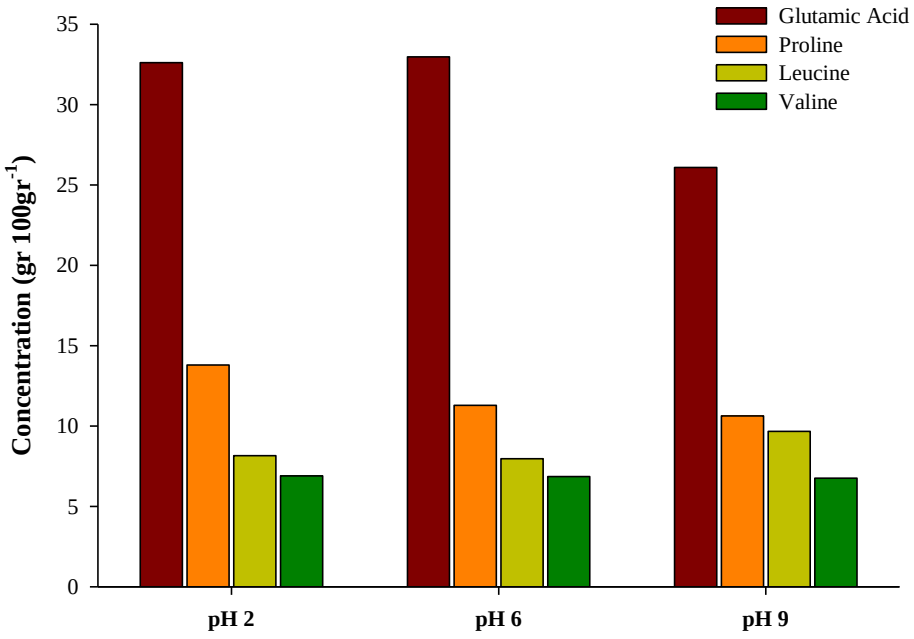


Figure 18. Prominent amino acids (%) in recovered hydrolysate of subcritical treated vacuum evaporated ice-cream wastewater.

5.5. CONCLUSION

Dairy manufacturign has been identifed as one the most ever growong industry in United States due to increasing demand for dairy products. Manufacturing bottled milk and dairy products including milk powder, cheese, yogurt, and concenrtade whey and whey

powedr generate considerable volume of byproducts and waste streams. Complexity of additives, sweeteners, establizers, emulsifiers, and other ingredints used in ice-cream processing generates wastewater which is highly rich in organic materials which induces environmental concerns. Among applied treatment methods for ice-cream WW, subcritical water hydrolysis has shown promising trend in hydrothermal biomass conversion to value-added materials. Subcritical water variables including temperature, pH, total solid content, and polarity of components in the reaction medium have a considerable effect on hydrolysis degree of organic coumpunds. The subcrtical hydrolysis of vacuum evaporated ice-cream WW process disclosed promising results, yielding values of hydrolysis degree from 47 to 106% . The recovered hydrolysates were high in glutamic acid, proline, leucine, and valine. The production of amino acids strongly depends on the hydrolysis conditions (time, temperature, and pH). Glutamic acid was the predominant amino acid in the hydrolyzed fraction (26-33%), followed by proline (11-14%), leucine (8-10%), and valine (6-8%). The recovery of amino acids such as aspartic acid, serine, cysteine, methionine, and histidine was less than 5%. The outcomes of this investigation proved the promising application of subcritical hydrolysis in conversion of organic compounds mainly proteins in ice-cream WW, and to produce a stream rich in amino acids.

5.6. REFERENCES

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CHAPTER 6

ONE-POT SYNTHESIS OF LACTOSE DERIVATIVES FROM WHEY PERMEATE [5]

MARYAM ENTESHARI

2020

6.1. ABSTRACT

The simultaneous production of lactulose (LAU), lactobionic acid (LBA), and organic acids from sweet and acid whey permeate (SWP and AWP) via catalytic synthesis (5% Ru/C) was studied in a continuous stirred-tank reactor. At selected conditions (60 °C, 60 bar, and 600 rpm), a maximum conversion of lactose (37 and 34%) was obtained after 90 min for SWP and AWP, respectively. The highest yield calculated with respect to the initial concentration of lactose for LAU was 22.98 ± 0.81 and 15.29 ± 0.81% after only 30 min for SWP, and AWP, respectively. For LBA, a maximum yield was found in SWP (5.23%) after 210 min, while about 2.2% was found in AWP. Six major organic acids (gluconic, pyruvic, lactic, formic, acetic, and citric acid) were quantified during the one-pot synthesis of lactose.

6.2. INTRODUCTION

Whey is the main byproduct obtained from the manufacture of cheese, yogurt, and milk protein concentrates (Nath et al. 2016). It is the yellowish liquid separated from curds, and it is mainly made of water (~94%), lactose (~5%), proteins (~1%), minerals (~1%), and milk fat (~0.5%) (Prazeres, Carvalho, and Rivas 2012). As a byproduct, whey is concentrated and further fractionated to produce a wide array of products and ingredients with food and pharmaceutical applications. Examples of ingredients derived from whey

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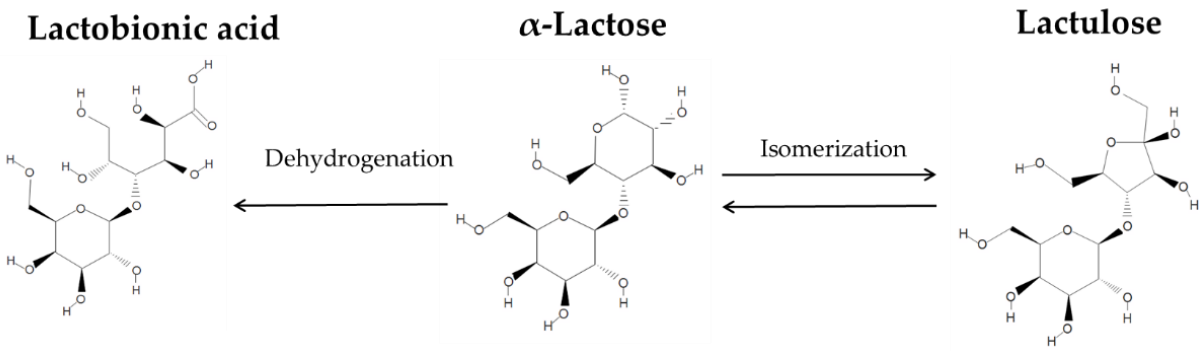
are milk serum protein concentrate, whey powder, whey protein concentrate, whey protein hydrolysate, and whey protein isolate. Manufacturing details on the production of milk protein ingredients can be found elsewhere (Smithers 2008, 2015).

Over the last few years, the number of food formulations containing milk proteins has considerably increased due to the functional and nutritional properties of milk proteins (Livney 2010). Nowadays, products such as sport drinks, desserts, beverages, snacks, dietary, and weight management supplements are formulated with milk proteins. Overall, the manufacture of concentrates of milk proteins involves numerous unit operations, such as thermal treatment, selective fractionation, concentration, and drying. Selective fractionation by means of membrane technology is the key processing step in which the protein fraction is sequentially separated from the whey stream. The separation is performed by multiple filtration stages, where the proteins are concentrated in the retentate, while lactose and minerals are concentrated in permeate stream. The byproduct derived from the production of milk proteins is known as whey permeate, and it contains a considerable amount of lactose (75–80% on dry basis).

Lactose (LA) presents technological challenges, such as poor solubility and low sweetness index, as well as malabsorption by a certain population, which has limited its further utilization (Cheng and Martínez-Monteagudo 2019). Consequently, there is a considerable industrial interest to further utilize LA as a feedstock for the production of lactose-based ingredients (Gänzle, Haase, and Jelen 2008; Martínez-Monteagudo, Enteshari, and Metzger 2019). As a feedstock, lactose has the potential to undergo different

reactions leading to the formation of value-added compounds. **Figure 19** illustrates the simultaneous formation of lactulose (LAU) and lactobionic acid (LBA), two of the most versatile lactose-derived ingredients, with applications in food, dairy, and pharmaceutical



formulations.

Figure 19. Reaction pathway for the formation of lactulose and lactobionic acid via isomerization and dehydrogenation, respectively.

Lactulose (4-O-β-D-galactopyranosyl-D-fructofuranose), an isomer of LA, is a disaccharide made of galactose and fructose. It is used in the treatment of hepatic encephalopathy and chronic constipation (Seki and Saito 2012). Industrially, LAU is synthesized by either homogenous catalysis under alkaline conditions, or by enzymatic synthesis using glycosyltransferases and glycosidases (Schuster-Wolff-Bühring, Fischer, and Hinrichs 2010). Reaction schemes and conditions for LAU synthesis can be found elsewhere (Aider and Halleux 2007). On the other hand, LBA (4-O-β-galactopyranosyl-D-gluconic acid) is an aldonic acid or sugar-acid made up of galactose linked to a gluconic acid (Gutiérrez, Hamoudi, and Belkacemi 2012). The LBA molecule has various functionalities, such as antioxidant, chelating, and humectant, arising from the high number

of hydroxyl groups. The partial oxidation of LA results in the formation of LBA, where the aldehyde group is converted into its corresponding carbonyl group via selective oxidation (Chia, Latusek, and Holles 2008). Most of the LBA commercialized in the world is produced via 1) biocatalytic conversion using dehydrogenase or oxidoreductase, or 2) heterogeneous catalysis using Pd, Pt, or Ru as insoluble catalysts. The manufacturing methods of LBA can be found elsewhere (Gutiérrez, Hamoudi, and Belkacemi 2012).

One common strategy employed in the industrial production of lactose-derived ingredients is the use of purified lactose (monohydrate or pharmaceutical grade) as a feedstock, which minimizes the formation of secondary products. Fundamentally, such a strategy aims to produce a single compound via multi-step flow, involving the formation, separation, and isolation of secondary and primary products. Alternatively, the conversion of lactose through one-pot synthesis represents an innovative utilization of lactose. One-pot conversion involves the synthesis of a mixture of compounds that can be used with minimal separation in the final formulation. This has been exemplified by our group, where a sweetening syrup from aqueous lactose was produced via one-pot synthesis (enzymatic hydrolysis followed by catalytic isomerization) (Cheng, Metzger, and Martínez-Monteagudo 2020; Cheng et al. 2020). Similarly, Zaccheria et al. produced a mixture of sorbitol and dulcitol from lactose via one-pot catalytic conversion. In 2010, Gallezot conceptualized and exemplified the production of a pool of molecules from biomass via one-pot catalytic synthesis. Under the one-pot approach, the high concentration of lactose present in whey permeate can be used as an inexpensive feedstock for a variety of chemical modifications, such as hydrogenation, isomerization, and oxidation.

During the catalytic oxidation of lactose, the first products formed are LBA and LAU, followed by 2-keto-lactobionic acid as a secondary product (Murzina et al., 2008). Preliminary results showed that increasing the oxygen pressure improved the yield of LAU during the oxidation of lactose over Ru/C (Enteshari and Martínez-Monteagudo 2019). It should be highlighted that the production of LAU and LBA was evaluated using lactose monohydrate as a feedstock. Nevertheless, these results present an opportunity for the simultaneous production of LAU and LBA directly from whey permeate. This work aimed at producing LAU and LBA via a one-pot catalytic oxidation from sweet and acid whey permeate.

6.3. MATERIAL AND METHODS

Materials

α-D-Lactose monohydrate (98%, Acros Organics, Fair Lawn, NJ, USA), lactulose (99%, Alfa Aesar, Haverhill, MA, USA), lactobionic acid (97%, Sigma-Aldrich, St. Louis, MO, USA), D-(+)-glucose (99%, Sigma-Aldrich), D-(+)-galactose (99%, Sigma-Aldrich), D-gluconic acid sodium salt (99%, Sigma-Aldrich), pyruvic acid (98%, Sigma-Aldrich), L-(+)-lactic acid (85%, Sigma-Aldrich), formic acid (99%, Fisher Scientific, Waltham, MA, USA), citric acid (99%, Sigma-Aldrich), and orotic acid (98%, Sigma-Aldrich) were purchased from commercial suppliers. Reduced ruthenium supported on activated carbon (5% Ru/C, Alfa Aesar) was purchased from commercial suppliers, and it was used without further preparation. Sweet whey permeate (SWP) was obtained from a regional cheese factory (Valley Queen Co., Milbank, SD, USA), while the acid whey permeate (AWP) was obtained from a Greek yogurt plant (Chobani Co., Twin Falls, ID, USA).

Preparation of samples

A 10% wt./wt. solution of lactose (LAS) was prepared by dissolving D-lactose monohydrate in distilled water. Samples of SWP and AWP were analyzed for pH, total protein, fat content, total solids, and total non-volatile solids. The pH was measured in 10 mL of the sample using a triode epoxy electrode (Orion Versa Star Pro, Thermo Fisher Scientific). The protein content was determined by the Kjeldahl method, while the fat content was measured using the Mojonnier fat extraction, following the guidelines of the Association of Official Analytical Chemists (AOAC) methods 989.05 and 991.20, respectively. Total solids (TS) and total non-volatile solids (TNVS) were determined using the AOAC method 990.20. **Table 16** shows the compositional characteristics of SWP and AWP.

Table 16. Physicochemical characteristics of sweet whey permeate and acid whey permeate. Mean ± standard deviation (n = 3).

Parameters	Sweet Whey Permeate	Acid Whey Permeate
pH	6.23 ± 0.01	4.38 ± 0.02
Total solids (g L^{-1})	87.38 ± 0.25	85.35 ± 0.06
Total non-volatile solids (g L^{-1})	8.23 ± 0.17	9.22 ± 0.16
Fat (g L^{-1})	0.85 ± 0.23	1.81 ± 0.10
Total protein (g L^{-1})	3.02 ± 0.01	3.95 ± 0.08
Lactose (g L^{-1})	76.42 ± 4.3	61.73 ± 6.29
Organic acids (g L^{-1})	7.11 ± 0.35	17.91 ± 0.89

Experimental treatments

A set of experiments was first conducted to evaluate the influence of pressure (15, 40, 60, and 80 bar) and temperature (50, 60, 70, and 80˚C) on the conversion rate of lactose in a 10% wt./wt. solution of lactose. Initial rates (r_o) of lactose conversion were calculated using Equation (1):

$$\boldsymbol{r_o = \frac{d[La]}{dt}\Big|_{t=0}} \quad (1)$$

At selected conditions, the second set of experiments was conducted to evaluate the formation of LAU and LBA directly from sweet whey permeate (SWP), and acid whey permeate (AWP).

One-pot synthesis

The one-pot conversion of LA was carried out in a continuous stirred-tank reactor (BR-300, Berghof Products & Instruments, Berghof, Germany). **Figure 20** schematically represents the one-pot conversion of lactose directly from whey permeate. Features and characteristics of the reactor, as well as the working conditions, have been explained thoroughly in previous work (Enteshari, Martínez-Monteagudo, and Processing 2018). Briefly, the reactor vessel was loaded with 400 mL of either LAS, SWP, or AWP, containing 0.5 g/L of Ru/C catalyst. Then, the vessel was lidded, clamped, and heated up to the target temperature. During heating, the vessel was pressurized with compressed air as an oxidizing agent (99.99% purity, Praxair, Sioux Falls, SD). The initial time of the catalytic reaction was considered when the solution reached the target temperature and pressure. Once the working conditions were obtained, the reaction was monitored over 210

min, withdrawing samples (~15 mL) every 30 min and stored at −20 °C until further analysis. Samples were withdrawn from the sampling port ((6) in **Figure 20**), consisting of a set of needle valves that allowed the subtraction of samples without losing pressure inside the reactor.

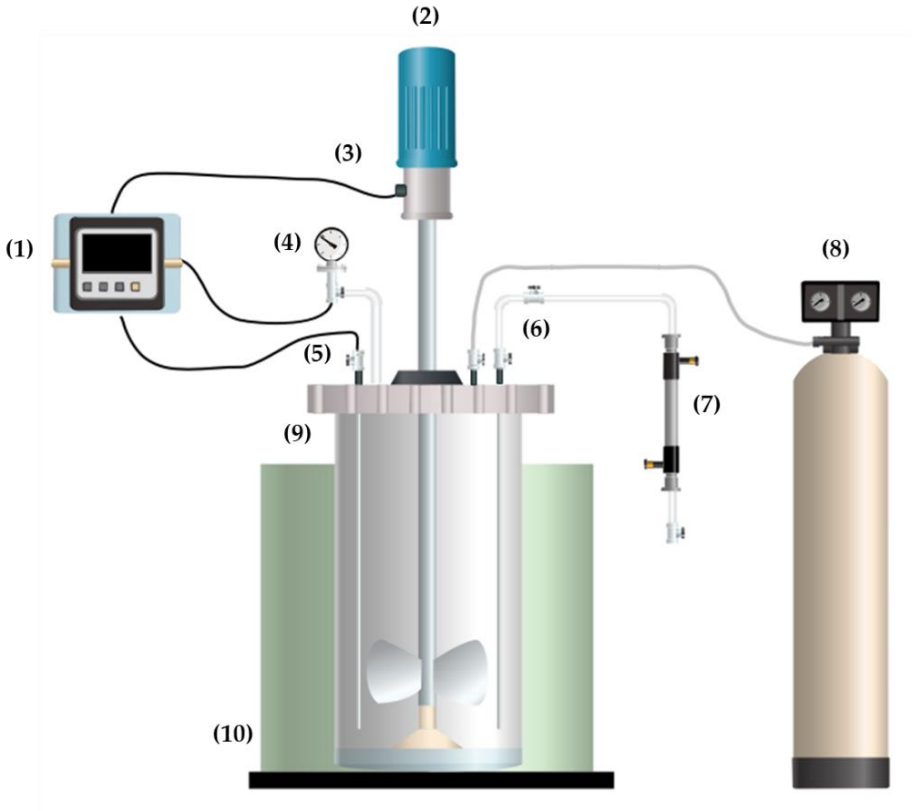


Figure 20. Schematic of the continuous stirred-tank reactor used for the one-pot conversion of lactose permeate: (1) data logger, (2) stirrer, (3) tachometer, (4) pressure gauge, (5) thermocouple, (6) sampling port, (7) cooling system, (8) compressed air cylinder, (9) stainless-steel reactor, (10) electrical heater.

Quantification of reaction products

The reaction products derived were quantified by liquid chromatography-mass spectrophotometry (LC-MS). Firstly, samples withdrawn from the reactor were filtered through a 20-25 μm filter (NO 541 Hardened Ashless, and 110 mm diameter, WhatmanTM

Co., Marlborough, MA, USA) to remove the spent catalyst. Then, the filtered solution was diluted 10-fold with HPLC grade water, followed by centrifugation using ultracentrifugation filters (Amicon® filters, Merc Millipore, Billerica, MA, USA) at 9000 rpm for 10 min (accuSpin Micro 17R, Fisher Scientific, Waltham, MA, USA). After centrifugation, the precipitate was discarded, while the supernatant was used for analysis. An aliquot of 10 µL from the supernatant was injected into a Shimadzu LC system (LC-20AD, Shimadzu Corp, Kyoto, Japan), combined with a Qtrap 5500 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA). Analytes were eluded by means of an HPX-87C column (250x4.0 mm, Bio-Rad Aminex, Hercules, CA, USA) operated at 80 ˚C with a mobile phase consisted of acetonitrile: water (20:80 v/v), at a flow rate of 0.2 mL/min. Mass spectrophotometer analysis was operated in negative mode at a temperature of 500 ˚C, a curtain gas of 30 psi, an ion source gas of 15 psi for nebulizer (GS1), and heater (GS2). Samples were quantified according to HPLC-grade analytical standards.

Quantification of organic acids

The organic acids formed during the catalytic oxidation of LA were analyzed by HPLC, according to the methodology reported by Zeppa et al., with some modifications. An HPLC instrument (Beckman Coulter, Inc., Fullerton, CA) equipped with a solvent delivery module (System Gold® 125), a multichannel wavelength scanning detector (190-600 nm, System Gold 168 detector), and a refractive index detector (RI-2031, Jasco Corporation, Hachioji, Japan) were used for the analysis. The separation of organic acids was performed using an ion exclusion column (ROA-Organic Acid H+ 8%, Phenomenex,

Torrance, CA, USA) heated at 60 ˚C. The mobile phase was a sulfuric acid solution (0.013 N), at a flow rate of 0.5 mL min^{-1}.

Data analysis

Lactose conversion was expressed as a percentage of converted LA into its derivatives, according to Equation (2).

$$\boldsymbol{C_{LA} = \frac{[LA_o] - [LA_t]}{[LA_o]} \cdot 100} \quad (2)$$

where C_{LA}—conversion of lactose (%), $[LA_o]$—initial concentration of lactose (mM), and $[LA_t]$—concentration of lactose at a given reaction time. The formation of LAU and LBA was determined as the product yield (Y_i) according to Equation (3).

$$\boldsymbol{Y_i = \frac{Concentration\ of\ target\ product}{Initial\ concentration\ of\ lactose} \cdot 100} \quad (3)$$

Experimental runs were conducted in duplicate, and all the figures were made with SigmaPlot software V11 for Windows (Systat Software, Inc, Chicago, IL, USA).

6.4. RESULTS AND DISCUSSION

Reaction conditions

Figure 21 shows a graphical representation of the temperature-pressure history during the conversion of LA. Each experimental treatment started with the heading time,

which includes loading, pressurization (arrow (1)), and heating of the vessel. Start of the reaction time (arrow (2)) was considered when both the target temperature and pressure were achieved. Afterward, samples (~15 mL) were withdrawn for analysis at interval times of 30, 90, 150, and 210 min. The end of the reaction time (arrow (3)) marked the beginning of the cooling and depressurization (arrow (4)). Similar characterization of the temperature-pressure history was reported elsewhere (Enteshari & Martínez-Monteagudo, 2018) detailed description of the main variables involved during the one-pot conversion allows one to meaningfully interpret the formation of reaction products, and facilitates comparison with the literature.

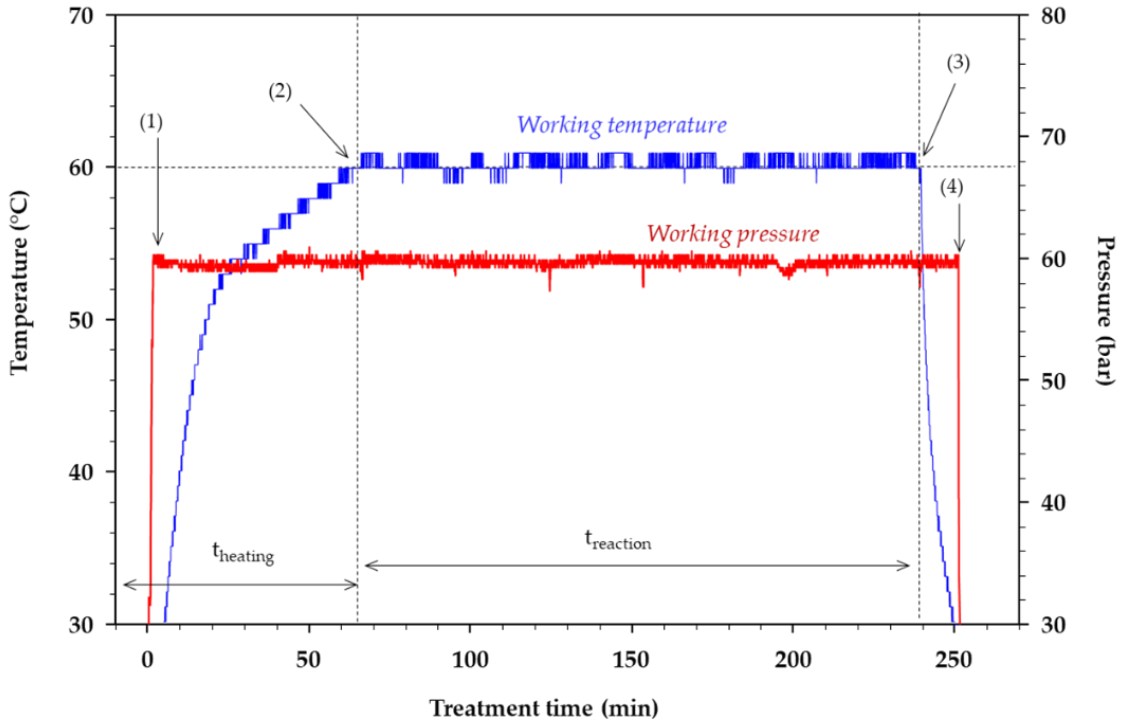


Figure 21. Representative temperature-pressure history during the one-pot conversion of lactose. Reaction temperature was 60˚C and pressure was 60 bar. $t_{heating}$—heating time (min), and $t_{reaction}$—reaction time (min). Arrows (1–4) represent the start of pressurization, the beginning of the reaction time, the end of the reaction time, and the depressurization, respectively.

Initial rates

The initial rate of LA conversion is shown in **Figure 22.** Pressure influence on the initial rates was evaluated at a constant temperature (60˚C, **Figure 22a**), where the initial rates increased with the pressure until it reached a maximum value of 1.95 ± 0.09 mmol/min at 60 bar, while a further increment of pressure resulted in lower values of initial rates (0.70 ± 0.06 mmol/min at 80 bar). This finding is important from an economic and operational perspective, since the application of high pressure will increase the operational costs and be difficult to operate. Influence of temperature on the initial rates of lactose conversion was evaluated at a constant pressure (60 bar, **Figure 22b**). The highest values of initial rates were obtained at 60˚C, and further elevation of the temperature did not significantly increase the initial rates. Overall, the magnitude of the initial rates was higher by increasing the temperature than by the increment of the pressure. This observation is in agreement with the Arrhenius and Eyring theory (Martinez-Monteagudo and Saldana 2014).

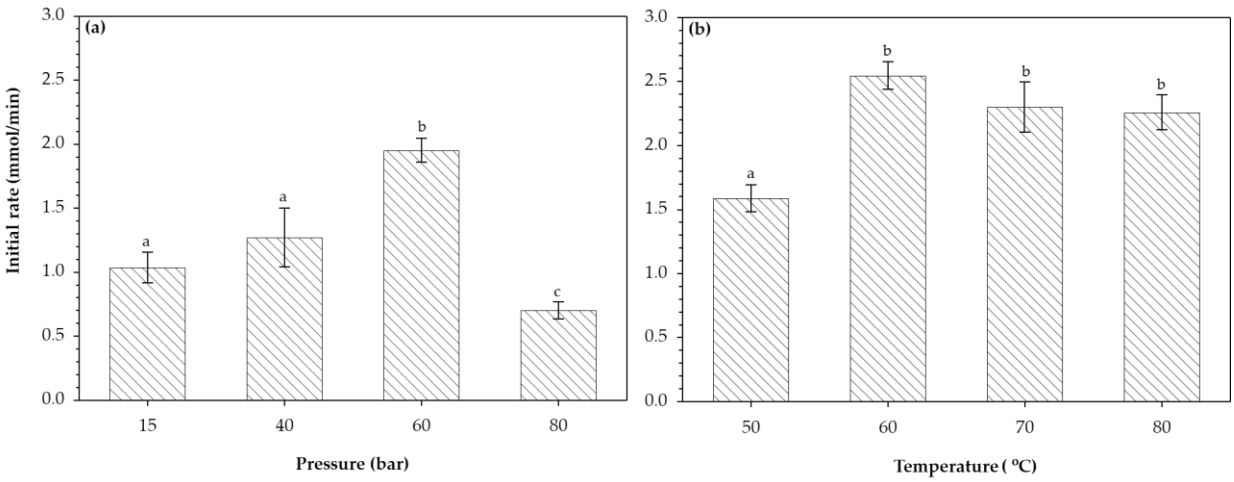


Figure 22. Initial rate of lactose conversion: (**a**) pressure effect (temperature = 60 °C) and (**b**) temperature effect (pressure = 60 bar). Mean ± standard deviation within treatments with different letters are (**a**–**c**) significantly different (P< 0.05) according to Tukey's test.

Conversion and yield

Figure 23 shows the kinetic curves of lactose conversion for SWP and AWP. During the first 30 min of reaction, about 30 and 24% of lactose was converted for SWP and AWP, respectively. After 210 min, the conversion of lactose reached a maximum value of about 37 and 34% for SWP and AWP, respectively. Mäki-Arvela et al. (Mäki-Arvela et al. 2010) reported conversion values of about 90% during the oxidation of LA (0.86 M) under alkaline conditions (pH = 8), using Pd/C as a catalyst. The difference in the conversion values can be explained by the deactivation of the catalyst and the pH of the solution. Indeed, Mäki-Arvela et al. (Mäki-Arvela et al. 2011) reported that Ru/C rapidly underwent deactivation during the oxidation of lactose monohydrate solution (0.86 M), achieving conversion values of 28–32%. In SWP and AWP, the presence of minerals and residual proteins seems to impact the activity of the catalyst negatively. However, the deactivation of Ru/C due to minerals and residual protein needs further experimental evidence.

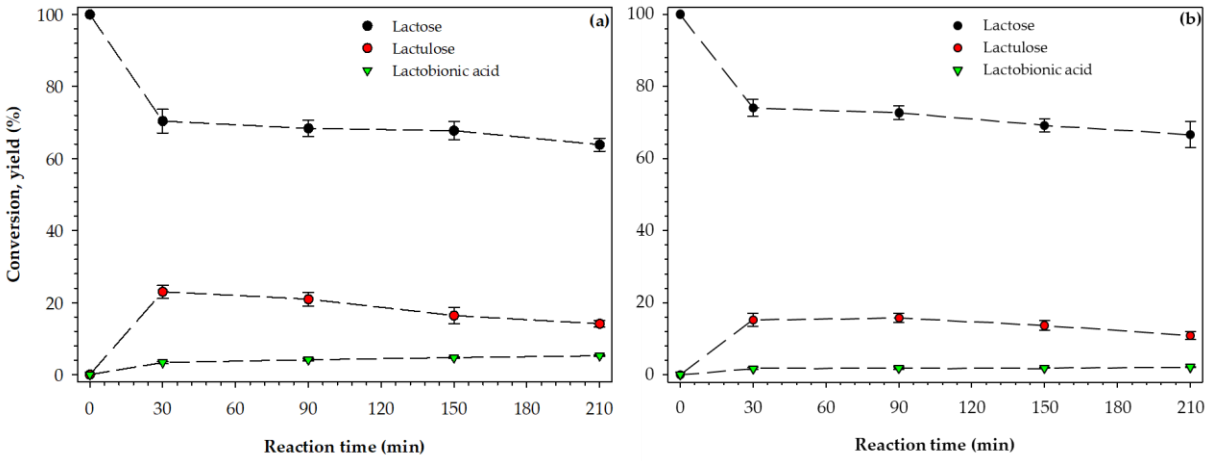


Figure 23. Conversion of lactose and yield of lactulose and lactobionic acid over time in: (**a**) sweet whey permeate (SWP), and (**b**) acid whey permeate (AWP). Temperature = 60˚C and pressure = 60 bar, and stirring rate 600 rpm.

Figure 23a,b showed the formation curves for LAU and LBA corresponding to SWP and AWP, respectively. In untreated samples of SWP and AWP, the concentration of LAU and LBA was below the detection limit. An increment in the yield values of LAU was observed within the first 30 min of reaction, 22.99 ± 0.81 and 17.29 ± 0.96% for SWP and AWP, respectively. The relatively high yield values for SWP is not surprising, since the pH of the SWP (6.23 ± 0.01) favored the formation of LAU. Seo et al. (Seo, Park, and Han 2015) reported yield values of LAU of 29% (90˚C and 20 min of reaction) during the isomerization of cheese whey, using Na_2CO_3 (0.5%) as a catalyst. As the reaction proceeded, the yield values decreased to 14.13 ± 0.13 and 10.93 ± 0.07% for SWP and AWP, respectively. The observed reduction in the yield of LAU by increasing the reaction time is due to the hydrolysis of LAU and subsequent formation of organic acids. The mechanism of lactose isomerization consisted of epimerization and aldose–ketose interconversion. Such a network of reactions is known as Lobry de Bruyn-van Ekenstein transformation. Hajek et al. (Hajek et al. 2013) have studied the isomerization of lactose in an alkaline environment.

In the case of LBA, the yield for the SWP (**Figure 23a**) increased asymptotically with the reaction time, reaching a value of 5.23 ± 0.02%. Similar behavior, but less pronounced, was observed in the yield values of LBA for AWP (2.15 ± 0.15%). The relatively high yield of LBA for SWP, nearly 2-fold than AWP, is not surprising, since the pH of SWP (6.23 ± 0.01) favored LBA formation. It is thought that acidic conditions hinder the interaction between molecular oxygen and LA moieties, affecting LBA formation. The role of pH during the catalytic oxidation of LA has been discussed elsewhere (Tokarev et al.

2007; Gutierrez, Hamoudi, and Belkacemi 2011). Mechanistically, the oxidation of aldehydes is consensually thought to occur in two steps, hydration of the aldehyde group and subsequent dehydrogenation to produce their corresponding acid (Besson and Gallezot 2000).

Formation of glucose, galactose, and organic acids

The conversion values of lactose, as well as the yield values of LAU and LBA (**Figure 23**), suggest the formation of other products derived from hydrolysis, oxidation, and degradation. These products were divided into three groups—monosaccharides, sugar acids, and organic acids (**Figure 24**).

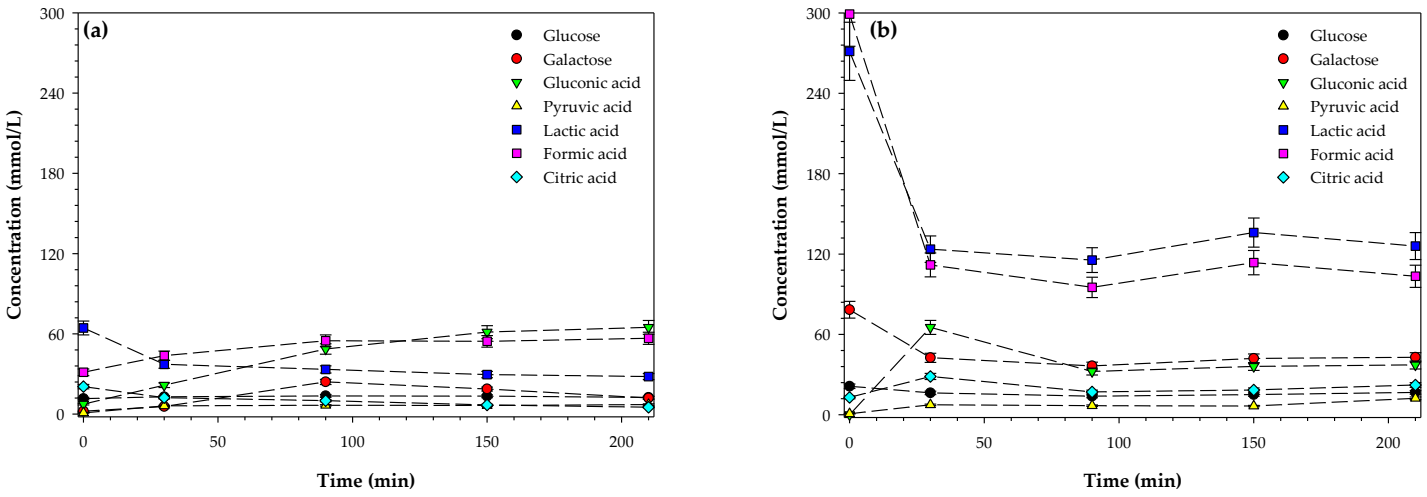


Figure 24. Formation of organic acids over time in: (**a**) sweet whey permeate (SWP), and (**b**) acid whey permeate (AWP). Temperature 60˚C, pressure 60 bar, and stirring rate 600 rpm.

The first group of compounds is monosaccharides (glucose and galactose) that are formed due to the hydrolysis of lactose, breakage of the glycosidic linkage. The initial concentration of glucose and galactose was higher in AWP (about 21 and 78 mmol/L, respectively) than that of SWP (about 4.61 and 2.21 mmol/L, respectively). Acid whey permeate is the byproduct of yogurt manufacture (Talebi et al. 2020), and it is characterized by a relatively high concentration of organic acids. As the reaction proceeded, the glucose in SWP (**Figure 24a**) gradually increased with time, reaching a maximum of about 13 mmol/L after 90 min. Subsequent progression of the reaction resulted in a lower concentration of glucose (about 9 mmol/L after 210 min). A similar trend was observed for galactose in SWP (Figure 6a), where a maximum concentration (24 mmol/L) was obtained after 90 min, followed by a reduction in the concentration with the progression of the reaction. On the other hand, the concentration of glucose and galactose in AWP (**Figure 24b**) gradually decreased with time from about 21 and 78 mmol/L at the beginning of the reaction to 16 and 42 mmol/L after 210 min, for glucose and galactose, respectively. It appears that lactose underwent hydrolysis to produce glucose and galactose that further oxidized to form their respective sugar acids, gluconic and galactonic acid.

The sugar acids represent the second group of components derived from the catalytic conversion of lactose. In SWP, the concentration of gluconic acid increased with time until it reached a plateau at 150 min (60 mmol/L). Afterward, the concentration of gluconic acid marginally increased up to 66 mmol/L after 210 min. In the case of AWP, the concentration of gluconic acid reached a maximum value of 65 mmol/L only after 30 min, followed by a reduction to about 30 mmol/L. Chemical process for the production of gluconic acid

consisted of catalytic oxidation of a concentrated glucose solution under alkaline conditions (Pal, Kumar, and Banerjee 2016).

The third group of components under consideration corresponds to organic acids. Overall, the concentration of organic acids in AWP was higher than that of SWP. Formic and lactic were the most predominant organic acids (299 and 270 mmol/L, respectively) in AWP. This observation is not surprising, since acid whey is derived from the formation of lactic acid bacteria, whose main product is lactic acid. A general trend was observed in SWP, where the concentration of organic acids increased slightly with the reaction time (**Figure 24a**). Citric, acetic, and uric acids were found at relatively low concentrations. These findings are in agreement with the earlier studies on the formation of isosaccharinic acids and organic acids (Seo, Park, and Han 2015).

Organic acids are formed from the breakdown of glucose and galactose. More specifically, lactic acid is formed as the primary byproduct of the catalytic oxidation of glucose (Comotti et al. 2006). The catalytic oxidation of glucose over bimetallic catalysts has been recognized as an efficient alternative for the production of gluconic acid. The oxidation of glucose occurs via a carbonyl conjugated radical mechanism due to the formation of H_2O_2, which in turns is formed by the presence of oxygen in the aqueous media (Yan et al. 2020). However, the mechanisms of glucose oxidation strongly depend on the pH of the medium and the type of catalysts (Comotti et al. 2006). On the other hand, the concentration of organic acids in AWP showed an increase with time for citric and pyruvic acid, while the concentration of formic and lactic acid decreased with time (**Figure**

24b). Citric acid and pyruvic acid are formed due to glucose breakdown, which subsequently formed oxalic acid and others.

Product distribution

Figure 25 exemplifies the one-pot synthesis of lactose derivatives, where a pool of molecules synthesized from SWP (**Figure 25a**) and AWP (**Figure 25b**) was plotted at different reaction times.

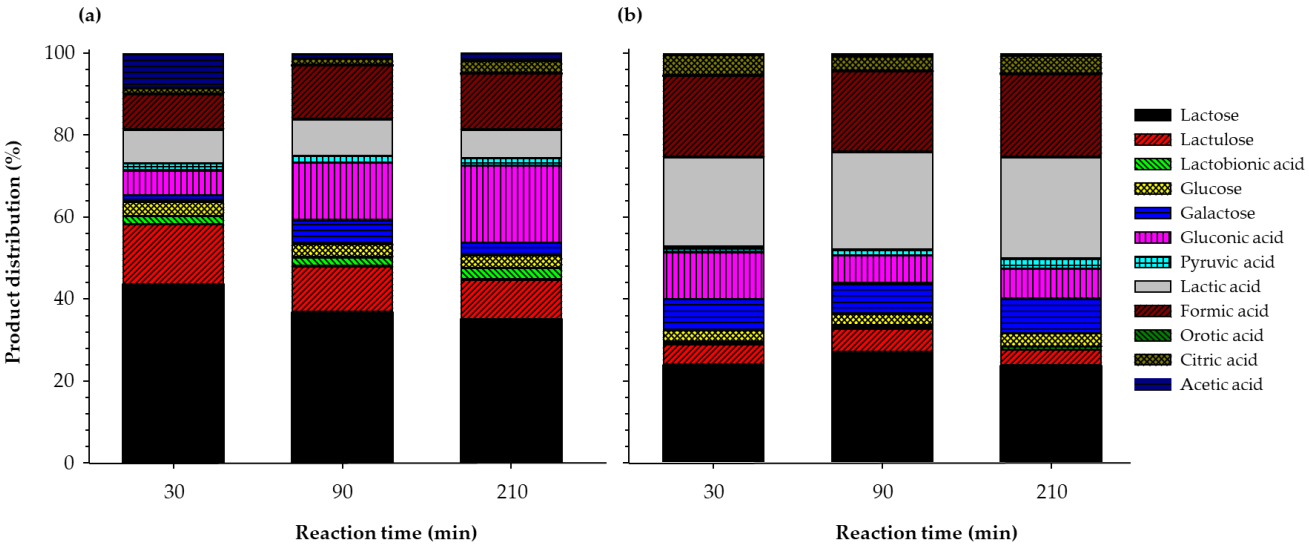


Figure 25. One-pot synthesis of lactose derivatives from (**a**) sweet whey permeate and (**b**) acid whey permeate.

In SWP, the more predominant compounds were lactose (43–35%), gluconic acid (6–18%), LAU (14–9%), formic acid (8–13%), and lactic acid (8–7%), whose concentration varied with the reaction time. For AWP, lactose (27–23%), lactic acid (21–24%), formic acid (19–20%), gluconic acid (11–7%), and LAU (5–3%) were the most predominant compounds. It is worth mentioning that these results should be considered cautiously because the final concentration is influenced by a number of factors, including pH,

temperature, the composition of the stream, the type of catalyst, and catalyst load. Thus, the concentration of a given group of components may be optimized in order to maximize their production according to the study of reaction kinetics. The concept of one-pot was first introduced by Kolb et al. (Kolb, Finn, and Sharpless 2001), who described a click-chemistry in which a set of chemical transformations yielded higher efficiency, fast rates, and simple product isolation. Soon after, the concept of click chemistry quickly found widespread application in various research areas, including organic chemistry, polymer synthesis, petroleum, and biorefinery.

Currently, there are several terminologies to describe multi-step reactions that take place in one pot, such as domino reaction, cascade reaction and tandem reaction. One-pot synthesis is effective because several chemical transformation steps can be carried out in a single pot, while circumventing several purification procedures at the same time. Thus, a one-pot procedure can minimize chemical waste, save time, and simplify practical aspects.

6.5. CONCLUSION

The one-pot conversion of lactose streams resulted in the formation of four main group of components—rare carbohydrate (lactulose), monosaccharides (glucose and galactose), sugar acids (gluconic acid), and organic acids. The final distribution and their concentration differ from SWP and AWP, where lactulose was favored in SWP, and organic acids were favored in AWP. The synthesis of a pool of molecules through a one-pot approach represents an alternative approach for the utilization of streams of lactose.

Upon further separation, organic acids can be used as building blocks of numerous applications in the manufacture of herbicides, bioplastics, and biofertilizers.

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CHAPTER 7

OVERAL CONCLUSION

An increase in consumer demand of dairy food products has welcomed advanced technologies into dairy industry to enhance the production yield. Perhaps, success of dairy industry and its sustainability is strongly linked to implementing advanced operation units. In this regard, the role of membrane processing in growth of dairy industry is unavoidable. Implementing filtration technologies and fractionation methods to produce concentrated milk protein fractions requires consumption of massive volume of water and accordingly considerable amount of wastewater could be generated during processing. Therefore, with increasing trend in manufacturing high-protein dairy products including yogurt, cheese, and ice-cream to meet consumer needs, dairy industries are facing with management of byproducts and wastewater treatment. Hence, identifying and implementing valorization methods to utilize dairy byproducts for production of value-added ingredients has proposed new profitable corridors for dairy manufacturers. Also, wastewater management through conversion of dairy streams to functional materials and feedstocks have opened new windows for dairy producers to comply with environmental regulations and guarantee their sustainability.

Ice-cream production effluent is identified as one of the most important sources of organic contamination from dairy industry. Presence of fat, emulsifier, proteins, sweeteners, fruits, and nut particles in ice-cream wastewater makes its treatment so challenging. Combination of biological and physiochemical methods have been used to breakdown the emulsion of ice-cream wastewater and removing its organic materials

before discharging into the sewage system. So, identifying appropriate treatment methods are highly demanded to assist ice-cream manufactures follow with environmental regulations. Recently, subcritical water hydrolysis has brought advantages for converting biomass to valuable materials. Proteins and carbohydrates such as casein and lactose, are identified as two main sources of organic matter content in ice-cream wastewater which contributes in high biological and chemical oxygen demands. The effectiveness of subcritical technique in hydrolysis of ice-cream wastewater has been disclosed in this study. Also, functional properties of recovered hydrolysates including antioxidant and antihypertensive activities have shown potency of subcritical treatment for converting wastewater into valuable compounds.

Cheese manufacturing has been recognized as one of high-ranking effluent from dairy industry. The high level of organic materials in cheese effluent is derived from its lactose, protein, and fat content. With aid of filtration and membrane processing, the protein content or mainly whey proteins can be recovered in retentate for further applications as whey protein concentrate and isolate. After protein separation, the remained liquid is called whey permeate which is rich in lactose and causes many severe environmental problems. Lactose can be separated from whey stream as lactose crystals for food and pharmaceutical applications. However, lactose powder manufacturing is not profitable due to its poor solubility and relevant issues for lactose intolerance. Among available methods for lactose conversion, one-pot catalytic reactions have proposed promising trend in lactose utilization directly from whey stream.