Instituto Tecnológico y de Estudios Superiores de Monterrey

Campus Monterrey

School of Engineering and Sciences

SOLID-STATE FERMENTATION FOR ENHANCED EXTRACTABILITY OF NUTRACEUTICAL COMPOUNDS OF TOMATO BY-PRODUCTS

A thesis presented by

Luis Octavio Cano y Postigo

Submitted to the

School of Engineering and Sciences

in partial fulfillment of the requirements for the degree of

Master of Science

In Biotechnology

Monterrey Nuevo León, December 2nd, 2020

DEDICATION

“The mediocre teacher tells. The good teacher explains. The superior teacher demonstrates. The great teacher inspires.”

-William Arthur Ward

I dedicate this thesis to all the teachers who have touched my life.

And to the greatest teachers of all, my parents, for it is due to their combined effort that I am where I stand now, and the accomplishments of this work are as much theirs as they are mine. I am thankful for every teacher's inspiration and where it has brought me, and I can’t wait to see where it will take me next. I will always carry a part of them with me wherever I go.

ACKNOWLEDGEMENTS

"No one who achieves success does so without the help of others. The wise and confident acknowledge this help with gratitude."

-Alfred North Whitehead

First, I would like to express my deepest gratitude and admiration to Dr. Tomás García for being both a guide and an inspiration through all this journey, always encouraging me and patiently teaching me new things. This project could never have been accomplished without his constant effort and support. You showed me what makes a passionate educator and a good mentor, and I am confident that I could not have been paired with someone better. For that, I will always be thankful.

To my co-advisor, Dr. Daniel Alberto Jacobo, for sharing his knowledge, expertise, and passion. You are a clear example of what Mexico needs to keep growing and learning. I would also like to thank the committee members, Dr. Luis Eduardo García Amézquita and Dr. Diego Eloyr Navarro López, for accepting to be part of this and for your advice and kindness.

Thanks to CONACyT for the scholarship and the Tecnológico de Monterrey for the academic scholarship.

To Ale Hurtado, for your constant support and unconditional help with the UHPLC analysis. I genuinely appreciate your help, patience, and friendship. To those excellent scientists and friends who started this journey along with me: Alberto, Mari, and Rebeca thank you all for the fun moments and congratulations. We did it!

Thanks to my friends Seferino, Juan Pablo, Diego, Benjamin, Yoshua, for being always there to listen to me and help me in my lowest points, your companionship, and friendship is a treasure that I will forever cherish. Thank you all for being such an inspiration to me, for being by my side at all times, for your constant motivation and support.

And thanks to my parents and family for always believing in me, for always celebrating my successes, and accompanying me through all this process. I could not have done it without you. I love you all

SOLID-STATE FERMENTATION FOR ENHANCED EXTRACTABILITY OF NUTRACEUTICAL COMPOUNDS OF TOMATO BY-PRODUCTS

BY

LUIS OCTAVIO CANO Y POSTIGO

ABSTRACT

Tomato pomace is a food industry by-product produced in significant quantities, mainly composed of the tomato seeds and peel, and pulp residues. This organic by-product is both rich in functional nutraceutical compounds like carotenes and phenolic compounds, which have proven to offer antioxidant, anti-inflammatory and anti-carcinogenic properties. Nutraceutical compounds can be extracted by a wide variety of techniques, like with the use of organic solvents, enzymatic hydrolysis, supercritical fluid extraction, or ultrasound-assisted extraction. Solid-state fermentation (SSF) is a bioprocessing technique that has previously been used to generate compounds of interest. However, it has not been used as a specialized pretreatment for extracting nutraceutical compounds already present in the substrate. Based on that, this study aimed to evaluate the potential effectiveness of an SSF as part of pretreatment to increase the extraction of nutraceutical compounds like lycopene and phenolic compounds. The extractions of SSF were directly compared with other pretreatments, including industrial-grade enzymes and enzymatic cocktails from fungi. Two fungal strains, *Aspergillus niger* ATCC 6275 and *Rhizopus oryzae* BIOTEC00X (isolated from agave pomace and identified by MALDI-TOF mass spectrometry), were used for the fermentation of tomato pomace for three 3 days at 30 °C and 80% humidity. Then, the tomato pomace was dried until constant weight and was subsequently subjected to an extraction using hexane for total carotenes and methanol for total phenolic compounds. Spectrophotometric measurements were used as a quick screening method to determine the best extraction performance. Results showed that SSF as a pretreatment helped to obtain between 50-60% more carotenes than the control, while commercial enzymes offered an increase of 107%. The best treatment for phenolics was obtained with an enzyme-rich broth, with an increase of 90-97% in extracted total phenolics, followed by the SSF with an increase of a 24-38% over the controls. Individual phenolic compounds were identified and quantified by the application of ultra-high-performance liquid chromatography (UHPLC).

This technique allowed the tentative identification of 15 compounds, from which naringenin derivative III, protocatechuic acid and feruloylquinic acid were the most susceptible to variate depending on the pretreatment applied to the tomato pomace. It is concluded that SSF increases the extraction of nutraceutical compounds present in tomato by-products and could become an alternative method of extraction at an industrial scale, given that it is an innovative and green technology.

USO DE LA FERMENTACIÓN EN ESTADO SÓLIDO PARA MEJORAR LA EXTRACTABILIDAD DE COMPUESTOS NUTRACÉUTICOS DE SUBPRODUCTOS DE TOMATE

POR

LUIS OCTAVIO CANO Y POSTIGO

RESUMEN

El bagazo de tomate es un subproducto que se genera en cantidades importantes durante el procesado industrial del tomate, y que está compuesto principalmente por las semillas y piel de tomate, además de restos de pulpa. Estos subproductos son ricos en compuestos nutracéuticos funcionales como carotenos y compuestos fenólicos con propiedades antioxidantes, antiinflamatorias y anticancerígenas, entre otras. La extracción de compuestos nutracéuticos se puede realizar mediante una amplia variedad de técnicas y tratamientos, como el uso de disolventes orgánicos, la hidrólisis enzimática, la extracción con fluidos supercríticos o la extracción asistida por ultrasonidos. En este sentido, se propone la fermentación en estado sólido (SSF) como una técnica de bioprocesamiento a explorar. Aunque la SSF se ha utilizado para generar compuestos bioactivos de interés, no se ha empleado como un pretratamiento especializado para extraer compuestos nutracéuticos ya presentes en el sustrato. Basado en los anterior, este estudio tuvo como objetivo evaluar la efectividad potencial de una SSF como parte de un pretratamiento sobre bagazo de tomate para aumentar la extracción de nutracéuticos como el licopeno y los compuestos fenólicos. Las extracciones de muestras tratadas con una SSF se compararon directamente con otros pretratamientos, que incluyen enzimas comerciales de grado industrial y cócteles enzimáticos de hongos obtenidos por fermentación sumergida. Se simuló la generación de bagazo de tomate resultante de un procesado industrial, del cual se analizaron diversas cualidades fisicoquímicas como el pH, la acidez titulable, sólidos solubles y totales, así como el contenido de licopeno y compuestos fenólicos totales. Posteriormente, se realizó un estudio del crecimiento y capacidad enzimática de dos cepas

de hongos, *Aspergillus niger* ATCC 6275 y una cepa aislada de bagazo de agave, *Rhizopus oryzae* BIOTEC00X, la cual fue identificada mediante espectrometría de masas MALDI-TOF. Ambas cepas se utilizaron para la fermentación de bagazo de tomate durante tres 3 días a 30 °C y 80% de humedad. Luego, el tomate se secó hasta peso constante y, posteriormente, se sometió a una extracción utilizando hexano para carotenos totales y metanol para compuestos fenólicos. Se utilizaron mediciones espectrofotométricas como método de detección rápida para determinar el mejor rendimiento de extracción. Los resultados mostraron que la SSF como pretratamiento ayudó a obtener entre un 50-60% más de carotenos que el control, mientras que las enzimas comerciales ofrecieron un aumento del 107%. El mejor tratamiento para la extractabilidad de compuestos fenólicos se obtuvo con un caldo rico en enzimas, con un incremento del 90-97% en los fenoles totales extraídos, seguido del SSF, con un incremento del 24-38% sobre los controles. La identificación y cuantificación de componentes fenólicos individuales también se llevaron a cabo mediante la aplicación de cromatografía líquida de ultra alta eficiencia (UHPLC). Esta técnica permitió la identificación tentativa de 15 compuestos, de los cuales el derivado de naringenina III, el ácido protocateico y el ácido feruloilquínico fueron los más susceptibles de variar dependiendo del pretratamiento aplicado al bagazo de tomate. Se concluye que la SSF incrementa la extracción de compuestos nutracéuticos presentes en los subproductos del tomate y podría convertirse en un método alternativo de extracción a escala industrial, dado que es una tecnología innovadora y verde.

LIST OF FIGURES

LIST OF TABLES

CONTENTS

CHAPTER 1 GENERAL INTRODUCTION

1.1 Introduction

Nowadays there is an increasing amount of food industry by-products that end up discarded without further processing and utilization. One such by-product is the tomato pomace, a conglomeration of seeds and skin of the tomato. This by-product has been reported to harbor a high concentration of nutraceutical compounds, such as lycopene and phenolic compounds, which could be extracted and concentrated for its subsequent application as either a food additive or a nutritional supplement. There is still a lot of explorable options in terms of extraction optimization and alternative techniques that could tackle the typical problem of feasibility and scalability, while still retaining an environmentally friendly application.

Pectinase represents a high value enzyme due to its ability to degrade pectin, which are part of the structural polysaccharides present in the vegetable cells, a key component of the plant tissue integrity. This has been applied with the objective of clarifying fruit juices and wine, remove the peel from citrus fruit and to improve oil extraction and bioactive compounds from plant tissue as part of a pretreatment (Farinas, 2015). Many substrates can be used to recreate a SSF with fungus, like coffee husk, soy bean, cranberry, cocoa, orange and lemon peels, which serves as a precedent that points to a potential utilization of the tomato pomace in the same manner (Soccol et al., 2017).

A novel proposition for the utilization of SSF, in contrast to simply utilize it as a production process for bioactive compounds, is to apply it as pretreatment process, with the objective of increasing the yield of extraction for both classic and emerging extraction strategies. The fundamentals of using SSF as a pretreatment are exactly the same as the case of an enzyme assisted extraction (degradation of the cell wall), but instead of adding enzymes that were previously extracted from another source, SSF would utilize the very same extracellular enzymes that are produced as part of the fermentation (Bhanja Dey & Kuhad, 2014). SSF by design does not require constant agitation for a proper propagation of the fungal mycelium which simplifies its execution compared to an enzymatic degradation. At the same time, SSF can be done without any kind of acclimatation of the substrate, other

than temperature and water content, whereas an enzymatic process will usually require the addition of buffers and other co-agents for proper enzymatic activity.

Aspergillus niger is known for its ability to produce large amounts of both cellulolytic and pectinolytic enzymes as part of the fermentation. On general terms, the fermentation only requires the presence of the substrate with the appropriate water content and an initial inoculation with the fungi spores (previously quantified and dissolved to optimal levels) (Sundarram & Murthy, 2015). Subsequently, the fungi will start to grow and form mycelia over the surface of the substrate. The mycelia expansion, both in terms of space occupied and area of contact, will become the main parameter that will determine the effectiveness of the pretreatment, as it from the mycelia where the enzymes will be excreted and will star to work (Ramos Sanchez, 2015). Unexplored parameters like growth speed, mycelia density and mycelia strength through time will need to be considered, given that on the pretreatment phase it will represent the channel of distribution for the enzyme, so the desired behavior would be a fast and far reaching mycelium, without forgetting that that the same mycelium could end up becoming an important physical barrier that needs to be dealt with at the time of extraction.

Phenolic compounds are found inside the matrix of plants in three ways: free, insoluble-bound, and soluble-bound (conjugated). The conjugated fraction is esterified to soluble compounds like some low molecular weight carbohydrates, lipids, or proteins, while insoluble fraction is found to be esterified to more complex cell wall components (Londoño-Hernández et al., 2017). Unfortunately, insoluble-bound phenolics cannot be readily extracted with most conventional extraction protocols. They can only be liberated from the matrix by the process of hydrolysis, such as an acid, alkaline, or enzymatic hydrolysis (Williams et al., 2017). Thus, SSF involving an enzymatic hydrolysis can be applied to improve the extraction yield of phenolic compounds.

Until now, very little has been explored in the field of using SSF as an actual pretreatment for subsequent extraction of bioactive compounds. The common application of SSF as a production process, both for active enzymes and compounds of interest, represents the main focus for this technique, which opens up a new and interesting line of investigation. The only relevant example for the potential use of SSF is presented by (Jamal et al., 2017), where they utilized SSF with *A. niger* as a pretreatment process for the subsequent extraction of lycopene from tomato pomace. The isolated results of this research

present a positive prospect for this new application of SSF. However, there are still some avenues worth researching, particularly in the areas of optimization for the incubation and fermentation parameters, the implications for the utilization of this pretreatment with other types of extraction processes, and the potential utilization of different strains of microorganisms.

1.2 Hypothesis

This dissertation hypothesized that tomato pomace is an accessible by-product rich in nutraceuticals like lycopene and phenolic compounds. By applying an SSF with different strains of fungi as part of a pretreatment, the tomato pomace will release a higher number of nutraceuticals due to the release of cell wall-bound compounds present in the substrate. Therefore, nutraceuticals present in the SSF-treated tomato pomace could be more easily extractable, resulting in a higher recovery of valuable components.

1.3 General Objective

The general objective of this thesis was to evaluate the extractability of lycopene and phenolic compounds of tomato pomace by using solid-state fermentation, in comparison with other pretreatments like submerged fermentation or commercial enzymes.

1.4 Specific Objectives

The specific objectives for the present thesis were the following:

1) To characterize the physical and physico-chemical (total weight, by-product content, total solids, soluble solids, pH, titratable acidity) properties, and bioactive content (lycopene and total phenolics) of a tomato pomace batch, obtained in the laboratory simulating the same conditions as the industrial tomato processing.

2) To study the growth and mycelium generation of *Aspergillus niger* ATCC 6275 and a fungal wild strain (*Rhizopus oryzae* BIOTEC00X) when growing under solid-state and submerged fermentation conditions with tomato pomace as substrate, as well as determine the optimal incubation period for maximum enzymatic activity (quantified by DNS methodology).

3) To evaluate the pretreatment effects of commercial enzymes (cellulase, pectinase and Rapidase ®), submerged fermentation (enzyme rich fermentation broth) and SSF in the extractability of lycopene and total phenolic compounds of tomato pomace by spectrophotometry techniques.

4) To characterize and quantify the individual phenolic content of treated tomato pomace extracts by ultra-high-performance liquid chromatography (UHPLC).

1.5 Thesis Structure

The present thesis is composed of 4 chapters. Chapter 1 presents a general introduction to the topic addressed in this thesis. Chapter 2 consists of a review of the current literature regarding the nature of the agro-industrial by-product generation, its different types, and the potential to obtain derived high added value products. There is also an in-depth description of different extraction and pretreatment techniques to obtain nutraceutical compounds, highlighting the lycopene extraction with different techniques. An overview of SSF is also covered.

Chapter 3 presents the results of this thesis investigation regarding the impact of different pretreatments on the extractability of nutraceutical compounds, as well as the characterization of the growth and degradative activity of two fungi strains (*A. niger* ATCC 6275 and *R. oryzae* BIOTEC00X*).*

Finally, based on the results presented herein, Chapter 4 summarizes a series of general conclusions and recommendations for this and future studies.

CHAPTER 2 LITERATURE REVIEW

2.1 Food industry by-products

A great number of food by-products, also known as by-products, are produced annually by the world's food industries. This is a result of the expansion of the global population at an exponential rate. As such, there is a significant demand for food products to meet the population needs. Food by-products are any edible food products that have been discarded, deteriorated, or consumed by pests and the non-edible or undesirable portion of the food. Food industries suffer from a lack of innovation in developing effective waste management strategies and more sophisticated processing strategies to generate less waste volume. Food industry by-products are characterized for having high nutritional content, which can become a severe problem in the long run, given that they can putrefy and become a breeding ground for disease-causing organisms (Ravindran and Jaiswal 2016). In addition, these wastes cannot be safely and efficiently disposed of in the environment, given their poor biological stability, high concentration of organic compounds, poor oxidative stability, and optimum enzymatic activity. Constant technological advancements help reduce food by-product generation, but it is equally important to focus on existing accumulated food by-products.

Industries usually decide to dispose of the generated waste, while the minority looks to reprocess the waste and obtain a functional food ingredient. This disparity is generally caused by the lack of a proper processing line for the food by-products and a technological gap for an efficient and profitable extraction yield of the functional compound. A percent of the food by-product (38%) occurs during the food processing stage and is divided into three big groups (Martins et al., 2017). The first is food by-product from seafood, including skins, bones, oils, and blood. The second is the wastes from animal-based industries, which generate fats, bones, viscera, and hides. Finally, there are the wastes that come from fruit and vegetable processing, which generate peels, stems, shells, bran and seeds, and trituration residues after the extraction and separation of sugars, juice, starch, and oils (Kruczek et al., 2016).

The fruit and vegetable industry are probably the most immediately exploitable in terms of usable food by-products. For example, apple pomace is a by-product mainly

generated in the juice industry due to biomass generated after the apple fruit processing. The pomace contains high quantities of total dietary fibers (74%), which have been demonstrated to have a critical role in improving and managing the gastrointestinal system. Orange pomace is known to be rich in fibers, which can be utilized as a part of an additive for products that lack water/oil holding capabilities, as well as having a low-fat content and high mineral content (Daliu et al., 2018). In general, more than 90% of the generated biomass from the juice industry is skin or pulp tissue which is rich in cell wall polysaccharides (like cellulose, hemicellulose, lignin, and pectin) and phenolic compounds bound (flavanols and phenolic acids). Unfortunately, due to the nature of high moisture of pomace residues, they are particularly susceptible to developing an undesired microorganism growth, so their useful lifespan is short unless dried, increasing shelf life and expanding the possible storage possibilities. Recycling the pomace or processing it for its nutritional components will result in a direct cost saving in a factory (Ben-Othman et al., 2020).

2.1.1 Tomato by-products

The tomato is one of the most widely cultivated vegetables worldwide, and more than 80% is processed and consumed in various products like tomato juice, paste, purée, ketchup, sauce, and salsa. For processing, tomatoes are washed, sorted, and sliced. Sliced tomatoes undergo a hot- or cold-break method for juice preparation (Shi & Le Maguer, 2000). Juice from tomatoes is usually obtained using a screw or paddle extractor. In the manufacturing of other tomato products such as pulp, puree, paste, and ketchup, tomato juice is concentrated with steam coils or vacuum evaporators. Either thermal or mechanical treatments are often involved in these processes, which may affect tomato product quality. During the tomato processing, the majority by-product will be the tomato pomace, representing at most 4% of the fruit weight. As previously explained, tomato pomace consists mainly of seeds and peel (pericarp), and fiber is the primary compound of tomato pomace, consisting form 25%-50% on a dry weight base. Its value is directly related to its rich concentration of nutraceutical components like lycopene, responsible for many fruits' red color, and phenolic compounds, as well as a significant amount of dietary fiber.

2.1.2 Nutraceutical compounds (carotenoids and phenolic compounds)

Nutraceuticals are bio-compounds that are either present or originated from food, which offers health benefits beyond primary nutritional effects. Nutraceuticals can be categorized based on their chemical structure and nature, like carbohydrate derivatives, phenolic compounds, isoprenoid derivatives, fatty acids, and structural lipids (Santana-Gálvez et al., 2019). Most of these compounds are present in plants as phytochemicals and serve as a type of immune system by offering a wide range of multi-targeted responses to external stress.

Carotenoids are a family of over 600 fat-soluble plant pigments, of which approximately 20 are present in the human blood and tissues. They are potent reactive oxygen scavenger (ROS), protecting against some types of cancer and overall oxidative stress. The value of these compounds comes from the fact that both humans and animals cannot synthesize carotenoids, and as such, they must ingest foods with them in a concentrated quantity and a type of supplement if possible. Two of the higher concentrated carotenoids present in the tomato pomace are β-carotene and lycopene. β-carotene is a precursor of vitamin A that protects the cells from oxygen-induced lipid peroxidation and inhibit free radicals. Lycopene, on the other hand, does not have vitamin A activity but is considered to be the best single oxygen scavenger and quencher in the carotenoid family. A quick example of its efficiency is how studies have shown that tomato paste consumption, which has a high concentration of lycopene, significantly lowers UV-induced erythema, and prevents collagen breakdown (Stahl & Sies, 2012). If these positive effects can be correlated to lycopene-rich foods' ingestion, it could be interesting to see the effects of a more concentrated presentation of lycopene as a type of food supplement.

The other important type of nutraceuticals present in the tomato pomace is the polyphenols. These are secondary metabolites that have amassed great attention in the health industry for their anticarcinogenic, anti-inflammatory, antimutagenic, and antioxidant properties. Different polyphenols can be protective against numerous health concerns, including cancer, hypertension, asthma, diabetes, cardiovascular disease, and infection. Tomatoes contain naringenin, quercetin, rutin, and chlorogenic acid as the main phenolic compounds. The concentration of individual phenolic compounds and lycopene in tomato is

determined by many agronomic, geographical, and seasonal factors in addition to variety (Martínez-Valverde et al., 2002).

The nutraceutical market is centralized mainly in the United States and Europe, with a percentage of 37% and 33%, respectively, followed by Japan (18%) and the rest of the world. Based on a recent news report from the Grand View Research Inc, the global nutraceuticals market is projected to reach USD 578.23 billion by 2025 (Daliu et al., 2018). India and China will lead as the fastest expanding nutraceutical markets due to the strong economic growth that allows them to diversify and upgrade foods, drugs, and beverage production infrastructure (Souyoul et al., 2018).

2.2 Extraction of nutraceutical compounds

The extraction and utilization of various bioactive compounds present in plants and various foods, like carotenoids and polyphenols, have been of great interest in both the scientific community and the health and nutrition industry for the past couple of decades. As such, extraction techniques have been developed to extract the bioactive compounds that are accumulated on the different plant parts such as the fruits, roots, leaves, seeds, and skin (Singh et al., 2015). With this in mind, a series of extraction techniques have been developed and scaled up to meet its optimized parameters of extraction, the most common being distillation, and solvent-based extraction. Is essential noting that the selected solvent to extract a particular bioactive compound will directly depend on the solid matrix, meaning, the shape and state of the raw material, the polarity of the compounds of interest, and the desired concentration and purity of the extract (Nandagopal et al., 2016).

2.2.1 Organic solvent extraction

In a general overview, most extraction processes revolve around a solid-liquid extraction with a variety of solvents, with the notable drawbacks of requiring long extraction times and large quantities of solvents used. Despite the well-known drawbacks of the classic technics, they are still used as long as they are optimized for each particular process and are properly adjusted for an industrial scale (Li et al., 2014). Given that the solvent power of a solvent in sub-critical conditions increases with temperature, classic extraction methods tend to use high processing temperatures as a way to optimize the extraction procedure.

This approach comes with some noticeable drawbacks, mainly in the form of thermal decomposition of the target compound, a problem that is accentuated by the fact that free form of solutes of interest would not be as stable as to when it was previously bound to the solid matrix of the plant.

The Soxhlet technique is one of the most well-known methods of extraction of bioactive compounds found within a plant tissue matrix. It allows the unmonitored operation of recycling solvent over long periods of time, resulting on greater amounts of extracted compounds without the need of excessive volumes of solvent. Its basic and functional approach has also made the Soxhlet extraction method a main point of reference for the comparison of newer and more effective methods of extraction (Cheok et al., 2014). A successful extraction with the Soxhlet technique depends on the characteristics of the matrix and on the particle, size given that the diffusion is typically the limiting step during the extractions.

Recent investigations concerning the extraction of lycopene do not really focus on optimization for Soxhlet extraction, but this method is still popular as a relative comparison (difference between new and old type of extractions), or as a way of obtaining total carotenoid content and using said data as a base line for comparison. (Kehili et al., 2019) conducted an experiment for the extraction of lycopene utilizing supercritical CO2 from tomato pomace. To determine the maximum carotenoid content in the tomato peels, Soxhlet extraction with hexane as the solvent was used for 12 h. They recorded a maximum extractable amount of lycopene from the tomato pomace of 1198 ± 71.86 mg/kg, on dry basis, an amount on the higher side of the expected yield. (Cadoni et al., 1999) and (Nobre et al., 2009) both did similar experiments, where lycopene rich oleoresin was extracted from tomato by-products using supercritical CO2, while simultaneously comparing their results with a Soxhlet extraction. Cadoni (2000) obtained a total lycopene concentration of 770 ± 0.7 mg/Kg on dry basis using Soxhlet for 6 hours and hexane as solvent, while Nobre (2009) obtained 755 mg/kg, 560 mg/kg and 578 mg/kg with a solvent mixture of acetone: hexane (1:1) for 6 h, based on different tomato batches and time of collection. All cases were carried at room temperature, as time was not a factor for the extraction of lycopene, and 6 hours seem to be more than enough to obtain the total amount concentrated in the pomace. Variation of the extraction yield is highly dependent on the type of tomato and the time of collections, so direct comparison between methods can be difficult, still, a decent

workaround is the utilization of a comparative %, where Soxhlet is assumed to be able to extract all posible lycopene and the obtained yield will be used as the 100% from that point forward.

2.2.2 Enzymatic hydrolysis extraction

Some bioactive compounds found in plant tissue are concentrated and retained in the polysaccharide-lignin network by hydrogen or hydrophobic bonding. As a result, they are not easily accessed by the solvent, causing significant increments in the time of extraction and decreasing the overall final yield. An alternative to the traditional physical pretreatments that break the cell-wall, like grinding or cutting, one can opt to apply an enzymatical degradation to the extraction material. This is achieved by adding pectinase/cellulase (alone or in mixtures) to the raw material, causing that breakage of the cell wall and the hydrolysis of the lipid bodies and structural polysaccharides (Sharma et al., 2014).

The effectiveness of this method depends directly on the total enzymatic concentrations used, the pretreatment temperature and the time of degradation. Taking into account that all enzymes have an optimal reaction temperature, the pretreatment should always be performed with temperatures as close as posible to this target, without forgetting that high temperatures can cause thermal degradation of the compounds of interest, which can sometimes force a compromise between the enzymatic activity (dependent on temperature) and the stability of the compound of interest (Huynh et al., 2014). Degradation time on a lab scale will usually not represent a problem, given that the economic feasibility of the process is not a priority in that context, but can very quickly become an impediment on an industrial scale due to the increase in costs when high temperatures must be maintained through long periods of time. Finally, both concentration and mixture proportions must be analyzed on a case-by-case basis, as there is a possibility of causing inhibiting effects when two enzymes are used in tandem and the total quantity of enzyme utilized should be justified by a cost/benefit balance.

(Amiri-Rigi & Abbasi, 2016) did an extensive essay for the optimization of enzymatic pretreatment to enhance lycopene extraction efficiency from tomato industrial waste. The analyzed parameters were enzyme concentration (1, 5, 9, 13, 17, 21, 30, 60, 90, 120 and 150 µl pectinase/gram of industrial tomato waste powder), pH (4, 4.5, 7 and 10), temperature

(35, 45 and 55 °C) and the time of incubation (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 min). After preliminary testing with the enzymatic concentration, they found out that its best to utilize lower enzyme concentration, where the optimal value was 9 µl pectinase/gram given that any more than that caused a steady decline in the extraction yield. This is due to an inhibition effect from the enzyme itself, where high concentrations cause the overall population of enzymes to stop working. Regardless of which enzyme is being utilized, it is recommended to identify the maximum point of extraction yield present on the cusp of the bell shape of the graph (yield X concentration). Optimal time was defined as 1 h, optimal pH was given a range of 4.5 to 5.5 and optimal temperature was 45°C. Optimal parameters reflected a total increase of extraction of total lycopene in 17.31%, from the control (18.9%) to the enzymatic pretreatment (36.21%), meaning almost twice the original mount.

2.2.3 Ultrasound assisted extraction

The fundamentals of an Ultrasound Assisted Extraction (UAE) technique can be divided in two stages; first, cavitation phenomena throughout and near the cell walls and second, the recovery of the cell contents after the cell walls are successfully broken. On a basic level, the application of frequencies that go from 20-2000 kHz on a liquid media will result in high shear forces that will affect the solid matrix (Ghitescu et al., 2015). This is the result from the implosion of the cavitation bubbles that occur in the vicinity of the solid, resulting in microjets that hit the cell surfaces and result in the erosion, peeling and breakdown of the plant tissue, as well as particle generation (Martinez-Solano et al., 2020). As an added effect, the implosion of the cavitation bubbles will generate a localized turbulence that result in micro mixing of the media and the substrates. Most noticeably, ultrasound causes an increase in hydration and swelling of the tissue, as well as the enlargement in the pores of the cell wall. This is beneficial for the extraction, as it improves the diffusion process and, as an extension, increases the mass transfer rate (Chemat et al., 2017).

The clear benefit of increased yield of extraction becomes very apparent after looking at the direct effects the technique has over the solid matrix, but ultrasound also presents itself as an attractive alternative to classic extraction techniques based on other benefits present in the extraction process. Mainly, it facilitates the extraction of thermolabile compounds under conditions (lower temperature) that would otherwise result in lower

quantity and/or quality (Xu et al., 2017). Still, it's important to remember that high frequencies can and will generate heat over time, so the process temperature must be kept in check to prevent thermal degradation. The enhancement of the extraction performance of the solvent utilized, without the need to add external heat to increase its solvent power, presents the opportunity of utilizing alternative solvents included in the "Generally recognized as safe" (GRAS) list, like ethanol, as part of the environmentally friendly mindset that has become very important in the recent years.

(Amiri-Rigi et al., 2016) presented an alternative use of the ultrasound pretreatment, focusing more on a quick application of the technology, followed by additional treatments or just a typical solvent extraction, than to rely on ultrasound as the single leading factor for the increase of extraction of lycopene. By applying 50W of power and 20 kHz, at 25°C and for just 30 seconds, they were able to obtain an extraction increase of 14.52 % (from 18.88% to 33.4%). Clearly, the final extraction yield is far too small compared to the results that previously discussed techniques have obtained (>90%), but the fact that the process requires just a small investment of time and power, could make this process a potentially attractive addition, as a type of a flash pretreatment, that could be incorporated into other extraction equipment or become part of a processing line.

2.2.4 Microwave assisted extraction

Microwave Assisted Extraction (MAE) is a technique that utilizes electromagnetic fields (microwaves) in the frequency of 300MHz to 300 GHz. It is usually directly compared to the classical extraction method of hydro-distillation, due to their similar nature, where MAE offers the advantage of obtaining final oil yields with similar compositions but with shorter extraction times and a lower energy consumption. This is achieved due to the microwaves heating the interior of the plant cells compared to the conventional heating process that originates on the outside (Ekezie et al., 2017). The fact that the heating process avoids the cell walls and begins in the interior volume means that there is no need for heat conduction form the outside in contact with an external heat source. In other words, the water inside the plant cells initially heat up under the irradiation of the microwaves, which in turn creates an immediate internal change of increased pressure, and subsequently, end in the breakdown of the cell walls and the release of the bioactive compounds (Adetunji et al., 2017). The extraction mechanism can then be summed up in 3 sequential steps; first, the solutes detach

form their active sites in the solid matrix due to the sudden increase in pressure and temperature, second, the solvent diffuses across the newly open pores of the matrix, and third, the solutes dissolve into the solvent and exit the matrix (Ranjith Kumar et al., 2015).

Similar to the flash pretreatment application of ultrasound, MAE can also be applied in very brief time periods to achieve worthwhile increase in the extraction of lycopene (Ho et al., 2015) presented a rather comprehensive study of the effects of extraction of lycopene using MAE, where he described the effects on how temperature and solvent used affected the quantity and type of isomer of the extract, which could be in the form of trans of cis-lycopene. While the scope of this document doesn't cover issues of molecular structure and transformation, it's important to note that extraction methods can have a direct impact on the bioavailability and bio-accessibility, both important parameters in the health and food industry. In Ho's research the optimum MAE conditions were determined as: 1:10 solvent ratio at 400 W in a 30 seconds period with a yield of 13.592 mg/100 g of extracted all-trans-lycopene. While not particularly impressive in terms of total lycopene extracted, this quick and simple pretreatment offered an increase in final yield of 53% compared to the control. Once again, the quick and rather simple requirements to execute this pretreatment makes it an attractive option to add to other types of extraction protocols and work in combination with other pretreatment options.

2.2.5 Supercritical fluid extraction

Finally, one of the most promising emerging technologies for bioactive compounds extraction due to is scalability to an industrial scope, SFE offers increase extraction yield, faster extraction times and the utilization of a green process for the environment. SFE consists on the utilization of CO2 on its critical state, which corresponds to temperatures and pressures above its respective critical point. The result of this is a fluid phase with intermediate properties between a gas and a liquid (Prado et al., 2014). Most noticeably, supercritical CO2 shows a significant increase in its solubilization power of solutes present on a solid matrix. This occurs due to the fact that the density of a gas that it's at its critical limit increase with higher pressures, increasing the solvent power without the need to increase the temperature. While the viscosity for the supercritical CO2 stays around the same values when the density increases, the diffusivity changes and the mass transfer rate between the solvent and the solute increases (Durante et al., 2014). The result of all these

properties is a fluid with enhanced solvent power but without the surface tension that all liquids present.

SFE represents an emerging alternative to the typical organic solvent extraction processes for the obtention of a lycopene rich oleoresin, which can later be transformed into many sub products. With all these in mind, a series of investigations have been made with the purpose of developing and better understanding how SFE can be applied for the obtention of lycopene, its optimized parameters, direct comparison with other techniques and the addition of supplementary pretreatments, like enzymatic degradation, for this type of extraction. Optimization parameters like temperature and pressure are always the first to be studied, with typical ranges that go from 40°C to 80°C and 30 MPa to 50 MPa. (Hatami et al., 2019) presented a research focused on the optimal extraction of lycopene with this same technique, in which they obtained the maximum lycopene yield at 80°C and 50 MPa for a total of 1.32 mg/Kg of raw material. As expected, increase of temperature and pressure will cause an increase the solvent power for lycopene at this supercritical conditions, with the main limiting factor being the maximum amount of pressure realistically reachable and sustainable, and the potential risk of causing thermal degradation of the compound when using too high temperatures, defeating one of the main reasons of using SFE in the first place. The presented units of mg/Kg of raw material makes it difficult to assess how truly effective the extraction was, as the amount of water present in the raw material can greatly vary, preventing more direct comparisons between other extraction techniques and researches that report their results on mg/Kg of dry tomato.

2.3. Solid-state fermentation

2.3.1 General aspects

SSF is the reproduction of naturally occurring microbiological activity, like ensiling or composting, with the purpose of producing some type of desired product in a controlled and monitored way. More specifically, SSF is defined as any type of fermentation process performed on a non-soluble material, which acts as both a solid base for growth and as the main source of nutrients due to the lack of a liquid medium. Contrary to what the name could suggest, the process of SSF does not occur on a totally dry solid, as there is still a need to have some level of water activity for the growth of the microorganism. SSF provides an

environment close to the natural habitat of the microorganisms. This advantage is especially relevant for fungi, which are better adapted to work on a solid matrix, where mycelium can better grow and expand (Thomas et al., 2013). Yeast and bacteria can also be used as microorganisms for SSF processes like *Bacillus subtilis* (Torino et al., 2013), *Lactobacillus delbrueckii* (John et al., 2006), or *Saccharomyces cerevisiae* (Chen et al., 2007). However, they require a higher moisture content for proper growth, and their overall yields are comparatively lower than that of fungi, mainly for the lack of fast-growing and expanding mycelia that increases the area of contact and enzyme distribution over the substrate (Lizardi-Jiménez & Hernández-Martínez, 2017). The result of a SSF is the production of bioactive compounds that can later be added as food supplement and additives (sweeteners, antioxidants, colorants, preservatives), as well as enzymes, like pectinase or cellules, that can be recovered and concentrated for industrial use (juice clarification, paper pulp whitening) (Bhanja Dey et al., 2016).

In recent years, SSF has captured the attention of the scientific community for the development of industrial bioprocesses, due to its advantages in the form of lower energy requirements without compromising high production yields. It also offers less wastewater production that could carry the risk of bacterial contamination. It also aligns with the eco-friendly focus of present trends, due to it utilizing solid agro-industrial by-products as the substrate (source of carbon). A graphical overview of how SSF works can be seen in **Fig 1**. Among the wide variety of nutraceuticals, phenolic compounds are the most researched by using SSF. However, as different substrates are explored, there is also a surge of new research focusing on different nutraceuticals (Sadh et al., 2018).

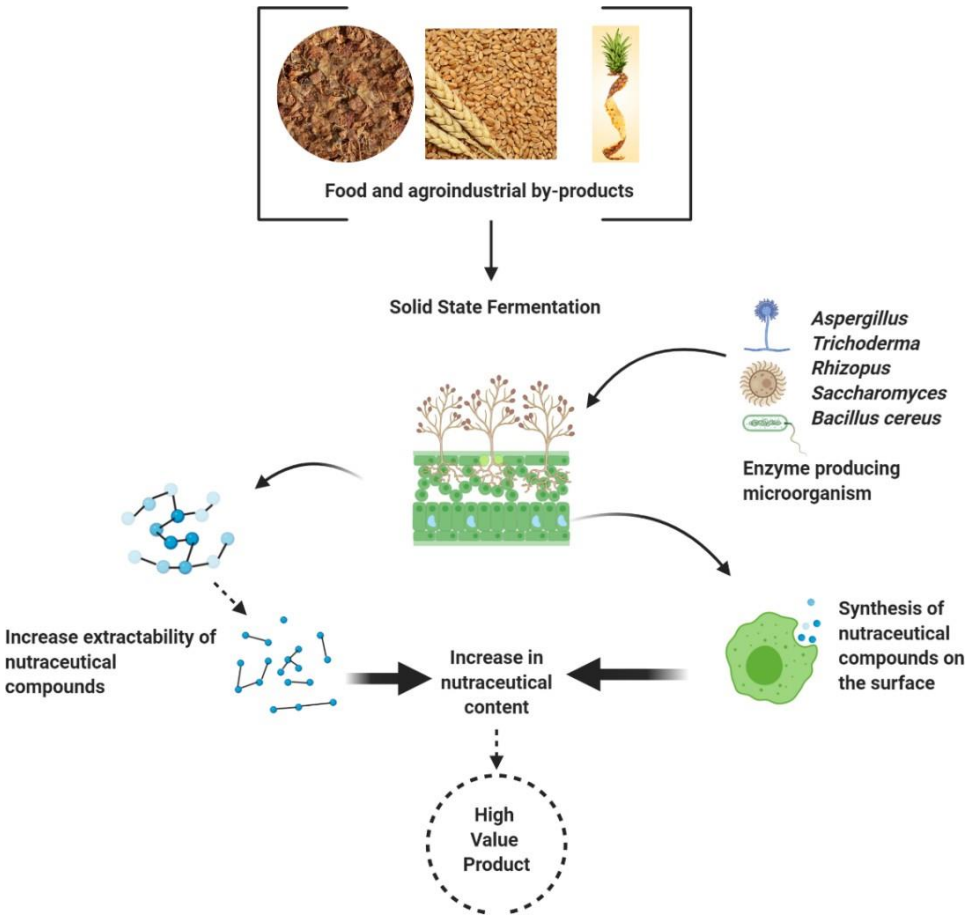

Fig. 1 Schematic representation of a SSF process by using food and agro-industrial by-products to enhance the nutraceutical content.

2.3.2 Substrate

As seen in **Fig. 1**, the selected substrate is mostly an industrial by-product, which still retains a significant number of nutritional components like potato peel waste (Al-Weshahy & Rao, 2012), orange peel (Rivas et al., 2008), coffee skin (Ballesteros et al., 2014), or pineapple peel (Paepatung et al., 2009). It is important to highlight that substrates used in SSF differ significantly in composition, chemical nature, mechanical properties, particle size (including inter and intra-particle spaces), water retention capacity, and surface area. These factors affect the overall process design and product development and must be

taken into careful considerations to ensure that the following process is both optimal and efficient. Some pretreatments can be applied for the improvement of the availability of the bound nutrients, like in the case of milling or shredding the vegetable material. Once the substrate is inoculated, enzyme-producing microorganisms will grow on the surface and will start to hydrolysis the primary polymeric substrates like polysaccharides and proteins. Further fermentation will provide beneficial effects like the liberation of bound nutraceutical compounds or the biosynthesis of some bioactive compounds. Finally, depending on the objective of the fermentation, downstream processing for the purification and quantification of the end product can be carried out (Sadh et al., 2017).

2.3.3 Key parameters

SSF involves a matrix with low water content, which directly differs from submerged fermentation (SmF). Thus, careful consideration of various parameters needs to be taken into account, given that the process is susceptible to small changes, and maximum yield will significantly depend on proper optimization. Key factors to be considered include the adequate selection of the microorganisms and their substrate or solid matrix composition, the correct selection of moisture and temperature, as well as an effective selection of extraction and purification techniques.

To develop a new bioprocess involving SSF, it is required to analyze the relationship between the microorganism physiology and the end product of interest. Factors that can be optimized are pH, aeration, temperature, water activity, humidity, matrix consistency, type of solid substrate, particle size, and a series of potential pre-treatments to the matrix. The most critical parameters tend to be moisture and the nature of the solid substrate, as these have a direct link to the speed and quality of the culture (Farinas, 2015). Fungi, in particular, require moisture levels between 40-60%, but the type of substrate or its availably could alter this critical factor. It is recommended to perform a thorough screening of potentially usable food and agro-industrial by-products to select the proper matrix which requires minimum manipulation to be used.

2.3.4 Types of SSF applications

The most notable application of SSF is the production of enzymes like pectinases, cellulases, and amylases. Other examples of products of value are biopolymers such as exopolysaccharides (EPS), polyhydroxyalkanoates (PHA), and dextran (Abu Yazid et al., 2017); however, they come with the drawback of requiring expensive media components. Biosurfactants have also gained recent attention due to their vast, and increasing application in healthcare and environment (Soccol et al., 2017). It is widely known that their production in submerged conditions possesses problems associated with severe foaming and, at times, an increase in the viscosity of the medium due to the associated formation of EPS, which in turn makes SSF a viable alternative.

A less explored application of SSF is its utilization as a method to increase the concentration of nutraceutical compounds by enhancing their biosynthesis and/or extractability. This application is possible due to the following 3 main reasons: (i) the structural breakdown of the cell walls due to the colonization of the fungus; (ii) the liberation of bound nutraceuticals due to the action of different hydrolytic enzymes produced by the microorganisms during the fermentation; and (iii) the production of some nutraceutical compounds synthesized by the microorganism. In the first two cases, the innovative application of SSF mainly focuses on an enzymatic fermentation, where the objective is the production of enzymes along the surface of the substrate. Usually, these enzymes are then recovered with a downstream process and subsequently used as part of a pre-treatment in a SmF, given that enzymes increase the extraction yield of secondary metabolites due to their release from complex substrates (Verduzco-Oliva & Gutierrez-Uribe, 2020). As a new approach, these enzymes produced in a SSF act directly into the cellular structure of the substrate, releasing the phytochemical compounds bound to the solid matrix and increasing the extractability of those bioactive compounds.

2.3.5 Strategies to enhance the biosynthesis and extractability of nutraceuticals

As explained in the previous sections, SSF already has a plenty of applications for the generation of bioactive compounds of commercial interest thorough different strategies. When a compatible substrate is selected with the proper microorganism, it is possible to generate nutraceutical compounds through new biosynthesis, which can add value to the resulting product or could be utilized as a bioactive rich source ready for extraction. It has been reported that occasionally the bioactive compounds, especially flavonoids, phenolics and saponins, initially biosynthesized by vegetables, may be found in endophytes fungi colonies which are considered as innovative and feasible antioxidant (Verduzco-Oliva & Gutierrez-Uribe, 2020). The SSF is often applied for the synthesis of bioactive compounds, but using conventional defined media and genetic engineering, as in the case of production of lycopene with Saccharomyces cerevisiae (Hong et al., 2019). However, there are very few studies reporting a SSF for the biosynthesis of specific nutraceutical compounds using agro-industrial by-products or residues.

On the other hand, SSF can also impact on the extractability of nutraceutical compounds. This nutraceutical extractability depends directly on some physicochemical properties and molecule interactions with the environment, including the cellular structures that may compartmentalize the bioactive compounds (Bhanja Dey et al., 2016). For example, phenolic compounds are secondary metabolites that are synthesized by intracellular organs and are subsequently liberated and moved into the cell-matrix or the vacuole through a vesicle transfer system (Anokwuru et al., 2018). Phenolic compounds can also end up in the cell-matrix by ATP-binding cassette transporters, where they can be linked (by covalent links) to bigger molecules like pectin or cellulose (Pan et al., 2017). Based on that, phenolic compounds are found inside the matrix of plants in three ways: free, insoluble-bound, and soluble-bound (conjugated). The conjugated fraction is esterified to soluble compounds like some low molecular weight carbohydrates, lipids, or proteins, while insoluble fraction is found to be esterified to more complex cell wall components (Londoño-Hernández et al., 2017). Unfortunately, insoluble-bound phenolics cannot be readily extracted with most conventional extraction protocols. They can only be liberated from the matrix by the process of hydrolysis, such as an acid, alkaline, or enzymatic hydrolysis (Williams et al., 2017). Thus,

SSF involving an enzymatic hydrolysis can be applied to improve the extraction yield of phenolic compounds.

SSF starts when microorganisms have limited accessibility to cellulose, hemicellulose and other components in plant cell walls due to its chemical connection with lignin. As a natural part of its biology, microorganisms generate large quantities of various enzymes, so it can more easily access the content of the lignocellulosic compounds. During SSF, microorganisms degrade lignin with the effect of ligninolytic enzymes, like lignin peroxidase, so that it can access the energy-rich polysaccharides for their metabolism and growth (Gupta et al., 2016). In the case of fruits and vegetables, phenolic compounds can be easily released from pectin and cellulose conjugates by the direct application of SSF (Zambrano et al., 2018). In the following sections, the application of SSF to obtain high value molecules from cereals, fruit and vegetables industrial by-products is described. Also, **Table 1** shows a summary of recent SSF applications for the enhancement of nutraceutical content from foods and agro-industrial by-products.

Table 1. Summary of recent SSF for the enhancement of nutraceutical content from foods and agro-industrial by-products.

Agroindustrial products	**Bioactive compounds**	**Fermentation conditions**	**Major findings**	**References**
Cereals and legumes				
Barly grains	Polyphenols Flavonoids	*Aspergillus awamorinakazawa* 30°C, 5 days	Increase in total phenolic and flavonoid content (56%)	(Sandhu & Punia, 2017)
Brewers spent grain	Xylooligosaccharides (XOS)	*Trichoderma reesei* 30°C, 3 days	Production of XOS comparable with the use of commercial enzymes	(Amorim et al., 2019)
Mung Beans	Polyphenols	*Cordyceps militaris* SN-18 25°C, 7 days	Insoluble phenolic compounds transformed into soluble phenolics Increase in TPC (89%) in acetone extraction	(Xiao et al., 2015)

Oats	Polyphenols	*Monascus anka* 30°C, 14 days	Significant increase (100-fold) in free, bound and conjugated phenolics	(Bei et al., 2017)
Purple Rice	Monacolin K Antioxidant red pigments	*Monascus purpureus* CMU002U 30°C, 5 days	High level of monacolin K (13,482 ppm) and red pigment production (388.25 units/g)	(Prodpran et al., 2017)
Soybean okara	Polyphenols Isoflavones Fiber	*Saccharomyces cerevisiae* 24°C, 24 h	Improved nutritional quality Reduction of raw fiber content (40%)	(Queiroz Santos et al., 2018)
Soybean	Menaquinone 7 (Vitamin K)	*Bacillus subtilis* NCIM 2708 37°C, 24 h	The highest MK-7 concentration (39.039 µg/g) was obtained after 24 h of fermentation	(Singh et al., 2015)
Soybean	Polyphenols Isoflavones	*Rhizopus oligosporus* RT-3; 35°C, 12-30 h	Enhanced TPC and isoflavone contents (167%)	(Xiao et al., 2016)
Soybean	Polyphenols Isoflavones	*Tricholoma matsutake* 30°C, 0-12 days	Enhanced TPC and isoflavone contents (275% at 12 days) Amino acid and fatty acid slightly increased (18%)	(Lee et al., 2019)
Wheat bran	Ferulic acid	*Aspergillus niger* 28°C, 7 days	Increase of ferulic acid content (43%)	(Yin et al., 2019)
Wheat grains	Polyphenols Flavonoids	*Aspergillus awamorinakazawa* 30°C, 6 days	Release and/or biosynthesis of some bioactive compounds Increase of TPC by 170% in the best conditions.	(Sandhu et al., 2016)
Fruits and vegetables				
Apple Pomace	Polyphenols	*Phanerocheate chrysosporium* 37°C, 14 days	Mobilization of the polyphenolic compounds Increase in TPC by 70% average, with a best case of 180%	(Ajila et al., 2011)
Fig by-products	Polyphenols	*Aspergillus niger* HT4; 30°C, 72 h	Increase of TPC release and antioxidant activity (5.48 and 98.54 times, respectively)	(Buenrostro-Figueroa et al., 2017)
Garden cress seeds	Polyphenols	*Trichoderma reesei* 30-35°C, 4-5 days	Increase of TPC and antioxidant activity (9-fold)	(Abdel-Aty et al., 2019)

Mexican mango seed	Polyphenols	*Aspergillus niger* GH1; 30°C, 60 h	Effective release of bound phenols Increase in TPC by 235%	(Torres-León et al., 2019)
Pineapple and guava fruit	Polyphenols	*Rhizopus oligosporus* 22°C, 12 days	Phenol rich extracts TPC increase in 62% for pineapple and 105% for guava	(Sousa & Correia, 2012)
Pineapple by-products	Polyphenols	*Kluyveromyces marxianus NRRL Y-8281;* 30°C, 3 days	Increase in phenolic content and antioxidant activity (7%)	(Rashad et al., 2016)
Plum pomace	Polyphenols Flavonoids	*Aspergillus niger* *Rhizopus oligosporus* 30°C, 14 days	TPC increased by over 30% with *R. oligosporus* and 21% for SSF with *A. niger*	(Dulf et al., 2016)
White grape pomace	Carotenoids γ-linolenic acid	*Actinomucor elegans* *Umbelopsis isabelline* 28°C, 12 days	β-carotene and lutein gradually increased until the end of the fermentation	(Dulf et al., 2020)

2.3.6 Advantages and drawbacks of SSF: Is it industrially feasible?

SSF can serve as both an extraction pre-treatment or a biological process for the biosynthesis of bioactive compounds, which could make it an attractive operation for its application at an industrial scale. It can be applied to solid materials, as long as the surface is relatively solid and with sufficient moisture for the utilized microorganism. The direct and immediate alternative is the process of submerged fermentation (SmF), a process that is already widely utilized in the industry. SmF is a term that compromises a large variety of stirred and non-stirred microbial processes, where a liquid medium surrounds the substrate (Couto & Sanromán, 2006). The main differences between SSF and SmF are determined by various physicochemical features, such as the mixing and diffusion of substrates and products in relation to biomass, which is much smaller in SSF; the solubility and diffusion of oxygen and other non-polar gases, which is greater in SSF; heat conduction, which is much smaller in SSF; and water content, which is smaller in SSF. All of these processing conditions are what will define if SSF is truly applicable at an industrial scale and if it is worth

applying SSF over SmF. **Table 2** shows a summary of the most relevant advantages and drawbacks of SSF compared to SmF, and each point will be further explored closely.

Table 2. Overview of SSF advantages and drawbacks as compared with submerged fermentation.

Solid state fermentation process	
Advantages	**Drawbacks**
• Low waste water output • No foaming phenomenon • Low cost media and substrates • Low energy cost • Small volumes of substrate (lower operational cost) • Low moisture content of the substrate avoiding contamination	• Media are heterogeneous, defaulting proper mixing • Substrate moisture level is difficult to control • pH, temperature and dissolved oxygen need precise control • Continuous medium mixing is required for longer fermentations • Continuous medium agitation can damage mycelia (slower growth)

SSF offers the possibility of a higher fermentation productivity, higher end-concentration of products, higher product stability, lower catabolic repression, cultivation of microorganisms specialized for the water-insoluble substrates or mixed cultivation of various fungi (Singhania et al., 2009). It is essential not to forget that most of these advantages are mainly exploited at a laboratory scale, like the lower demand for sterility due to the low water activity used in SSF. Still, this technology has plenty to offer in terms of production.

Some of the significant challenges that SSF implementation brings is usually in the form of scale-up, purification of end products, and biomass estimation. However, with the quick development in the field of biochemical engineering, several bioreactors have been designed with the purpose of overcoming the problem of scale-up, and to an extent, the on-line monitoring of critical parameters, as well as heat and mass transfer. Substrates, which commonly are agro-industrial residues, bring a series of challenges due to its heterogeneous

nature, in the form of kinetics and modeling studies, which are required to develop for the implementation and design of the bioreactors and its operation (Soccol et al., 2017).

One of the essential characteristics of a microorganism-based process is its incubation and propagation. In the case of fungi, sporulation is the critical phenomenon that dictates how fast the fermentation process can develop, as well as directly influence the process of mycelia production. Sporulation is a rather complicated process to control in SmF, while it is relatively easy to obtain spores by SSF (Gomes et al., 2018). This acts as a double edge sword, given that early sporulation is also a significant cause of concern for the safety of SSF and can also be a focal point for the generation of health hazards (Viniegra-Gonzàlez, 1997). Also, an intrinsic advantage of sporulation is that solid surface cultures are the natural habitat of fungal organisms. It is easier to conserve and control the morphological cycle, which in turn makes SSF a more proper procedure to produce spores for several types of industrial applications, like in food fermentation or biopesticide production (Abdul Manan & Webb, 2017).

Another clear advantage of SSF is the amount of water that is necessary to carry out the process. For starters, when utilizing microorganisms adapted to low water activity levels, they are capable of thriving selectively in many SSF processes, thereby diminishing the cost of upstream processing and passively prevent other microorganisms from growing in the same medium. The reduced level of water content in the fermentation mix for the SSF process favors a greener industrial operation, with lower levels of wastewater. Many agro-industrial by-products and residues come already with a rather high level of humidity, like in the case of many pomaces generated in the extraction of juices, meaning that there will not be a need for the extra addition of water to the substrate, although a light drying process might be required instead (Rudakiya, 2019).

The fact that SSF is not widespread in the industry is because there are several complications involving essential process parameters like mixing, heat exchange, oxygen transfer, gradients of pH, moisture control, and nutrient and product distribution due to the nature of heterogeneity of the substrate and culture. In comparison, environmental control is relatively simple in SmF due to the homogeneity of the suspension of the microorganism, as well as nutrients in the liquid phase. Thus, SSF presents the natural complication regarding environmental factors, which tend to be difficult, laborious, and often inaccurate

to control, limiting the industrial potential of these technologies (Lizardi-Jiménez & Hernández-Martínez, 2017).

Finally, another important aspect to consider for the application of SSF is the type of bioreactor that can be potentially used on an industrial scale, with their respective advantages and disadvantages. First of all, the majority of SSF processes are done in batch. Other processes do not require a particular container and instead will have the substrate spread on a suitable floor surface. As a result, SSF processes require relatively more simple bioreactor designs than for liquid fermentations. Such bioreactors can have continuous or occasional agitations (slow rotating drums), or they can be completely static (tray systems and airflow systems) (Mitchell et al., 2019). For rotating drums, they tend to be cylindrical containers mounted on its side onto spinning support. They require an inlet and outlet circulation of humid air. They are mainly used for the production of enzymes or biomass, which makes them a viable option for the scalable process of generating nutraceutical compounds or simply enhance the nutritional/antioxidant nature of the substrate. Still, they come with some notorious disadvantages, like the fact that the drums cannot be filled above 30% capacity or mixing becomes ineffective, as well as precise control of the rotatory movement, as to prevent and minimize damage to the mycelial growth due to shear force. The alternative design of a tray fermenter is also viable, especially when oriented for the fermentation of foods and the production of enzymes (Arora et al., 2018). They are also fed with humidified air, and the bed temperature is continuously monitored and adjusted based on the recycling airflow. As expected, there is less shear force in this design, reducing the possibilities of cell damage, at the cost of mixed results and yields across the whole surface of fermentation.

Currently, there is a lack of information regarding the utilization of direct SSF as a pretreatment for the enhanced extraction of nutraceutical compounds like lycopene and phenolic compounds of tomato pomace. As SSF could enhance the nutraceutical extractability, it would be of great interest to study the SSF impact in the extractability of these compounds of interest, as the end result could be a high value product from tomato pomace with a potential industrial application.

CHAPTER 3 Comparative study between submerged and solid-state fermentations, and commercial enzymes on tomato pomace for the extraction of lycopene and phenolic compounds

The objective of this work was to evaluate the extractability of lycopene and phenolic compounds of tomato pomace by using solid-state fermentation (SSF), in comparison with other pretreatments like submerged fermentation (SmF) or commercial enzymes. In order to do this, first it was necessary to generate a homogeneous batch of tomato pomace by simulating the industrial processing from the fresh fruit. Then, different assays were carried out to determine the development and enzymatic capabilities of two fungal strains (*Aspergillus niger* ATCC 6275 and *Rhizopus oryzae* BIOTEC00X), as well as their fermentation capabilities with tomato pomace. Next, it was necessary to characterize the activity of commercially available enzymes (cellulase, pectinase, and Rapidase ®). All pretreatments were applied on tomato pomace, followed by the quantification of carotenoids and total phenolic compound by spectrophotometry. Finally, to characterize and quantify the individual phenolic content of treated tomato pomace extracts, ultra-high-performance liquid chromatography (UHPLC) was employed with samples taken from the extracts from each type of pretreatment.

3.1 Methodology

3.1.1 Processing and characterization of tomato pomace

Ripe tomato fruit with a similar appearance and no evident damage were subjected to superficial cleaning with cold water before applying a blanching treatment, where the fruit was subjected to a quick submersion of no more than 15 seconds in water with a temperature of 85°C. The tomato fruit was then chopped down with a conventional knife and liquified using a laboratory-scale blender. The resulting mixture was then filtrated with the use of a commercial strainer, and the filtrate, which was composed of the skin and seeds of the tomato (tomato pomace), was dried using a DX402C Drying Oven (Yamato, Japan) at 55°C for two days. The dried tomato pomace was then pulverized using a commercial

Hamilton Beach coffee mill set at max settings. Particle size was homogenized by using a commercial strain with a #9 mesh. The fine tomato pomace powder was then placed inside a sealed plastic bag at 3°C under no-light conditions until further use.

A sample of the liquified tomato was used for the characterization of its fundamental physical properties. Humidity was determined by drying 10g of liquified tomato using a small aluminum tray, previously weighted, inside a drying oven at 55°C. The tomato tray was weighted every hour using an analytical balance until a constant weight was achieved. Brix was determined by pouring 1 ml of the liquified tomato on a portable refractometer set at 24°C. The pH was determined on 10 g of sample by using a previously calibrated potentiometer. Titratable acidity was measured by placing 10 g of sample under constant agitation, on a 50 mL beaker and drop feeding a NaOH 0.1 M solution until a pH of 8 was achieved. Lycopene content and total phenolic compounds were also measured from a sample of the tomato pomace and quantified by spectrophotometry (see section 3.1.7).

3.1.2 Commercial enzymes characterization

3.1.2.1 Cellulose as substrate

The optimal enzymatic proportion studies of maximum enzymatic activity were initially carried over with two commercially available enzymes (cellulase and pectinase). For this, multiple microtubes (by triplicate) were prepared with concentrations ranging from 2 to 14 g/L of cellulose using a stock solution of 2%, a set 20 µL of a stock enzyme solution 7.5%, and water for a final reaction volume of 200 µL. The reaction mix was placed for 1 hour in an incubator at 30°C to allow the cellulose's hydrolysis. After the incubation, 300 µL of DNS was added, and the new mixture was placed on a water bath at 55°C for 12 minutes. Finally, the mixture was placed on ice for 15 minutes to stop the reaction. The mixture's absorbance was then read using a Varioskan LUX multimode microplate reader (ThermoFisher, USA) at 515 nm. Total reducing sugars were used as a quantifiable parameter for the enzyme's activity and were determined by comparing the essays' absorbance with the results from a calibration curve using glucose.

3.1.2.2 Pectin as substrate

A secondary study was carried over with the same goals, but now the two used enzymes were a pectinase and Rapidase ® (pectinolytic enzyme), and the glucose was changed for citric pectin. Multiple microtubes (by triplicate) were prepared with concentrations ranging from 2 to 14 g/L of pectin using a stock solution of 2%, a set 20 µL of a stock enzyme solution 7.5%, and water for a final reaction volume of 200 µL. The reaction mix was placed for 1 hour in an incubator at 30°C to allow the pectin's hydrolysis. After the incubation, 300 µL of DNS was added, and the new mixture was placed on a water bath at 55°C for 12 minutes. Finally, the mixture was placed on ice for 15 minutes to stop the reaction. The mixture's absorbance was once again read at 515nm. Total sugars were again the quantifiable factor to determine enzymatic activity.

3.1.3 Fungi incubation and propagation

3.1.3.1 General Aspects

Two fungal strains were used in this study, *Aspergillus niger* ATCC 6275 and a wild strain isolated from agave bagasse (later identified as *Rhizopus oryzae* BIOTEC00X). Initial incubation of fungi was carried out by depositing spores from each strain into potato dextrose agar medium and incubating at 30 °C for 3 days, where visually one could appreciate a total cover of the surface with mycelium and spores. All the following experiments that involved the two strains were inoculated by a stock spore solution with a final spore count of 1×10^6 spores/mL. The stock solution for each strain was prepared by first recovering a spore volume using distilled water and the gentle touch of a wood brush, which helped resuspend the present spores in the mycelium into the distilled water. A Neubauer chamber was used to quantify the total amount of spores present in the volume, and the required dilutions allowed to achieve a final concentration of 1×10^6 spore/mL.

3.1.3.2 Taxonomic identification

To identify the wild fungal strain, a protein extract was generated from a mycelium pellet following the protocol "Formic acid extraction method" (Bruker Daltonics GmbH). One µL of this extract was transferred to a stainless-steel plate, allowed to dry, and covered with 1 µL of the matrix (10mg / mL of HCCA dissolved in ACN: H2O: TFA 50: 47.5: 2.5), and mass spectra were generated with the method "MBT_FC.par" in a Microflex L.T. equipment (Bruker Daltonics GmbH). The spectra obtained were compared with reference spectra from the filamentous fungi and BDAL databases (Bruker Daltonics GmbH) using the MALDI BIOTYPER 3.1 software. The identification results were expressed as log score values indicating the similarity of the unknown sample profile to available database entries.

3.1.3.3 Fungal growth with no available glucose

Initial tests were done with both strains to see if they could grow on a medium with no directly available glucose, forcing them to generate pectinolytic and cellulitic enzymes to degrade a complex substrate to obtain its carbon source. For this purpose, a minimal medium (Kaminskyj, 2001) was elaborated. The medium consisted of nitrate salts, trace elements, 1% thiamine, and a final pH of 6.5 (adjusted by NaOH 1M); when not in use, the medium was stored at 3°C. The test consisted of preparing by triplicate a set of flasks with a total volume of 20 mL of minimal medium with 1% pectin. Each flask was then inoculated with 1 ml of the stock spore solution for each strain and left at 30°C and 150 RPM for three days. At the end of the incubation period, visual confirmation was made to determine if fungal growth was successful.

3.1.4 Submerged fermentation (SmF) with tomato pomace and estimation of the optimal fermentation period

3.1.4.1 Determination of optimal tomato pomace concentration for SmF

SmF with tomato pomace was initially performed to estimate the optimal fermentation time and optimal concentration of tomato pomace in the medium. The best parameters were determined by measuring total reducing sugars present on the

fermentation broth. First, the optimal concentration was estimated by preparing minimal medium supplemented with 0.025%, 0.05%, and 0.075% tomato pomace powder and incubating multiple flasks (by triplicate) with 20 ml of said medium. Two sets of 9 flasks were prepared, a set per fungi strain, and each triplet within each tomato concentration set. The flasks were inoculated with 1 ml of the stock spore solution with each strain. All inoculated flasks were incubated at 30°C at 150 RPM. Each day, 2 ml were extracted with a syringe from the fermentation broth and were filtrated by a 0.2-micron filter. To quantifying total reducing sugars, 200 µL of the filtrated broth was combined with 300 µL of DNS to generate a new reaction mix for each flask. This reaction mix was subjected to the exact conditions and measurements described in Subsection 3.1.2.1.

3.1.4.2 Optimal incubation period for SmF

Once the optimal proportion was determined, a new set of flasks were prepared with 20 ml of the minimal medium supplemented with the best tomato pomace concentration. Two sets of 4 flasks were inoculated, each with 1ml of their respective fungal spore solutions. All inoculated flasks were incubated at 30°C at 150 RPM. Each day, for the following five days, 1ml of fermentation broth was extracted with a syringe and filtrated with a 0.2-micron filter. To quantifying total reducing sugars, 200 µL of the filtrated broth was combined with 300 µL of DNS to generate a new reaction mix for each flask. This reaction mix was subjected to the exact conditions and measurements described in section 3.1.2.1.

3.1.4.3 Total protein determination by Bradford

To further understand the nature of the enzymatic activity, a Bradford protein assay was applied to a sample of the fermentation broth on days 2 to 5 to quantify an estimate of the total enzyme concentration present in the solution, which would later help define the specific enzyme activity of the fermentations enzymes and compare them to the industrial enzymes. First, a calibration curve using BSA (1.25–10 µg/ml), was done to validate the method and establish a linear correlation between absorbance and protein content. A sample (2 ml) of the broth from each day was recovered and filtrated with a 0.2-micron filter. Then, a volume of 150 µl was placed on a microplate well by triplicate for each day. Finally, each well was filled with 150 µl of 1x Bradford dye reagent and incubated for 10 minutes at

room temperature. The microplate mixture's absorbance was then read using a Varioskan LUX multimode microplate reader (ThermoFisher, USA) at 595 nm.

3.1.4.4 DNS assay to determine total glucose released by fermentation broth

Once the best tomato pomace concentration and the optimal amount of incubation days were determined, the process of SmF with both strains was repeated under optimal conditions. At the end of the third day, 5 ml of the fermentation broth was recovered with a syringe and filtrated by a 0.2-micron filter. The same essay for total reducing sugars liberated from pectin previously done in section 3.1.2.1 was repeated, with the slight modification of using 20 µl of filtered broth in place of the enzyme solution. This was done for both strains incubated at the same conditions.

3.1.5 Growth conditions in solid-state fermentation (SSF) using tomato pomace

Some test runs were made to determine the best conditions and understand the fungal growth behavior when applied to an SSF with tomato pomace. First, inoculation was done by adding 1ml of the stock spore solution to several Petri dishes with 1 gram of tomato rehydrated to 85%, which were then left for three days at 30°C with no light conditions. A control sample under the same conditions but without inoculation was also incubated to see if the endemic microorganisms already present in the tomato would grow and develop. After the incubation, visual confirmation was done to determine if the tomato pomace's fungal growth was only one type of strain, and if the control presented signs of microorganism growth. An attempt was made to sterilize the tomato pomace powered by humid heat before inoculation with the spores, but the procedure naturally rehydrated the pomace, making control of the water content difficult.

The resulting standard procedure and conditions for the solid-state fermentation are as follows. One gram of tomato pomace powder was rehydrated by adding 5 ml of distilled water, effectively upping the humidity to 85%. The powder was then placed on a sterile Petry dish, and 1 ml of the stock spore solution was added. The dish was then left in the incubator at 30°C for the required period of incubation.

3.1.6 Evaluation of pretreatments for enhancing the bioactive extractability

Extractions of both lycopene and phenolic compounds were done with tomato pomace, which was previously pretreated by commercial enzymes and enzymes from the fermentation broth incubated at optimal conditions, as well as by a SSF with both strains. Three different types of controls were used for each extraction, one with pure tomato pomace powder, one where SSF conditions were simulated but without inoculation, and one where tomato pomace was dissolved in distilled water and placed under enzymatic incubation conditions. Pretreatments were done as follows, with internal triplicate and with two true replicates in all cases.

3.1.6.1 Commercial enzymes pretreatment

In the case of industrial enzyme pretreatment, 1 g of tomato pomace was resuspended with 20 ml of distilled water inside a Falcon tube (50 ml), followed by the addition of 0.025 g Rapidase ® per tube. All Falcon tubes were incubated for 1 hour at 30°C and with 150 RPM. Once the pretreatment finished, each tube's content was emptied on a clean open petri dish and left to dry at 50°C for 24 hours using a DX402C Drying Oven (Yamato, Japan). The dry tomato was recovered and placed on new Falcon tubes, where preliminary grinding was done using a kitchen knife. The resulting tomato pomace was then ready for the extraction of bioactive compounds.

3.1.6.2 Submerged fermentation broth pretreatment

Treatment with enzymes from SmF consisted of first recovering the enriched broth from the fermentation. The SmF process using both strains were repeated on Falcon tubes of 50 ml by inoculating 20 ml of minimal medium supplemented by 0.075 tomato pomace with 1 ml of stock spore solution. After an incubation made at 30°C and 150 RPM for three days, all falcon tubes were centrifugated at 4000 x g and 24°C for 10 minutes. 10 ml of each falcon tube was transferred to a new set of clean tubes where 1 g of tomato pomace powder was added and incubated for 1 hour at 30°C and 150 RPM. Once the pretreatment finished, each tube's content was emptied on a clean open petri dish and left to dry at 50°C for 24

hours using a DX402C Drying Oven (Yamato, Japan). The dry tomato was recovered and placed on new Falcon tubes, where preliminary grinding was done using a kitchen knife. The resulting tomato pomace was then ready for the extraction of bioactive compounds. The process was done for both strains of fungi.

3.1.6.3 Solid-state fermentation pretreatment

The last pretreatment was the one with SSF. For this, 1 g of tomato pomace powder was placed on a clean petri dish, where 5 ml of distilled water was added. Inoculation was done under sterile conditions by adding 1×10^6 spores to the pomace while surrounded by two flames. The Petri dishes were sealed and incubated at 30°C under no-light conditions for three days. Once incubation was finished, each petri dish was opened and left to dry at 50°C for 24 hours using a DX402C Drying Oven (Yamato, Japan). The dry tomato was recovered and placed on new Falcon tubes, where preliminary grinding was done using a kitchen knife. The resulting tomato pomace was then ready for the extraction of bioactive compounds. The process was done for both strains of fungi.

3.1.7 Extraction procedures and total bioactive compound determination

To determine the impact of the pretreatments in the extractability of the nutraceutical compounds, a rapid screening was performed by determination of lycopene and phenolic compounds using spectrophotometry.

3.1.7.1 Extraction of lycopene and spectrophotometry quantification

Each tube with 1g of dry sample (control and pretreated samples) of tomato pomace had 10 mL of hexane added, followed by an ultra-homogenization using a Tissuemizer (IKA Ultra Turrax, Germany), consisting of 15 s at 50,000 rpm, 15 s of cold-water cooling, 15 s at 50,000 rpm, another 15 s of cooling and finally 20 s at 35,000 rpm. The resulting mixture of hexane with tomato pomace was subjected to centrifugation at 4000Xg and 24°C for 10 minutes. The lycopene-enriched hexane was recovered by decantation into a new clean set of tubes.

Total lycopene concentration was estimated by the application of a spectrophotometric assay using a SmartSpec Plus Spectrophotometer (BIO-RAD, USA) and the following equation:

$$c\left(\frac{mg\ Lic}{Kg\ d.t}\right) = \frac{\frac{Abs\ at\ 470}{185}\left(\frac{mmol}{L}\right) * 0.01L * \frac{537\ mg}{mmol}}{g\ dry\ tomato} * 1000\ \left(\frac{g}{kg}\right) * dilution\ factor$$

Where $\frac{537\ mg}{mmol}$ is the extinction coefficient of lycopene in hexane at 470 nm.

3.1.7.2 Extraction of total phenolic compounds and spectrophotometry quantification

Each tube with dry tomato pomace was mixed with 10 mL of methanol, followed by an ultra-homogenization using a tissuemizer (IKA Ultra Turrax, Germany), consisting of 15 s at 50,000 rpm, 15 s of cold-water cooling, 15 s at 50,000 rpm, another 15 s of cooling and finally 20 s at 35,000 rpm. The resulting mixture of methanol with tomato pomace was subjected to centrifugation at 4000Xg and 4°C for 20 minutes. The enriched methanol was recovered by decantation into a new clean set of tubes.

Quantification of total phenolic content was done using Folin reagent and spectrophotometric measurement. All measurements were done by triplicate and for each treatment. First, 15 µl of extraction methanol was added to 240 µl of distilled water. Then, 15 µl of Folin (0.25 N) reactive were added, and the resulting mixture was incubated for 3 minutes. Finally, 30 µl of Na2CO3 (1N) was added and incubated for 2 hours in no light conditions. Each treatment's resulting solution was then deposited into a microplate, and absorbance was read at 765 nm using the Varioskan LUX multimode microplate reader (ThermoFisher, USA). Results were reported as chlorogenic acid equivalents by comparing the resulting absorbances with a calibration curve previously done with chlorogenic acid.

3.1.8 Determination of individual phenolic compounds by ultra-high-performance liquid chromatography (UHPLC)

The samples from all pretreatments were subjected to UHPLC for more precise quantification of individual phenolic compounds from tomato pomace extracts. The

equipment used was a UHPLC-PDA Acquity Arc system (Waters, Milford, MA, USA). Compounds were separated on a Waters Cortecs reverse phase C18 (4.6 × 50 mm, 2.7 µm pore size) column. Chromatographic data were processed with Empower software (Waters).

The identification and quantification of individual phenolic compounds were performed as described by Becerra-Moreno et al. (2015). The methanol extract of each treatment was filtered using a nylon syringe filter (0.22 µm) and injected (2 µL) in a vial chamber and column temperatures were 4 and 40 °C, respectively. Mobile phases consisted of water adjusted to pH 2.4 with orthophosphoric acid (phase A) and methanol (phase B). The gradient solvent system used was 0/90, 11/65, 13/2, 16/90 (min, % phase A) at a constant flow. Phenolic compounds were detected at 280, 320 and 360 nm. Identification of individual phenolic compounds was performed on the basis of retention time, elution order and UV spectra as compared with authentic standards and reported data (Perea-Domínguez et al., 2018; Valdez-Morales et al., 2014; Gómez-Romero et al., 2010). To quantify phenolic compounds, standard curves of chlorogenic acid (0–20 ppm) were prepared. Thus, the concentration of individual phenolic compounds was expressed as µg chlorogenic acid equivalents per g of tomato pomace (dry weight, dw). Similarly, the concentration of total phenolics (mg/kg dw) was determined as the sum of all individual phenolic compounds.

3.1.9 Statistical analysis

Replication was achieved by repeating a treatment under the same conditions. All treatments were run concurrently. There were three replicates per treatment (n = 3). Statistical analyses were performed using the three replicates with data representing the mean values of samples and their standard error. Turkey's method was to create confidence intervals for all pairwise differences between treatments to determine which ones were statistically different ($P < 0.05$) between them with a 95% certainty. Statistical analyses were conducted with Minitab version 19.20 software.

3.2 Results and discussion

3.2.1 Characterization of tomato pomace and simulation of industrial processing

Selection of fruits with uniform maturity, size, and no defects was carried out. General results for each valued property of the tomato are summarized in **Table 3**. The tomato processing resulted in the conglomeration of both seeds and skin with a high-water content. Given that the pretreatments were going to be done in the following weeks, the tomato pomace was successfully dried and pulverized, which formed a final batch of tomato pomace powder. Any material that was not actively used was left to rest at 3°C and no light conditions for adequate preservation. A visual representation of the aforementioned process can be observed in **Fig 2**.

The tomato pomace was relatively acid and with high amount of water content (4.28 and 89.59% respectively). This means that the matrix was more than suitable for the fungal development during SSF. That being said, while high water content does facilitate the development of the fungus, reduction of the overall water content could help further improve the production of enzymes during the fermentation process. While a lower water content does in fact present a high stress medium for the fungus, it has also been reported that lower values may induce further sporulation of the organisms (Hamidi Esfahani et al., 2004), resulting on a higher production of mycelium and as such, a higher surface of contact. Depending on the batch size and volume of the tomato pomace, hydration of the tissue may represent an unfavorable condition of the system, as it reduced the porosity and result in oxygen transfer limitations.

The rest of the tomato pomace properties were in line with what has been previously reported (Teka, 2013; Khandaker et al., 2008) for the tomato fruit at a ripe stage of development. This suggest that the approximate composition of tomato pomace is also in its majority carbohydrates (more than 57%), which are manly composed of lining, hemicellulose, pectin and cellulose (Lu et al., 2019). As such, this polymeric structure will require either physical stress or a biological enzymatic pretreatment to properly enhance the lycopene extractability from the cell matrix.

Table 3. Physical and physicochemical characteristics, and phenolic and lycopene content (spectrophotometric determinations) of tomato pomace.

Parameters	Value
Physical and physicochemical characteristics	
Total weight of whole fruit (g)	125.15±10.00
By-product recovered (g/100 g fw*)	5.08±0.40
Total solids (g/100 g fw*)	11.41±1.00
Soluble solids (° Brix at 24°C)	2.92±0.38
pH	4.28±0.09
Titratable Acidity (g citric acid Kg-1 fw*)	4.50±0.12
Phenolic and lycopene content	
Lycopene (mg of lycopene/ Kg fw*)	3.68±0.32
Phenolic compounds (mg of chlorogenic acid/ Kg fw*)	217.36±12.87
*Values are the mean of at least three independent determinations ± standard deviation	
** fw, fresh weight	

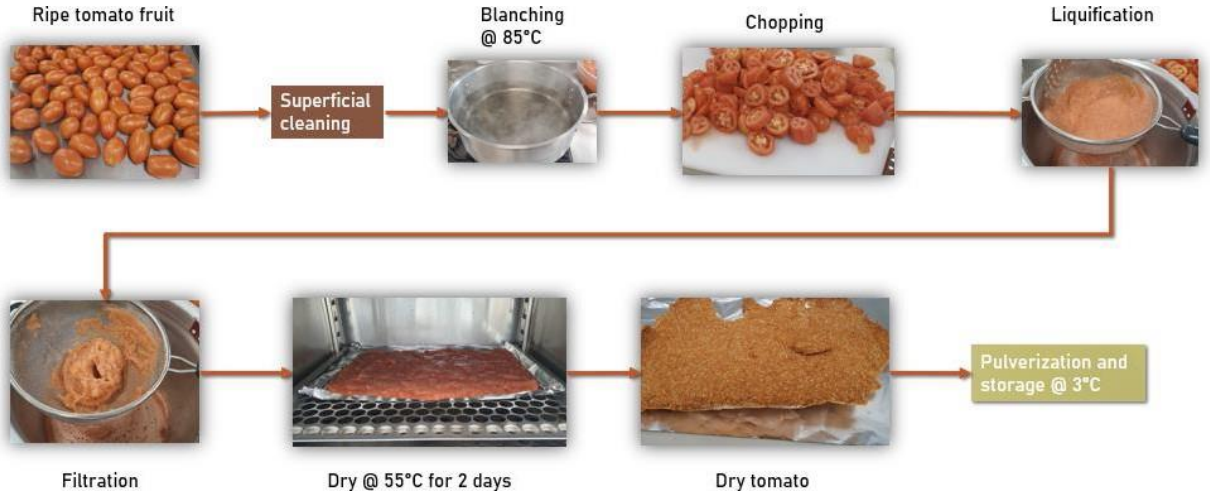

Fig. 2 Simulation of processing of tomato pomace generation.

3.2.2 Commercial enzymes characterization

The first set of experiments using a cellulase and a pectinase returned a series of unsatisfactory results. **Fig. 3** shows that the total amount of glucose liberated as the cellulose concentration increased stayed relatively static, meaning that the available enzymes quickly saturated with what initially appeared to be a minimal substrate concentration. The nKat unit was defined as 1nm of glucose liberated per nanomole of cellulose present per second, allowing for a more accurate unit to compare both the activity between these two enzymes and the enzymes present in future assays (see sections 3.2.4). The quick saturation of the enzymes is reflected by the decreasing slope present in **Fig. 3B**, where both enzymes' highest activity was observed at the first concentration of cellulose (1 g/L). These enzymes' cellulitic activity was low and easy to saturate, which caused a switch of focus towards another commercially available enzyme known as Rapidase ® and its pectinolytic capabilities.

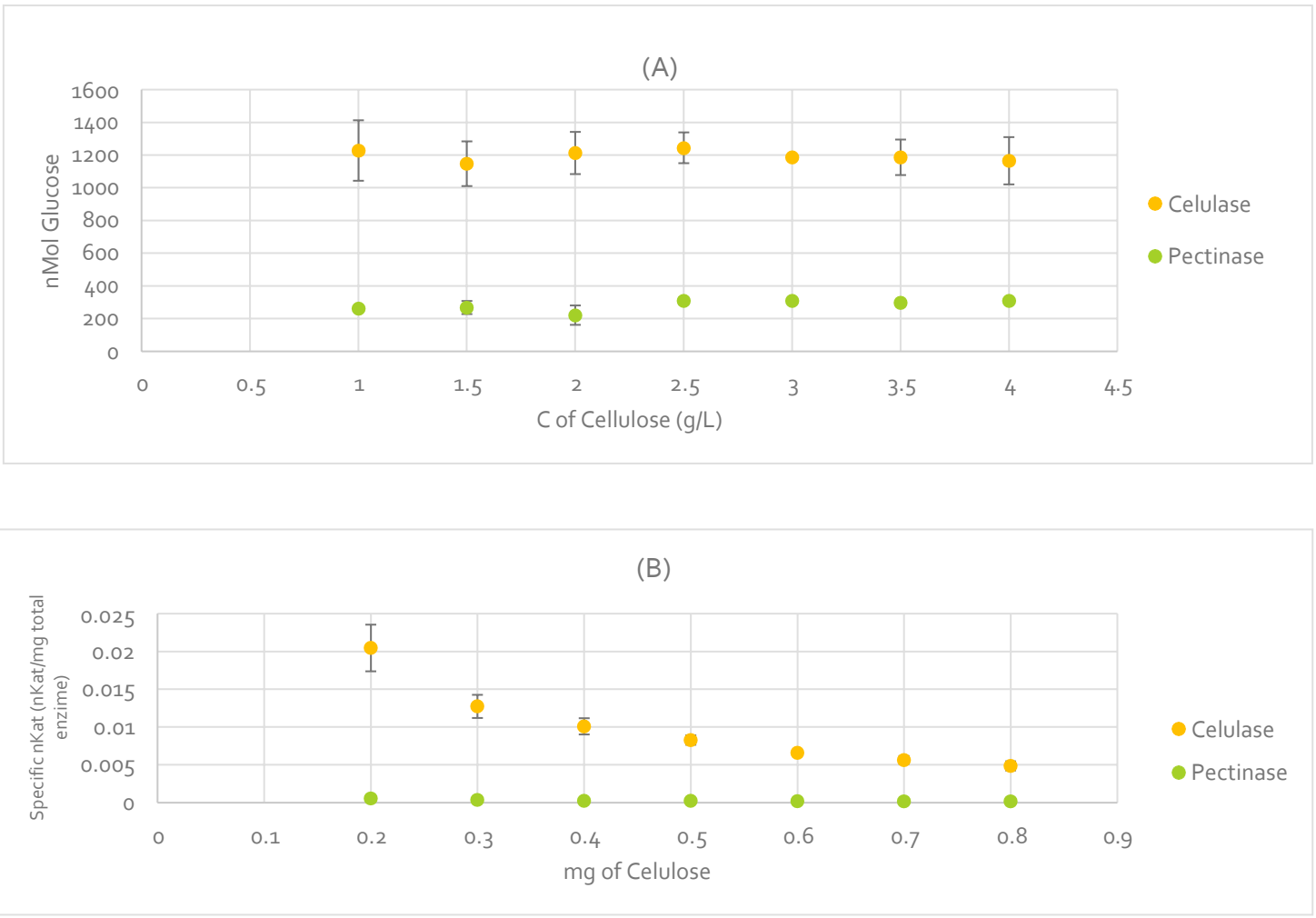

Fig. 3 Results of enzymatic activity characterization for cellulase and pectinase enzymes. A: total nanomoles of glucose liberated based on an initial concentration of substrate and a fixed amount of enzyme utilized for both cellulase and pectinase. B: specific nKat for each enzyme, which is taken by dividing nKat by the total amount of enzyme used in the reaction. Data represent the means of three replicates and their standard errors.

The second set of experiments for available commercial enzymes were done with Pectinase and Rapidase ®. The results are shown in **Fig. 4**, wherein **Fig. 4A** one can see an increase of glucose liberated for the Rapidase ®, up to a maximum (10 g/L of pectin), while the pectinase presents a positive slope with no discernable inflection point in the tested range. Comparing the different nKat for each enzyme shows that the highest activity is present in the first two amounts (0.4 and 0.8 mg of pectin), form which point both enzymes proceed to arrive to a plateau. Said stage appears to be indefinite for the pectinase, while the Rapides ® presents another decrease once the amount of pectin surpasses 2 mg. It was established that the optimal ratio of enzyme/substrate for the Rapidase ® was 1/40, which can be seen in **Fig. 4A**, where the concentration of substrate reads 10 g/L of pectin.

A similar study was carried over (Wilkins et al., 2007), where both cellulase and pectinase were used to degrade grapefruit peel waste to produce sugars for subsequent use in fermentation. They found that the optimal proportion of enzyme/peel dry matter to obtain the highest yield with the lowest loadings for pectinase was 1/200 and for cellulose was 1/500. This proportions are significantly smaller than the ones obtained in the present study, which could explain the reason why the results seen in **Fig. 4** do not show an increase in yield with an increase in available substrate. Still, the tendency of pectinase having a higher capacity of degrading peel wastes compared to cellulase is maintained. An important difference was that the treatment periods were considerably higher (24 hours) which allowed for a smaller enzyme concentration. An economical study should be carried out to determine if the increase in incubation time is worth the smaller required enzyme concentration, given that there is a significant cost attached to the maintenance of the optimal enzymatic parameters for such a long period of time, which could prove difficult to replicate at an industrial scale.

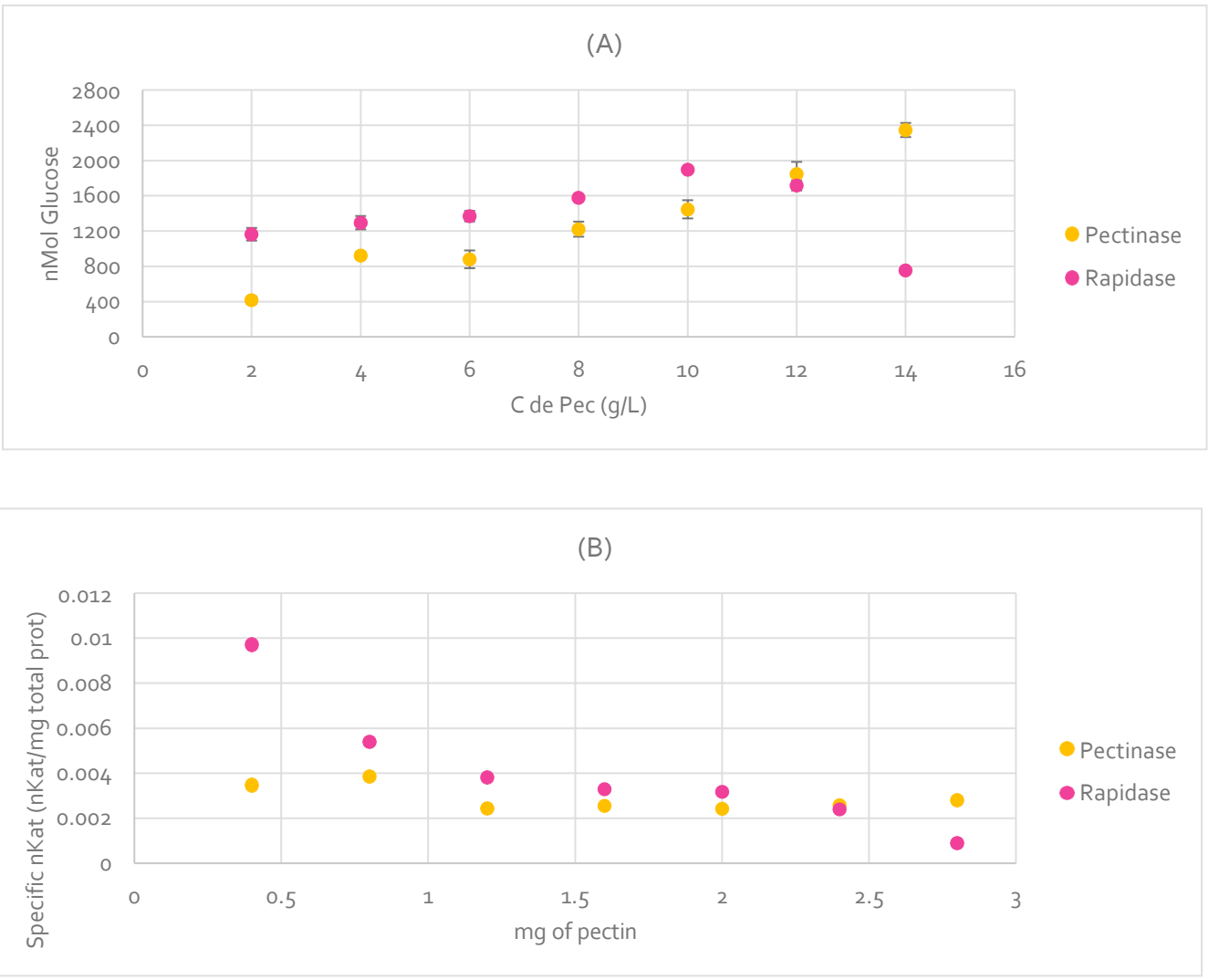

Fig. 4 Results of enzymatic activity characterization for pectinase and Rapidase ® enzymes. A: total nanomoles of glucose liberated based on an initial concentration of substrate and a fixed amount of enzyme utilized for both Pectinase and Rapidase ®. B: specific nKat for each enzyme, which is taken by dividing nKat by the total amount of enzyme used in the reaction. Data represents the mean of three replicates and their standard errors.

3.2.3 Fungal growth and identification of wild strain of filamentous fungus

Both strains were able to grow on PDA medium in a matter of days while also showing a substantial mycelium development. For the unidentified fungus, the corresponding mass spectra by MALDI-TOF were successfully obtained. **Fig. 5** compares the obtained spectra with the closest reference spectra present in the database employed, whose closest match was determined to be *Rhizopus oryzae*.

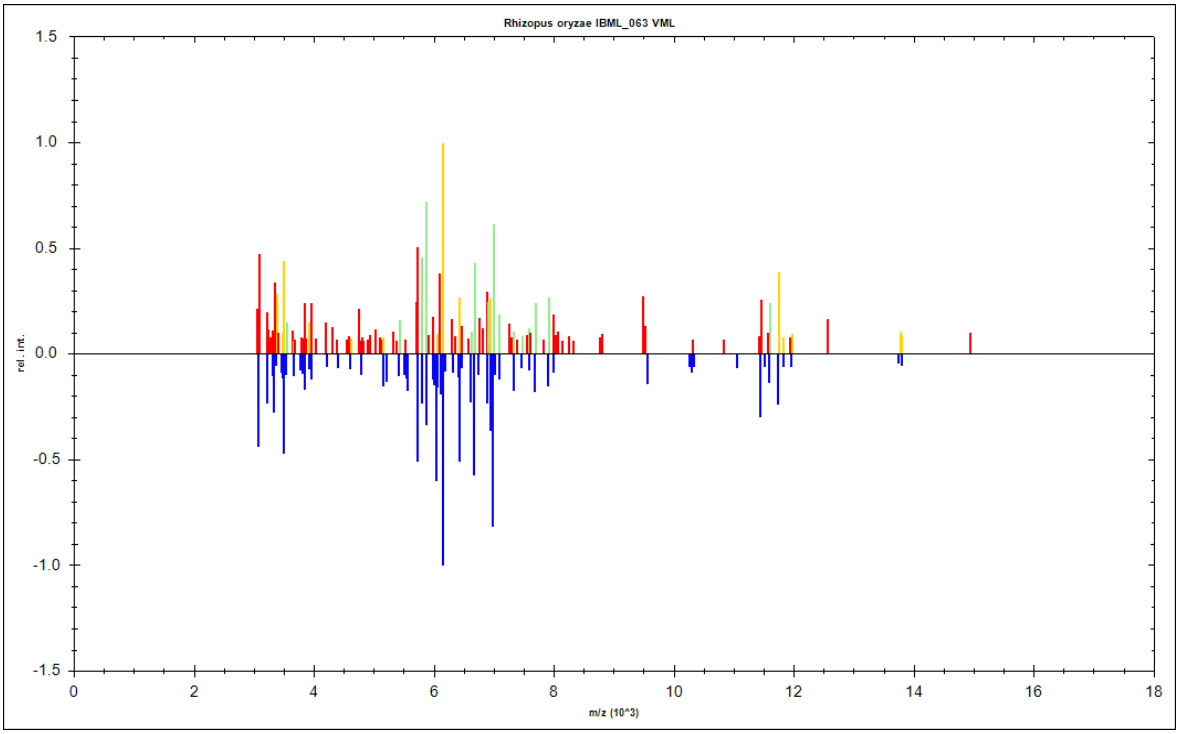

Fig. 5 Normalized MALDI-TOF mass spectra for fungal identification. The obtained spectrum for the wild strain (bottom) is compared with the reference spectrum (highest similarity found in the database).

Rhizopus oryzae is a heterothallic fungal species commonly found in soil, dung and rotting vegetation. Most strains of this fungi are active components in the production of oriental foods or alcoholic beverages in countries like china, japan and Indonesia (Oda et al., 2002). It has also been used for quite some time for the production of enzymes like lipases and glucoamylases, as well as a part of a bioreactor for the production of organic acids and a plethora of fermented foods (Cantabrana et al., 2015). It has been reported to have a high capacity for the generation of industrial enzymes, like cellulase, pectinase and xylanase, which makes this organism highly valuable for its fermentative applications at an industrial

scale. It also has the capacity of generating carbohydrate-active enzymes (CAZy) which allows the degradation of easily digestible plant cell wall mono and polysaccharides (Battaglia et al., 2011). This quality could directly correlate to an increase in extractability of bioactive compounds resulting from a fermentative pretreatment.

On the other hand, in the preliminary test carried out with minimal medium with supplemented pectin, both strains showed mycelium formation within the medium by day two and continued to grow in size until the experiment was stopped on day 5. A visual example of mycelium pellets formed by *Aspergillus* can be seen in **Fig. 6**.

Fig. 6 Images of *A. niger* ATCC 6275 strain grown on minimal medium with only pectin as source of carbon after 3 days.

3.2.4 SmF with tomato pomace

Various concentrations of tomato pomace were tested to determine the optimal ratio of substrate required for the best enzymatic activity present in the fermentation broth. This was achieved by the quantification of reducing sugars liberated post fermentations using DNS methodology and spectrophotometry. **Fig. 7** shows that the highest absorbance was achieved with a 0.75 **(**W/V) concentration of tomato pomace. Higher or lower concentration of tomato pomace was responsible of the decrease of the enzyme production. This concentration was established as the standard concentration for any subsequent essays involving SmF with tomato pomace.

The variation of days of incubation had a direct effect on the reducing sugars measured in the fermentation broth, which is shown in **Fig. 8**. Maximum concentration was obtained at day three (7.5 µg/mL), from which point on it showed a decrease. A high presence of reducing sugars is attributed to a higher present enzymatic activity. At the same time, optimal incubation time is crucial in minimizing energy and cost in a fermentation process. A comparable experiment was carried over (Oberoi et al., 2012) where cellulase and pectinase were employed to hydrolase banana peels. They were able to obtain a liberation of 48000 µg/mL of reducing sugars from the banana peel by using a combination of both enzymes during an incubation period of 15 h. Once again, the extended time of incubation allowed a much higher increase in reducing sugars.

To determine the specific activity in the fermentation broth, it was necessary to estimate the total concentration of protein present in the broth, which was determine by carrying over a Bradford test. The results (**Fig. 9**) showed an increase in total protein content as the fermentation period continued. By using the information present in **Fig. 8** and **Fig. 9**, it was decided that the best day in terms of effective enzymatic activity was at day three, given that this was when a higher amount of glucose was present in the broth while maintaining a relatively low concentration of protein, meaning a much-concentrated enzyme activity. An example of a vial where the SmF was carried out can be seen in **Fig 10**.

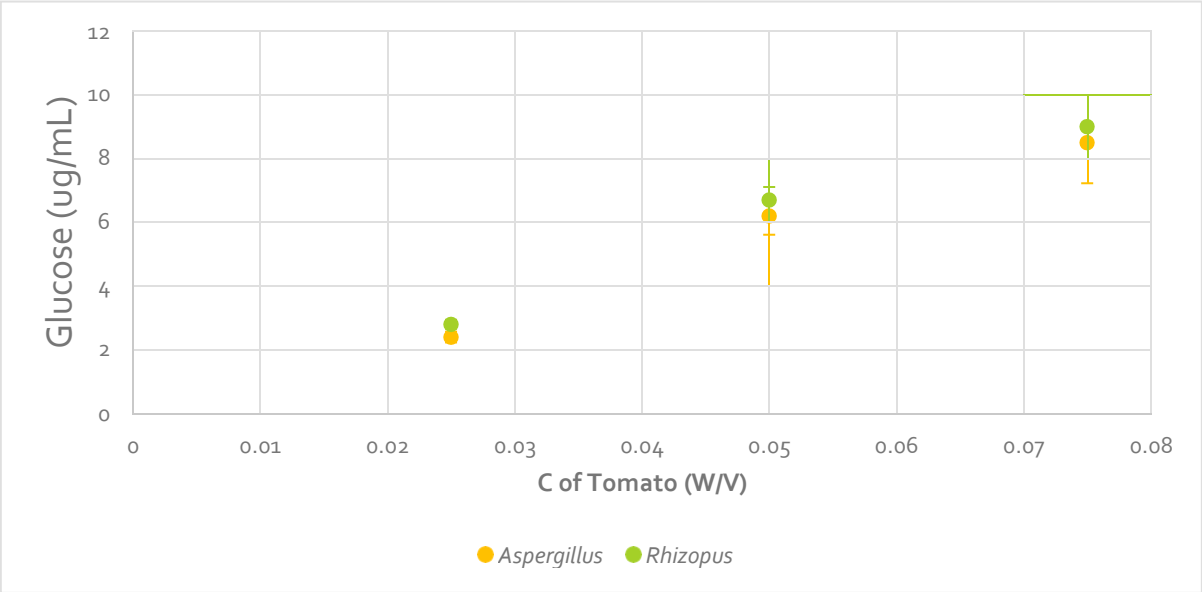

Fig. 7 Glucose liberated obtained by DNS test from fermentation (3 days) with minimal medium supplemented by 3 different concentrations (0.25,0.50,0.75 W/V) of tomato pomace. Data represent the means of three replicates and their standard errors.

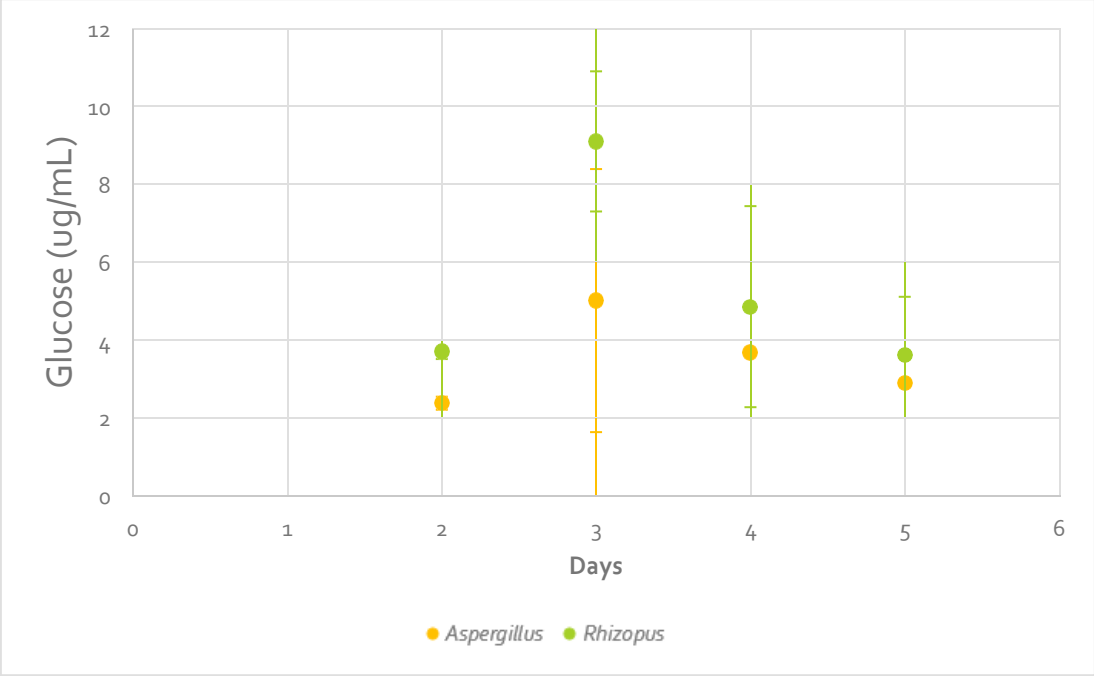

Fig. 8 Different concentrations of glucose liberated by the enzymatic effect of the fermentation broth form each strain for each day. Data represent the means of three replicates and their standard errors.

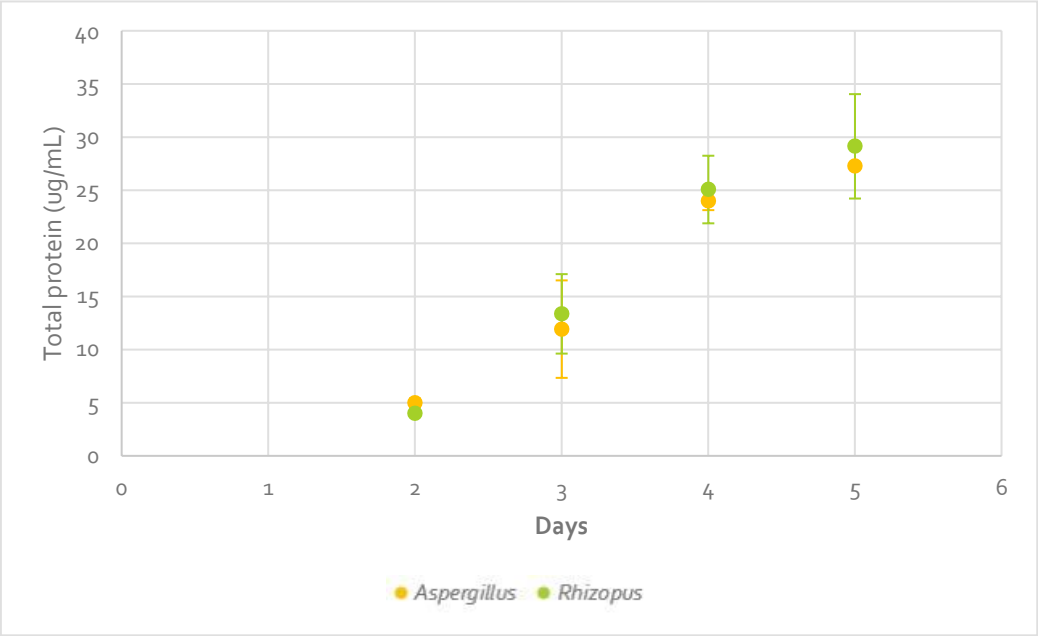

Fig 9. Results from the Bradford essay to determine the concentration of total protein present in the fermentation broth for each given day. Data represent the means of three replicates and their standard errors.

When comparing the pectinolytic activity of Pectinase and Rapidase ® with the enzymatic broth of both strains (**Fig. 11**), one can observe that the industrial enzymes show a higher amount of free glucose in all cases. When comparing the nKat of all treatments (**Fig. 11B**), the industrial enzymes show significantly higher maximums and averages than the enzymatic broth. This could be attributed to the fermentation process liberating a higher amount of cellulitic enzymes than pectinolytic enzymes, resulting in reduced pectin hydrolysis. In all cases, both strains show comparable results at all stages and tests so far, suggesting that up to this point, they have virtually the same effect as a fermentation microorganism.

As explained on an alternative investigation (Kuo et al., 2019), the use of cellulase and pectinase can produce a significant liberation of reducing sugars (81,000 µg/mL), like in the case of using said enzymes on orange peel wastes. The resulting hydrolyzed biomass can then be used to feed a fermentation for the production of bacterial cellulose, which was increased by 4-6 times higher than by using traditional Hestrin and Schramm medium.

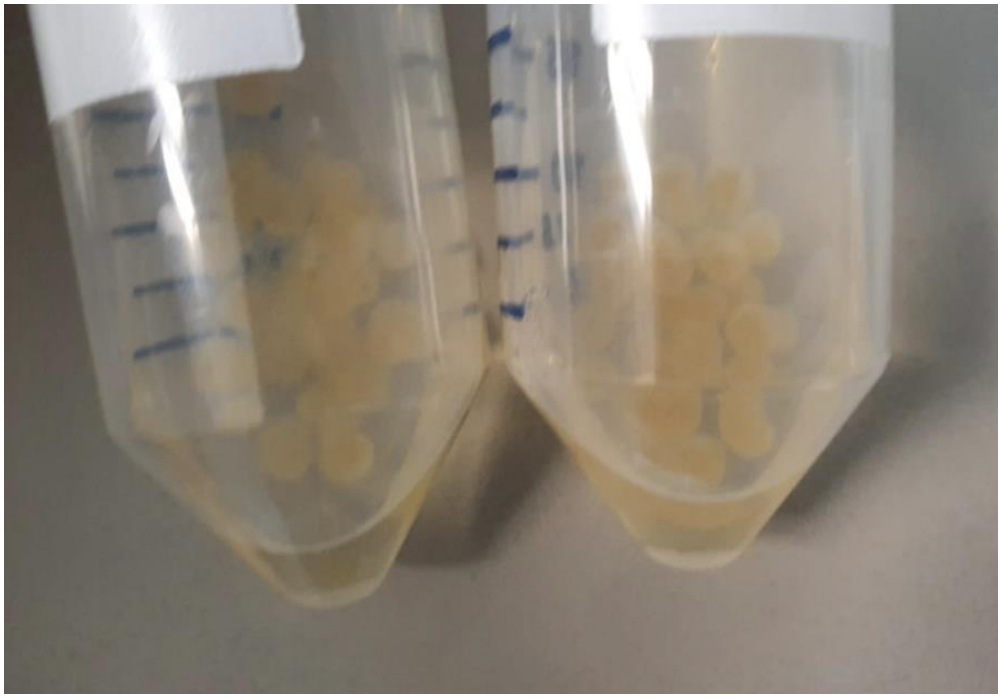

Fig. 10 SmF of *A. niger* ATCC 6275 after 5 days of incubation

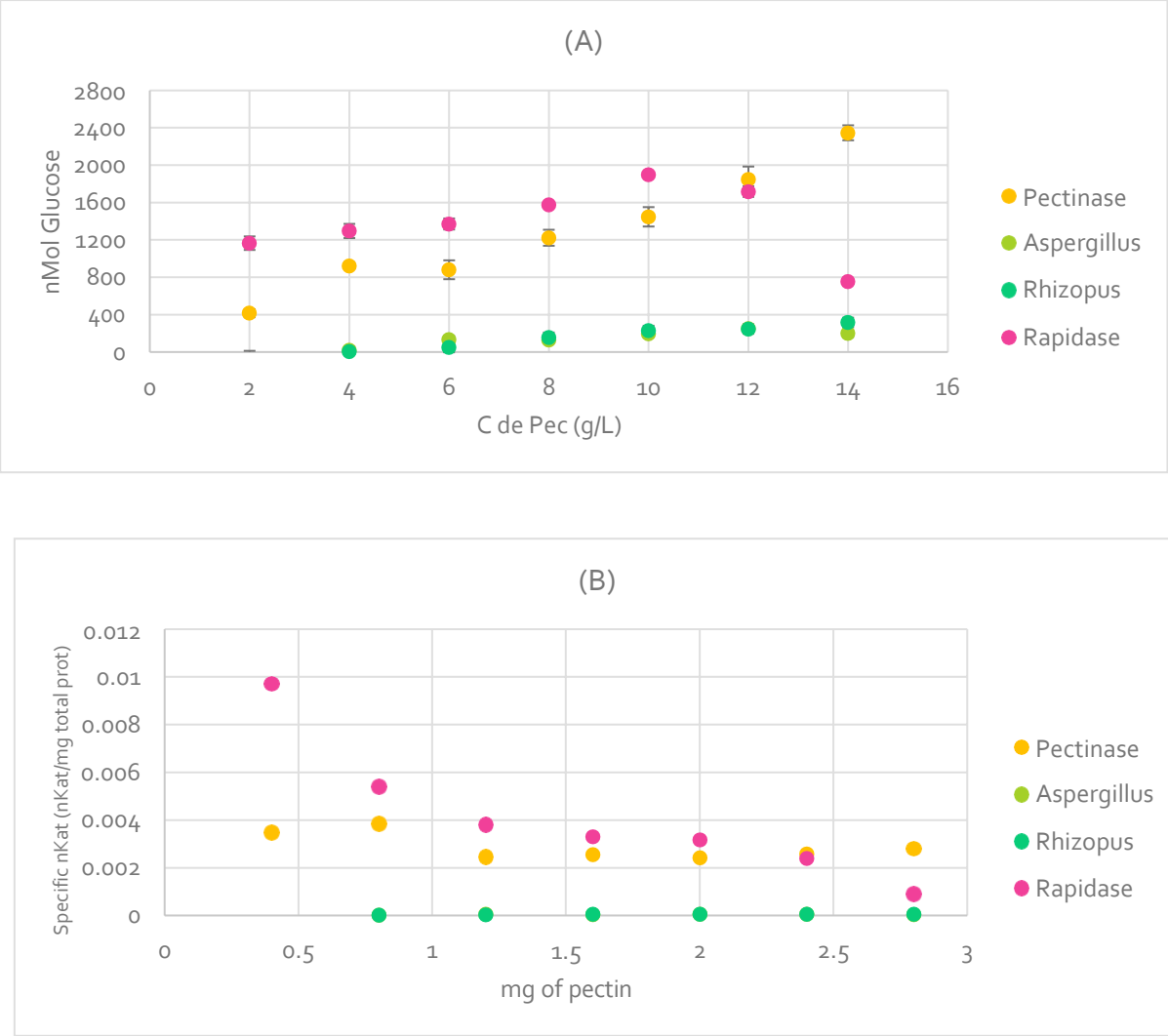

Fig. 11. Results of enzymatic activity characterization of enzyme rich fermentation broth from both strains A: total nanomoles of glucose liberated based on an initial concentration of substrate and a fixed amount of enzyme or fermentation broth. B: specific nKat for each treatment, which is taken by dividing nKat by the total amount of protein used in the reaction. Data represent the means of three replicates and their standard errors.

3.2.5 SSF with tomato pomace

Successful fermentation of rehydrated tomato (to 80% humidity) with both strains was carried over. There was no need to add any kind of additive to the tomato for the fungi's significant growth, and in all cases, there was only one strain of fungi at all times. The particle size (between 0.8 and 1.25 mm) resulted optimal for effective growth of the fungus, which was not big enough that contact surface decreased but nether too small that the particles would agglomerate and decrease the solvent penetration in the matrix (Meireles, 2008). Examples of successful fermentation with both strains can be observed in **Fig. 12**. Controls with rehydrated tomato (no inoculation) were also incubated, and in 35% of the cases, an already present filamentous fungi were shown to grow. There was an attempt to sterilize the tomato pomace by humid heat before inoculation, but in all attempts, the tomato naturally absorbed water, making the control of the humidity a problematic task. Still, the fact that there was never the presence of more than one fungus at a time indicates that both *Aspergillus* and *Rhizopus* have an aggressive enough development to compete and win in the use of the tomato pomace as a substrate. Finally, an SSF was carried over five days, from which an evident depletion of the initial tomato could be observed, meaning that a significant portion of the tomato was digested as seen in **Fig. 13**. This meant that the total possible recoverable lycopene and phenolic components could only be less than that of the initial tomato substrate, making lengthy fermentation period an ill advanced strategy for the extraction of these nutraceutical components.

A study compared the possibility of generating proteases using tomato pomace as a substrate for both solid-state fermentation and submerged fermentation (Belmessikh et al., 2013). The selected microorganism was *Aspergillus oryzae* for its high capacity of protease generation. The optimal incubation time for both cases was determined to be 96 hours, which is very similar to the results obtained in this study. Humidity was significantly reduced (60 %), which would imply an applied drying process for the fresh tomato pomace. There was also the enrichment of the tomato pomace with both casein and sodium chlorate for better production and growth. While the addition of additives to the pomace results in an increased production of enzymes, it also means an increase in cost and requires a homogenization of said compounds across all the fermentation surface, which could prove difficult at an industrial scale. While the enzymes of interest were not the same, it was reported that SSF generated 9 times more enzymes that the SmF. Another example of a

fermentation with tomato pomace was carried over by using *Pleurotus ostreatus* and *Trametes versicolor* for the production of protease (Landolo et al., 2011). This was possible to achieve without any kind of optimization in the culture conditions, and without the addition of O_2, while still been able to produce 34,000 U/g.

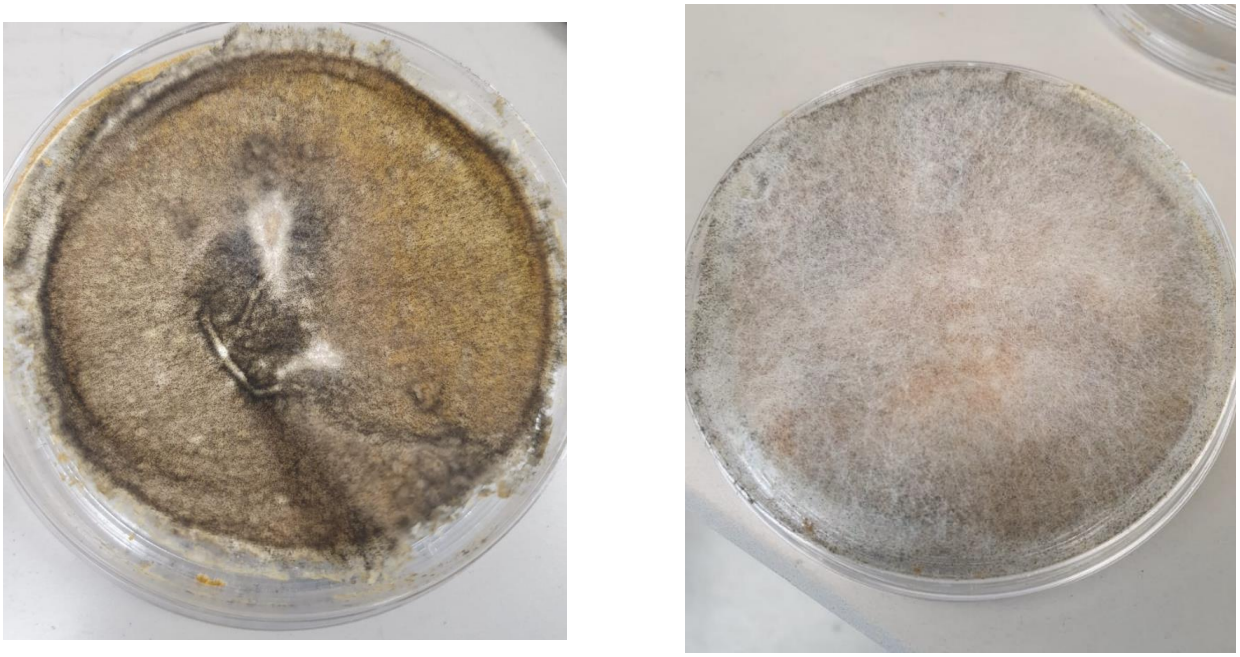

Fig. 12 Examples of SSF using tomato pomace and *A. niger* ATCC 6275 (left) and *R. oryzae* BIOTEC00X (right) after 3 days.

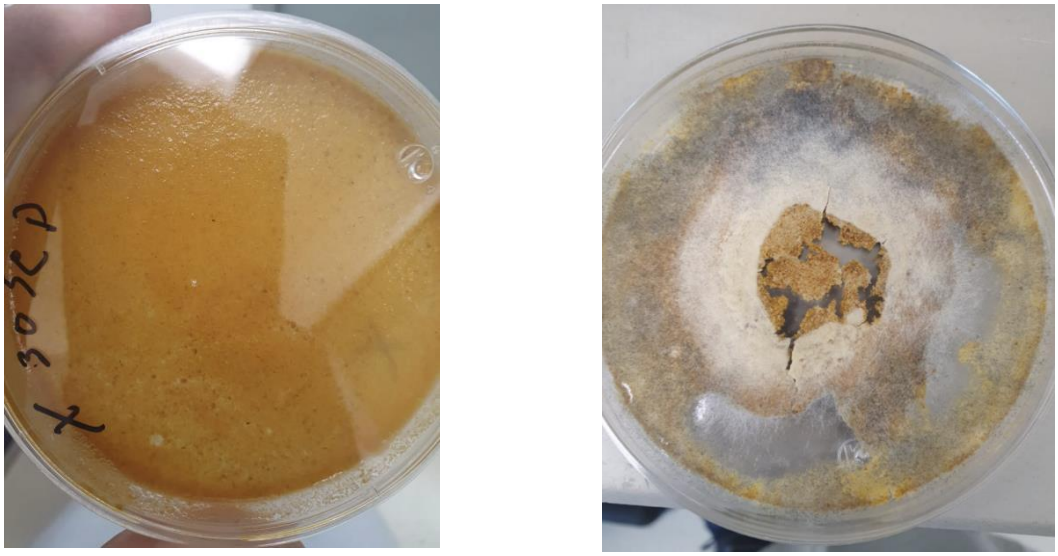

Fig. 13. SSF of tomato pomace using *R. oryzae* BIOTEC00X after 1 day of incubation (left) and after 7 days of incubation (right).

3.2.6 Extraction and quantification of total nutraceutical compounds

Three controls were used as a reference during the comparison of all the different pretreatments. The summarized results can be seen in **Fig. 14**. The first control (tomato pomace powder) was used to compare the Rapidase ® pretreatment for both types of extraction (lycopene and phenolic compounds). We can see a significant increase in the extraction of lycopene (227% increase), corresponding to the best treatment for the extraction of lycopene, while in the case of phenolic components, we see a decrease of 21%. This result is similar to other investigations (Choudhari & Ananthanarayan, 2007), where they obtained an increase of 188% of lycopene extraction using a 0.5% w/w, compared to the 2.5% w/w used in this thesis. The extraction increase of 90.6 µg/g is also similar to the one obtained here (76 µg/g). There is a direct benefit of applying pectinase to the tomato pomace, given that pectinases have the capacity of disintegrating the pectin compounds found in both the middle of the lamella and primary walls, as well as and the chromoplast inside the cell, which are more easily penetrated by the solvent when cell-wall polysaccharides are degraded (Neagu et al., 2014).

The extracted lycopene from tomato pomace (3.3 mg/100g) was comparable to other reported extraction (2.8 mg/100g) (Toor & Savage, 2005), but less than the ones found in pure peels (8.7 mg/100 g). This is expected, given that while the outer skin of the fruit has a high lycopene content, the seeds have significantly less (Efthimiadou et al., 2014). Non the less, the present rich concentration of lycopene in the tomato pomace offers a product of interest for its antioxidant properties and potential application as a functional ingredient (Luisa García et al., 2009).

Both SSF of *Aspergillus* and *Rhizopus*, an increase of lycopene extraction can be appreciated (more than 45%), while a more modest increase is also present for phenolic compounds (38% and 24%, respectively). The treatments with enzymatic broth were the ones with the most polarizing results, as in the case of lycopene, *Aspergillus* showed a small increase of 36%, while *Rhizopus* showed no real difference with its control. In comparison, the enzymatic broth of both strains increased the total phenolic compounds by 80%, by far the best pretreatment for phenolic extraction. The significant increase of polyphenol extraction with the fermentation broth could be attributed to the broth carrying over some of the naturally forming polyphenols from the original fermentation (Sepúlveda et al., 2020),

which when entering in contact with the tomato pomace for the enzymatic pretreatment, get absorbed by the porous of the cell wall, causing a increases in total polyphenol present in the surface of the matrix, which in turn increase the readily extractable phenolic compounds.

A similar study utilizing crude enzymes form a solid-state fermentation using *Fusarium solani* was able to obtain 152 mg of gallic acid equivalents per gram of tomato pomace (Azabou et al., 2016) compared to the 4.15 mg of chlorogenic acid equivalents obtained using the crude fermentation broth form the submerged fermentation. The significant increase (approximately 103%) of polyphenols recovered from tomato pomace compared to the polyphenols present in the fresh fruit could be attributed to the fact that the epidemic tissue receives a much higher intensity of bright adiation, particularly in the pericarp, which stimulates the synthesis of polyphenols (Toor & Savage, 2005).

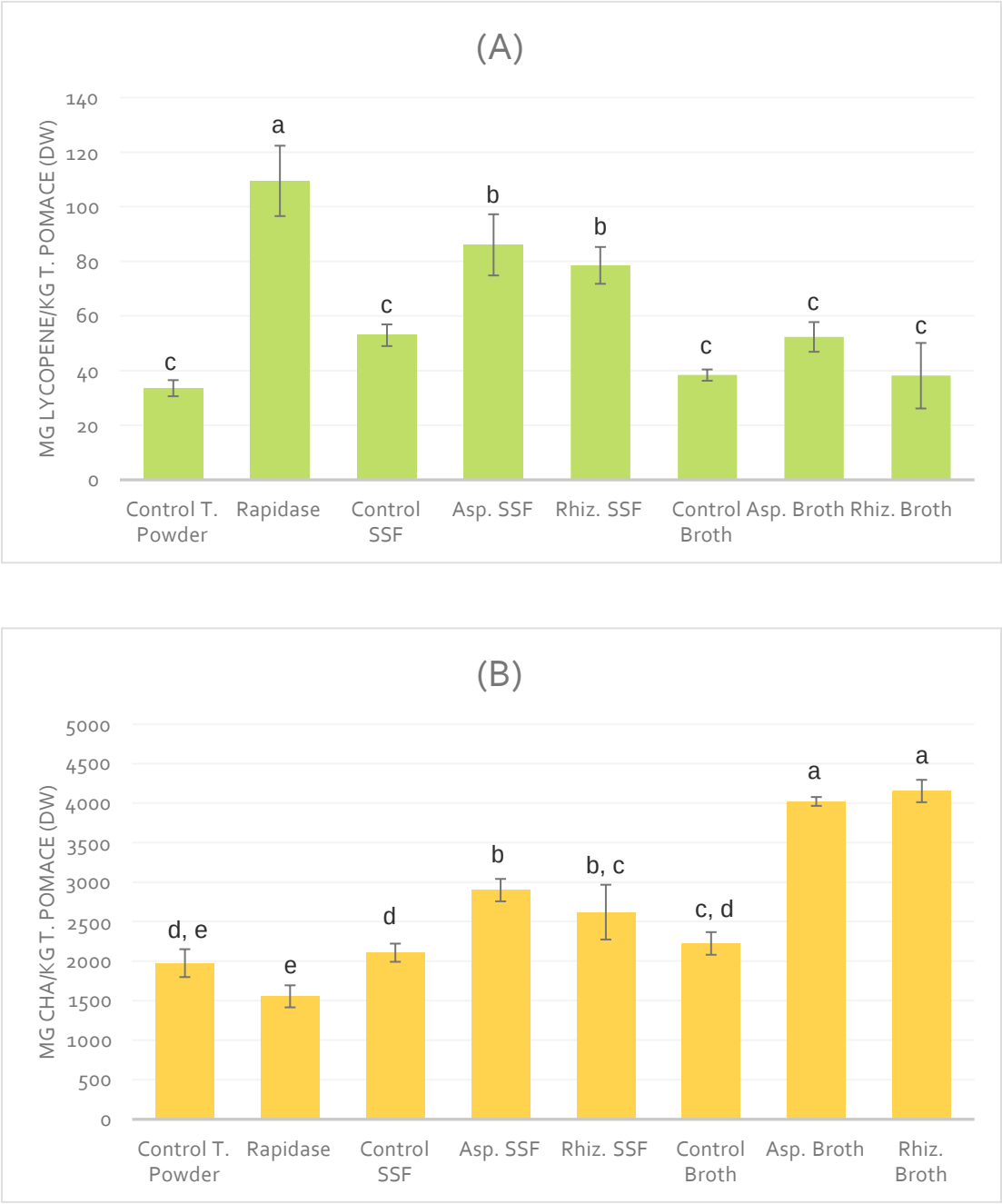

Fig. 14 Bioactive content (spectrophotometric determinations) from tomato pomace for each pretreatment. A: lycopene content expressed as mg of lycopene/ Kg of tomato pomace dw. B: total phenolic compounds expressed as mg of chlorogenic acid/ Kg of tomato pomace dw. Data represent the means of 6 replicates and their standard errors. Bars with different letters indicate statistical difference by the Tuckey test ($P < 0.05$).

3.2.7 Tentative identification and quantification of phenolic compounds

The UHPLC analysis monitored at 280, 320 and 360 nm allowed the tentative identification of 15 phenolic compounds, which have been summarized in **table 4.** Three chromatograms are shown in **Fig. 15;** these are representative chromatograms from tomato pomace samples at 280, 320, and 360 nm. They were chosen from all the available chromatograms to more noticeably highlight each one of the peaks corresponding to the 15 identified compounds. This was achieved by comparing UV/Vis spectra data for each peak with available literature (Perea-Domínguez et al., 2018; Valdez-Morales et al., 2014; Gómez-Romero et al., 2010). Furthermore, **table 5** shows individual phenolic content (µg CHA/g dry weight) in pretreated tomato pomace by UHPLC. The predominant phenolic compounds detected in tomato pomace, as well as the most susceptible to variate based on the type of pretreatment were naringenin derivative III (peak #15), protocatechuic acid (peak # 1) and feruloylquinic acid (peak # 12). This vast variety of phenolic compounds present in tomato matches what has been previously reported in the literature (Valdez-Morales et al., 2014).

Table 4. UHPLC retention times, UV/Vis spectra data and assigned identity of phenolics from tomato pomace.

# Peak	Retention time (min)	UV λ_{max}	Assigned identity
1	1.20	261	Protocatechuic acid
2	2.14	288	Kaempherol-3-rutinoside
3	5.54	298	Sinapic acid hexose
4	6.38	288, SH 370	Coumaric acid derivative I
5	7.19	297	Coumaric acid derivative II
6	8.94	310	Coumaroylquinic acid
7	10.24	322	3- Caffeoylquinic acid
8	11.18	300, SH 370	Dihydroxycinnamic acid hexose
9	11.82	313, SH 370	4-Caffeoylquinic acid
10	14.82	290	Naringenin
11	15.54	281	Coumaric acid hexose
12	15.84	276, SH 230	Feruloylquinic acid
13	16.46	284, SH 331	Naringenin derivative I
14	16.58	284, SH 331	Naringenin derivative II
15	17.42	284, SH 331	Naringenin derivative III

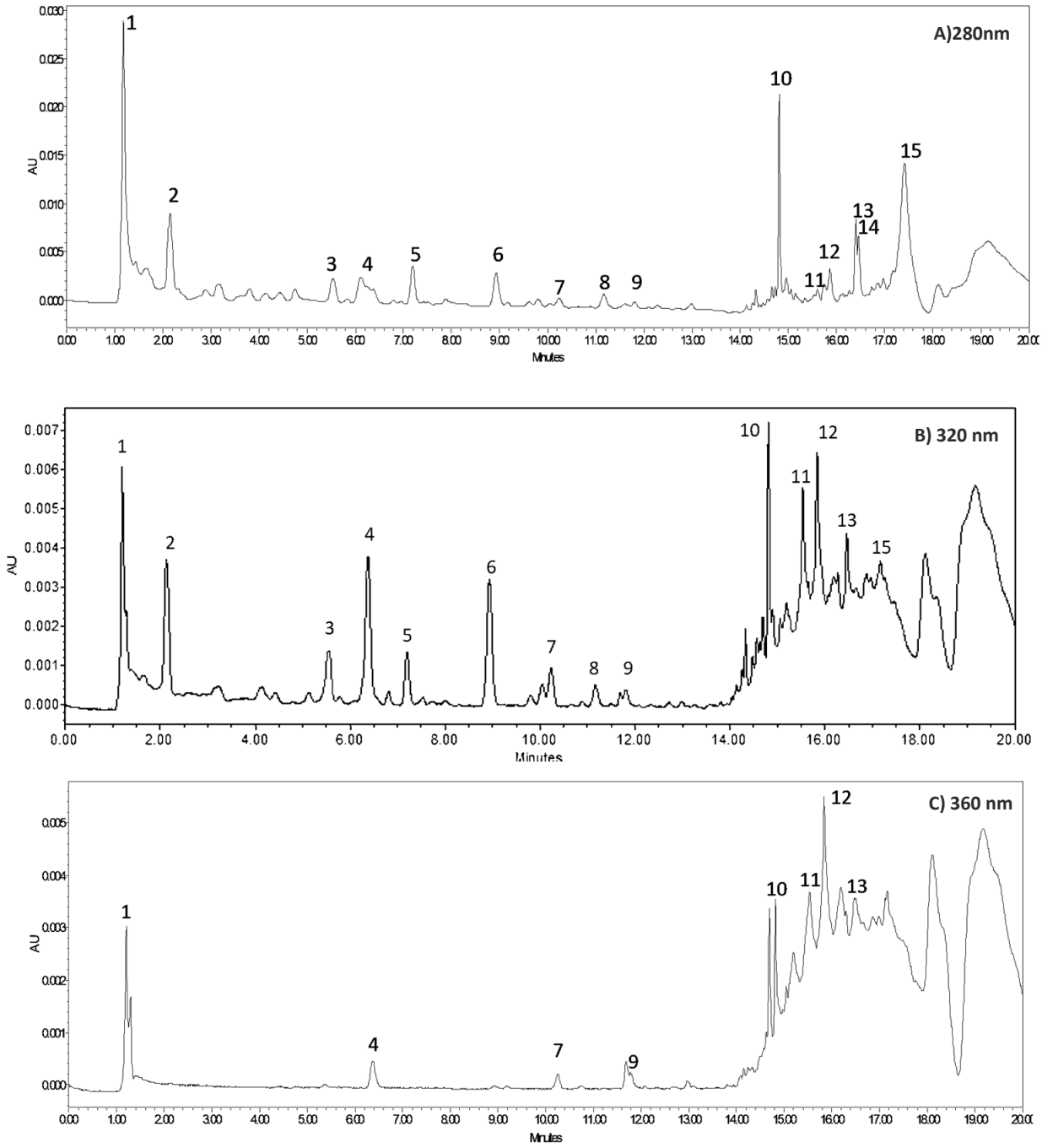

Fig. 15 Representative chromatograms obtained from a tomato pomace extract recorded at 280 (A) 320 (B) and 360 (C) nm. Peak identities are shown in Table 4.

Table 5. Individual phenolic content (µg CHA/g dry weight) in pretreated tomato pomace by UHPLC. Bold numbers highlight the most present phenolic compound for each pretreatment.

#	Tentative ID	Control powder	Rapidase	Control SSF	Asp. SSF	Rhiz. SSF	Control Broth	Asp. Broth	Rhiz. Broth
1	Protocatechuic acid	73.5±0.9 ^{d}	**141.8±14.0** ^{c}	80.1±3.9 ^{d}	194.0±17.7 ^{b}	159.6±6.8 ^{b,c}	63.7±2.6 ^{d}	**566.6±7.2** ^{a}	**531.5±47.0** ^{a}
2	Kaempherol-3-rutinoside	26.8±0.5 ^{b}	16.1±1.2 ^{c}	25.3±2.3 ^{b}	13.5±0.9 ^{c}	28.8±0.2 ^{b}	22.0±1.3 ^{b,c}	161.7±3.5 ^{a}	156.0±9.6 ^{a}
3	Sinapic acid hexose	5.6±0.0 ^{c}	2.8±0.2 ^{d}	5.7±0.7 ^{c}	8.1±0.7 ^{b}	Tr.	6.7±0.3 ^{b,c}	19.3±0.2 ^{a}	19.3±1.5 ^{a}
4	Coumaric acid derivative I	2.7±0.0 ^{f}	10.9±0.9 ^{c}	4.3±0.3 ^{e,f}	8.5±0.8 ^{d}	5.9±0.4 ^{e}	4.1±0.0 ^{e,f}	46.8±1.3 ^{a}	36.0±1.8 ^{b}
5	Coumaric acid derivative II	10.0±0.1 ^{b}	10.7±0.6 ^{b}	8.8±0.2 ^{b}	7.7±0.7 ^{b}	7.9±0.5 ^{b}	9.2±0.3 ^{b}	37.0±1.6 ^{a}	39.5±3.2 ^{a}
6	Coumaroylquinic acid	7.0±0.1 ^{c,d}	18.7±1.8 ^{a}	6.7±0.5 ^{c,d}	6.2±0.5 ^{d}	6.1±0.5 ^{d}	9.3±0.5 ^{c}	13.6±1.1 ^{b}	16.9±1.4 ^{a}
7	3- Caffeoylquinic acid	0.3±0.0 ^{b}	Tr.	0.1±0.0 ^{b}	n.d.	n.d.	0.1±0.0 ^{b}	51.0±3.7 ^{a}	49.9±1.7 ^{a}
8	Dihydroxycinnamic acid hexose	3.2±0.1 ^{c}	18.2±1.4 ^{b}	3.5±0.2 ^{c}	2.3±0.2 ^{c}	3.4±0.1 ^{c}	2.5±0.0 ^{c}	23.8±2.4 ^{a}	22.0±1.3 ^{a}
9	4-Caffeoylquinic acid	Tr.	Tr.	Tr.	n.d.	n.d.	Tr.	n.d.	n.d.
10	Naringenin	18.1±0.1 ^{b}	20.6±0.4 ^{a}	20.6±1.5 ^{a}	9.4±0.5 ^{c}	7.1±0.5 ^{d}	20.6±0.9 ^{a}	22.0±1.6 ^{a}	22.1±0.2 ^{a}
11	Coumaric acid hexose	n.d.	Tr.	Tr.	2.0±0.2 ^{a}	1.0±0.1 ^{b}	Tr.	Tr.	Tr.
12	Feruloylquinic acid	2.1±0.1 ^{c}	21.6±2.0 ^{c}	2.0±0.1 ^{c}	**503.3±42.0** ^{a}	**371.8±27.0** ^{b}	2.3±0.1 ^{c}	Tr.	0.2±0.0 ^{c}
13	Naringenin derivative I	8.7±0.1 ^{c}	3.6±0.1 ^{d}	7.9±0.1 ^{c}	2.9±0.2 ^{d}	4.2±0.4 ^{d}	7.5±0.4 ^{c}	15.6±0.3 ^{b}	23.4±1.5 ^{a}
14	Naringenin derivative II	8.7±0.1 ^{b}	n.d.	7.9±0.1 ^{c}	n.d.	n.d.	7.5±0.4 ^{c}	13.9±0.5 ^{a}	n.d.
15	Naringenin derivative III	**103.3±0.3** ^{a}	n.d.	**88.8±8.4** ^{a}	n.d.	Tr.	**81.1±5.7** ^{a}	2.3±0.2 ^{c}	63.1±4.7 ^{b}

Values are the mean of two independent determinations ± standard deviation. Lowercase letters indicate statistically significant differences ($p \leqslant 0.05$) between pretreatments for each given compound.
n.d. Not detected.
Tr. Traces.

When comparing the results of total phenolic content quantified by UHPLC (**Fig. 16**) and spectrophotometry (**Fig. 14B)** it can be notice that there is a significant spectrophotometric overestimation (250-800%) for all pretreatments. Disparity between quantification techniques for phenolic compounds is expected, given that there is no single analytical method that does not carry some kind of error in the measurement of total polyphenol content in biological tissue. This is explained by the structural diversity found among phenolic compounds and the large variation of content depending on the type of food and the plant it is derived from (Swallah et al., 2020). In the case of tomato pomace, the Folin reagent is expected to overestimate the content of phenolic compounds due to other present reducing agents, such as ascorbic acid (Martínez-Valverde et al., 2002). This problem is also present in other forms of tomato, like in the case of tomato juice, whose content of ascorbic acid can account for up to 46% of the estimated total phenols (Scalbert & Williamson, 2000).

While a more precise methods of quantification, like UHPLC, is required for a collective and accurate measurement of total phenolic compounds, quantification using spectrophotometry is still a useful technique for quick screening and comparison between samples, as also recommended by Schwartz et al. (1981). In this case, the proportions between treatments stayed relatively the same between spectrophotometry and UHPLC, as in both cases the best methods of extraction were the fermentation broth and SSF.

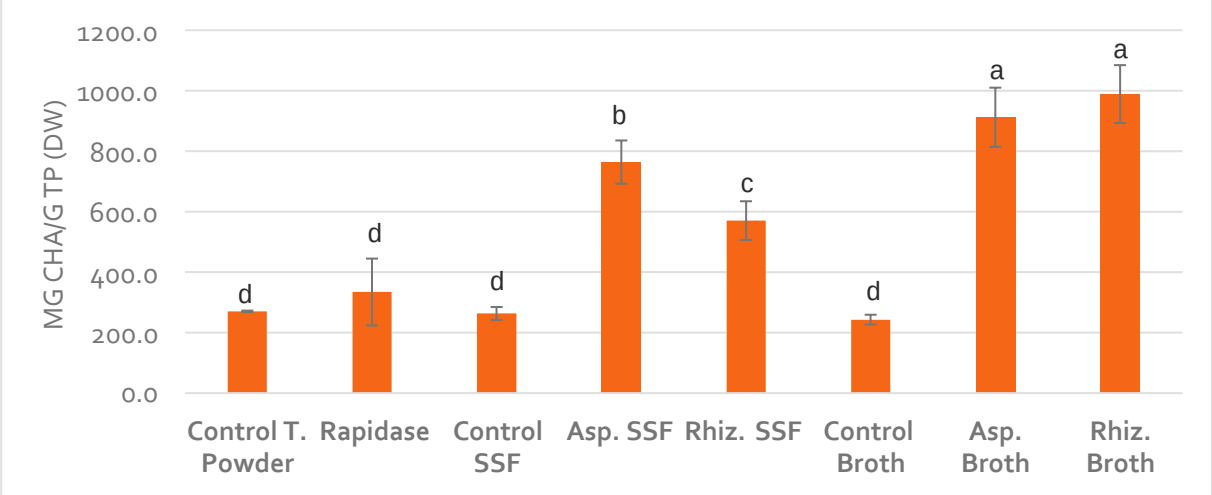

Fig. 16 Total phenolic content (µg CHA/g dry weight) obtained from each treatment with tomato pomace by UHPLC. Bars with different letters indicate statistical difference by the Tuckey test ($P < 0.05$).

CHAPTER 4 GENERAL CONCLUSIONS AND RECOMMENDATIONS

1. The tomato pomace that was used in this study presented physicochemical properties in line with those previously reported in the literature. It was also a suitable substrate for fermentation processes using both *Aspergillus niger* ATCC 6275 and *Rhizopus oryzae* BIOTEC00X, the latter being successfully identified by MALDI-TOF.

2. After studying the different conditions for submerged fermentation, it was found that the best tomato pomace concentration corresponded to 0.75 w/w, with an optimal incubation time of 3 days for the maximization of enzymatic activity present in the broth.

3. The studied industrial enzymes (Pectinase and Rapidase ®) showed a relatively constant specific activity across the tested substrate concentration range (1-4 g/L of pectin) while the submerged fermentation (SmF) broth showed low activity across the same range.

4. It was possible to carry out a solid-state fermentation (SSF) using both strains with tomato pomace as a substrate. It was not necessary to addy any kind of additive for the fungus to grow and develop mycelium in the surface. The optimal incubation time was determined to be 3 days. Fermentation periods of longer than 6 days will result in the loss of tomato pomace due to fungal metabolism, reducing the potentially recoverable bioactive compounds.

5. All pretreatments (industrial enzymes, SmF broth and SSF) showed a positive impact in the increase of extractability for both lycopene and phenolic compounds. The best treatments for the extraction of lycopene were SSF with both strains (50-60% increase) and the Rapidase enzyme degradation (107% increase). For the extraction of phenolic compounds, the best treatments were SSF (24-38% increase) and the SmF broths (90-97% increase).

6. Fifteen phenolic compounds were tentatively identified and quantified by UHPLC. Naringenin derivative III, protocatechuic acid and feruloylquinic acid were the most affected compounds by all pretreatment applied on tomato pomace samples.

7. During the quantification of total phenolic contents extracted from the tomato pomace, there was a significant spectrophotometric overestimation (250-800%) for all pretreatments when compared to the results obtained with UHPLC. While the quantities obtained with both methods differed, the proportions between treatments remained the same, demonstrating that spectrophotometric determination is a useful tool for quick comparison and screening.

The increased attention that SSF has gained in recent years comes from the constant and rapid change of today's environment and technological tendencies, where innovation is continuously searching for ways of doing industrial processes faster, cleaner, more efficient, and with higher returns. SSF is considered as a new and flexible alternative to the more common fermentation process (like SmF), with several biotechnological advantages, that while attractive, are directly linked to a laboratory scale (Sadh et al., 2018). At a pilot stage, it is expected a higher fermentation productivity, a higher product stability, and a higher final concentration of bioactive compounds, with the bonus of lower sterility demands due to the low water activity used in SSF. For the particular application of nutraceutical synthesis and enhanced extractability, SSF offers excellent results and potentially scalable process due to higher biomass production, high enzyme production, and lower protein breakdown (Cerda et al., 2019). There is a real competitive edge that SSF bring to the industry compared to other popular options like enzymatic degradation, considering that it is not necessary to use large volumes of water nor constant agitation.

SSF can enhance the production of a variety of nutraceuticals with the utilization of industrial food by-products. This marks an avenue where many potential products can be generated, with the added value of being a green technology which base materials are inexpensive and with high availability. Further efforts are needed in the direction of automation and optimization of the process, which could prove a similar efficiency to SmF.

Future research to further expand and complement the results obtained in this dissertation are the following:

1. An optimization process for all SSF parameters, including but not limited to: pH, additives, inoculum (spore concentration), humidity. These parameters are known to have a high impact in the overall effectiveness of the fermentation, so an optimization could greatly increase the impact in extractability.

2. Characterize the enzymes liberated during the fermentation process, using tomato pomace as a substrate, and compare with available industrial enzymes for a more complete profiling of the advantages and drawbacks of SSF.

3. An economical evaluation of the SSF application at industrial scale versus the traditional application of an enzymatic degradation for the enhancement of extractability of bioactive compounds. This is because SSF brings advantages over the enzymatic degradations that could translate to a feasible methodology.

4. The use of alternative extraction methods, like the application of super critical fluid extraction, coupled with any and all of the explored pretreatments could reveal an even more significant and viable technique for the extraction of nutraceutical compounds coming from tomato pomace.

5. Further mass spectrometry analyses would be useful for more accurate identification of phenolic compounds in tomato pomace. In addition, it is important to carry out the lycopene UHPLC determination especially regarding the use of specific lycopene standard for the compound quantification.

References:

Abdel-Aty, A. M., Bassuiny, R. I., Barakat, A. Z., & Mohamed, S. A. (2019). Upgrading the phenolic content, antioxidant and antimicrobial activities of garden cress seeds using solid-state fermentation by *Trichoderma reesei*. *Journal of Applied Microbiology*, *127*(5), 1454–1467. https://doi.org/10.1111/jam.14394

Abdul Manan, M., & Webb, C. (2017). Modern microbial solid state fermentation technology for future biorefineries for the production of added-value products. *Biofuel Research Journal*, *4*(4), 730–740. https://doi.org/10.18331/BRJ2017.4.4.5

Abu Yazid, N., Barrena, R., Komilis, D., & Sánchez, A. (2017). Solid-State Fermentation as a Novel Paradigm for Organic Waste Valorization: A Review. *Sustainability*, *9*(2), 224. https://doi.org/10.3390/su9020224

Adetunji, L. R., Adekunle, A., Orsat, V., & Raghavan, V. (2017). Advances in the pectin production process using novel extraction techniques: A review. *Food Hydrocolloids*, *62*, 239–250. https://doi.org/10.1016/j.foodhyd.2016.08.015

Ajila, C. M., Brar, S. K., Verma, M., Tyagi, R. D., & Valéro, J. R. (2011). Solid-state fermentation of apple pomace using Phanerocheate chrysosporium – Liberation and extraction of phenolic antioxidants. *Food Chemistry*, *126*(3), 1071–1080. https://doi.org/10.1016/j.foodchem.2010.11.129

Al-Weshahy, A., & Rao, V. A. (2012). Potato Peel as a Source of Important Phytochemical Antioxidant Nutraceuticals and Their Role in Human Health—A Review. *Phytochemicals as Nutraceuticals - Global Approaches to Their Role in Nutrition and Health*. https://doi.org/10.5772/30459

Amiri-Rigi, A., & Abbasi, S. (2016). Microemulsion-based lycopene extraction: Effect of surfactants, co-surfactants and pretreatments. *Food Chemistry*, *197*, 1002–1007. https://doi.org/10.1016/j.foodchem.2015.11.077

Amiri-Rigi, A., Abbasi, S., & Scanlon, M. G. (2016). Enhanced lycopene extraction from tomato industrial waste using microemulsion technique: Optimization of enzymatic and ultrasound pre-treatments. *Innovative Food Science & Emerging Technologies*, *35*, 160–167. https://doi.org/10.1016/j.ifset.2016.05.004

Amorim, C., Silvério, S. C., & Rodrigues, L. R. (2019). One-step process for producing prebiotic arabino-xylooligosaccharides from brewer's spent grain employing Trichoderma species. *Food Chemistry*, *270*, 86–94. https://doi.org/10.1016/j.foodchem.2018.07.080

Anokwuru, C., Sigidi, M., Boukandou, M., Tshisikhawe, P., Traore, A., & Potgieter, N. (2018). Antioxidant Activity and Spectroscopic Characteristics of Extractable and Non-Extractable Phenolics from Terminalia sericea Burch. Ex DC. *Molecules*, *23*(6), 1303. https://doi.org/10.3390/molecules23061303

Arora, S., Rani, R., & Ghosh, S. (2018). Bioreactors in solid state fermentation technology: Design, applications and engineering aspects. *Journal of Biotechnology*, *269*, 16–34. https://doi.org/10.1016/j.jbiotec.2018.01.010

Azabou, S., Abid, Y., Sebii, H., Felfoul, I., Gargouri, A., & Attia, H. (2016). Potential of the solid-state fermentation of tomato by products by Fusarium solani pisi for enzymatic extraction of lycopene. *LWT - Food Science and Technology*, *68*, 280–287. https://doi.org/10.1016/j.lwt.2015.11.064

Ballesteros, L. F., Teixeira, J. A., & Mussatto, S. I. (2014). Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. *Food and*

Bioprocess Technology, *7*(12), 3493–3503. https://doi.org/10.1007/s11947-014-1349-z

Battaglia, E., Benoit, I., van den Brink, J., Wiebenga, A., Coutinho, P. M., Henrissat, B., & de Vries, R. P. (2011). Carbohydrate-active enzymes from the zygomycete fungus Rhizopus oryzae: A highly specialized approach to carbohydrate degradation depicted at genome level. *BMC Genomics*, *12*(1), 38. https://doi.org/10.1186/1471-2164-12-38

Becerra-Moreno, A., Redondo-Gil, M., Benavides, J., Nair, V., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2015). Combined effect of water loss and wounding stress on gene activation of metabolic pathways associated with phenolic biosynthesis in carrot. *Frontiers in Plant Science*, *6*, 837. https://doi.org/10.3389/fpls.2015.00837

Bei, Q., Liu, Y., Wang, L., Chen, G., & Wu, Z. (2017). Improving free, conjugated, and bound phenolic fractions in fermented oats (Avena sativa L.) with Monascus anka and their antioxidant activity. *Journal of Functional Foods*, *32*, 185–194. https://doi.org/10.1016/j.jff.2017.02.028

Belmessikh, A., Boukhalfa, H., Mechakra-Maza, A., Gheribi-Aoulmi, Z., & Amrane, A. (2013). Statistical optimization of culture medium for neutral protease production by Aspergillus oryzae. Comparative study between solid and submerged fermentations on tomato pomace. *Journal of the Taiwan Institute of Chemical Engineers*, *44*(3), 377–385. https://doi.org/10.1016/j.jtice.2012.12.011

Ben-Othman, S., Jõudu, I., & Bhat, R. (2020). Bioactives from Agri-Food Wastes: Present Insights and Future Challenges. *Molecules*, *25*(3), 510. https://doi.org/10.3390/molecules25030510

Bhanja Dey, T., Chakraborty, S., Jain, K. Kr., Sharma, A., & Kuhad, R. C. (2016). Antioxidant phenolics and their microbial production by submerged and solid state fermentation

process: A review. *Trends in Food Science & Technology*, *53*, 60–74. https://doi.org/10.1016/j.tifs.2016.04.007

Bhanja Dey, T., & Kuhad, R. C. (2014). Enhanced production and extraction of phenolic compounds from wheat by solid-state fermentation with Rhizopus oryzae RCK2012. *Biotechnology Reports*, *4*, 120–127. https://doi.org/10.1016/j.btre.2014.09.006

Buenrostro-Figueroa, J. J., Velázquez, M., Flores-Ortega, O., Ascacio-Valdés, J. A., Huerta-Ochoa, S., Aguilar, C. N., & Prado-Barragán, L. A. (2017). Solid state fermentation of fig (Ficus carica L.) by-products using fungi to obtain phenolic compounds with antioxidant activity and qualitative evaluation of phenolics obtained. *Process Biochemistry*, *62*, 16–23. https://doi.org/10.1016/j.procbio.2017.07.016

Cadoni, E., Rita De Giorgi, M., Medda, E., & Poma, G. (1999). Supercritical CO2 extraction of lycopene and β-carotene from ripe tomatoes. *Dyes and Pigments*, *44*(1), 27–32. https://doi.org/10.1016/S0143-7208(99)00065-0

Cantabrana, I., Perise, R., & Hernández, I. (2015). Uses of Rhizopus oryzae in the kitchen. *International Journal of Gastronomy and Food Science*, *2*(2), 103–111. https://doi.org/10.1016/j.ijgfs.2015.01.001

Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Perino, S., Fabiano-Tixier, A.-S., & Abert-Vian, M. (2017). Review of Green Food Processing techniques. Preservation, transformation, and extraction. *Innovative Food Science & Emerging Technologies*, *41*, 357–377. https://doi.org/10.1016/j.ifset.2017.04.016

Chen, H. Z., Xu, J., & Li, Z. H. (2007). Temperature cycling to improve the ethanol production with solid state simultaneous saccharification and fermentation. *Applied Biochemistry and Microbiology*, *43*(1), 57–60. https://doi.org/10.1134/S0003683807010103

Cheok, C. Y., Salman, H. A. K., & Sulaiman, R. (2014). Extraction and quantification of saponins: A review. *Food Research International*, *59*, 16–40. https://doi.org/10.1016/j.foodres.2014.01.057

Choudhari, S. M., & Ananthanarayan, L. (2007). Enzyme aided extraction of lycopene from tomato tissues. *Food Chemistry*, *102*(1), 77–81. https://doi.org/10.1016/j.foodchem.2006.04.031

Couto, S. R., & Sanromán, M. Á. (2006). Application of solid-state fermentation to food industry—A review. *Journal of Food Engineering*, *76*(3), 291–302. https://doi.org/10.1016/j.jfoodeng.2005.05.022

Daliu, P., Santini, A., & Novellino, E. (2018). From pharmaceuticals to nutraceuticals: Bridging disease prevention and management. *Expert Review of Clinical Pharmacology*. https://doi.org/10.1080/17512433.2019.1552135

Dulf, F. V., Vodnar, D. C., & Socaciu, C. (2016). Effects of solid-state fermentation with two filamentous fungi on the total phenolic contents, flavonoids, antioxidant activities and lipid fractions of plum fruit (Prunus domestica L.) by-products. *Food Chemistry*, *209*, 27–36. https://doi.org/10.1016/j.foodchem.2016.04.016

Dulf, F. V., Vodnar, D. C., Toşa, M. I., & Dulf, E.-H. (2020). Simultaneous enrichment of grape pomace with γ-linolenic acid and carotenoids by solid-state fermentation with Zygomycetes fungi and antioxidant potential of the bioprocessed substrates. *Food Chemistry*, *310*, 125927. https://doi.org/10.1016/j.foodchem.2019.125927

Durante, M., Lenucci, M. S., & Mita, G. (2014). Supercritical Carbon Dioxide Extraction of Carotenoids from Pumpkin (Cucurbita spp.): A Review. *International Journal of Molecular Sciences*, *15*(4), 6725–6740. https://doi.org/10.3390/ijms15046725

Efthimiadou, A., Katsenios, N., Karkanis, A., Papastylianou, P., Triantafyllidis, V., Travlos, I., & Bilalis, D. J. (2014, July 6). *Effects of Presowing Pulsed Electromagnetic*

Treatment of Tomato Seed on Growth, Yield, and Lycopene Content [Research Article]. The Scientific World Journal; Hindawi. https://doi.org/10.1155/2014/369745

Ekezie, F.-G. C., Sun, D.-W., & Cheng, J.-H. (2017). Acceleration of microwave-assisted extraction processes of food components by integrating technologies and applying emerging solvents: A review of latest developments. *Trends in Food Science & Technology*, *67*, 160–172. https://doi.org/10.1016/j.tifs.2017.06.006

Farinas, C. S. (2015). Developments in solid-state fermentation for the production of biomass-degrading enzymes for the bioenergy sector. *Renewable and Sustainable Energy Reviews*, *52*, 179–188. https://doi.org/10.1016/j.rser.2015.07.092

Ghitescu, R.-E., Volf, I., Carausu, C., Bühlmann, A.-M., Gilca, I. A., & Popa, V. I. (2015). Optimization of ultrasound-assisted extraction of polyphenols from spruce wood bark. *Ultrasonics Sonochemistry*, *22*, 535–541. https://doi.org/10.1016/j.ultsonch.2014.07.013

Gomes, E., da Silva, R., de Cassia Pereira, J., & Ladino-Orjuela, G. (2018). Chapter 3 - Fungal Growth on Solid Substrates: A Physiological Overview. In A. Pandey, C. Larroche, & C. R. Soccol (Eds.), *Current Developments in Biotechnology and Bioengineering* (pp. 31–56). Elsevier. https://doi.org/10.1016/B978-0-444-63990-5.00003-7

Gómez-Romero, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. *Phytochemistry*, *71*(16), 1848–1864. https://doi.org/10.1016/j.phytochem.2010.08.002

Gupta, V. K., Kubicek, C. P., Berrin, J.-G., Wilson, D. W., Couturier, M., Berlin, A., Filho, E. X. F., & Ezeji, T. (2016). Fungal Enzymes for Bio-Products from Sustainable and

Waste Biomass. *Trends in Biochemical Sciences*, *41*(7), 633–645. https://doi.org/10.1016/j.tibs.2016.04.006

Hamidi Esfahani, Z., Shojaosadati, S., & Rinzema, A. (2004). Modelling of simultaneous effect of moisture and temperature on A. niger growth in solid-state fermentation. *Biochemical Engineering Journal*, *21*, 265–272. https://doi.org/10.1016/j.bej.2004.07.007

Hatami, T., Meireles, M. A. A., & Ciftci, O. N. (2019). Supercritical carbon dioxide extraction of lycopene from tomato processing by-products: Mathematical modeling and optimization. *Journal of Food Engineering*, *241*, 18–25. https://doi.org/10.1016/j.jfoodeng.2018.07.036

Ho, K. K. H. Y., Ferruzzi, M. G., Liceaga, A. M., & San Martín-González, M. F. (2015). Microwave-assisted extraction of lycopene in tomato peels: Effect of extraction conditions on all-trans and cis-isomer yields. *LWT - Food Science and Technology*, *62*(1), 160–168. https://doi.org/10.1016/j.lwt.2014.12.061

Hong, J., Park, S.-H., Kim, S., Kim, S.-W., & Hahn, J.-S. (2019). Efficient production of lycopene in Saccharomyces cerevisiae by enzyme engineering and increasing membrane flexibility and NAPDH production. *Applied Microbiology and Biotechnology*, *103*(1), 211–223. https://doi.org/10.1007/s00253-018-9449-8

Huynh, N. T., Smagghe, G., Gonzales, G. B., Van Camp, J., & Raes, K. (2014). Enzyme-Assisted Extraction Enhancing the Phenolic Release from Cauliflower (Brassica oleracea L. var. Botrytis) Outer Leaves. *Journal of Agricultural and Food Chemistry*, *62*(30), 7468–7476. https://doi.org/10.1021/jf502543c

Iandolo, D., Piscitelli, A., Sannia, G., & Faraco, V. (2011). Enzyme Production by Solid Substrate Fermentation of Pleurotus ostreatus and Trametes versicolor on Tomato

Pomace. *Applied Biochemistry and Biotechnology*, *163*(1), 40–51. https://doi.org/10.1007/s12010-010-9014-0

Jamal, P., Hashlamona, A., Jaswir, I., Akbar, I., & Nawawi, W. M. F. W. (2017). Extraction of lycopene from tomato waste using solid state fermentation. *International Food Research Journal*, *24*(Suppl.). https://www.cabdirect.org/cabdirect/abstract/20183161381

John, R. P., Nampoothiri, K. M., & Pandey, A. (2006). Solid-state fermentation for l-lactic acid production from agro wastes using Lactobacillus delbrueckii. *Process Biochemistry*, *41*(4), 759–763. https://doi.org/10.1016/j.procbio.2005.09.013

Kehili, M., Sayadi, S., Frikha, F., Zammel, A., & Allouche, N. (2019). Optimization of lycopene extraction from tomato peels industrial by-product using maceration in refined olive oil. *Food and Bioproducts Processing*, *117*, 321–328. https://doi.org/10.1016/j.fbp.2019.08.004

Kruczek, M., Drygaś, B., & Habryka, C. (2016). Pomace in fruit industry and their contemporary potential application. *World Scientific News*, *48*, 259–265.

Kuo, C.-H., Huang, C.-Y., Shieh, C.-J., Wang, H.-M. D., & Tseng, C.-Y. (2019). Hydrolysis of Orange Peel with Cellulase and Pectinase to Produce Bacterial Cellulose using Gluconacetobacter xylinus. *Waste and Biomass Valorization*, *10*(1), 85–93. https://doi.org/10.1007/s12649-017-0034-7

Lee, J. H., Hwang, C. E., Son, K. S., & Cho, K. M. (2019). Comparisons of nutritional constituents in soybeans during solid state fermentation times and screening for their glucosidase enzymes and antioxidant properties. *Food Chemistry*, *272*, 362–371. https://doi.org/10.1016/j.foodchem.2018.08.052

Li, Y., Fabiano-Tixier, A.-S., & Chemat, F. (2014). Essential Oils: From Conventional to Green Extraction. In Y. Li, A.-S. Fabiano-Tixier, & F. Chemat (Eds.), *Essential Oils*

as Reagents in Green Chemistry (pp. 9–20). Springer International Publishing. https://doi.org/10.1007/978-3-319-08449-7_2

Lizardi-Jiménez, M. A., & Hernández-Martínez, R. (2017). Solid state fermentation (SSF): Diversity of applications to valorize waste and biomass. *3 Biotech*, *7*(1), 44. https://doi.org/10.1007/s13205-017-0692-y

Londoño-Hernández, L., Ramírez-Toro, C., Ruiz, H. A., Ascacio-Valdés, J. A., Aguilar-Gonzalez, M. A., Rodríguez-Herrera, R., & Aguilar, C. N. (2017). Rhizopus oryzae – Ancient microbial resource with importance in modern food industry. *International Journal of Food Microbiology*, *257*, 110–127. https://doi.org/10.1016/j.ijfoodmicro.2017.06.012

Lu, Z., Wang, J., Gao, R., Ye, F., & Zhao, G. (2019). Sustainable valorisation of tomato pomace: A comprehensive review. *Trends in Food Science & Technology*, *86*, 172–187. https://doi.org/10.1016/j.tifs.2019.02.020

Luisa García, M., Calvo, M. M., & Dolores Selgas, M. (2009). Beef hamburgers enriched in lycopene using dry tomato peel as an ingredient. *Meat Science*, *83*(1), 45–49. https://doi.org/10.1016/j.meatsci.2009.03.009

Martinez-Solano, K. C., Garcia-Carrera, N. A., Tejada-Ortigoza, V., García-Cayuela, T., & Garcia-Amezquita, L. E. (2020). Ultrasound Application for the Extraction and Modification of Fiber-Rich By-Products. *Food Engineering Reviews*. https://doi.org/10.1007/s12393-020-09269-2

Martínez-Valverde, I., Periago, M. J., Provan, G., & Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (Lycopersicum esculentum). *Journal of the Science of Food and Agriculture*, *82*(3), 323–330. https://doi.org/10.1002/jsfa.1035

Martins, Z. E., Pinho, O., & Ferreira, I. M. P. L. V. O. (2017). Food industry by-products used as functional ingredients of bakery products. *Trends in Food Science & Technology*, *67*, 106–128. https://doi.org/10.1016/j.tifs.2017.07.003

Meireles, M. A. A. (2008). *Extracting Bioactive Compounds for Food Products: Theory and Applications*. CRC Press.

Mitchell, D. A., Pitol, L. O., Biz, A., Finkler, A. T. J., de Lima Luz, L. F., & Krieger, N. (2019). Design and Operation of a Pilot-Scale Packed-Bed Bioreactor for the Production of Enzymes by Solid-State Fermentation. In S. Steudler, A. Werner, & J. J. Cheng (Eds.), *Solid State Fermentation: Research and Industrial Applications* (pp. 27–50). Springer International Publishing. https://doi.org/10.1007/10_2019_90

Nandagopal, M. S. G., Antony, R., & Selvaraju, N. (2016). Comparative study of liquid–liquid extraction in miniaturized channels over other conventional extraction methods. *Microsystem Technologies*, *22*(2), 349–356. https://doi.org/10.1007/s00542-014-2391-5

Neagu, D., Leopold, L. F., Thonart, P., Destain, J., & Socaciu, C. (2014). *Enzyme-Assisted Extraction of Carotenoids and Phenolic Derivatives from Tomatoes*. 7.

Nobre, B. P., Palavra, A. F., Pessoa, F. L. P., & Mendes, R. L. (2009). Supercritical CO2 extraction of trans-lycopene from Portuguese tomato industrial waste. *Food Chemistry*, *116*(3), 680–685. https://doi.org/10.1016/j.foodchem.2009.03.011

Oberoi, H. S., Sandhu, S. K., & Vadlani, P. V. (2012). Statistical optimization of hydrolysis process for banana peels using cellulolytic and pectinolytic enzymes. *Food and Bioproducts Processing*, *90*(2), 257–265. https://doi.org/10.1016/j.fbp.2011.05.002

Oda, Y., Saito, K., Yamauchi, H., & Mori, M. (2002). Lactic Acid Fermentation of Potato Pulp by the Fungus Rhizopus oryzae. *Current Microbiology*, *45*(1), 1–4. https://doi.org/10.1007/s00284-001-0048-y

Paepatung, N., Nopharatana, A., & Songkasiri, W. (2009). *Bio-Methane Potential of Biological Solid Materials and Agricultural Wastes*.

Pan, F., Su, T.-J., Cai, S.-M., & Wu, W. (2017). Fungal endophyte-derived Fritillaria unibracteata var. wabuensis: Diversity, antioxidant capacities in vitro and relations to phenolic, flavonoid or saponin compounds. *Scientific Reports*, *7*(1), 42008. https://doi.org/10.1038/srep42008

Perea-Domínguez, X. P., Hernández-Gastelum, L. Z., Olivas-Olguin, H. R., Espinosa-Alonso, L. G., Valdez-Morales, M., & Medina-Godoy, S. (2018). Phenolic composition of tomato varieties and an industrial tomato by-product: Free, conjugated and bound phenolics and antioxidant activity. *Journal of Food Science and Technology*, *55*(9), 3453–3461. https://doi.org/10.1007/s13197-018-3269-9

Prado, J. M., Veggi, P. C., & Meireles, M. A. A. (2014). Extraction Methods for Obtaining Carotenoids from Vegetables—Review. *CURRENT ANALYTICAL CHEMISTRY*, *10*(1), 29.

Prodpran, P., Rapeepun, M., Chartchai, K., & Saisamorn, L. (2017). Antioxidant properties and production of monacolin k, citrinin, and red pigments during solid state fermentation of purple rice (Oryzae sativa) varieties by Monascus purpureus. *Czech Journal of Food Sciences*, *35*(No. 1), 32–39. https://doi.org/10.17221/154/2016-CJFS

Queiroz Santos, V. A., Nascimento, C. G., Schmidt, C. A. P., Mantovani, D., Dekker, R. F. H., & da Cunha, M. A. A. (2018). Solid-state fermentation of soybean okara: Isoflavones biotransformation, antioxidant activity and enhancement of nutritional quality. *LWT*, *92*, 509–515. https://doi.org/10.1016/j.lwt.2018.02.067

Ramos Sanchez, L. B. (2015). Fungal Lipase Production by Solid-State Fermentation. *Journal of Bioprocessing & Biotechniques*, *05*(02). https://doi.org/10.4172/2155-9821.1000203

Ranjith Kumar, R., Hanumantha Rao, P., & Arumugam, M. (2015). Lipid Extraction Methods from Microalgae: A Comprehensive Review. *Frontiers in Energy Research*, *2*. https://doi.org/10.3389/fenrg.2014.00061

Rashad, M. M., Mahmoud, A. E., Ali, M. M., & Nooman, M. U. (2016). *Antioxidant and Anticancer Agents Produced from Pineapple Waste by Solid State Fermentation*. *7*(6), 10.

Rivas, B., Torrado, A., Torre, P., Converti, A., & Domínguez, J. M. (2008). Submerged Citric Acid Fermentation on Orange Peel Autohydrolysate. *Journal of Agricultural and Food Chemistry*, *56*(7), 2380–2387. https://doi.org/10.1021/jf073388r

Rudakiya, D. M. (2019). Chapter 10—Strategies to Improve Solid-State Fermentation Technology. In N. Srivastava, M. Srivastava, P. K. Mishra, P. W. Ramteke, & R. L. Singh (Eds.), *New and Future Developments in Microbial Biotechnology and Bioengineering* (pp. 155–180). Elsevier. https://doi.org/10.1016/B978-0-444-64223-3.00010-2

Sadh, P. K., Duhan, S., & Duhan, J. S. (2018). Agro-industrial wastes and their utilization using solid state fermentation: A review. *Bioresources and Bioprocessing*, *5*(1), 1. https://doi.org/10.1186/s40643-017-0187-z

Sadh, P. K., Saharan, P., Duhan, S., & Duhan, J. S. (2017). Bio-enrichment of phenolics and antioxidant activity of combination of Oryza sativa and Lablab purpureus fermented with GRAS filamentous fungi. *Resource-Efficient Technologies*, *3*(3), 347–352. https://doi.org/10.1016/j.reffit.2017.02.008

Sandhu, K. S., & Punia, S. (2017). Enhancement of bioactive compounds in barley cultivars by solid substrate fermentation. *Journal of Food Measurement and Characterization*, *11*(3), 1355–1361. https://doi.org/10.1007/s11694-017-9513-6

Sandhu, K. S., Punia, S., & Kaur, M. (2016). Effect of duration of solid state fermentation by Aspergillus awamorinakazawa on antioxidant properties of wheat cultivars. *LWT - Food Science and Technology*, *71*, 323–328. https://doi.org/10.1016/j.lwt.2016.04.008

Santana-Gálvez, J., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2019). A practical guide for designing effective nutraceutical combinations in the form of foods, beverages, and dietary supplements against chronic degenerative diseases. *Trends in Food Science & Technology*, *88*, 179–193. https://doi.org/10.1016/j.tifs.2019.03.026

Scalbert, A., & Williamson, G. (2000). Dietary Intake and Bioavailability of Polyphenols. *The Journal of Nutrition*, *130*(8), 2073S-2085S. https://doi.org/10.1093/jn/130.8.2073S

Schwartz, S. J., Hildenbrand, B. E., & Elbe, J. H. V. (1981). Comparison of Spectrophotometric and HPLC Methods to Quantify Betacyanins. *Journal of Food Science*, *46*(1), 296–297. https://doi.org/10.1111/j.1365-2621.1981.tb14587.x

Sepúlveda, L., Laredo-Alcalá, E., Buenrostro-Figueroa, J. J., Ascacio-Valdés, J. A., Genisheva, Z., Aguilar, C., & Teixeira, J. (2020). Ellagic acid production using polyphenols from orange peel waste by submerged fermentation. *Electronic Journal of Biotechnology*, *43*, 1–7. https://doi.org/10.1016/j.ejbt.2019.11.002

Sharma, H. P., Patel, H., & Sharma, S. (2014). *Enzymatic extraction and clarification of juice from various fruits-A review*. *2*(1), 14.

Shi, J., & Le Maguer, M. (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Critical Reviews in Biotechnology*, *20*(4), 293–334. https://doi.org/10.1080/07388550091144212

Singh, R., Puri, A., & Panda, B. P. (2015). Development of menaquinone-7 enriched nutraceutical: Inside into medium engineering and process modeling. *Journal of Food Science and Technology*, *52*(8), 5212–5219. https://doi.org/10.1007/s13197-014-1600-7

Singhania, R. R., Patel, A. K., Soccol, C. R., & Pandey, A. (2009). Recent advances in solid-state fermentation. *Biochemical Engineering Journal*, *44*(1), 13–18. https://doi.org/10.1016/j.bej.2008.10.019

Soccol, C. R., Costa, E. S. F. da, Letti, L. A. J., Karp, S. G., Woiciechowski, A. L., & Vandenberghe, L. P. de S. (2017). Recent developments and innovations in solid state fermentation. *Biotechnology Research and Innovation*, *1*(1), 52–71. https://doi.org/10.1016/j.biori.2017.01.002

Sousa, B. A., & Correia, R. T. P. (2012). Phenolic content, antioxidant activity and antiamylolytic activity of extracts obtained from bioprocessed pineapple and guava wastes. *Brazilian Journal of Chemical Engineering*, *29*(1), 25–30. https://doi.org/10.1590/S0104-66322012000100003

Souyoul, S. A., Saussy, K. P., & Lupo, M. P. (2018). Nutraceuticals: A Review. *Dermatology and Therapy*, *8*(1), 5–16. https://doi.org/10.1007/s13555-018-0221-x

Stahl, W., & Sies, H. (2012). β-Carotene and other carotenoids in protection from sunlight. *The American Journal of Clinical Nutrition*, *96*(5), 1179S-84S. https://doi.org/10.3945/ajcn.112.034819

Sundarram, A., & Murthy, T. P. K. (2015). α-Amylase Production and Applications: A Review. *Environmental Microbiology*, 10.

Swallah, M. S., Sun, H., Affoh, R., Fu, H., & Yu, H. (2020, May 7). *Antioxidant Potential Overviews of Secondary Metabolites (Polyphenols) in Fruits* [Review Article]. International Journal of Food Science; Hindawi. https://doi.org/10.1155/2020/9081686

Thomas, L., Larroche, C., & Pandey, A. (2013). Current developments in solid-state fermentation. *Biochemical Engineering Journal*, *81*, 146–161. https://doi.org/10.1016/j.bej.2013.10.013

Toor, R. K., & Savage, G. P. (2005). Antioxidant activity in different fractions of tomatoes. *Food Research International*, *38*(5), 487–494. https://doi.org/10.1016/j.foodres.2004.10.016

Torino, M. I., Limón, R. I., Martínez-Villaluenga, C., Mäkinen, S., Pihlanto, A., Vidal-Valverde, C., & Frias, J. (2013). Antioxidant and antihypertensive properties of liquid and solid state fermented lentils. *Food Chemistry*, *136*(2), 1030–1037. https://doi.org/10.1016/j.foodchem.2012.09.015

Torres-León, C., Ramírez-Guzmán, N., Ascacio-Valdés, J., Serna-Cock, L., dos Santos Correia, M. T., Contreras-Esquivel, J. C., & Aguilar, C. N. (2019). Solid-state fermentation with Aspergillus niger to enhance the phenolic contents and antioxidative activity of Mexican mango seed: A promising source of natural antioxidants. *LWT*, *112*, 108236. https://doi.org/10.1016/j.lwt.2019.06.003

Valdez-Morales, M., Espinosa-Alonso, L. G., Espinoza-Torres, L. C., Delgado-Vargas, F., & Medina-Godoy, S. (2014). Phenolic content and antioxidant and antimutagenic activities in tomato peel, seeds, and byproducts. *Journal of Agricultural and Food Chemistry*, *62*(23), 5281–5289. https://doi.org/10.1021/jf5012374

Verduzco-Oliva, R., & Gutierrez-Uribe, J. A. (2020). Beyond Enzyme Production: Solid State Fermentation (SSF) as an Alternative Approach to Produce Antioxidant Polysaccharides. *Sustainability*, *12*(2), 495. https://doi.org/10.3390/su12020495

Viniegra-Gonzàlez, G. (1997). Solid state fermentation: Definition, Characteristics, Limitations and Monitoring. In S. Roussos, B. K. Lonsane, M. Raimbault, & G. Viniegra-Gonzalez (Eds.), *Advances in Solid State Fermentation* (pp. 5–22). Springer Netherlands. https://doi.org/10.1007/978-94-017-0661-2_2

Wilkins, M. R., Widmer, W. W., Grohmann, K., & Cameron, R. G. (2007). Hydrolysis of grapefruit peel waste with cellulase and pectinase enzymes. *Bioresource Technology*, *98*(8), 1596–1601. https://doi.org/10.1016/j.biortech.2006.06.022

Williams, B., Grant, L., Gidley, M., & Mikkelsen, D. (2017). Gut Fermentation of Dietary Fibres: Physico-Chemistry of Plant Cell Walls and Implications for Health. *International Journal of Molecular Sciences*, *18*(10), 2203. https://doi.org/10.3390/ijms18102203

Xiao, Y., Fan, J., Chen, Y., Rui, X., Zhang, Q., & Dong, M. (2016). Enhanced total phenolic and isoflavone aglycone content, antioxidant activity and DNA damage protection of soybeans processed by solid state fermentation with Rhizopus oligosporus RT-3. *RSC Advances*, *6*(35), 29741–29756. https://doi.org/10.1039/C6RA00074F

Xiao, Y., Zhang, Q., Miao, J., Rui, X., Li, T., & Dong, M. (2015). Antioxidant activity and DNA damage protection of mung beans processed by solid state fermentation with Cordyceps militaris SN-18. *Innovative Food Science & Emerging Technologies*, *31*, 216–225. https://doi.org/10.1016/j.ifset.2015.06.006

Xu, D.-P., Zheng, J., Zhou, Y., Li, Y., Li, S., & Li, H.-B. (2017). Ultrasound-assisted extraction of natural antioxidants from the flower of Limonium sinuatum: Optimization and

comparison with conventional methods. *Food Chemistry*, *217*, 552–559. https://doi.org/10.1016/j.foodchem.2016.09.013

Yin, Z. N., Wu, W. J., Sun, C. Z., Liu, H. F., Chen, W. B., Zhan, Q. P., Lei, Z. G., Xin, X., Ma, J. J., Yao, K., Min, T., Zhang, M. M., & Wu, H. (2019). Antioxidant and Anti-inflammatory Capacity of Ferulic Acid Released from Wheat Bran by Solid-state Fermentation of Aspergillus niger. *Biomedical and Environmental Sciences*, *32*(1), 11–21. https://doi.org/10.3967/bes2019.002

Zambrano, C., Kotogán, A., Bencsik, O., Papp, T., Vágvölgyi, C., Mondal, K. C., Krisch, J., & Takó, M. (2018). Mobilization of phenolic antioxidants from grape, apple and pitahaya residues via solid state fungal fermentation and carbohydrase treatment. *LWT*, *89*, 457–465. https://doi.org/10.1016/j.lwt.2017.11.025

VITA

Eng. Luis Octavio Cano is currently a Master of Science student with a specialty in biotechnology at the Tecnológico de Monterrey, Guadalajara campus. He completed his career study in Biotechnology Engineering in 2018 by the Tecnológico de Monterrey Campus Guadalajara. He is currently completing his research to determine if a solid-state fermentation has the ability to increase the extractability of lycopene and phenolic compounds from industrial tomato waste. He spent a stance at the Sup´Biotech School of Biotechonogy Engineers in Paris, where he carried out an innovative development project for new food products that were both palatable and with high nutritional content. He also led his team as captain in a competition sponsored by SIGMA for the development of innovative and healthy food products, where he won third place.

He also presented a poster in an international congress: *Emerging pre-treatments as promising strategies for the increment of nutraceutical extraction yield in plant foods: lycopene from tomato by-products as a case study*. IFT-EFFoST International Nonthermal Processing Workshop, Monterrey, Mexico, Nov-2019.

Currently, he is in the process of finalizing a review article entitled “Solid-state fermentation for enhancing the nutraceutical content from agrifood by-products: Recent advances and its industrial feasibility” conceived to be published in the journal *Food Bioscience* under the section of application of novel technology to foods.