Enhancing the Polyphenol profile and Antioxidant properties of *Crithmum Maritimum* Extract from Cascading Biorefinery by Lactic Acid fermentation

PECT4-1

Master Project

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STUDENT REPORT

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Preface

This Master thesis has been made at Aalborg University. The report and additional documents have been created in the period between September 2024 and June 2025.
This Report is directed to individuals with an interest in Biorefinerys, Optimization of Biomasses and usage of process simulation softwares like SuperPro intillegen. The Hope is that this project can serve as point of inspiration and as a resource for fellow students or researchers interested within related fields of study.

PECT-1
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Instructions for reading

The report is written in LaTeX, and each chapter is marked with a certain number and is divided into sections. All the references used throughout the report are indicated by the method referred to as the Institute of Electrical and Electronics Engineers (IEEE). The bibliography is made in Mendeley and BibTeX, and the citations used throughout the sections are noted in the text either at the beginning of a section or as each individual statement is made. Citations of figures and tables are mentioned in the caption.

Abstract

Crithmum Maritimum, which is a salt tolerant halophyte beach plant, is chosen to be tested on to see if its possible to enhance its polyphenol and antioxidant profile. This is done to to see if its can be enhanced for biorefiney purposes and to increase its value as a potential bio remedy for soil salinization. Therefore making the plant valuable for the pharmaceutical/ nutraceutical industry

The enhancement is done by solid fermentation with lactic acid bacteria, due to its probiotic chracteristics. Different strains and pretreatment methods were used to improve the yield, polyphenol content and antioxidant activity. Soxhlet extractions were used therafter to extract the contnents of the biomass. Thereafter all the results were applied in an *in silico* study of extracting polyphenols from the biomass on an industrial scale.

Based on the trials, Thermal pretreated *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* achieved the best results with an extraction yield of 32.93%, Polyphenol content of 25.18 mg/ml and IC50 of 3.32 mg/ml.

In the techno-economical analysis conducted, two cases, one based on former reports and the other on the laboratory experiments, were simulated which one is more feasible. Case 1 with fractionated biomass was in the end not feasible, but Case 2 with whole biomass was feasible. Case 2 can therefore be seen as possible plan for a biorefinery using *Crithmum Maritimum* as feedstock.

Acknowledgments

This report was able to be created with the help of laboratory assistant Lilian Bondig for her help in instructing how to use Laboratory equipment and how to conduct proper analysis of bio compounds. Also a thank you to the former Research Assistant Laura Sini Sofia Hulkko, who helped to start the project and instructed how to start the experiments. Special thanks to the project Supervisor Mette Hedegaard Thomsen, for giving me a space in her research group, helping me with the project and learning from her experience.

Contents

1 Introduction

1.1 Relevance of the study

In 2015 the United Nations adopted "the 2030 Agenda for sustainable Development", which shares a blueprint how to increase the prosperity for humanity while reducing the inequality of people and preserving the planet in the process. For this goal there are 17 Sustainable Development Goals (SDGs), which act as guidelines for developing and developed nations to achieve and uphold [1].
To achieve these SDGs new and old ideas must be reexamined and readjusted to see how they can be used. In this same spirit, plants that before only had some niche uses can have properties that could make it suitable for new purposes. Bioirefinerys can then take these plants and process them to extract the useful components or convert them into new products. These extracts and products can range from compounds for the pharmaceutical industry to producing biodiesel and hereby help the green transition away from fossil fuel.
Halophytes is a plant group that can fit these requirements and help different groups and areas due to its properties it possesses.

Figure 1.1: The 17 SDG of the UN. [1]

1.1.1 Biorefinery

In a biorefinery, biomass is processed to produce biofuels, chemicals, pharmaceutical products and other products. Biorefinerys can produce a lot of different products, which makes it a target for improvement and inclusion into circular economies, where the resources needed are used and recycled as much as possible, and can be included into the energy sector. Often producing only one product is not profitable, therefore value-added products increase the feasibility and these multi product biorefinerys are seen as the most sustainable and profitable option.
Biorefinerys can be classified into two main categories: Energy-driven biorefinery, where the main product is a energy carrier like fuel, heat and power. And Material driven biorefinery, where the product is biobased.
These two can also be combined, as often by-products or waste can be reused for different purposes.

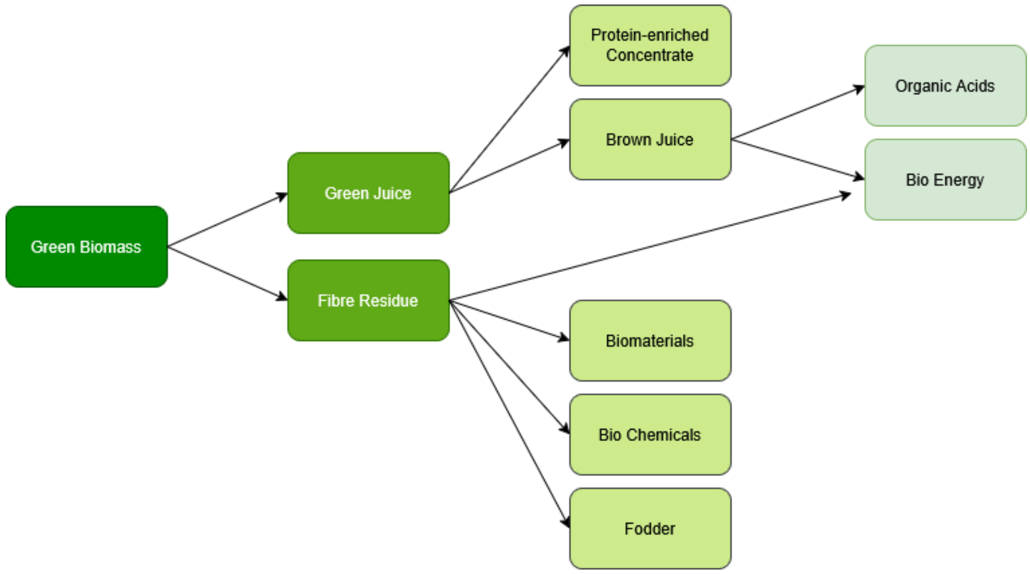

Figure 1.2: A simplified flowsheet of a green Biorefinery, which uses fresh unprocessed biomass, and its products.

To be able to combat and mitigate global warming, there is an interest to transition the current linear fossil-based economy towards a circular bio economics with an emphasis on sustainable agricultural cultivation. This will lead to a higher demand in biomass, which is driven by a demand in food security, bio energy/products. To achieve this goal, there is a big interest in finding bio-alternatives for current essential products, and make the concept of integrated biorefineries attractive, where multiple products and bioenergy is produced in a cascade to harness as much as possible what a potential biomass can provide [2], [3]. Therefore bio prospecting for high value applications have a high potential in circular bio economies, as it increases the value creation and increases the feasibility and profitability of the biorefinery [2].
One constraining factor for biorefineries are, that it can not use biomass that is considered as feedstock for human/ animal consumption. That is to ensure that there is enough edi-

ble biomass for human and animal consumption, and reduce competition in this market. Therefore it is essential to find biomass, where multiple parts of the plant can be used for different purposes. Halophytes can be potential biomass here, as often its leaves can be used for consumption while the rest of the plant can be used for other purposes.

1.1.2 Highvalue byproducts from Biorefineries

As already stated, due to the current low profitability of biorefineries, high value byproducts are added to increase the feasibility of the refineries, while producing multiple products. That means that the biomass has to be handled in a specific way, so to extract all potential of the biomass. Some biomass have therefore higher priorities, due their chemical profiles, but also because of that new biomass has been examined to find suitable prospects for this industry. The different products a biorefinery can produce, can be seen in figure 1.3:

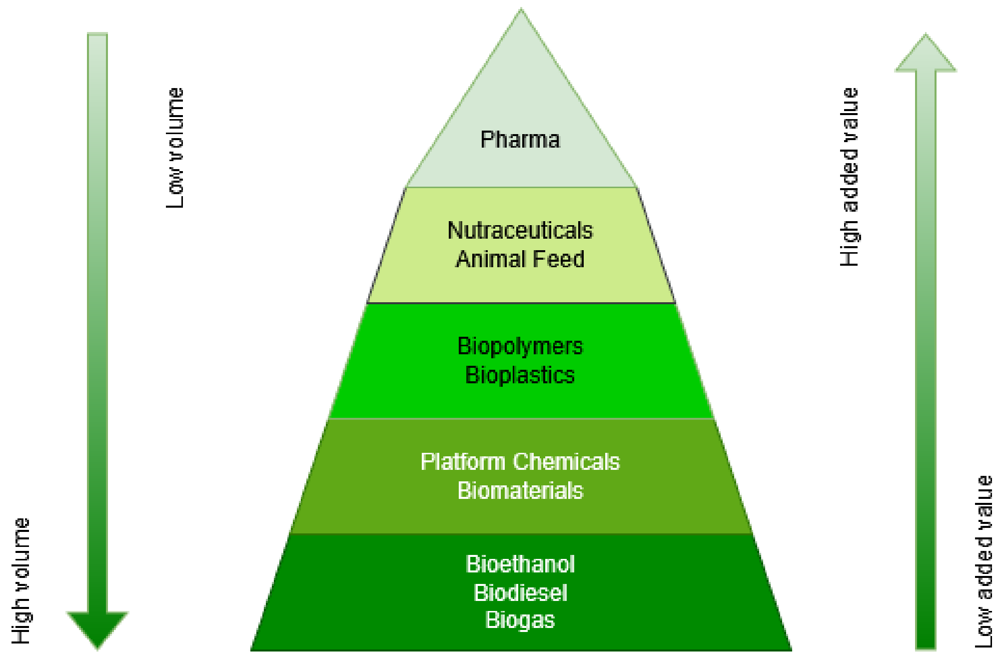

Figure 1.3: Value pyramid of a Biorefinery.

As it can be seen the highest value product, can often produce the least of and vice versa [4]–[6].

The highest added value for a biorefinery, is when it can produce a product for the pharmaceutical/cosmetics industry. For this purpose a biomass has to be treated in a specific way to extract compounds that can be used in these industries or work on biomass, where it is quite simple to extract these compounds.

As it can be seen in subsection1.1.1 biorefineries can work on multiple products at the same time and therefore it makes it quite simple to add new products into the workingflow. This project focuses mostly on the fiber residue of *Crithmum Maritimum* and the potential uses of it, while other projects have focused on other parts of the biorefinery processes, like the green juice part. One bio refinery has therefore the potential to produce multiple products with the potential of the rest of the used fibers being used for bioenergy.

1.1.3 Soil salinization

Through intensive agriculture, natural processes, water management systems and deforestation, the area of salt affected soils steadily increases around the globe. This salt salinization is a big contributor for agriculture land area degradation [7], [8].
There are three different levels of saline soil: Healthy soils, Saline soils and Sodic soils. Healthy soils have low salt concentrations and a balanced Nutrient composition. Saline Soils have an increased level of soluble salt, which result in Nutrient imbalance, which impacts plant growth and lowers biodiversity [9], [10]. Sodic Soils have high amount of adsorbed sodium, which inhibits almost all plant growth and biodiversity. It is often hard and dry, with almost a cement like composition, which results in waterlogging, where no water can be absorbed as seen in figure 1.4.

Figure 1.4: Heavy Salinized topsoil, which is not able to uptake water anymore.[11]

In total around 10 %, around 13.811.503 km^2,[8] of all soil in the world is affected by salinization, where different regions in the world have different amount of salt effected soil. All areas can suffer heavily from secondary salination, where either seawater infuses into coastal areas, overuse of fertilizers, mining activity and etc. can heavily increase salinity in areas, especially in farming areas. Primary salination can also occur, as it is linked to climate change and the increasing aridity and heavily affects in developing areas.[8]
All this salinity, results in less agricultural output with risque of desertification, due to the death of local plant life. Especially agricultural crops are salt sensitive, which results in less output with a possibility of a total collapse of local/ traditional farming in these areas. Normal crops have a saltsensitivity of less than 2 $ECds/M$, Electrical conductivity measured in deciSiemens per Meter, in the soil or 2 g/L of salt in the irrigation water[8]–[10]. This results economics problems and can also results in migration of the local populace to less saline areas, which creates other problems. Therefore other plants have to be used to replace the crops, that can not grow in these areas [8].

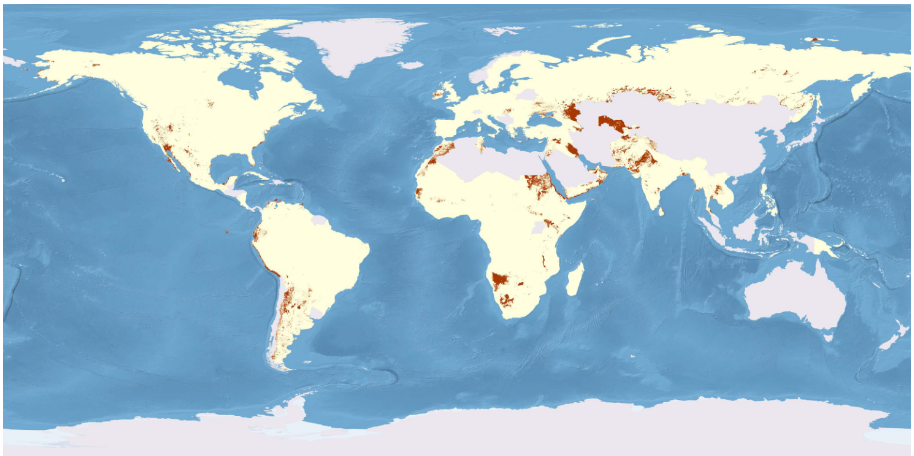

Figure 1.5: The top soil salinization level (0-30cm) in different areas of the world from 2021. Red is for high level salinization. Grey are areas where no data has been gathered.[7]

1.1.4 Halophytes

Halophytes are a types of plant that grows in saline areas. They mostly grow in marshes, seashores and saline deserts and could be used to combat the salinization of the before mentioned areas. Some of them are so salt tolerant that they can be irrigated with salt water. These halophytes have been used by humans for centuries already, as they can be used as a food source or as herbal remedies [12]–[14]. Many Halophytes have been part of the local food culture and could be used as either foodcrop/cashcrop or combined, due its potential for the pharmaceutical industry [15].
Therefore halophytes have big potential as a feedstock for bio refineries for energy driven or material driven bio refineries [16]. They can be a value added product for energy driven or be the main product for material driven.
Even with their potential as feedstock for bio refineries, they can also be used as feedstock for animals, which can reduce the antibiotic intake of livestock. That is due to that Halophytes are rich in Polyphenols and Antioxidants, which have been proven to have a probiotic effect when ingested. Polyphenols are a group of phenolics that only exist in plants. They are produced when plants are under environmental stress, pest or pathogens and are there to combat oxidative damages inside of the plant body [17], [18]. Plants that have high amount of polyphenols, grow in either harsh climates or have it as a defense mechanism[18]. In the case of Halophytes it is there as, they grow in high stress environments, where they have to combat the saline conditions of the ground. As they grow in saline conditions Halophytes also absorb the minerals in the ground and absorb NaCl from the ground [19]. Halophytes can therefore also be used as a bioremediation for saline soil, which can be used to improve/combat saline soil distribution [19].

Figure 1.6: Blooming *Crithmum Maritimum* part of the Halophyte group.[20]

1.1.5 Herbal Remedies

Mankind has long known that some plants have medicinal benefits, and used them to improve their lives before modern medicine was invented. Modern medicine is today mostly synthesized in laboratories, but still use extracts from plants or byproducts from other organisms. Under this development, some plants were set aside for others, but are getting rediscovered due their properties and their potential [21].
Many old remedies have been proven for their benefits and can help people and lessen the reliance on modern medicine. Most people know of some old remedies, that their mothers or grandmothers used to help or relief the body of problems. That is due to the probiotics and antibacterial properties of specific plants.
One such plant is *Crithmum Maritimum*, which for once had a place in the local food culture, where it grew, but had also medicinal uses in Herbal Medicine. Due its high amount of antioxidants and nutritional value, it was often used as stomach medicine [21].
Herbal Medicine, can be used to supplement modern medicine, but is not a replacement for it. One should also amass sufficient knowledge about it before using it, as some plants can be poisonous when not prepared correct.

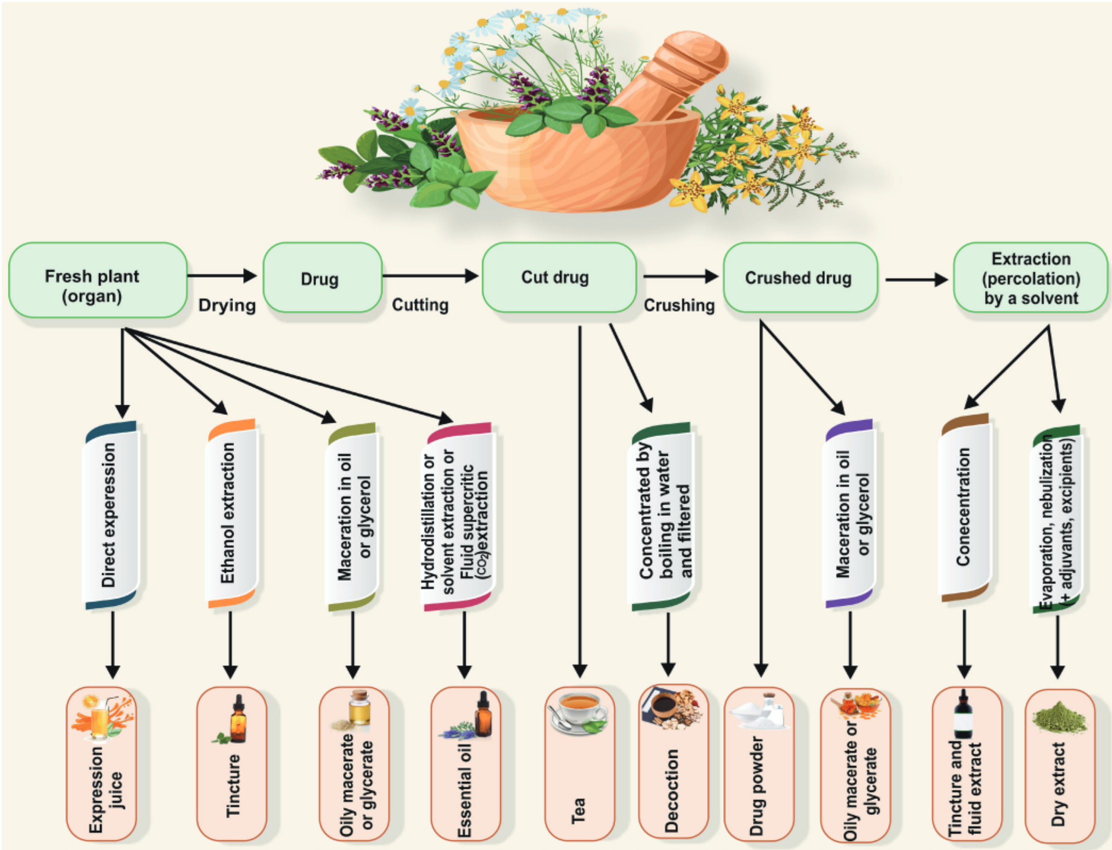

Figure 1.7: The usage of Herbal Remedies from Biomass to product.[21]

Another reason for the interest in herbal remedies/ probiotic compounds is the increasing bacterial resistance against antibiotics. That is due to over reliance and consumption of antibiotics, which has led to some bacteria developing resistances against it. Probiotics could replace in cases antibiotics, due to their antibacterial potential [22]. Botanics have therefore been used in the skincare industry for some time, as they are able to maintain a healthy skin culture. For harsher cases of bacteria, like acne, Lactic Acid bacteria, *Lactobacilli* family, have been detected to have inhibitory effect against these skin pathogens [23], [24]. It could therefore be beneficial to enhance the probiotic effects of some plants with Lactic Acid bacteria.

1.1.6 Fermentation

Humans have fermented different products for different purposes through out Human existence. This was done for food preservation purposes as they could by anerobic fermentation keep products longer preserved, like yogurt, cheese and kimchi [25]. That is due to the microorganism converting the glucose containing in the product into either ethanol or Lactic acid and thereby making the food uninhabitable by other bacteria, therefore increasing the shelf life of the food. This fermentation, is still cultivated around the world and in many cultures. For some food products it has been industrialized so that it could be mass produced like with cheese [26], while many still produce their own fermented food/drinks at home.

The principle of fermentation, is of interest as it could increase the probiotic effects of other products and therefore increase the anti-bacterial effect of existing products or enhance new [27]. It could therefore benefit the pharmaceutical/nutraceutical industry and decrease the reliance on antibiotics for humans and animals alike.

1.2 Problem statement

This report focuses on experiments to enhance specific compounds inside of the Halophyte species *Crithmum Maritimum*, which is a halophyte native to most rocky seasides in Europe and North Africa. The enhancement is done by solidstate fermentation experiments and the analysis of these extracts inside of a laboratory. The further process simulation will look at different cases based on the results of the experiment and former reports discussing the characteristics and fractionation of *Crithmum Maritimum*. Also it will look at value added streams, Lactic Acid fermentation and techno-economical assessment of the entire process. The problem statement is therefore:

- Is *Crithmum Maritimum* a suitable feedstock for a biorefinery?

2 Project objectives

To answer the problem statement given in section [REF], different project objectives are stated. These are based on international goals, previous research results and literature review of state-of-the-art research and literature. The main project objectives are:

- Examining different fermentation and treatment methods for the biomass
- Examination of the Polyphenol and antioxidant content of the Extraction
- A techno and economic evaluation of the entire process with the help of a flow sheet program.

To find the proper fermentation and treatment for the biomass *Crithmum Maritimum* a extensive literature study will be done and laboratory experiments to validate these findings. The results will thereafter be analysed to find the best results which will thereafter be simulated in a flowsheet program to test the feasibility of it. Here will be examined:

- Former reports and results of analyzing *Crithmum Maritimum* and its potential.
- Reports about enhancing valuable biocompounds inside of different biomasses.
- Looking into solidstate fermentation and what kind of fermentation are done to increase the probiotic characteristics of biomass.

After a potential fermenatation treatment for the biomass has been determined, fermentation experiments will be conducted to find the best way to increase the yield and increase the bioactivity. here will be examined:

- Normal fermentation conditions, and the proper moisture content necessary for this biomass.
- Fermentation with thermal pretreatment to see how it can improve the extraction.
- Fermentation with thermal pretreament and enzymes.

The techno-economic evaluation will look into if the entire report is feasible to conduct in reality. Here it will look into:

- Processing of the biomass
- Possible different cases for the biorefiney
- Feasibility study and possible scale ups

3 Literature review

3.1 Properties of Halophytes and *Crithmum Maritimum*

Halophytes does only contribute to around 1% of the total known plants species. These species of plants can survive in salinity conditions, which are toxic for other plant species [13]. These salt tolerant properties of the halophytes have made them popular as a new source of food and biomass, in areas wrought by droughts and high soil salinity, which makes other types of agriculture difficult or impossible [8].
Crithmum Maritimum, which are also known under the names "Rock samphire", "Sea Fennel" and "Samphire". The name "Samphire" is also shared with other unrelated edible halophyte species. They are found on the rocky coasts of Europe, West Asia, North Africa and on the Mediterranean and Black Sea rocky coastlines 3.1 [28]. It is an edible wild plant which is part of traditional culinary kitchens in most regions where it grows [12], [14]. Almost the entire plant can be eaten in various forms and can also in its dried form be used as a salt substitute. Another property, which makes it interesting as a biomass, is its high amount of antioxidants. This makes it attractive to the medical industry, as it is rich in polyphenols [15].

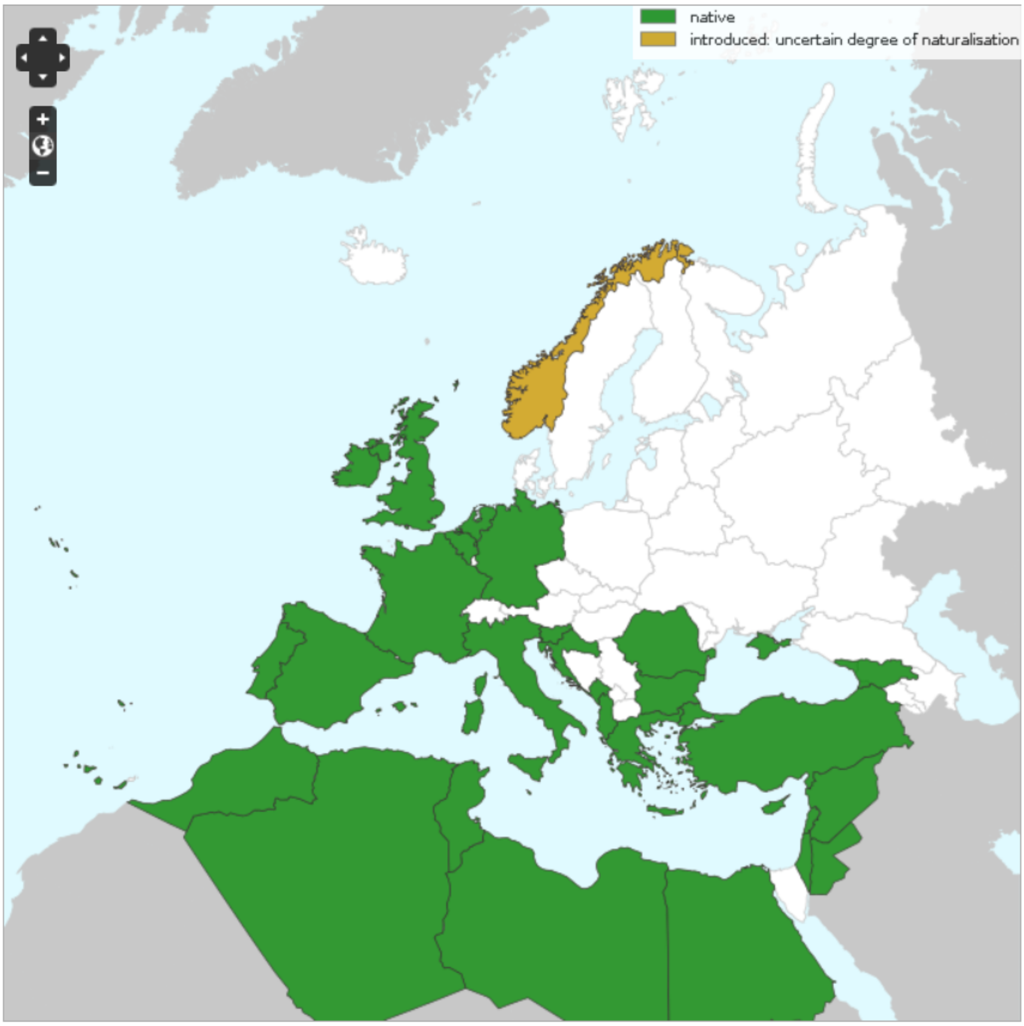

Figure 3.1: Documented natural habitat of *Crithmum Maritimum*[29].

3.1.1 Review of *Crithmum Maritimum*

This is a review about the bioactive compounds inside of C. Marithimum. Especially on polyphenolic and anti oxidant activity.

Crithmum Maritimum as already described is a Halophyte, and therefore has a high amount of polyphenols that protect it from its harsh salt environment [30]. These polyphenols are in high interest for researchers and the pharmaceutical industry, due to the many benefits of polyphenols. For the interest of this project only the polyphenol content and the antioxidant activity is of interest.
In the papers reviewed there is clear that a higher amount can be extracted through the use of methanol and ethanol or a mixture of these two with water. The amount extracted also differs depending on the amount of biomass used and what kind of extraction method has been used. But another big part is also if it has been harvested in nature or cultivated in a greenhouse/garden [31], [32]. And in nature there is also a difference how exposed it was to salt [30], [33]–[36].

Another part which can be interesting to go more into, is when and which part of the Halophyte to harvest, as there are different papers which have examined the polyphenol content and the antioxidant activity in different plant parts and in which part of its growth cycle it is [31], [37]–[39].

Cultivation Area	Extraction method	Solvent & Temperature	PP content	Antioxidant activity	Ref.
Croatia (adriatic coastline) [W]	Acid Hydrolysis	Hydrochloric Acid at 100 °C	4.72-9.48 %	-	[37]
Mallorca (Spain) [W]	Centrifugation extraction	Tris HCI buffer at 4 °C	0.56-0.72 mg L-tyrosine/ mg protein	172-292 mK/ mg protein	[34]
Brittany (France) [W]	Centrifugation extraction	Water/ Ethanol (1:2) at 4 °C	33.3 mg GAE / g DW	0.152 mg / mL	[40]
Tunis (Tunesia) [W]	Soxhlet Extraction & Maceration Extract	80% Aquaos Acetone at 80 °C	3.68 - 8.27 mg GAE / g DW	7.68 - 56.33 IC50 μg /mL	[38]
Eastern Libya [W]	Hydro Distillation	Water	-	34.3 IC50 μg / ml	[41]
Galicia (Spain) [W]	Centrifugation extraction	Water/ Ethanol (1:2) at 25 °C	23.6 mg GAE/ g DW	17.4-20.6 mg ascorbic acid / g DW	[42]
Greenhouse in Leipzig (Germany) [C]	Soxhlet	Water & Ethanol	14.97-64.7 mg GAE / g DM	2.84-3.53 EC50 mg/ ml	[32]
Volos (Greece) [W]	Centrifugation extraction	Ethanol/ Water mixtures from 20-80 °C	4.1-7.9 mg GAE/ g DW	1.83-23.69 μmol AAE / g	[43]

Table 3.1: The different areas of examined C. Maritimum and their properties. GAE is Gallic Acid Equivalent, DW is dryweight, AAE is Acetoacetic ester.[C] means that the plant has been cultivated, [W] means that it has been harvested in the wild.

Cultivation Area	Extraction method	Solvent & Temperature	Polyphenol content	Antioxidant activity	Ref.
Brittany (France) [W]	Centrifugation extraction	Water/ Methanol (1:1) at 4 °C	23-33 mg GAE / g DW	0.811-1.211 IC50 mg/mL	[35]
Croatia (adriatic coast) [W]	Hydro distillation	Methanol/ Water (80:20)	3.85-26.12 mg GAE/ g DW	0.63 - 2.14 μmol TE/ g	[36]
Garden in Tunis (Tunesia) [C]	Magnetic stirring	Methanol	9.426-17.11 mg GAE / g DW	7.68-56.33 IC50 μg /ml	[31]
Central Dalmatia (Croatia) [W]	Hydro distillation	Aqueous Ethanol (80%)	7.6-35.1 mg GAE/ g DW	2.6-61.8 %	[39]

Table 3.2: The different areas of examined C. Maritimum and their properties. GAE is Gallic Acid Equivalent, DW is dryweight, AAE is Acetoacetic ester, TE is Trolox. [C] means that the plant has been cultivated, [W] means that it has been harvested in the wild. Continuation of table 3.1

This shows that *Crithmum Maritimum* has high levels of Polyphenols and therefore has also a high antioxidant activity. But it can also be seen from the findings in the tables **??**, that the values can vary depending on what kind of extracting method used, but also where the plant was cultivated or foraged from. Therefore the extraction methods should be compared to see which on is the most efficient to extract the compounds, but also in which location *Crithmum Maritimum* grows best and then replicate the conditions of that locality to optimize growth conditions.

3.1.2 Salt tolerance of *Crithmum Maritimum*

As *Crithmum Maritimum* is a Halophyte, it has a high Salt tolerance, which ensures its survival in rocky coastal areas. But it is classified as a facultive halophyte, which means that it can not handle salt concentrations over 300 mM NaCl, compared to obligate halophytes, which need high salt concentrations to flourish, as these are often salt marsh plants [30].

As already stated *Crithmum Maritimum* grows on rocky cliffs/ grounds near the sea, which means that it is exposed to the sea but does not in itself come in contact with seawater. TO get optimal conditions for the plant the salinity concentrations should be around 100 mM NaCl. Salinity concentrations over 100mM NaCl starts to have a negative impact on the plant, which has a inhibition on the growth and the yield on the plant.[33], [44]

3.2 Effects of Fermentation

Here is a review about the common effects of fermentation. As humans have fermented different biomasses for a long time, there have been documented many of the different effects that fermentation can create. This review focuses on the positive effect that fermentation can have.

Feedstock	Micro-organism	Fermentation	Finding	Ref.
Dry Wheat Bran	*Bacillus coagulans, Lactobacillus plantarum* & *Candida utilis*	Solid State 24h-144h 25-30 °C	Improves the health of poultry and lessens organ damage	[45]
Canola Meal	*Lactobacillus salivarius*	Solid State 720h 32 °C	Improves Amino Acid while reducing Crude Fibre (16%) and glucosinalte content (38%)	[46]
Chickpea, Lentil, Faba bean	*A. oryzae, A. niger, L. plantarum*	Solid State 48h 30-37 °C	Increase in Phenolic Content and other compounds	[47]
Canola Meal	*Lactobacillus salivarius*	Solid State 720h 32°C	Reduction of Crude Fibre (16%) and glucosinalte content (38%)	[48]
Soybean	*L. acidophilus, L. delbruecki, L. salivarus, C. butyricum*	Solid State 48h-144h 37 °C	Increase in Nutritional value and anti microbial activity and further increase by protease supplements	[49]

Table 3.3: Impacts of fermentation to enhance Polyphenols and Antioxidant activity in different feedstocks.

Feedstock	Micro-organism	Fermentation	Finding	Ref.
Pomegranate peel, Thyme, Rosemary, Echinacae	*Lactiplantibacillus plantarum 129 J1/ P1*	48h 30 °C	Increase in phenolics, antioxidant and anti-inflammatory content	[50]
Acanthopanx senticous Harms	*Lactobacillus Plantarum, Saccharomyces cereviscae, Bacillus subtilis*	36h-60h 37°C	Increase of Polyphenol content (40%) and antioxidant activity	[51]
Kale (3 subtypes)	Spontaneous fermentation; *Lactiplantibacillus plantarum 332, Lactiplantibacillus paraplantarum G2114, Pediococcus pentosaceus 2211*	336h 18-20 °C	All fermentation triggered more polyphenols and anti oxidant activity	[52]
Maize	*Lactococcus lactis, Lactococcus lactis cremoris, Streptococcus thermophilus, Bifidobacterium thermopilium DSM 202212, Bifidobacterium choerinum K1/1, Bifidobacterium pseudolongum K4/4, Bifidobacterium animalis J311*	Fermentation 8h 37 °C, Storage 504h 6°C	Depending on strain phenolic increase between 46.1-94.6 % and antioxidant activity between 40.9-94.9 %	[53]

Table 3.4: Continuation of table 5.2

Feedstock	Micro-organism	Fermentation	Finding	Ref.
Black Tea and Kombucha	Mold, Yeast and Fungi	Liquid fermentation, few hours to multiple days	Femented tea has higher antioxidant capacity and anti-inflammatory effect	[54]
Highland barley bran	*B. subtilis*	Solid state 72h-120h 30-36 °C	Optimized femrentation for the type of Barley where the fermented version had an increase in polyphenol content of 203.17 %	[55]
Tea and different fruit juices	Lactic Acid bacteria	-	In all an increase in probiotic properties up to 90%	[56]
Pomegranate peel	*Aspergillus niger GH1*	Solid State 72h 30 °C	An increase of around 6 fold in polyphenol content and antioxidant activity	[57]

Table 3.5: Continuation of table 5.2

Fermentation of biomass, has been a consistent part of the human diet, through out the few last thousand years, and has been proven that it can improve the nutritional value of the biomass. Fermentation has also the potential to improve the polyphenol content and the antioxidant activity of the biomass and can therefore improve *Crithmum Maritimum* for the purposes of this project [27]. As *Crithmum Maritimum* has not that much data behind in that field, a review of other biomasses that have been fermented has been done, to see what fermentation can do to the polyphenol content and antioxidant activity of the biomass.
As it can be seen in the tables 3.3 3.4 3.5, the micro-organisms and the fermentation differs depending on the biomass and what they are examining. But in all cases a minimum fermentation time of 2 days is required, and depending on bacteria/ fungi strain a room temperature from 20-30 °C. Else all biomasses had a benefit, by being fermented for a period of time with a few excecptions, else the amount of polyphenol content and antioxidant activity increased in different amounts.
While doing this review, other reviews have been found, which show a similiar picture. These reviews either looked at a specific food group or looked at just different biomasses. Biomass:

- Multiple Biomass (10 in total). [58]
  - Soybeans, Black Soybeans, Cocoa Seed, Mungbean and soybean milk, Plum fruit byproducts, Brown rice flour, Kiwifruit wine, Defatted wheat germ, Liquorice root extract and Faba beans.

- Multiple Crusciferous vegetables. [59]
  - Potherb (Mustard), Pak Choi (Chinese Leaf Mustard), Broccoli Puree, Curly kale leaves, red cabbage (shredded) and Ethiopian kale.
- Multiple Whole grains. [60]
  - Maize, Millet, Quinoa, Rye, Sorghum, Wheat, Brown Rice and oat.
- Multiple polyphenol rich biomass. [61]
  - Citrus, Black wolfberry, Tartary Buckwheat (leaves), Soy Sauce, Cheonggukjang, Soy bean, Fermented Soymilk and Fermented Blueberry juices.

3.3 Lactic Acid fermentation

Lactic Acid fermentation is an anaerobic fermentation, which converts glucose and other six-carbon sugars into lactic Acid. As stated it is anaerobic which means it is a fermentation ocurring without the presence of oxygen. Here three major fermentations can occur which have different start and end products [62]:

- Homofermantative process

$$Glucose + 2ADP + 2P_i \rightarrow 2Lactate + 2ATP \tag{3.1}$$

- Heterofermantive process

$$Glucose + ADP + P_i \rightarrow Lactate + Ethanol + CO_2 + ATP \tag{3.2}$$

- Bifidium pathway

$$2Glucose + 5ADP + 5P_i \rightarrow 3Acetate + 2Lactate + 5ATP \tag{3.3}$$

Where ADP is adenosine diphosate, ATP is adenosine triphosphate and P_i is inorganic Phosphate. As it can be seen all the different pathways can produce Lactic Acid, but in different ways and in different quantities.
The most common lactic Acid fermentation done with food products is the homofermantive fermentation, while hetero fermantive is used for some beer types in Germany and Belgium, as it produces ethanol.
Lactic Acid fermentation is often used in food preservation, as through the generation of lactic acid the acidity rises and kills of harmful pathogens and increases the shelf life of these products. It has also shown to have probiotic effects in these products.

3.3.1 Effects of fermentation with *Lacto Bacillus*

There has been for a long time an interest for improving the different properties of food. This can be for different purposes, improving feedstock products for animals to reduce their medical intake or improving food products for human consumption. One proven method is to ferment biomasses fit for human and animal consumption with different strains of *Lacto Bacillus* [45]. Another benefit that *Lacto Bacillus* has, is that it can improve skin conditions and improve acne in patients [23], [24], [63]. Therefore there is a possibility that biomass that has been fermented with *Lacto Bacillus* has enhanced biocompounds that can reduce skin problems.
The two most common strains are *Lacto Bacillus Salivarus* and *Lacto Bacillus Plantarum*. The fermentation normally used is Solid Stated Fermentation, as this allows for a higher use of Biomass usage [51].
For solid state fermentation one of the big factors that can influence microbial development and fermentation, is moisture content of the batch working with. This moisture content has a high range of between 50-90% and often depends on the biomass used and what purpose the fermentation has.[64]–[66]
Lacto Bacillus is found in most naturally fermenting food and drink products around the world and is linked to their many probiotic benefits. This is one of the reasons it is being examined if it can be used to enhance the probiotic effects of animal fodder and thereby reduce the antibiotic intake of agricultural animals.[45]
But as it can be seen from 3.33.43.5 there has been done studies on a large number of biomasses, fermentation methods and different bacteria strains. Therefore it is of interest to make different fermentation trials with the chosen biomass, as every biomass reacts different while fermenting and need different fermentation times.

3.4 Literature review conclusion

Through this literature review, it has been determined that *Crithmum Maritimum* has a high amount of polyphenols and antioxidant activity, which makes it attractive as source of biomass for biorefineries. It has also been determined that solidstate fermentation with bacteria, especially Lactic Acid bacteria like *Lactobacillus*, are able to enhance the bio compounds of biomasses and increase the probiotic effects, which polyphenol rich biomass already had. It is therefore of interest how much the polyphenol content of *Crithmum Maritimum* can be increased due a solidstate fermentation with Lactic Acid bacteria.

4 Materials and Methods

The Material used for the process is called *Crithmum Maritimum* and can be harvested in its different growth stages from May to October throughout the Mediterranean and North Africa, by farmers if it is cultivated or foraged from cliffsides as described in section 3.1. The entire plant is used and was dried at first before it can examined. The Plants were thereafter shredded to a size of 5-12mm to achieve better access to the properties of the plant.

Figure 4.1: *Crithmum Maritimum* in its dried and shredded form.

4.1 Extraction & Fermentation

As the different properties of *Crithmum Maritimum* had to be examined, the biomass had to be extracted. In the same context there was an interest in optimizing the extracted properties, as *C. Mari.* is rich in Polyphenols and Antioxidants. For that different Pre-treatments and Fermentations have been used.

Three different Methods were used to extract the contents of *C. Mari.* and examine which one had the highest yield, polyphenol content and antioxidant activity.
The first was a basic Soxhlet extraction of the biomass without any pretreatment and fermentation:

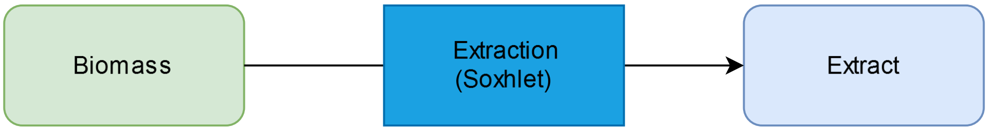

Figure 4.2: Basic Extraction of *Crithmum Maritimum* without any fermentation

In the second version the biomass has been fermented for 72 hours and thereafter extracted:

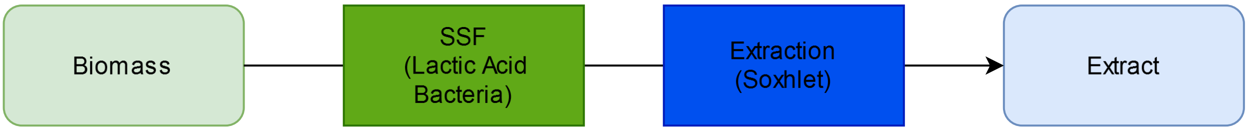

Figure 4.3: Fermentation and extraction of *Crithmum Maritimum* with Lactic Acid bacteria

In the third version the biomass was pretreated and thereafter fermented. On part of this was also been subjected to an enzyme to see if that can improve the fermentation:

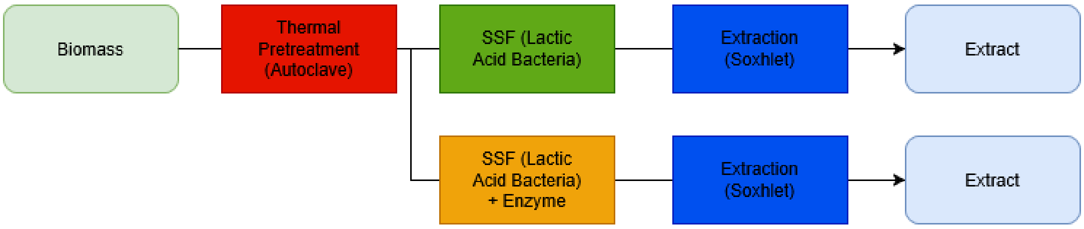

Figure 4.4: Fermentation and extraction of *Crithmum Maritimum* with Lactic Acid Bacteria and pretreatment.

As already stated for the Extraction a Soxhlet Extraction has been used, while the fermentation a Solid State Fermentation (SSF) was used, as to use as much biomass as possible. The Enzyme used in one of the batches is Cellic CTec3, which is a standard commercially available Enzyme.
For the Extraction as already stated a Soxhlet Extraction was used, with Water as the solvent. For the Extraction 5 gram of the dried biomass was used and run through in a 5 hour Extraction with 250 ml of solvent. After the Extraction has been done the Liquid has to cool down, before it can be analysed. The Extraction was analysed to determine the Drymatter content and hereafter the Glucose content was determined with the help of HLPC. The Extract was therafter rotary evaporated, to remove much of the water inside of it, and then freeze dried so that it can be further examined at a later date.

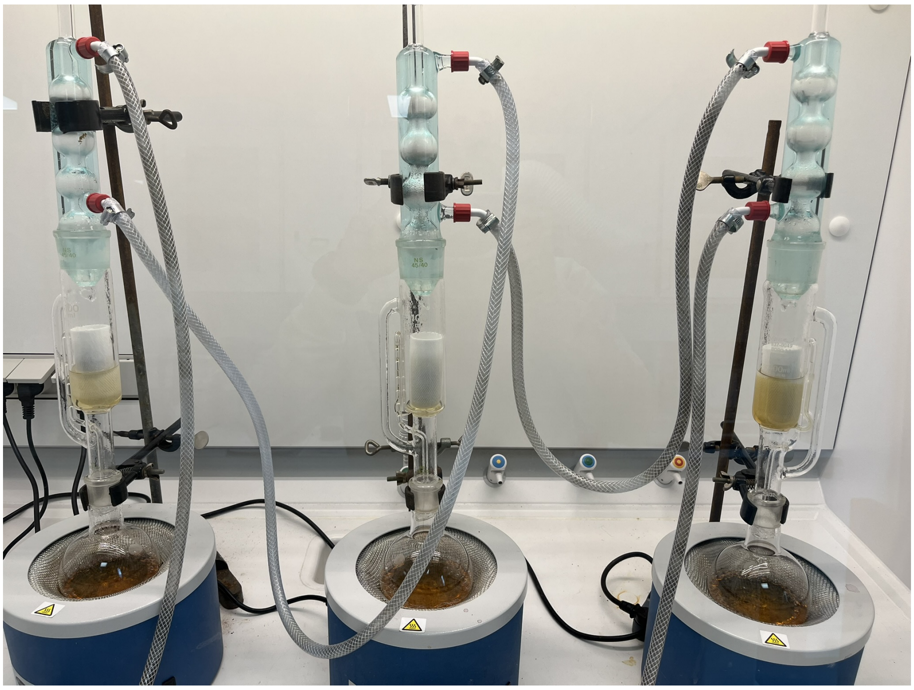

Figure 4.5: Soxhlet Extraction of the dried biomass.

The Fermentation was done with *Lacto Bacillus*, as discussed in the the former chapter, as it has the potential to improve the pro biotic properties of different biomasses. Therefore it was used to examine if it also is able to improve the properties of halophytes and in this case C. Mar.
Two strains of the *Lacto Bacillus* strain were cultivated *L. Bac. Salivarus* and *L. Bac. Plantarum*, as these are the most commonly used strains of this Bacteria Group and the most documented.
For the Fermentation itself 15g of dried *C. Mar.* has been moisturized with water up to a moisture content of 75% and later 85% as the dried Biomass was able to soak a lot of water. The soaked biomass was then added with 10% of its weight with the cultivated strains and allowed to ferment for 72 hours in a 37 °C Water bath. After it was removed from the water bath, the fermented biomass was dried in a 60 °C oven for 2-3 days, and hereafter it was ready to be used for Extraction in a Soxhlet Extractor.

4.2 Polyphenol Analysis

For the polyphenol analysis the freeze dried samples was rehydrated in a ratio of 10 mg/ml and can therafter be analyzed for its content of polyphenols. For the total phenolics analysis, 10μg of the rehydrated sample will be put into a 96 well plate in sixplicate standard. Hereafter 100μl of diluted Folin-Ciocalteu is added. It needs to rest in a dark room for 10 minutes before 100μl of sodium chloride is then added. This needs to rest for 90 minutes in a dark room. After that its absorbance will be analysed at 725 nm.
To be able to use the results of this sample, a calibration curve was also done. To start 20μl of sodium chloride was added in the first wells, always double the size of the orig-

inal samples. Hereafter 10μl of purified water was added to the other wells, depending on how many samples the original well plate has. The Sodium Chloride has to be diluted now, so 10μl of the first wells was then added to the second well, and then 10μl of this mixture was then added to the next wells. This continues until all the samples have been mixed and diluted that way. Now it had to rest for 10 minutes in a dark room. After that 100μl of diluted Folin-Ciocalteau was added and it had to rest for 90 minutes in a dark room again.
Now it could be analysed and the calibration for the original samples could also be done. To do that, the mean absorbance for each standard compound was calculated, and the results was be plotted in a graph. Now a function for these plot points had to be determined and the R^2 for the equation was found.
This equation can then be used to calculate the polyphenol content of the original samples.

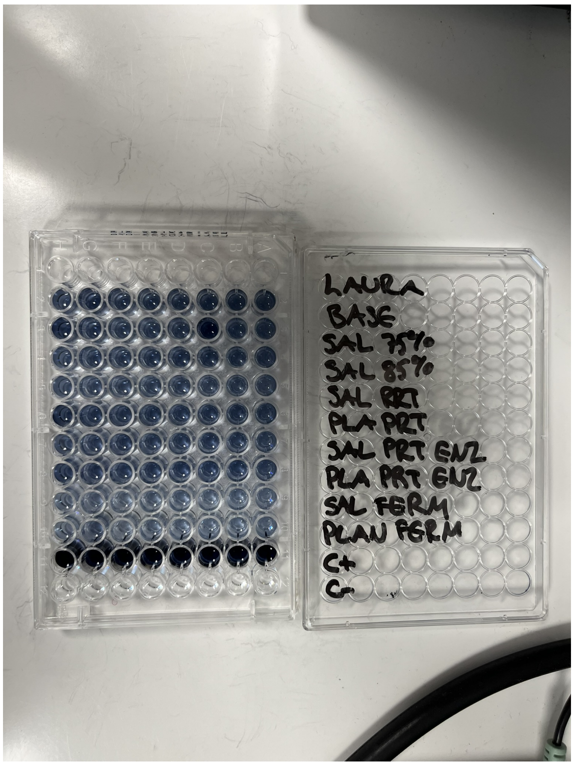

Figure 4.6: Polyphenol analysis of the different extracts

4.3 DPPH analysis

For the DPPH analysis a 96 wells was used to to make enough samples so that they could be analyzed. For the analysis there was a positive and negative control and the samples that had to be analysed. There were 6 replicates of each with 2 extra samples that were used for colour correction. The coloured correction samples were there to eliminate the heavy colours of pigments or phenolics in the extracts. For the Setup $8 \cdot 22$ μl GA solution were used for the positive control, $8 \cdot 22$ μl of the extraction solvent for the negative control. Hereafter $8 \cdot 22$ μl of the sample was then added, a column for each sample that has to be analysed. The six replicates that were to be analyzed got added 200 μl of DPPH solution, while the colour correction samples got 200 μl of methanol. After these solution were added the plate had to incubated in a dark room for 30 minutes. When the 30 minutes were gone the plate were then read at a wavelenght at 517nm.
To be able to analyse the readings, first the average of two colour corrections in the same column were calculated, and then used to substract from the read samples.

$$Absorbance\ of\ sample = Absorbance\ of\ test\ sample - Absorbance\ of\ colour\ correction \tag{4.1}$$

The average of all the samples were then calculated. The average of the absorbance of the negative control was then used to calculate the Radical Scavenging Activity (RSA). It was given in percentage and was calculated by subtracting the fraction of the absorbance of a sample with the average absorbance of the negative control times 100, from 100.

$$RSA[\%] = 100 - (Absorbance\ of\ sample/Absorbance\ of\ average\ negative\ control\ \cdot 100) \tag{4.2}$$

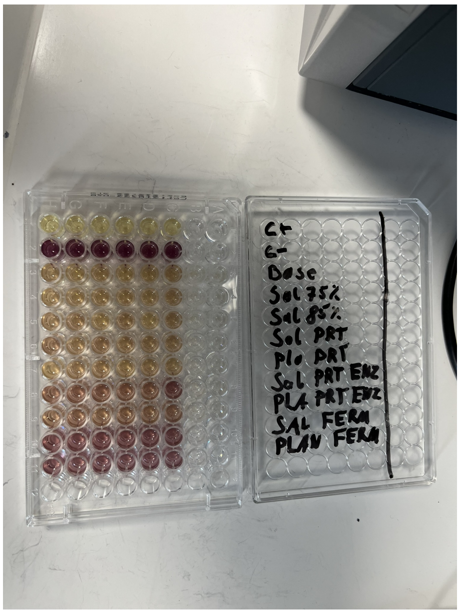

Figure 4.7: DPPH analysis of the different extracts

4.4 IC50 analysis

IC50, short for "Half maximal inhibitory condition", is the measure of the potency of a substance to be able to halt or inhibit a biological or biochemical function. It can be a better value to see the antioxidant activity of a compound and as it gives precise numbers, it is able to be compared with other sources.
This test was done when the RSA of a DPPH analysis is over 50%. Here it is to note as it measures how much of a concentration is needed to achieve the half maximal inhibitory effect, the lower the result is, the better of a result it is.
The analysis was done by a serial dilution of the sample that has to be analysed. 44 μl of the sample was added to the first well, while 22 μl of the solvent was added to the other wells. Now 22 μl of the sample in the first well was then taken and added to the other well, and then this is continued, so that the concentration was halved everytime. Else the same procedure as in the DPPH essay had to be followed, so that the absorbance of the sample could be read. Thereafter the mean Absorbance was calculated for each column and then the IC50 was calculated by an online tool by AAT Bioquest Inc [67].
As the interest was to see the concentration of the compound compared to its inhibitory effect, the liquid should had a ratio of around 50mg/ml to achieve proper readings.

5 Biomass Extraction Results

This Chapter shows the results of the Extraction process of *Crithmum Maritimum* and the results of the different fermentation methods. These results are used as a basis for Lactic Acid bacteria will be used and what kind of pretreatment the biomass will have in the process simulation part.

5.1 Soxhlet Extraction yield

As it can be seen from the flowsheet in section 4.1 three different extraction and fermentation trials were done to examine how to increase the extraction yield, while also trying to increase the bioactive compounds inside of *Crithmum Maritimum*.
After all fermentation trials were soxhlet extracted, the extract yield was examined. The results are given in percentage based on 5g dried biomass that was extracted, which can be seen in figure 5.1 :

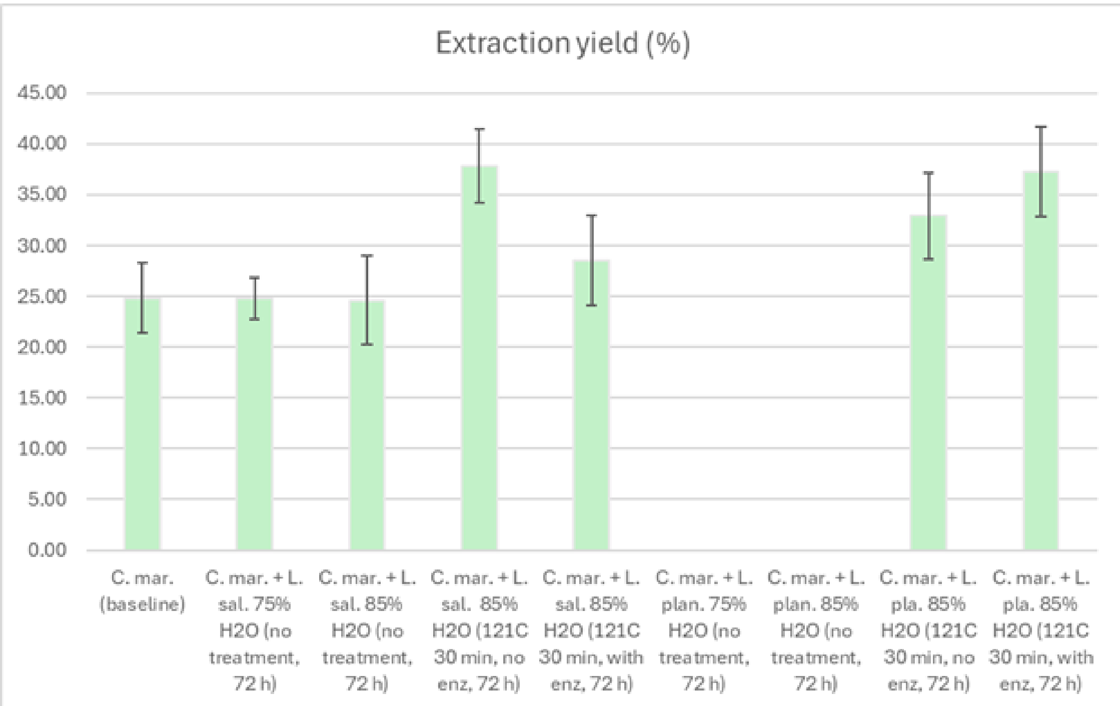

Figure 5.1: The Mean Extraction yields in % for the different treated Biomasses

From these results it can be seen that the fermentation has increased the extraction yield, while also showing the differences between the two bacterial strains of *lactobacillus* that have been used. It can be seen that *L. Sal.* has the highest extraction yield while also having a higher survivability compared to *L. Plan.*, which failed under basic fermentation conditions. But *L. Plan.* has generally a higher extraction yield, when it was pretreated. The big interest here is that extraction yield differs when the biomass has been treated with the enzyme. For *L. Sali.* has a lower Extraction yield than the one with *L. Plan.* while the samples which have only been pretreated have more similiar results.

5.2 Polyphenolic analysis of the extract

After the Extract has been analysed based on its extraction yield, the bioactive compounds of the extract has to be analysed. As the goal was to examine if fermentation can increase the polyphenolic activity of the extract. The method of the analysis has been shown in section 4.2. The results of the analysis can be seen in figure **??**:

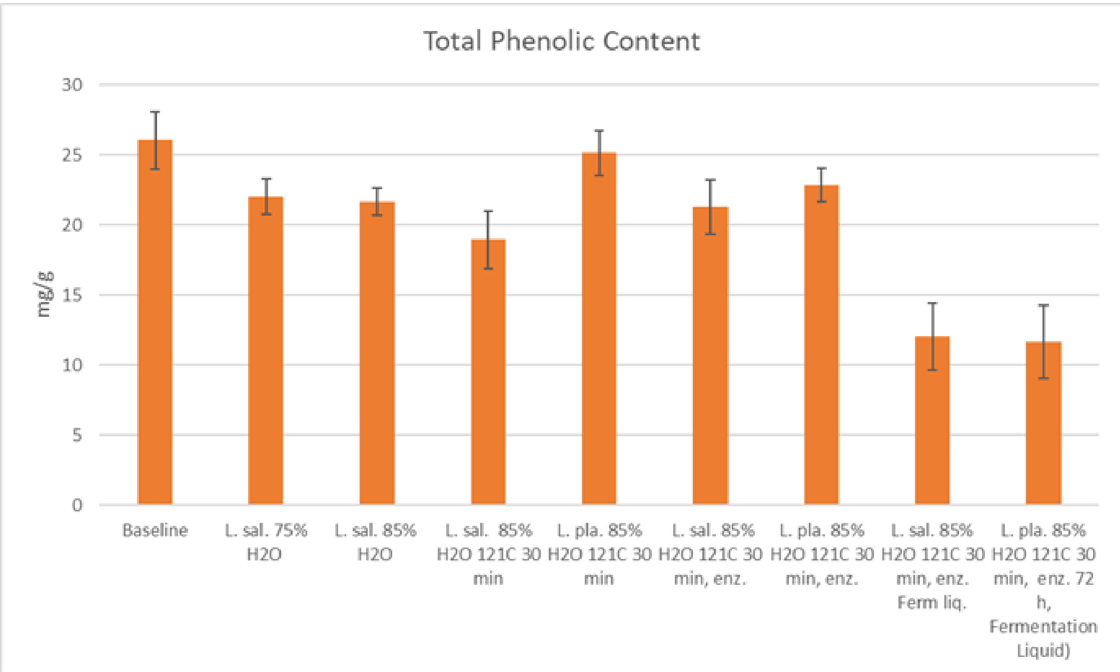

Figure 5.2: The Polyphenol Content in the Extracts.

As it can be seen from the results, the phenolic content of the extracts are quite similar to each other, with the highest being the base extract, without any treatment and fermentation. Else it seems that Extracts from the *L. Plan.* fermentation have a higher phenolic content compared to the *L. Sali.*
The fermentation liquids of the Fermentation samples with enzymes, have also been examined to see if they were rich in polyphenols and thereby see if those version even need extractions, as those samples were quite liquified. As if they already released all the

bioactive compound in the fermentation stage they would not need any extractions.

5.3 DPPH analysis of the extracts

As it could be seen in the former section in figure 5.2 the polyphenolic content is quite similiar to each other and as polyphenols have antioxidant properties, the RSA reading from the DPPH analysis should follow in a similiar way.

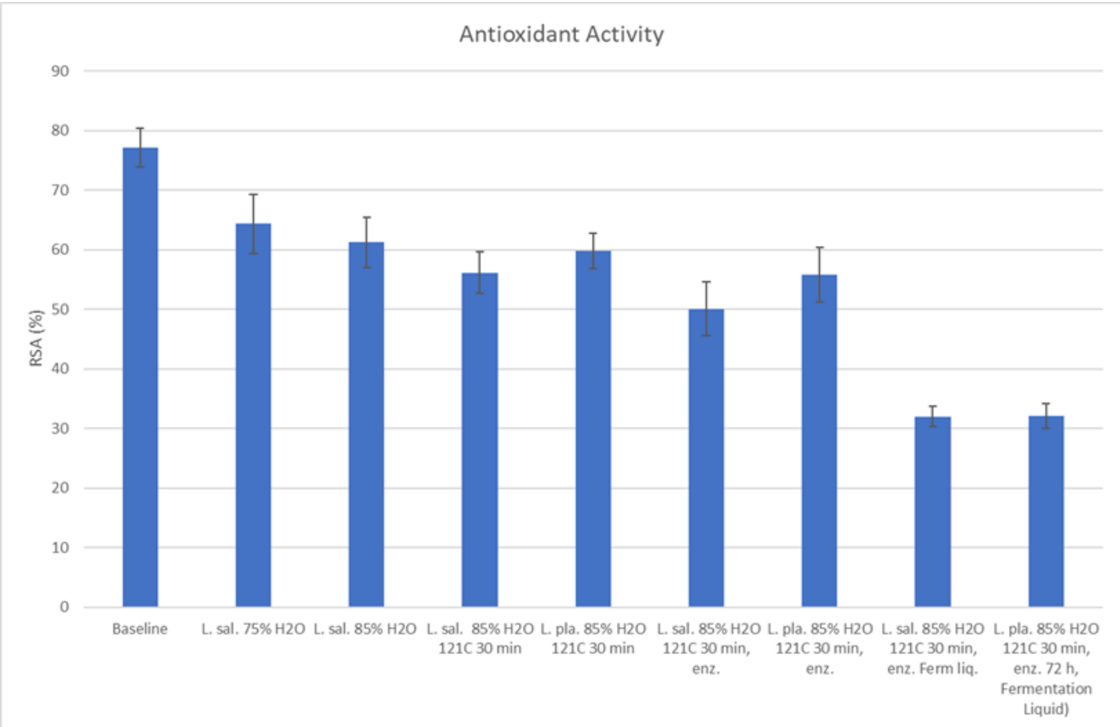

Figure 5.3: The antioxidant activity of the samples

As it can be seen from the results the base extract has again the highest amount of RSA activity. And again following the polyphenol results the samples fermented with L. Plan. have a higher RSA activity then their L. Sali. counterparts. But as these results show that almost all samples, have a high RSA reading over 50%, an IC50 anlysis will be done to find how effective each sample actually is.

5.4 IC50 analysis of the extracts

As stated in section 4.4 an IC50 analysis, based on the DPPH analysis will be done to see its half maximal inhibitory concentration, thereby seeing how effective each sample actually is.

Biomass + Treatment	IC50 concentration (mg/mL)
C. mar. (baseline)	4.1865
C. mar. + L. sal. 75% H2O (no treatment, 72 h)	12.9179
C. mar. + L. sal. 85% H2O (no treatment, 72 h)	3.0875
C. mar. + L. sal. 85% H2O (121C 30 min, no enz, 72 h)	5.9626
C. mar. + L. sal. 85% H2O (121C 30 min, with enz, 72 h)	15.0886
C. mar. + L. pla. 85% H2O (121C 30 min, no enz, 72 h)	3.3167
C. mar. + L. pla. 85% H2O (121C 30 min, with enz, 72 h)	7.1336

Table 5.1: The Biomass with their IC50 concentration

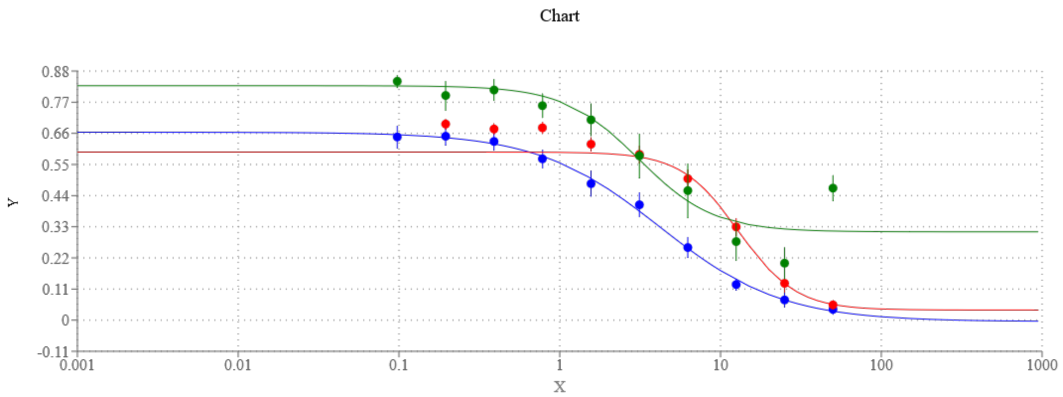

Figure 5.4: IC50 comparison between the Baseline and the fermented C. Mari L. Sali at 75% and 85% each.

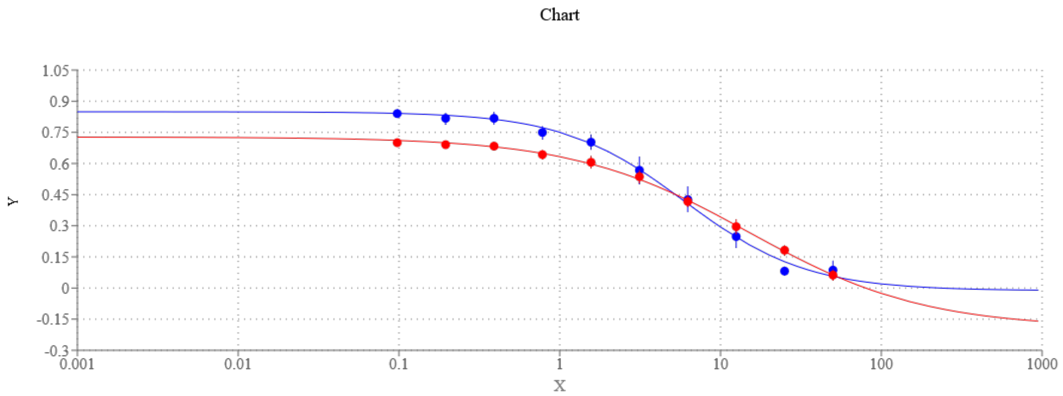

Figure 5.5: IC50 comparison between C. Mari L. Sali., one which has only pretreated and one where it has been pretreated and enzyme added.

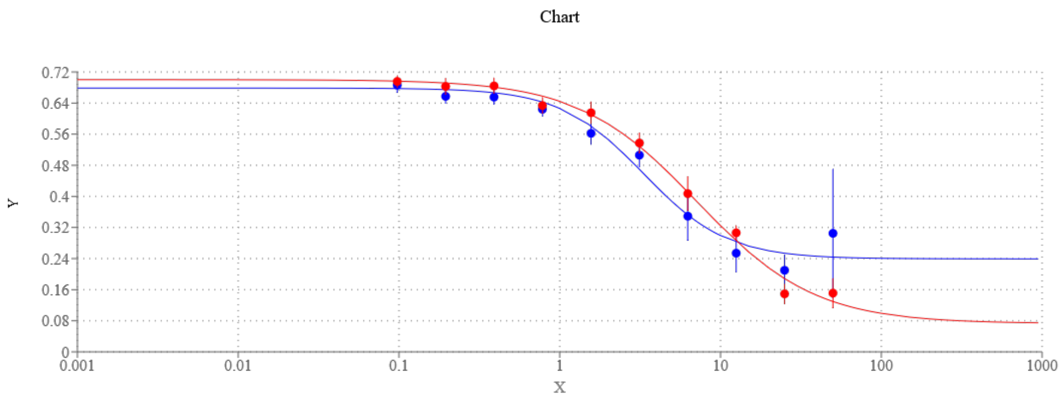

Figure 5.6: IC50 comparison between C. Mari L. Plan., one which has only pretreated and one where it has been pretreated and enzyme added.

To understand the charts properly, the important point is to see from where the graph starts and where it drops down and how big the slope of the graph is. The bigger the difference in drop of concentration and the steeper the slope the better IC50 result it is.
The results from the graphs, as seen in numerical format in table 5.1, the different extracts have a different inhibitory effect, as stated in section 4.4. Here is to note that Biomass treated with enzymes, had a worse inhibitory effect compared to the other extracts. While the biomass with only thermal pretreatment had some of the best inhibitory effect. Here is it interesting to note, that the sample which had only 75% moisture content needs a high concentration to achieve half inhibitory effect. This could be due to human error or an other mistake done during testing.

5.5 Total Comparison

After all the results from the above sections have been analyzed, they need to compared to each other to see which treatment method is the most effective in total. To do that the data from the Extraction yield, Phenolic Analysis and the IC50 gathered and compared to each other.

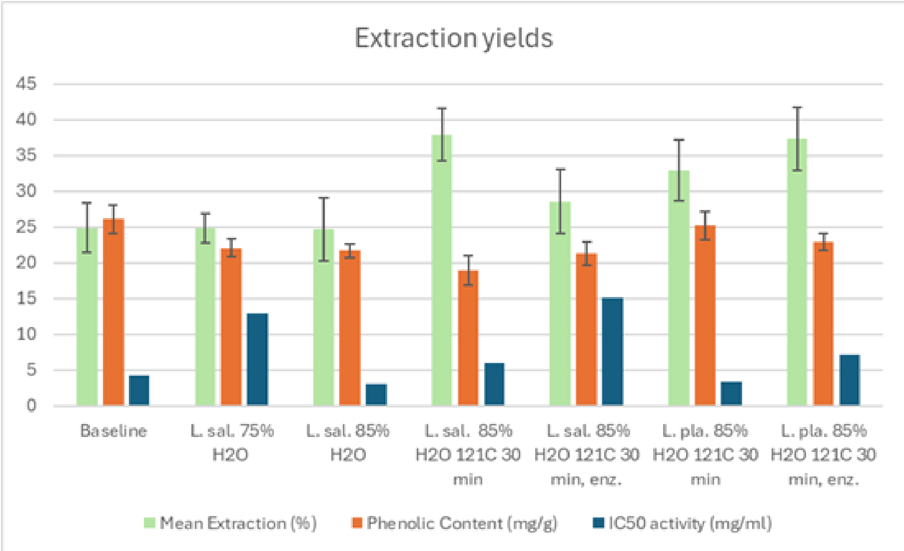

Figure 5.7: All the Extraction yields of the different extracts.

Here to note is, that the lower the IC50 value is, the better its effectivity is, compared to the other values where they should be as high as possible.

From these numbers, it can be seen that *C. Mari.* which was pretreated and had the *L. Plan.* strain, had the highest Extraction yield, while having as high phenolic content and low IC50 number.

Therefore for the process simulation *C. Mari.* + *L. Plan.* 85% H2O (121C 30 min, no enz, 72 h) is going to be used and worked on.

6 Process Simulation

For the Process Simulation Intillegen SuperPro Designer (SPD) has been used as a process Design tool [68]. This was done to simulate the Fermentation process ocurring in the extraction process. There will be two simulations:

- Case 1: A fractionated biomass refinery
- Case 2: A whole biomass refinery (Green Biorefienery)

In this chapter they will be compared in their processes and how they differ from each other.

6.1 Preparation for the simulation

To use SPD, which is detailed process designer tool, more information about the biomass needs to be gathered. As this project mostly focused on the extraction process of *Crithmum Maritimum* and analyzing its content, some information about the plant could not be examined. Therefore necessary information that is required will be gathered from other reports. The information that is going to gathered is the general composition of the plant. The data comes from this report,[44] , where the composition of *Crithmum Maritimum* at different salt concentrations was analyzed.

Salt Concentration (mM NaCl)	Juice (w/w%)	Fibers (w/w%)	Drymatter Fiber (%)	Drymatter Juice (%)	Drymatter Whole (%)
0	70.35	29.65	27.22	5.58	11.06
86	70.46	29.54	27.15	5.39	11.77
171	66.97	33.03	26.06	7.44	13.56

Table 6.1: The amount of fractionated and drymatter content of the biomass.[44]

Average amount of compound in green juice fraction (g/100g DM)					
Salt Concentration (mM NaCl)	Organic Acid	Crude protein	Lipids	Carbohydrates	Ash
0	0.34	30.98	1.66	40.77	29.39
86	0	30.14	2.11	29.68	34.16
171	0	26.32	1.77	29.25	31.99

Table 6.2: The average composition of the green juice at different salt concentrations.[44]

Average amount of compound in fiber residue fraction (g/100g DM)					
Salt Concentration (mM NaCl)	Klason Lignin	Crude protein	Lipids	Carbohydrates	Ash
0	39.82	19.62	2.08	25.85	9.49
86	40.80	19.76	2.26	38.79	10.10
171	35.3	18.38	1.69	27.33	10.84

Table 6.3: The average composition of the fiber residue at different salt concentrations.[44]

These values were taken directly from the data sheet associated with [44], the following values for the compounds in the entire biomass were calculated based on the before mentioned data sheet.

Average amount of compound in whole biomass (g/100g DM)						
Salt Concentration (mM NaCl)	Klason Lignin	Crude protein	Lipids	Carbohydrates	Organic Acids	Ash
0	26.43	23.09	1.94	17.39	0.23	16
86	27.85	23.09	2.21	26.47	0	17.85
171	20.96	21.29	1.72	16.22	0	18.6

Table 6.4: The average composition of the entire biomass at different salt concentrations.[44]

Average amount of sugar compounds (g/100g DM)				
Salt Concentration (mM NaCl)	Glucose	Xylose	Arabinose	Lactic Acid
0	25.33	17.92	1.58	0.23
86	27.86	16	2.87	0
171	19.29	12.27	2.03	0

Table 6.5: The average composition of the total sugar profile at different salt concentrations.[44]

6.2 Processing of *Crithmum Maritimum*

Through the use of an in silico analysis approach with the simulation software Intillegen SuperPro Designer (SPD), a biomass processing plant using the biomass *Crithmum Maritimum* was done.
The biomass will be refined into the value added streams, where the end products of the simulation are the extract and the fibers of the biomass. By using the data from the former sections and chapters, the extraction yield, the operational costs (OPEX), the capital expenditures (CAPEX), the equipment size and the efficiency will be simulated and calculated. After this simulation a techno-economic analysis will be done to find the optimal condition for processing. The flowsheets for the simulations can be seen in the figures 6.1,6.2:

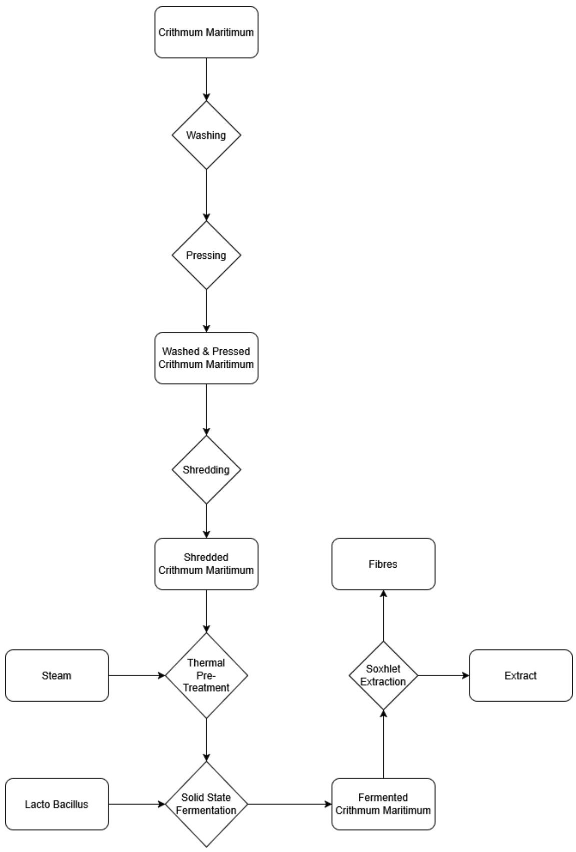

Figure 6.1: A flowsheet of a Biorefinery based on the experiments done. The rectangle shaped boxes are products, the Diamond shaped boxes are processes.

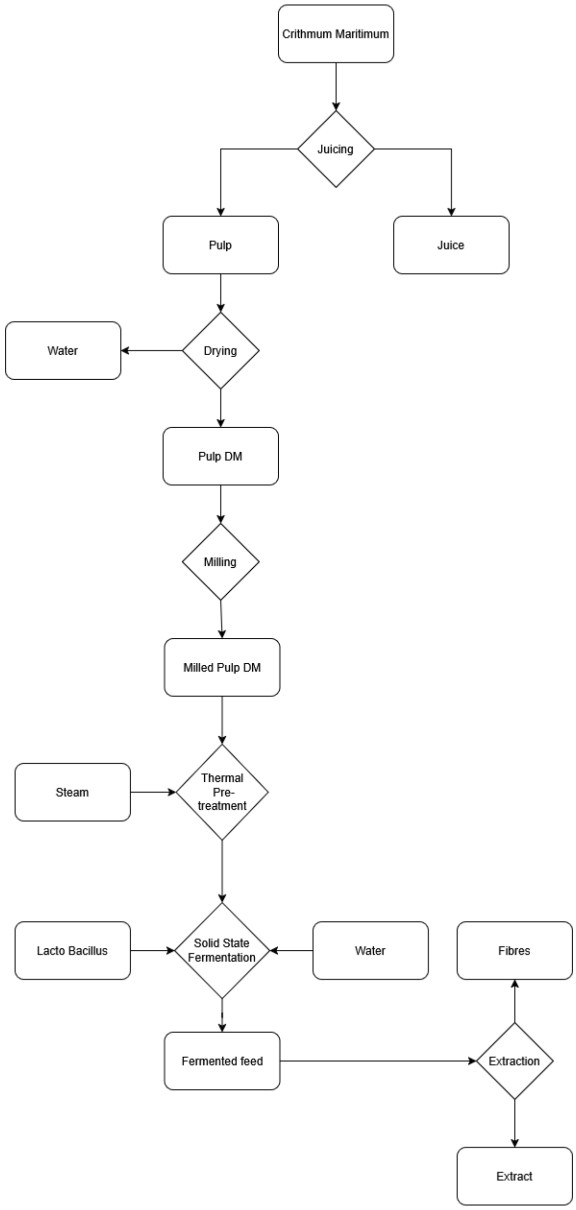

Figure 6.2: A flowsheet of a realistic biorefienry using Critmum Maritimum as a raw ressource. The rectangle shaped boxes are products, the Diamond shaped boxes are processes

The main difference between the these two flowsheets is that in the first the biomass is not fractionised in a fibre pulp and juice, as this project is based on whole biomass. But that would not be realistic as the juice has a potential as a protein enriched by product. It could therefore be an interesting value added product for the biorefinery.

6.2.1 Biomass Preparation

The biggest difference between the two cases, is how the biomass is prepared for extraction. In the first case the entire biomass is washed and thereafter shredded directly in its entirety.

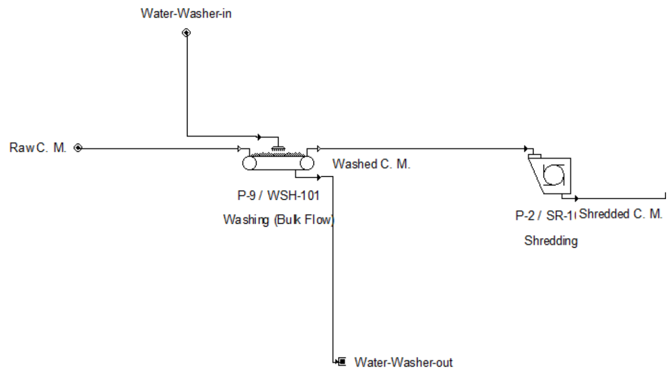

Figure 6.3: The start of the processing for the whole biorefinery

In case two the biomass is first fractionated in its juice and pulp fraction, which is done with the help of a screwpress and the pulp thereafter dried in a drum dryer. The juice fraction is seen as a value added stream and not further examined. This pulp is then shredded and will thereafter be processed.

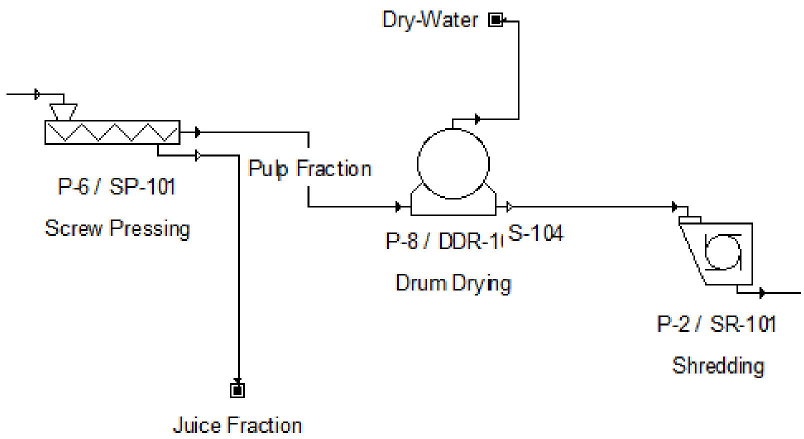

Figure 6.4: The start of the fractionated biorefinery

Parameter	Value	Unit
Whole Biomass		
Biomass input	1	ton / batch
Biomass output	1	ton / batch
Outlet Temperature	25	°C
Fractionated Biomass		
Biomass input	1	ton / batch
Biomass output	0.103	ton / batch
Outlet Temperature	70	°C
Juice Fraction output	0.674	ton / batch

Table 6.6: The Input and Output conditions of the Biomass before the main fermentation.

6.2.2 Thermal pretreatment

The shredded biomass has to undergo a Thermal pretreatment, so that under the main fermentation, the lactic Acid bacteria has easier access to the glucose inside of the biomass. The termal pretreatment does that by breaking the lignocellulose barriers of the shredded biomass even more down. For that it is heated to 121 °C and kept at that temperature for 20 minutes before it gets cooled down again.

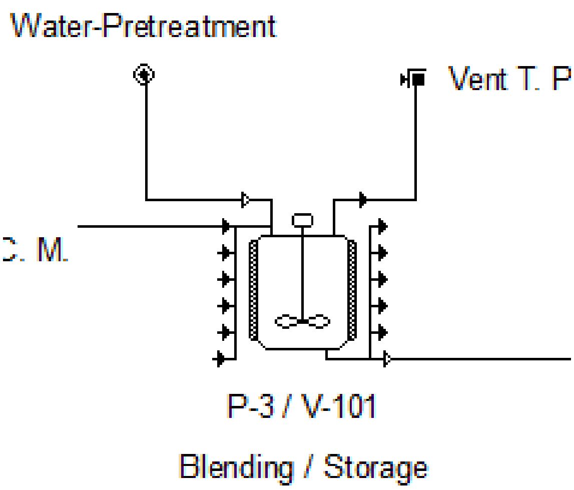

Figure 6.5: The Thermal pretreatment of the Biomass.

Here is to note, as *Crithmum Maritimum*, has already a natural moisture content of over 85%, no extra water is added to the Biomass, where it is not fractionated as seen in the start of the section 6.1.

Parameter	Value	Unit
Whole Biomass Shredded		
Biomass input	1	ton / batch
Inlet Temperature	25	°C
Biomass output	1	ton / batch
Outlet Temperature	30	°C
Fractionated Biomass Shredded		
Biomass input	0.103	ton / batch
Water input	0.548	ton / batch
Inlet Temperature	70	°C
Biomass output	0.651	ton / batch
Outlet Temperature	30	°C

Table 6.7: The Input and Output conditions of the Biomass after it has undergone Thermal pretreatment.

6.2.3 Lactic Acid bacteria preparation

As the fermentation is a big part of the process, enough Lactic Acid bacteria have to be prepared for it. As it is based on the lab setup in section 4.1, around 10% of the weight of the biomass, will be added in the form of Lactic Acid bacteria. Depending on the size of this operation a varying of the bacteria has to be supplied. Therefore an Inoculation preparation and fermentation step will be included, so that it is possible to produce the necessary bacteria on site. This step will consist of a shake flask to inoculate the bacteria and a seed fermentor to create the necessary amount of bacteria. Depending on the amount needed, more or less seed fermentors can added or removed.
For this purpose MRS Broth, which has been used to cultivate *Lactobacillus Plantarum* in the lab, will be simulated as close as possible in SPD. This Broth will consist of [69]:

- Cells, which are used as inoculation and are simulated as *Lacto Bacillus Plantarum* cells.
- Peptone, which in the industry used to encourage microbial growth.
- Media salts, which is the medium buffering modeled as a single component.
- Extracts, consisting of beef and yeast extracts
- Glucose, the main carbon source

To start the media and water will be added to a shake flask and heated to 121°C, to sterilize it. Hereafter it will be cooled down to 30°C, and the inoculation liquid will be added. The fermentation will then be simulated. The Mass Stoichiometry of the fermentation reaction will be this [69]:

$$35\,Extracts{+}55\,Glucose{+}8\,Media\ salts{+}12\,Peptone \rightarrow 15\,Cells{+}35\,CO_2{+}35\,Lactic\ Acid{+}25\,Water \tag{6.1}$$

This reaction simulates the the creation Lactic Acid and and Lactic Acid bacteria like *Lactobacillus Plantarum*.

Thereafter the Broth will be transferred to a Seed fermentor, where the same process occurs to increase the amount of MRS Broth. The conversion rate for this reaction is at 98%.

This step has been inspired by an example of SuperPro Intillegen, where they simulate the creation of Probiotics [69].

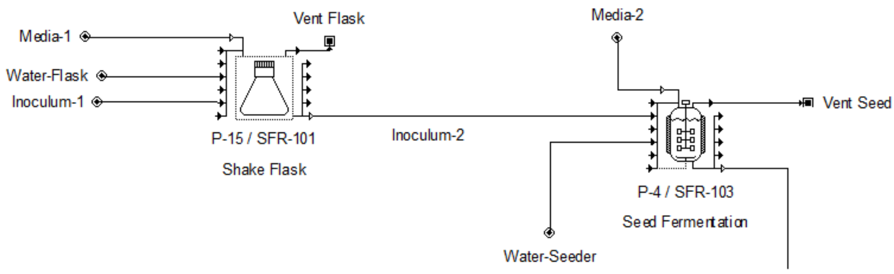

Figure 6.6: The inoculation and preparation of *Lactobacillus*

Due to the nature of the inoculation running in batches and not being able to run in continuously, it was decided to run it in batches. That can reduce the overall output of the refinery, but makes it up that it is easier to maintain the the equipment when it is offline, or if it finished its part of the batch.

Parameter	Value	Unit
Inoculation of Lactic Acid bacteria		
Media Input	0.5	kg / batch
Water Input	2	kg / batch
Inoculation input	0.02	kg / batch
Inoculation Output	2.49	kg / batch

Table 6.8: The Input and Output of the Shake flask to inoculate the media.

Parameter	Value	Unit
Whole Biomass Seed Fermentation		
Media Input	19.85	kg / batch
Water Input	79.4	kg / batch
Inoculation Input	2.49	kg / batch
Lactic bacteria output	100	kg / batch
Fractionated Biomass Seed fermentation		
Media Input	12.72	kg / batch
Water Input	50.88	kg / batch
Inoculation Input	2.49	kg / batch
Lactic bacteria output	65.14	kg / batch

Table 6.9: The lactic acid bacteria preparation for the main fermentation.

6.2.4 Fermentation of *Crithmum Maritimum*

For the fermentation of the biomass the green biomass and the probiotic Lactic Acid bacteria are combined in fermentation tank, where the main fermentation occurs. The procedure is simulated like the lab experiment explained in section 4.1.
For the simulation depending on the case the entire green biomass or the fiber fraction enters the tank, where it will be combined with 10% of its weight of Lactic Acid bacteria and ferment for 3 days. After the fermentation is done the contents of the fermentation tank will transported to a Soxhlet extractor where the extraction will occur.

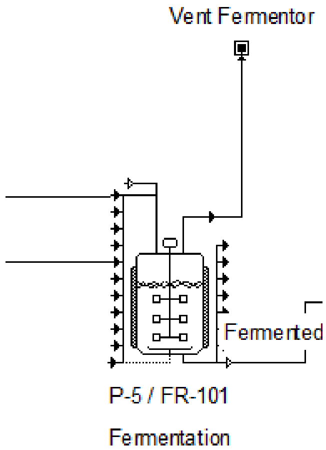

Figure 6.7: The fermentor unit responsible for fermenting the biomass.

Parameter	Value	Unit
Whole Biomass Fermentation		
Biomass Input	1	ton / batch
Lactic Acid Bacteria input	0.1	ton / batch
Temperature Input	30	°C
Fermented Biomass output	1.1	ton / batch
Temperature output	37	°C
Fractionated Biomass Fermentation		
Biomass Input	0.651	ton / batch
Lactic Acid Bacteria input	0.065	ton / batch
Temperature Input	30	°C
Fermented Biomass output	0.716	ton / batch
Temperature output	37	°C

Table 6.10: The values of the main fermentation.

6.2.5 Extraction of *Crithmum Maritimum*

As the Polyphenol contents of the Fibre residue of *Crithmum Maritimum* is the main priority of this project, these fibres have to be extracted. In the laboratory a Soxhlet Extractor has been used, and therefore a Soxhlet extraction will be modeled in SPD.
As there is no Soxhlet Extractor in SPD, a modified cycle has to be built that can replicate the functions of a industrial Soxhlet Extractor. This Soxhlet Extractor would consist of two units, a storage unit, where water is continuously added, and a evaporation unit. These replicate in sequence a Soxhlet extraction chamber, a Siphon, continuously added solvent and a container storing the extraction, usual a round bottom flask. This cycle is to ensure that there will continuously new solvent added to the extractor, where the solid biomass will have retention time of 5 hours, following the experimental setup in the lab. After the process has passed the evaporation chamber it will be condensed to ensure that the extraction is in a liquid phase.
By default in SPD around 99 w% of the solvent will be evaporated in the Evaporator, where hereafter again 99 w% of the solvent and solvent solubles will be decanted and stored in a storage Unit, hereby having dry biomass and solvent extract. To make sure that there is a pure concentration of the extract ready to be used, the rest of the solvent inside of the extract will be removed. The remainder of the solvent existing in the extract will evaporate leaving a pure concentrate. This step has been inspired by this Master report[70].

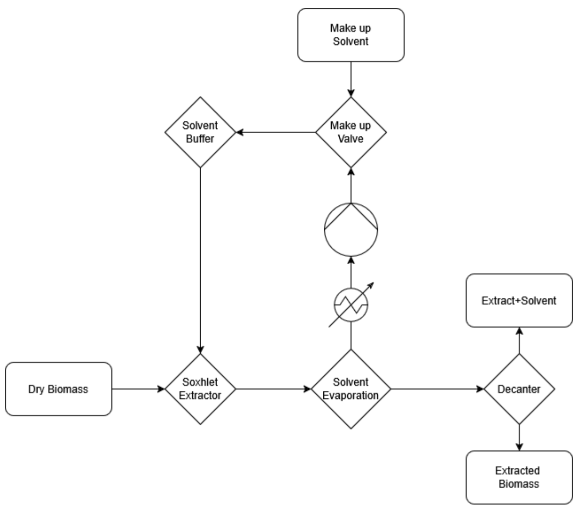

Figure 6.8: Flowsheet of a Soxhlet Extractor in SPD. The solvent unit is followed by a condenser and a pump unit. Rectangle Boxes are Products and diamond shaped boxes are processes.

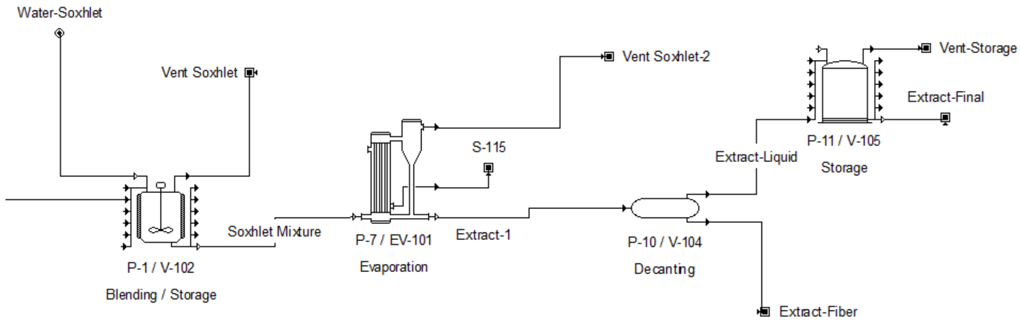

Figure 6.9: The Extractor as it is modeled in the simulation

As the experimental setup used 5g of biomass to 250ml of Solvent, as stated in section 4.1, this is replicated in process simulation.

Parameter	Value	Unit
Whole Biomass extraction		
Biomass input	1.1	ton / batch
Water input	5.923	ton / batch
Residence time	5	hours
Evaporation Rate	99	%
Decanting of Liquids	99	%
Fibre output	0.106	ton / batch
Extract output	0.102	ton / batch

Table 6.11: The values for the soxhlet extraction for the whole biomass

Parameter	Value	Unit
Fractionated Biomass extraction		
Biomass input	0.716	ton / batch
Water input	4.249	ton / batch
Residence time	5	hours
Evaporation Rate	99	%
Decanting of Liquids	99	%
Fibre output	0.097	ton / batch
Extract output	0.05	ton / batch

Table 6.12: The values for the soxhlet extraction for the fractionated biomass

From these results it can be seen that, by using a whole biomass refinery, 10 % of the total biomass in its weight is converted into an extract, while using a fractionated biomass refinery only 5% of its total weight is converted into an extract. In both cases around another 10 % of its weight will be dry fiber residue, that can also be sold.

6.3 Processing Costs

Another part that has to be modeled is the processing costs of the simulation, as based on that a profitability analysis can be conducted to see if the simulation is feasible. For that two main factors have to be analyzed for the cost:

- The building and equipment cost (CAPEX)
- Material and manpower cost (OPEX)

SPD uses mostly standard values for the CAPEX and OPEX, but they can be adjusted if necessary. The important part that has to be defined manually are the materials cost and their selling prices, as SPD sets them to zero at the start. To find proper values for these two parameters different reports have been analyzed, that worked with a similar biomass and are selling a similar end product. AQUACOMBINE, which was a EU initiated Halophyte biomass biorefinery, has been used as a baseline for the cost and possible revenue of this process simulation[3], [71].

Material	Value (Euro per kilo)	REF
Buying Prices		
Crithmum Maritimum Biomass	6.7	[3]
Water	0.002	[70]
Cultivation Media	142.9	[72]
CIP Caustic	0.04	[69]
Selling Prices		
Protein Juice	1.94	[3]
Fiber pulp	0.62	[3]
Extract	1504.37	[3]

Table 6.13: The Buying and selling prices of the materials and Chemicals.

7 Techno-economic analysis

To make an economic evaluation of the two refinery types presented in this report, the Economic Evaluation Report (EER) in SPD is used. This tool is able to show detailed information about the revenue streams and the cost of the equipment and materials used inside of the process simulation. Thereby the CAPEX and OPEX of each refinery will be shown.

For both cases the first economic analysis, the input mass flow rate of the biomass *Crithmum Maritimum* was chosen to be 1 ton per batch, with a total of 106 batches a year. After that a sensitivity analysis will be done to see when the the refinery will be profitable.

The revenue and material cost are based on the numbers of the AQUACOMBINE project[3].

The operating hours are the standard number given by SPD for the specific component.

7.1 Case 1: Fractionated Biomass refinery

For case 1 the refinery is focused on refining the fibre fraction of the biomass, where the juice fraction is seen as a value added stream. This refinery is inspired by the AQUACOMBINE project, where they combine fish cultivation and Halophyte cultivation with a biorefinery.

First the Cashflow anlysis and the summary of the process economics. Here it can be seen that the operating cost are higher than the total revenue and therefore the process based on 1 ton biomass input is not feasible.

1. EXECUTIVE SUMMARY (2025 prices)

Total Capital Investment	13,887,000 €
Capital Investment Charged to This Project	13,887,000 €
Operating Cost	5,656,000 €/yr
Main Revenue	289,000 €/yr
Other Revenues	10,790 €/yr
Total Revenues	300,000 €/yr
Batch Size	50.09 kg MP
Cost Basis Annual Rate	5,310 kg MP/yr
Unit Production Cost	1,065.27 €/kg MP
Net Unit Production Cost	1,065.27 €/kg MP
Unit Production Revenue	56.53 €/kg MP
Gross Margin	- 1,784.41 %
Return On Investment	- 29.70 %
Payback Time	N/A
IRR (After Taxes)	N/A
NPV (at 7.0% Interest)	- 43,559,000 €

MP = Total Flow of Stream 'Extract-Final'

Figure 7.1: The Executive summary of the Fractionated Biomass ERR

In total the equipment cost is at 2.176M€. The most expensive equipment in this refinery are the fermentation tanks that are used to ferment the biomass and to prepare the *Lactobacillus* for the main fermentation, costing at 584k€ and 454k€. These fermentation tanks are also expensive as they are large. It would therefore interesting to see if the cost could be decreased for using multiple smaller tanks. For Soxhlet extraction the equipment is overestimated, as every single component had to be modeled by hand. The other main cost are the storage tank used for the Thermal pretreatment and for the Soxhlet extractor, each costing at 224k€ and 168k€.

2. EQUIPMENT SPECIFICATION AND FOB COST (2025 prices)

Main Equipment				
Quantity/ Standby/ Staggered	**Name**	**Description**	**Unit Cost (€)**	**Cost (€)**
1 / 0 / 0	FR-101	Fermentor Vessel Volume = 889.35 L	584,000	584,000
1 / 0 / 0	SFR-103	Seed Fermentor Vessel Volume = 82.77 L	454,000	454,000
1 / 0 / 0	V-102	Blending Tank Vessel Volume = 5537.70 L	224,000	224,000
1 / 0 / 0	V-101	Blending Tank Vessel Volume = 716.10 L	168,000	168,000
1 / 0 / 0	EV-101	Multi-Effect Evaporator Mean Heat Transfer Area = 0.44 m2	101,000	101,000
1 / 0 / 0	SR-101	Shredder Rated Throughput = 4.29 kg/h	67,000	67,000
1 / 0 / 0	DDR-101	Drum Dryer Drum Area = 0.93 m2	45,000	45,000
1 / 0 / 0	SP-101	Screw Press Throughput = 1000.00 kg/h	39,000	39,000
1 / 0 / 0	V-104	Decanter Tank Vessel Volume = 0.01 L	30,000	30,000
1 / 0 / 0	V-105	Flat Bottom Tank Vessel Volume = 2.33 L	29,000	29,000
		Unlisted Equipment		435,000
			TOTAL	2,176,000

Figure 7.2: The Equipment cost of the Fractionated Biomass ERR

For the capital expenditure of the biorefinery as seen in figure 7.3, which consist of land area, equipment, construction and etc. while building the biorefiney. These cost can fluctuate in reality, as SPD often uses standard choices concerning constructing a new refinery, while different choices can be chosen while planning and constructing this refinery. These choices can range from using a preexisting building, to needing to reinforce the ground due to bad terrain. These numbers can also change depending on which country the refinery will be built, due to the economies and regulations of the country.

3. FIXED CAPITAL ESTIMATE SUMMARY (2025 prices in €)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	2,176,000
2. Installation	783,000
3. Process Piping	762,000
4. Instrumentation	871,000
5. Insulation	65,000
6. Electrical	218,000
7. Buildings	979,000
8. Yard Improvement	326,000
9. Auxiliary Facilities	871,000
TPDC	7,050,000
3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	1,763,000
11. Construction	2,468,000
TPIC	4,230,000
3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	11,280,000
3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	564,000
13. Contingency	1,128,000
CFC = 12+13	1,692,000
3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	12,972,000

Figure 7.3: The Fixed Capital Cost of the Fractionated Biomass ERR

In the following figure the labor cost for this refinery can be seen. These numbers are the standard numbers from SPD, as these are industry standard. These labor cost as seen in figure 7.7 are 35.22% of the total OPEX.

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost (€/h)	Annual Amount (h)	Annual Cost (€)	%
Operator	50.47	39,468	1,991,922	100.00
TOTAL		39,468	1,991,922	100.00

Figure 7.4: The Labor Cost of of the Fractionated Biomass ERR

For the materials used in this refinery, the numbers are based on the start of the section 7. The raw materials cover 16.14% of the total OPEX, as the main component of the refinery process *Crithmum Maritimum* covers 77.8% of all the materials used. The next biggest is the Media used to inoculate and create more of *Lactobacillus* bacteria covering 21.93% of all the materials. This is based on the MRS Broth used in the laboratory, to cultivate Lactic Acid bacteria and the cost of it could decrease when used industrially, as it would go through a vendor dealing with industrial level stocks.

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Cell	0.00	0	kg	0	0.00
CIP - Caustic	0.04	30,534	kg	1,074	0.12
Crithmum Mariti	6.70	106,000	kg	710,518	77.80
Media	142.90	1,401	kg	200,242	21.93
Water	2.00	686	MT	1,373	0.15
TOTAL				913,207	100.00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

Figure 7.5: The Materials cost of the Fractionated Biomass ERR

The utilities as seen in figure 7.6, are based on industry standards and are fixed values inside of SPD. Here the highest usage is Steam consisting of total 66.58% of the total utilities. This cost can be reduced if the heat could be produced locally by burning the rest of the fibre pulp or finding a different pretreatment that can create the same results as the the thermal pretreatment currently used.

8. UTILITIES COST (2025 prices) - PROCESS SUMMARY

Utility	Unit Cost (€)	Annual Amount	Ref. Units	Annual Cost (€)	%
Std Power	0.07	21,391	kW-h	1,565	18.47
Steam	8.78	642	MT	5,639	66.58
Chilled Water	0.29	4,325	MT	1,265	14.94
TOTAL				8,469	100.00

Figure 7.6: The Utilities cost of the Fractionated Biomass ERR

The total OPEX can be seen in figure 7.7, where the yearly OPEX is at 5.656 M€. As it can be seen the highest share of the cost are facility dependent consisting of 43.2% at around 2.443 M€. As already discussed this is an average estimate from SPD, as the number can fluctuate depending on the location and country where the facility will be built. The second highest contributor to the OPEX is the labor cost at 35.22%. These numbers could change when looking at each component and see how many man hours it actually needs. The salary can also change depending on the country where the facility is located.

9. ANNUAL OPERATING COST (2025 prices) - PROCESS SUMMARY

Cost Item	€	%
Raw Materials	913,000	16.14
Labor-Dependent	1,992,000	35.22
Facility-Dependent	2,443,000	43.20
Laboratory/QC/QA	299,000	5.28
Consumables	0	0.00
Waste Treatment/Disposal	1,000	0.01
Utilities	8,000	0.15
Transportation	0	0.00
Miscellaneous	0	0.00
Advertising/Selling	0	0.00
Running Royalties	0	0.00
Failed Product Disposal	0	0.00
TOTAL	5,656,000	100.00

Figure 7.7: The Annual operating cost of the Fractionated Biomass ERR

In figure 7.8, all the total revenues form the refinery can be seen and their different selling prices. From the figure it can also be seen what the highest revenue stream is and how much it is able to produce every year, with the extract consisting of 96% of the total revenue of 289380€ a year. It can also be seen that the juice fraction yield is 14 times higher than the extract yield, while only having a revenue of 2386€ a year.

There is therefore a case that the protein juice has not enough value in its current state in warranting its own stream and the biomass should not have been fractionated. But as the stream consist only of the protein of *Crithmum Maritimum* and should be enriched to achieve a higher concentration of protein. This idea should be looked into as the the Juice fraction is the most produced product of the biorefienry.

As also already stated in the start of this section in the current state of the refinery this setup is not feasible.

10. PROFITABILITY ANALYSIS (2025 prices)

A.	Direct Fixed Capital	12,972,000 €
B.	Working Capital	266,000 €
C.	Startup Cost	649,000 €
D.	Up-Front R&D	0 €
E.	Up-Front Royalties	0 €
F.	Total Investment (A+B+C+D+E)	13,887,000 €
G.	Investment Charged to This Project	13,887,000 €
H.	**Revenue/Savings Rates**	
	Extract-Fiber (Revenue)	10,378 kg /yr
	Extract-Final (Main Revenue)	5,310 kg /yr
	Juice Fraction (Revenue)	71,470 kg /yr
I.	**Revenue/Savings Price**	
	Extract-Fiber (Revenue)	0.81 €/kg
	Extract-Final (Main Revenue)	54.50 €/kg
	Juice Fraction (Revenue)	33.39 €/1000 kg
J.	**Revenues/Savings**	
	Extract-Fiber (Revenue)	8,403 €/yr
	Extract-Final (Main Revenue)	289,380 €/yr
	Juice Fraction (Revenue)	2,386 €/yr
1	Total Revenues	300,169 €/yr
2	Total Savings	0 €/yr
K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	5,656,000 €/yr
2	Net AOC (K1-J2)	5,656,000 €/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	1,065.27 €/kg MP
	Net Unit Production Cost	1,065.27 €/kg MP
	Unit Production Revenue	56.53 €/kg MP
M.	Gross Profit (J-K)	- 5,357,000 €/yr
N.	Taxes (25%)	0 €/yr
O.	Net Profit (M-N + Depreciation)	- 4,124,000 €/yr
	Gross Margin	- 1,784.41 %
	Return On Investment	- 29.70 %
	Payback Time	N/A

MP = Total Flow of Stream 'Extract-Final'

Figure 7.8: The Profitability Analysis of the Fractionated Biomass ERR

7.2 Case 2: Whole Biomass refinery

Case 2 is based on using the entirety of the plant as a biomass for the biorefinery process, where it follows the lab experiments and conditions as close as possible.
Compared to the fractionated biomass, the whole biomass has only a small deficit of revenue compared to the operating cost and states that it can be paid back.

1. EXECUTIVE SUMMARY (2025 prices)

Total Capital Investment	13,694,000 €
Capital Investment Charged to This Project	13,694,000 €
Operating Cost	5,755,000 €/yr
Main Revenue	5,456,000 €/yr
Other Revenues	62,980 €/yr
Total Revenues	5,519,000 €/yr
Batch Size	102.50 kg MP
Cost Basis Annual Rate	10,865 kg MP/yr
Unit Production Cost	529.63 €/kg MP
Net Unit Production Cost	529.63 €/kg MP
Unit Production Revenue	507.97 €/kg MP
Gross Margin	- 4.26 %
Return On Investment	7.15 %
Payback Time	13.99 years
IRR (After Taxes)	N/A
NPV (at 7.0% Interest)	- 7,551,000 €

MP = Total Flow of Stream 'Extract-Final'

Figure 7.9: The Executive Summary of the whole biomass

For the Equipment cost, which can be seen in figure 7.10, the largest cost are the fermentation tanks where the Lactic Acid Bacteria fermentation and the main fermentation occurs, costing 454k€ and 620k€. These fermentors are that expensive due to their large sizes and therefore it should be looked into using multiple smaller fermentors to reduce the cost. All the numbers are quite similar to case 1, as mostly the same equipment is used, but in the end case 1 is more expensive as it has more equipment that needs to be purchased.

2. EQUIPMENT SPECIFICATION AND FOB COST (2025 prices)

Main Equipment				
Quantity/ Standby/ Staggered	Name	Description	Unit Cost (€)	Cost (€)
1 / 0 / 0	FR-101	Fermentor Vessel Volume = 1361.29 L	620,000	620,000
1 / 0 / 0	SFR-103	Seed Fermentor Vessel Volume = 127.41 L	454,000	454,000
1 / 0 / 0	V-102	Blending Tank Vessel Volume = 7826.45 L	235,000	235,000
1 / 0 / 0	V-101	Blending Tank Vessel Volume = 1096.54 L	178,000	178,000
1 / 0 / 0	EV-101	Multi-Effect Evaporator Mean Heat Transfer Area = 0.62 m2	101,000	101,000
1 / 0 / 0	SR-101	Shredder Rated Throughput = 41.67 kg/h	67,000	67,000
1 / 0 / 0	V-104	Decanter Tank Vessel Volume = 0.01 L	30,000	30,000
1 / 0 / 0	V-105	Flat Bottom Tank Vessel Volume = 4.51 L	29,000	29,000
		Unlisted Equipment		429,000
			TOTAL	2,143,000

Figure 7.10: The Equipment cost of the whole biomass

For the capital estimate cost as seen in figure 7.11, the numbers are almost identical as seen in figure 7.3. But that is to be expected as there was no change to the settings of SPD.

3. FIXED CAPITAL ESTIMATE SUMMARY (2025 prices in €)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	2,143,000
2. Installation	774,000
3. Process Piping	750,000
4. Instrumentation	857,000
5. Insulation	64,000
6. Electrical	214,000
7. Buildings	964,000
8. Yard Improvement	321,000
9. Auxiliary Facilities	857,000
TPDC	6,944,000
3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	1,736,000
11. Construction	2,430,000
TPIC	4,167,000
3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	11,111,000
3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	556,000
13. Contingency	1,111,000
CFC = 12+13	1,667,000
3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	12,777,000

Figure 7.11: The Fixed Capital cost of the whole biomass

For the Labor cost, which can be seen in figure 7.12, the labor cost is slightly higher compared to figure 7.4. That is to the larger size of the fermentors in case 2 which SPD determined needed to have more hours. Else this number could be reduced, when analyzing if each equipment actually needs the amount of hours that SPD assigns it, and depending on the country the Salary will also fluctuate.

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost (€/h)	Annual Amount (h)	Annual Cost (€)	%
Operator	50.47	39,837	2,010,544	100.00
TOTAL		39,837	2,010,544	100.00

Figure 7.12: The Labor cost of the whole biomass

For the material cost, as seen in figure 7.13, the total cost of the materials is higher compared to figure 7.5, due to the higher demand of Media. In total the Crithum Maritimum consist of 69.55 % of the total material demand and the Media consist of 30.17 % of the demand. This higher Media demand is also there as there is more biomass that needs to be fermented. In case 2 the entire biomass is fermented, while in case 1 only 29.54% of the total biomass will be processed and extracted on.

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Cell	0.00	0	kg	0	0.00
CIP - Caustic	0.04	35,256	kg	1,240	0.12
Crithmum Mariti	6.70	106,000	kg	710,550	69.55
Media	142.90	2,157	kg	308,240	30.17
Water	2.00	826	MT	1,653	0.16
TOTAL				1,021,683	100.00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

Figure 7.13: The Materials cost of the whole biomass

For the utilities, which can be seen in figure 7.14, there is a higher demand for chilled water compared to the numbers seen in figure 7.6. That is due to the larger production of extract, due to a higher availabitlity of biomass. Therefore more biomass will be pre-treated and more of that biomass needs to be cooled down, so that it can be fermented at the right temperatures.

8. UTILITIES COST (2025 prices) - PROCESS SUMMARY

Utility	Unit Cost (€)	Annual Amount	Ref. Units	Annual Cost (€)	%
Std Power	0.07	38,266	kW-h	2,799	21.91
Steam	8.78	870	MT	7,638	59.79
Chilled Water	0.29	7,994	MT	2,339	18.31
TOTAL				12,775	100.00

Figure 7.14: The Utilities cost of the whole biomass

For the operating cost, which can be seen in 7.15, the is a higher operating cost compared case 1, which can be seen in figure 7.7. As already mentioned there is a higher labor and material demand due to the processing of more biomass.
The highest cost beside the Facility dependent are the Labor cost at 34.94%, which as already discussed have potential to be reduced when analysing the biorefinery closer.

9. ANNUAL OPERATING COST (2025 prices) - PROCESS SUMMARY

Cost Item	€	%
Raw Materials	1,022,000	17.75
Labor-Dependent	2,011,000	34.94
Facility-Dependent	2,407,000	41.83
Laboratory/QC/QA	302,000	5.24
Consumables	0	0.00
Waste Treatment/Disposal	1,000	0.01
Utilities	13,000	0.22
Transportation	0	0.00
Miscellaneous	0	0.00
Advertising/Selling	0	0.00
Running Royalties	0	0.00
Failed Product Disposal	0	0.00
TOTAL	5,755,000	100.00

Figure 7.15: The annual operating cost of the whole biomass

For the profitability analysis, which can be seen in figure 7.16, there is much larger revenue compared to case 1. But still there is a larger operating cost compared to the revenue, which result in a small deficit. Therefore this facility starts only to be profitable when it starts to depreciate.
This Biorefinery with an in put of 1 ton of biomass, can become profitable, with small adjustments to the operating cost. But as seen by these numbers this refinery has potential to become feasible, by either adjusting the operating cost or scaling up and thereby increasing the revenue of this facility.

10. PROFITABILITY ANALYSIS (2025 prices)

A.	Direct Fixed Capital	12,777,000 €
B.	Working Capital	278,000 €
C.	Startup Cost	639,000 €
D.	Up-Front R&D	0 €
E.	Up-Front Royalties	0 €
F.	Total Investment (A+B+C+D+E)	13,694,000 €
G.	Investment Charged to This Project	13,694,000 €
H.	**Revenue/Savings Rates**	
	Extract-Fiber (Revenue)	11,320 kg /yr
	Extract-Final (Main Revenue)	10,865 kg /yr
I.	**Revenue/Savings Price**	
	Extract-Fiber (Revenue)	5.56 €/kg
	Extract-Final (Main Revenue)	502.18 €/kg
J.	**Revenues/Savings**	
	Extract-Fiber (Revenue)	62,980 €/yr
	Extract-Final (Main Revenue)	5,456,407 €/yr
1	Total Revenues	5,519,387 €/yr
2	Total Savings	0 €/yr
K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	5,755,000 €/yr
2	Net AOC (K1-J2)	5,755,000 €/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	529.63 €/kg MP
	Net Unit Production Cost	529.63 €/kg MP
	Unit Production Revenue	507.97 €/kg MP
M.	Gross Profit (J-K)	- 236,000 €/yr
N.	Taxes (25%)	0 €/yr
O.	Net Profit (M-N + Depreciation)	979,000 €/yr
	Gross Margin	- 4.26 %
	Return On Investment	7.15 %
	Payback Time	13.99 years

MP = Total Flow of Stream 'Extract-Final'

Figure 7.16: The profitability analysis of the whole biomass

7.3 Sensitivity analysis

For this section a sensitivity analysis will be done, to find when the different cases can become profitable. For that the input of biomass will be adjusted and thereafter EER generated to find the CAPEX, OPEX, Revenue, ROI and the payback time of the different versions of the cases.
All the different EER reports can be found in the Appendix A.1.
These are the following economical values of the different versions of the cases:

Biomass input (t per batch)	CAPEX (M€ / year)	OPEX (M€ / year)	Revenue (M€ / year)	ROI (%)	Paybacktime (years)
Case 1: Fractionated Biomass					
0.1	12.048	4.34	0.029	-26.78	N/A
1	13.887	5.656	0.3	-29.7	N/A
2	15.017	6.955	0.602	-33.47	N/A
Case 2: Whole Biomass					
0.1	11.459	4.195	0.551	-22.89	N/A
1	13.694	5.755	5.52	7.15	14
2	14.852	7.249	11.04	27.95	3.58

Table 7.1: The total Revenue and costs depending on the biomass input

As it can be seen from the table 7.1, Case 1 has no possibility to become feasible, as it is not able to produce enough of its main product: A polyphenol rich extract. In comparison case 2 becomes quickly feasible, as long as there is enough biomass input. As already stated in the former section the OPEX and CAPEX could be reduced by using a different setup, smaller and multiple fermentation/ storage tanks, and looking deeper into labor cost, to either reduce the necessary hours or the salary.

8 Discussion

As soil salinization is increasing throughout the world there is a need to combat this, as the increasing salinization reduces arable land and in the worst case can force people from their homes. For this halophytes have a potential to combat this trend, as, as they are salt marsh plants, require salinized soil to grow and and remove salt from the ground. The only problem with that is that the farmers have to be able to live of these plants and at this current stage it is not feasible, as there is not enough of a demand of halophytes as a food crop and as a cash crop.
This can however change as more and more research is conducted over halophytes, as they are rediscovered as herbal medicine and rich in polyphenols. As these halophytes are probiotic and are rich in antioxidants, because of these polyphenols, have a potential for the pharmaceutical industry and nutraceutical sector.
Crithmum Maritimum, a halophyte biomass, was chosen as it is rich in polyphenols and antioxidants, which have been proven in several reports. Because of these reports it was decided to test if the concentration of polyphenols could be increased through the use of solid state fermentation with Lactic Acid bacteria. This was done after a literature study looked into the solid state fermentation, and the benefits of Lactic Acid fermentation which occur naturally.
For the solidstate fermentation two *Lactobacillus* types were used: *Lactobacillus Plantarum* and *Lactobacillus Salivarus*. Both of them have been shown to have probiotic effects either in fermentation or direct application, like skincare.
For the experiments three main points were examined, the increase in drymatter yield, the polyphnenol amount inside of the extracts and the antioxidant activity of these extracts. For that four ways were established, the baseline were the *Crithmum Maritimum* was just extracted, hereafter a solidstate fermentation with the two *lactobacillus* types at different moisture contents. For the third the biomass was heat pretreated in an autoclave to help breaking the cell walls down inside of the biomass and again solid state fermented with *Lactobacillus*. For the fourth test it was pretreated and also an enzyme added to help breaking the cell walls down.
These experiments showed that *Salivarus* is a hardier strain than *Plantarum*, as in the standard fermentation all *Plantarum* test failed. Hereafter the tests showed that in most cases the extraction yield increased, but in all cases the polyphenol content slightly decreased or had a higher decrease. The antioxidant readings were also different, case by case, where it seems that the *Plantarum* strain has a better antioxidant effect on the biomass. As many of these test are done by pipetting the extract samples, there is a risk of human

failure, therefore they were repeated multiple times to achieve proper results. After all these test the treatment method and *Lactobacillus* strain that had the best effect on the biomass was determined to be Thermal pretreatment at 85% moisture content with a solid state fermentation with *Lactobacillus Plantarum*.
After these experiments were done, a techno-economic analysis was conducted to see if a biorefinery based on the lab results and experiments were feasible. For that two cases were done one based on former reports and a EU project, that worked with fractionated Halophyte Biomass and the other based on the Lab experiment and the material available. These two cases are almost identical with a Soxleth Extractor, a *Lactobacillus* incubator and seed fermentor, and the main fermentor tank, which ferments the biomass. The only difference is that the fractionated case has a screwpress and a dryer to fractionate the biomass and to dry the pulp thereafter, as it is done in the AQUACOMBINE project and to use the values from the former reports.
The first big problem for the fractionated biomass is that around 60-70% of the entire biocomponents inside of the biomass are getting removed inside of the juice fraction and only around 30-40% of the pulp fraction is left for the rest of the refinement. This lowers the demand for *Lactobacillus* as only 10% of the biomass weight will be added, but at the same time lowers the amount of biomass that can be extracted on, thereby reducing the amount of the potential extract. This is slightly mitigated as the juice fraction is seen as a value added stream, which can be used for protein enriched juice, but in this case, from the prices given in the AQUACOMBINE project, not enough to create enough revenue to offset the lost potential amount of extract.
To improve this a protein enrichment section could be added to Case 1 to increase the concentration of protein in the Juicefraction and therefore make it a more viable value added stream, which can generate enough revenue to offset the lower production of polyphenol rich extract. The whole biomass in comparison, can generate a better revenue as it can use the entirety of the biomass and therefore extract more of the extract. In comparison around 10% of the biomass will be converted into the main revenue source, in the case of a whole biomass refienry, compared where only 5% of the biomass will be converted into the main revenue source, for the fractionated biomass. In this case the question is how much to upscale it for whole biomass refinery, to make it as profitable as possible.

9 Conclusion

Based on the literature studies and the experimental results, the fermentation of *Crithmum Maritimum* with *Lactobacillus*, is able to enhance the biocompounds of the biomass. Through the use of Lactic Acid fermentation the yield and the antioxidant activity were enhanced while the polyphenol content stayed around the same. The yield was increased by 8%, the polyphenol count had a drop by 0.9 mg/g and the IC50 was reduced by 0.8 mg/ml. Based on these results, biomass already rich in polyphenols, like Halophytes, can be enhanced while normal biomass, like wheat, could be improved.
Based on the process simulation, the biggest limiting factor in the fermentation process is the supply of Lactic Acid bacteria. But that can be overcome by producing the necessary Lactic Acid bacteria on site and cultivating it. Else the biggest feasibility occurred when fermenting the entire biomass instead of fractionating it first. Here the necessary revenue to make it feasible is lost in the Juice fraction, as it constitutes of almost 70% of the biomass.
The processing of whole *Crithmum Maritimum* through the use of Lactic Acid fermentation is able to pay itself back in 14 years with an ROI of 7%, while only having biomass input of 1 ton per batch. This can improved by up scaling the input already by 1 ton, where the payback period is reduced to 3.5 years. Compared to the fractionated biomass refinery even when the revenue doubled from 0.3M€ to 0.6M€ the CAPEX and OPEX would increase even more, so that the ROI fell even more from -26.78% to -33.47%.

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A Appendix

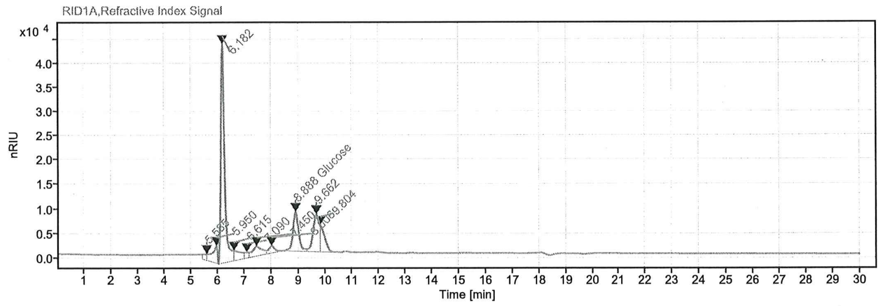
A.1 Appendix A HPLC Reports

A.1.1 Free Sugar Reports

Anton

Single Injection Report Agilent

Sample name:	C.MAR BL1		
Data file:	2024-08-29 sugars jte.amx03-09-240calibration of free sugars.pmxC.MAR BL120241016 181222-r001.dx	**Operator:**	SYSTEM
Instrument:	HPLC_01	**Injection date:**	2024-10-16 23:15:21+02:00
Inj. volume:	20.000 µL	**Location:**	P1-E1
Acq. method:	2024-08-29 sugars jte.amx	**Type:**	Sample
Processing method:	03-09-240calibration of free sugars.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration [g/L]	Group
Glucose	8.89	293919.812	118829.141	0.404	0.404	
			Sum	**0.404**		

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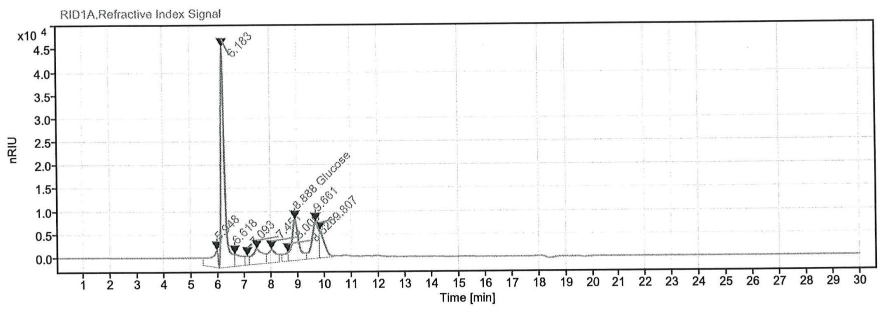
Figure A.1: Free Sugar Reading of the Baseline 1

Single Injection Report Agilent

Sample name:	C.MAR BL2		
Data file:	2024-08-29 sugars jte.amx03-09-240calibration of free sugars.pmxC.MAR BL220241017 001734.dx	**Operator:**	SYSTEM
Instrument:	HPLC_01	**Injection date:**	2024-10-17 00:19:30+02:00
Inj. volume:	20.000 µL	**Location:**	P1-E2
Acq. method:	2024-08-29 sugars jte.amx	**Type:**	Sample
Processing method:	03-09-240calibration of free sugars.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration [g/L]	Group
Glucose	8.89	296034.665	157379.183	0.532	0.532	
			Sum	**0.532**		

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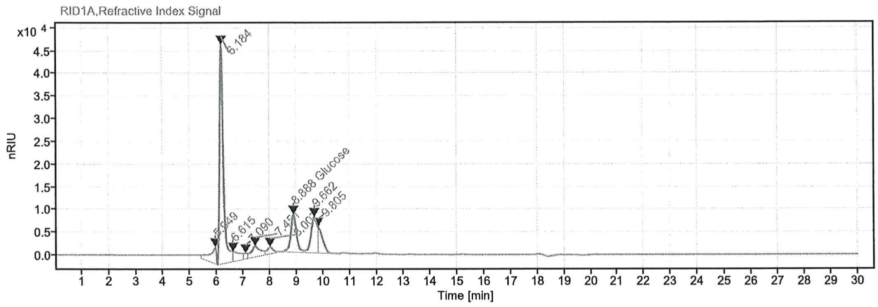
Figure A.2: Free Sugar Reading of the Baseline 2

Single Injection Report Agilent

Sample name:	C.MAR BL3		
Data file:	2024-08-29 sugars jte.amx03-09-240calibration of free sugars.pmxC.MAR BL320241017 004938.dx	**Operator:**	SYSTEM
Instrument:	HPLC_01	**Injection date:**	2024-10-17 00:51:35+02:00
Inj. volume:	20.000 µL	**Location:**	P1-E3
Acq. method:	2024-08-29 sugars jte.amx	**Type:**	Sample
Processing method:	03-09-240calibration of free sugars.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	**RT [min]**	**RF**	**Area**	**Amount []**	**Concentration [g/L]**	**Group**
Glucose	8.89	294021.410	120253.907	0.409	0.409	
			Sum	**0.409**		

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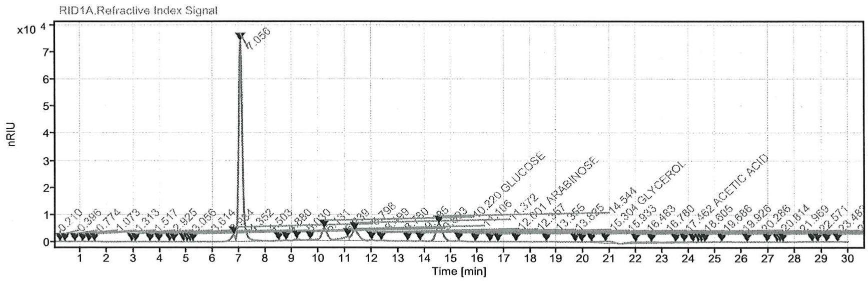
Figure A.3: Free Sugar Reading of the Baseline 3

Anton

Single Injection Report Agilent

Sample name:	C.MAR.L.SAL 1		
Data file:	C.MAR.L.SAL 1Sugars-AM-18102024.amx20241104 142919FREESUGARS-PM-04112024.pmx.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-04 14:32:06+01:00
Inj. volume:	20.000	**Location:**	P1-A1
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-04112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration [g/L]	Group
GLUCOSE	10.22	352906.429	73836.914	0.209	0.209	
ARABINOSE	12.00	-1045973.855	3073.736	-0.003	-0.003	
GLYCEROL	15.30	246255.439	4977.515	0.020	0.020	
ACETIC ACID	17.46	167577.183	1180.849	0.007	0.007	
ETOH	25.23	-16787.558	7430.136	-0.443	-0.443	
			Sum	**-0.209**		

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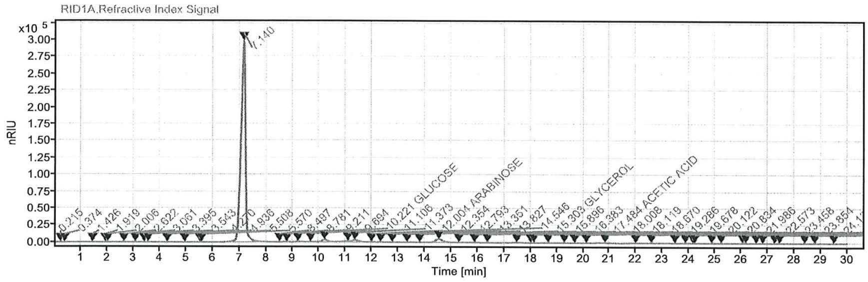
Figure A.4: Free Sugar Reading of *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 75% Moisture content sample 1.

Single Injection Report Agilent

Sample name:	C.MAR.L.SAL 2		
Data file:	C.MAR.L.SAL 2Sugars-AM-18102024.amx20241104 150308FREESUGARS-PM-04112024.pmx.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-04 15:05:55+01:00
Inj. volume:	20.000	**Location:**	P1-A2
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-04112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration [g/L]	Group
GLUCOSE	10.22	367744.483	51151.715	0.139	0.139	
ARABINOSE	12.00	-2875489.868	3657.571	-0.001	-0.001	
GLYCEROL	15.30	238142.977	4117.244	0.017	0.017	
ACETIC ACID	17.48	167388.988	958.710	0.006	0.006	
ETOH	25.23	-992.616	498.795	-0.503	-0.503	
			Sum	**-0.342**		

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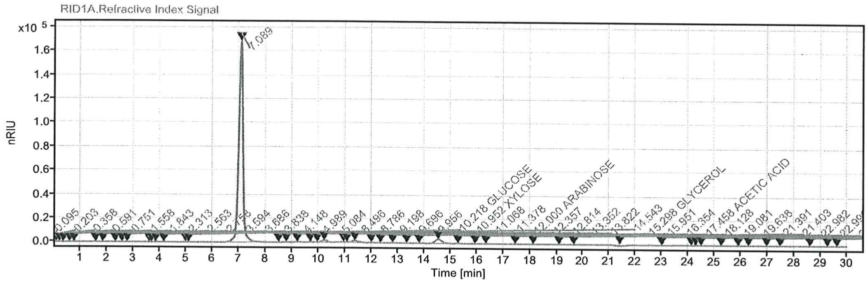
Figure A.5: Free Sugar Reading of *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 75% Moisture content sample 2.

Single Injection Report Agilent

Sample name:	C.MAR.L.SAL 3		
Data file:	C.MAR.L.SAL 3Sugars-AM-18102024.amx20241104 153656FREESUGARS-PM-04112024.pmx.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-04 15:39:42+01:00
Inj. volume:	20.000	**Location:**	P1-A3
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-04112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	Manual Integration		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration [g/L]	Group
GLUCOSE	10.22	435477.773	23941.288	0.055	0.055	
XYLOSE	10.95	2054339.846	5725.175	0.003	0.003	
ARABINOSE	12.00	-419381.606	2235.701	-0.005	-0.005	
GLYCEROL	15.30	202364.312	2135.978	0.011	0.011	
ACETIC ACID	17.46	167759.519	1521.709	0.009	0.009	
ETOH	25.25	-941.440	473.286	-0.503	-0.503	
			Sum	**-0.431**		

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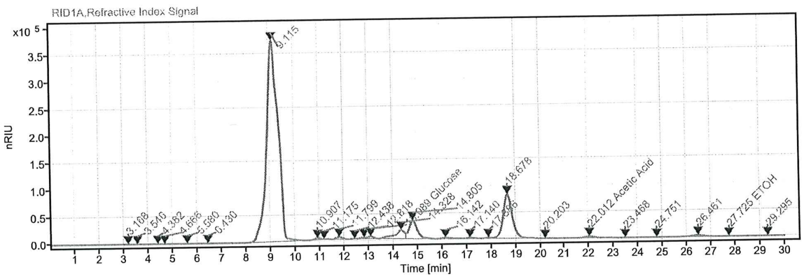
Figure A.6: Free Sugar Reading of *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 75% Moisture content sample 3.

Single Injection Report Agilent

Sample name:	CMLS 1.1		
Data file:	CMLS 1.1Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241121 165801.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-21 17:00:45+01:00
Inj. volume:	20.000	**Location:**	P2-F4
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	**RT [min]**	**RF**	**Area**	**Amount** []	**Concentration** []	**Group**
Glucose	13.09	405684.427	69746.724	0.172	0.172	
Acetic Acid	22.01	224538.425	86490.934	0.385	0.385	
ETOH	27.72	-437.773	11217.380	-25.624	-25.624	
			Sum	**-25.067**		

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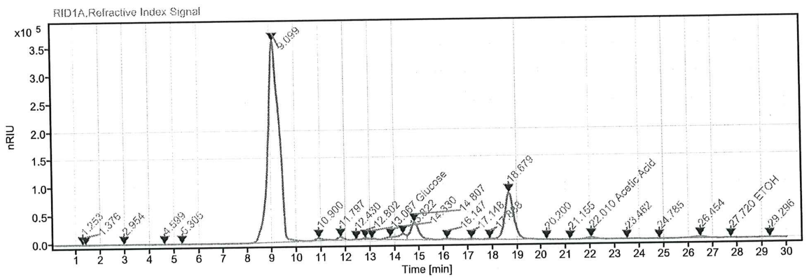
Figure A.7: Free Sugar Reading of *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content sample 1.

Single Injection Report — Agilent

Sample name:	CMLS 1.2		
Data file:	CMLS 1.2Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241121 173147.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-21 17:34:31+01:00
Inj. volume:	20.000	**Location:**	P2-F5
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Glucose	13.07	348609.128	24496.733	0.070	0.070	
Acetic Acid	22.01	225051.776	98271.398	0.437	0.437	
ETOH	27.72	-392.723	10273.856	-26.161	-26.161	
			Sum	**-25.654**		

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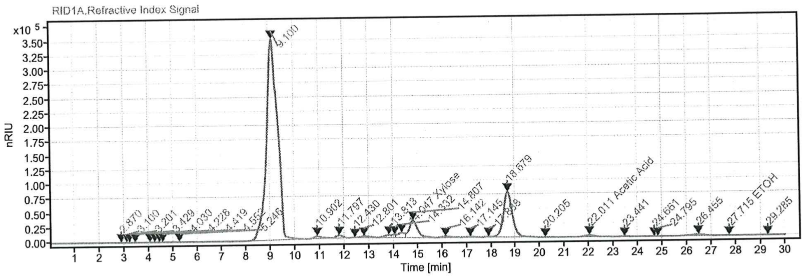
Figure A.8: Free Sugar Reading of *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content sample 2.

Single Injection Report Agilent

Sample name:	CMLS 1.3		
Data file:	CMLS 1.3Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241121 180534.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-21 18:08:17+01:00
Inj. volume:	20.000	**Location:**	P2-F6
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	**RT [min]**	**RF**	**Area**	**Amount** []	**Concentration** []	**Group**
Xylose	14.05	394784.463	55490.990	0.141	0.141	
Acetic Acid	22.01	224664.690	89123.281	0.397	0.397	
ETOH	27.72	-394.030	10301.791	-26.145	-26.145	
			Sum	**-25.607**		

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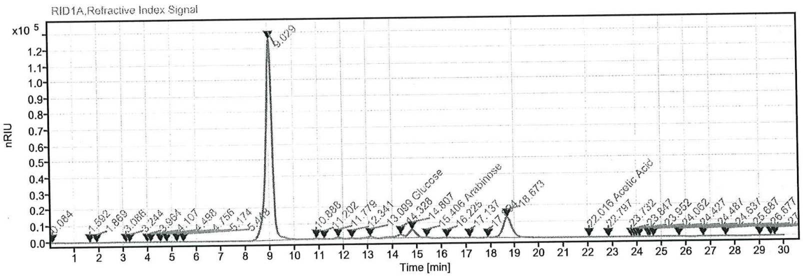
Figure A.9: Free Sugar Reading of *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content sample 3.

Single Injection Report Agilent

Sample name:	PRE LSCM 1.1		
Data file:	PRE LSCM 1.1Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241121 223548.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-21 22:38:32+01:00
Inj. volume:	20.000	**Location:**	P2-E7
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	**RT [min]**	**RF**	**Area**	**Amount** []	**Concentration** []	**Group**
Glucose	13.10	340992.197	22209.028	0.065	0.065	
Arabinose	15.41	114185.893	3266.251	0.029	0.029	
Acetic Acid	22.02	149437.961	3155.335	0.021	0.021	
ETOH	27.60	-412.019	10682.786	-25.928	-25.928	
			Sum	**-25.813**		

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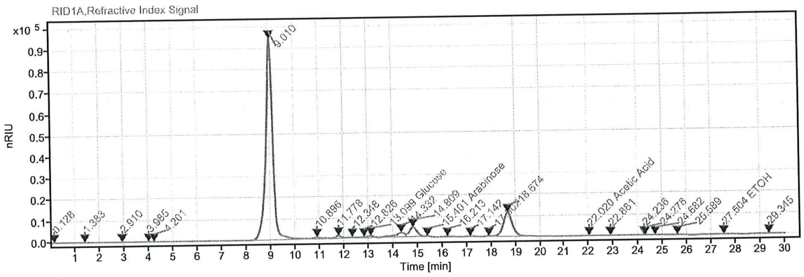
Figure A.10: Free Sugar Reading of Thermal pretreated *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content sample 1.

Single Injection Report Agilent

Sample name:	PRE LSCM 1.2		
Data file:	PRE LSCM 1.2Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241121 234320.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-21 23:46:04+01:00
Inj. volume:	20.000	**Location:**	P2-E8
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	**RT [min]**	**RF**	**Area**	**Amount []**	**Concentration []**	**Group**
Glucose	13.10	304442.400	14677.507	0.048	0.048	
Arabinose	15.40	87186.291	2323.868	0.027	0.027	
Acetic Acid	22.02	132455.508	2304.257	0.017	0.017	
ETOH	27.50	-1056.650	21121.207	-19.989	-19.989	
			Sum	**-19.897**		

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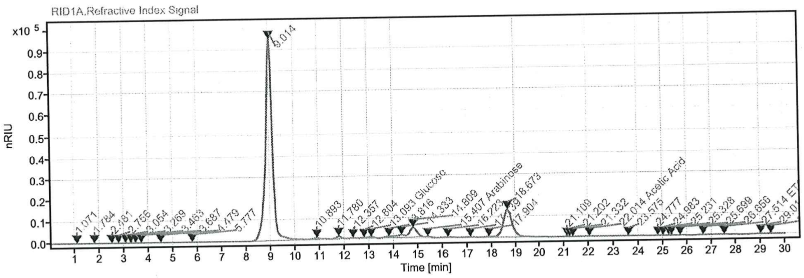
Figure A.11: Free Sugar Reading of Thermal pretreated *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content sample 2.

Single Injection Report Agilent

Sample name:	PRE LSCM 1.3		
Data file:	PRE LSCM 1.3Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241122 001706.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-22 00:19:51+01:00
Inj. volume:	20.000	**Location:**	P2-E9
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	**RT [min]**	**RF**	**Area**	**Amount []**	**Concentration []**	**Group**
Glucose	13.09	253999.585	9013.912	0.035	0.035	
Arabinose	15.41	105347.860	2942.941	0.028	0.028	
Acetic Acid	22.01	158166.334	3751.771	0.024	0.024	
ETOH	27.51	-173.017	5041.305	-29.138	-29.138	
			Sum	**-29.051**		

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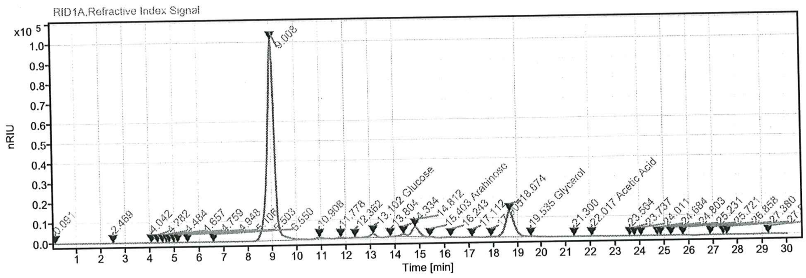
Figure A.12: Free Sugar Reading of Thermal pretreated *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content sample 3.

Single Injection Report Agilent

Sample name:	PRE LPCM 2.1		
Data file:	PRE LPCM 2.1Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241122 005053.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-22 00:53:37+01:00
Inj. volume:	20.000	**Location:**	P2-E10
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	**RT [min]**	**RF**	**Area**	**Amount []**	**Concentration []**	**Group**
Glucose	13.10	380051.917	39607.718	0.104	0.104	
Arabinose	15.40	88721.937	2374.007	0.027	0.027	
Glycerol	19.54	251373.984	6059.478	0.024	0.024	
Acetic Acid	22.02	156508.501	3627.421	0.023	0.023	
ETOH	27.54	-239.702	6751.161	-28.165	-28.165	
			Sum	**-27.987**		

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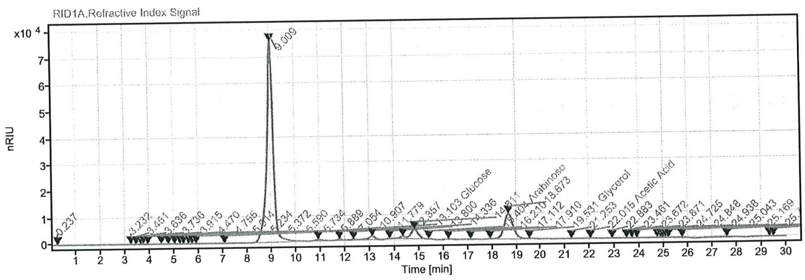
Figure A.13: Free Sugar Reading of Thermal pretreated *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture content sample 1.

Single Injection Report Agilent

Sample name:	PRE LPCM 2.2		
Data file:	PRE LPCM 2.2Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241122 012441.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-22 01:27:26+01:00
Inj. volume:	20.000	**Location:**	P2-E11
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Glucose	13.10	356675.114	27348.785	0.077	0.077	
Arabinose	15.40	67482.851	1713.423	0.025	0.025	
Glycerol	19.53	224799.195	4593.584	0.020	0.020	
Acetic Acid	22.01	150943.663	3248.691	0.022	0.022	
ETOH	27.58	-873.414	18674.435	-21.381	-21.381	
			Sum	**-21.237**		

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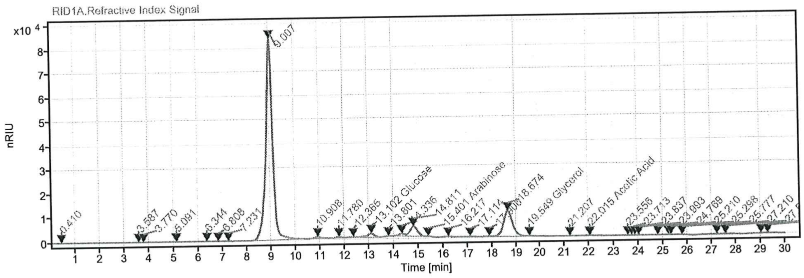
Figure A.14: Free Sugar Reading of Thermal pretreated *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture content sample 2.

Single Injection Report Agilent

Sample name:	PRE LPCM 2.3		
Data file:	PRE LPCM 2.3Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241122 015828.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-22 02:01:12+01:00
Inj. volume:	20.000	**Location:**	P2-D6
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Glucose	13.10	366306.084	31518.674	0.086	0.086	
Arabinose	15.40	71032.635	1819.090	0.026	0.026	
Glycerol	19.55	227693.728	4731.216	0.021	0.021	
Acetic Acid	22.01	152847.891	3372.050	0.022	0.022	
ETOH	27.56	-258.190	7205.197	-27.907	-27.907	
			Sum	**-27.752**		

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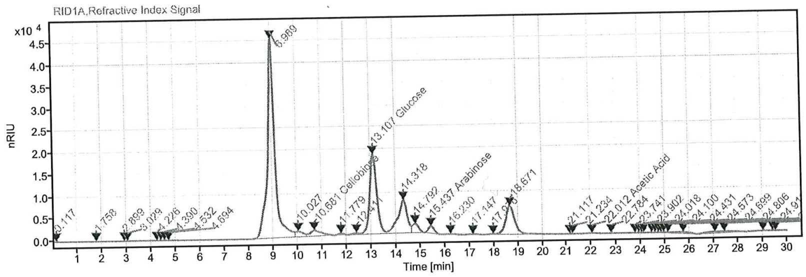
Figure A.15: Free Sugar Reading of Thermal pretreated *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture content sample 3.

Single Injection Report Agilent

Sample name:	PRE+enz LSCM 3.1		
Data file:	PRE+enz LSCM 3.1Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241122 041331.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-22 04:16:15+01:00
Inj. volume:	20.000	**Location:**	P2-D10
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Cellobiose	10.68	-97820.038	42124.497	-0.431	-0.431	
Glucose	13.11	436837.013	356928.204	0.817	0.817	
Arabinose	15.44	345589.897	26518.813	0.077	0.077	
Acetic Acid	22.01	143498.605	2819.192	0.020	0.020	
			Sum	**0.483**		

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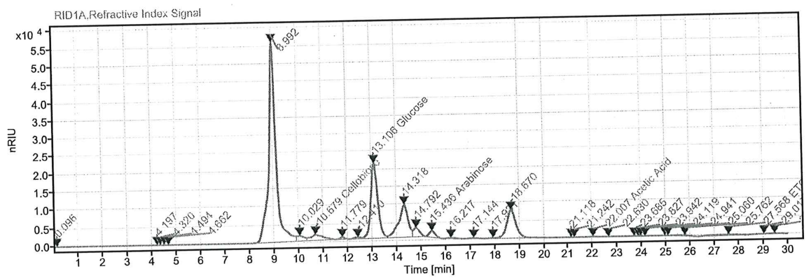
Figure A.16: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content sample 1.

Single Injection Report Agilent

Sample name:	PRE+enz LSCM 3.2		
Data file:	PRE+enz LSCM 3.2Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241122 044717.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-22 04:50:02+01:00
Inj. volume:	20.000	**Location:**	P2-D11
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Cellobiose	10.68	-136143.289	55969.662	-0.411	-0.411	
Glucose	13.11	437907.821	410789.248	0.938	0.938	
Arabinose	15.44	355153.614	29289.485	0.082	0.082	
Acetic Acid	22.01	156701.202	3641.581	0.023	0.023	
ETOH	27.57	-1026.095	20735.586	-20.208	-20.208	
			Sum	**-19.576**		

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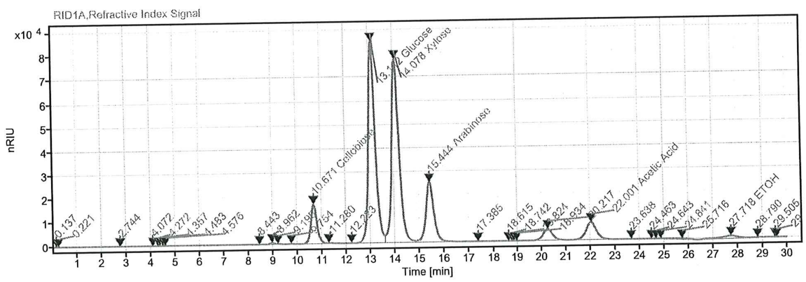
Figure A.17: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content sample 2.

Single Injection Report Agilent

Sample name:	PRE+enz LSCM 3.3		
Data file:	PRE+enz LSCM 3.3Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241122 052104.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-22 05:23:47+01:00
Inj. volume:	20.000	**Location:**	P2-D4
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Cellobiose	10.67	-3705902.600	291685.222	-0.079	-0.079	
Glucose	13.11	443366.883	1697321.223	3.828	3.828	
Xylose	14.08	465124.627	1640913.382	3.528	3.528	
Arabinose	15.44	474404.219	574577.964	1.211	1.211	
Acetic Acid	22.00	227219.753	227706.568	1.002	1.002	
ETOH	27.72	13372.151	64765.862	4.843	4.843	
			Sum	**14.334**		

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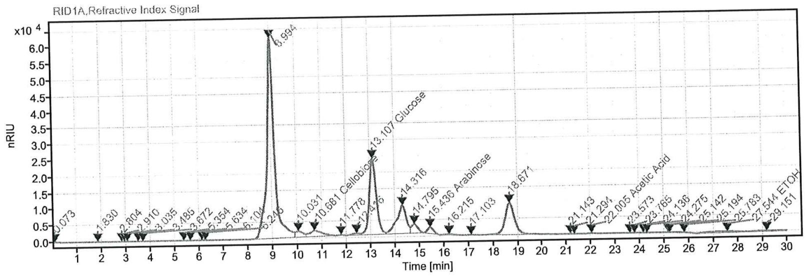
Figure A.18: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content sample 3.

Single Injection Report Agilent

Sample name:	PRE+enz LPCM 4.1		
Data file:	PRE+enz LPCM 4.1Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241122 023213.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-22 02:34:56+01:00
Inj. volume:	20.000	**Location:**	P2-D7
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Cellobiose	10.68	-160006.297	63973.896	-0.400	-0.400	
Glucose	13.11	438691.655	461557.535	1.052	1.052	
Arabinose	15.44	363167.971	31951.548	0.088	0.088	
Acetic Acid	22.01	164988.902	4331.426	0.026	0.026	
ETOH	27.54	-2422.729	32601.976	-13.457	-13.457	
			Sum	**-12.690**		

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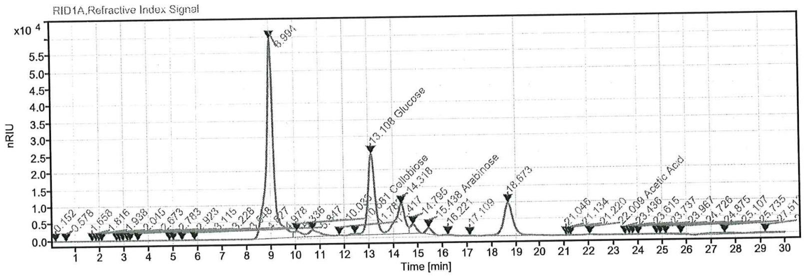
Figure A.19: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture content sample 1.

Single Injection Report Agilent

Sample name:	PRE+enz LPCM 4.2		
Data file:	PRE+enz LPCM 4.2Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241122 030558.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-22 03:08:41+01:00
Inj. volume:	20.000	**Location:**	P2-D8
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	**RT [min]**	**RF**	**Area**	**Amount []**	**Concentration []**	**Group**
Cellobiose	10.68	-158484.494	63476.591	-0.401	-0.401	
Glucose	13.11	438880.232	475669.255	1.084	1.084	
Arabinose	15.44	363520.484	32076.831	0.088	0.088	
Acetic Acid	22.01	168009.284	4629.528	0.028	0.028	
ETOH	27.51	-2399.685	32470.874	-13.531	-13.531	
			Sum	**-12.732**		

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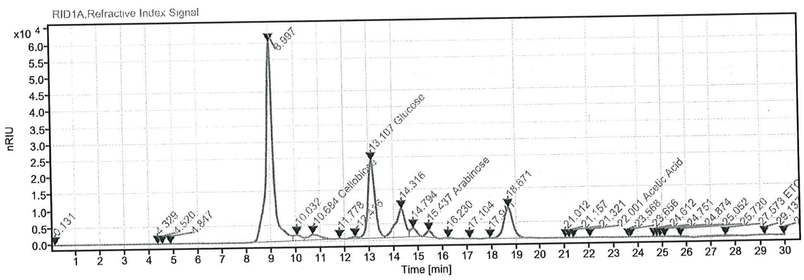
Figure A.20: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture content sample 2.

Single Injection Report Agilent

Sample name:	PRE+enz LPCM 4.3		
Data file:	PRE+enz LPCM 4.3Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241122 033944.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-22 03:42:28+01:00
Inj. volume:	20.000	**Location:**	P2-D9
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	**RT [min]**	**RF**	**Area**	**Amount []**	**Concentration []**	**Group**
Cellobiose	10.68	-145319.055	59100.342	-0.407	-0.407	
Glucose	13.11	438648.044	458410.755	1.045	1.045	
Arabinose	15.44	365754.100	32888.149	0.090	0.090	
Acetic Acid	22.00	162771.133	4129.879	0.025	0.025	
ETOH	27.57	-1085.721	21480.413	-19.784	-19.784	
			Sum	**-19.031**		

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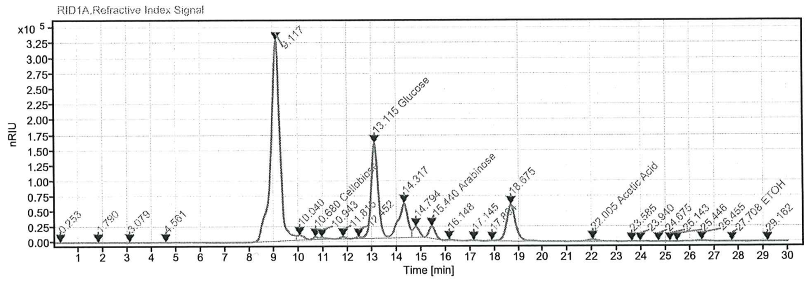
Figure A.21: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture content sample 3.

Single Injection Report Agilent

Sample name:	CMLS +ENZ 2.1		
Data file:	CMLS +ENZ 2.1Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241121 205427.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-21 20:57:12+01:00
Inj. volume:	20.000	**Location:**	P2-F10
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Cellobiose	10.68	-143923.625	58628.577	-0.407	-0.407	
Glucose	13.11	444144.556	3030308.870	6.823	6.823	
Arabinose	15.44	469038.780	351583.132	0.750	0.750	
Acetic Acid	22.00	224854.093	93380.444	0.415	0.415	
ETOH	27.71	-332.958	8959.382	-26.908	-26.908	
			Sum	**-19.328**		

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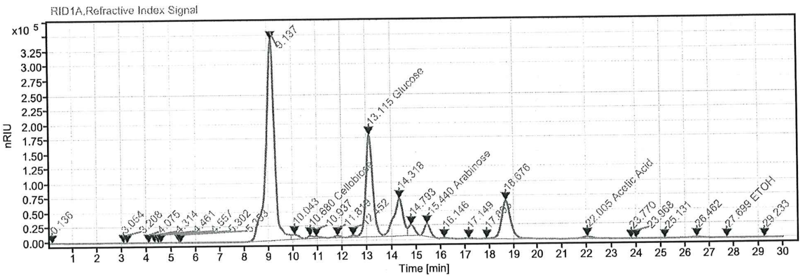
Figure A.22: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content fermentation liquid sample 1.

Single Injection Report — Agilent

Sample name:	CMLS +ENZ 2.2		
Data file:	CMLS +ENZ 2.2Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241121 212814.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-21 21:31:00+01:00
Inj. volume:	20.000	**Location:**	P2-F11
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Cellobiose	10.68	-167792.446	66491.303	-0.396	-0.396	
Glucose	13.12	444251.975	3398208.134	7.649	7.649	
Arabinose	15.44	470215.220	384602.969	0.818	0.818	
Acetic Acid	22.01	225233.022	103219.823	0.458	0.458	
ETOH	27.70	-357.210	9501.743	-26.600	-26.600	
			Sum	**-18.071**		

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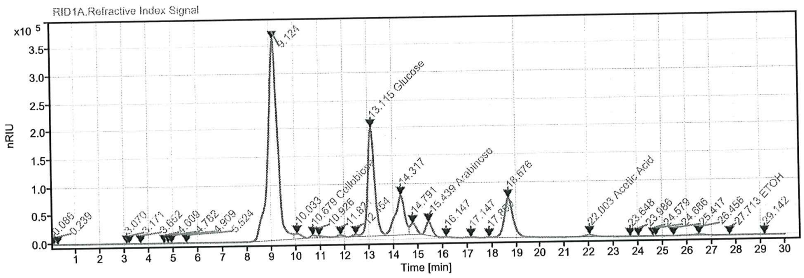
Figure A.23: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content fermentation liquid sample 2.

Single Injection Report Agilent

Sample name:	CMLS +ENZ 2.3		
Data file:	CMLS +ENZ 2.3Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241121 220202.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-21 22:04:45+01:00
Inj. volume:	20.000	**Location:**	P2-E6
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Cellobiose	10.68	-191305.185	73829.160	-0.386	-0.386	
Glucose	13.12	444345.785	3801033.591	8.554	8.554	
Arabinose	15.44	471524.309	429223.430	0.910	0.910	
Acetic Acid	22.00	225554.805	113328.787	0.502	0.502	
ETOH	27.71	-291.363	7999.280	-27.455	-27.455	
			Sum	**-17.874**		

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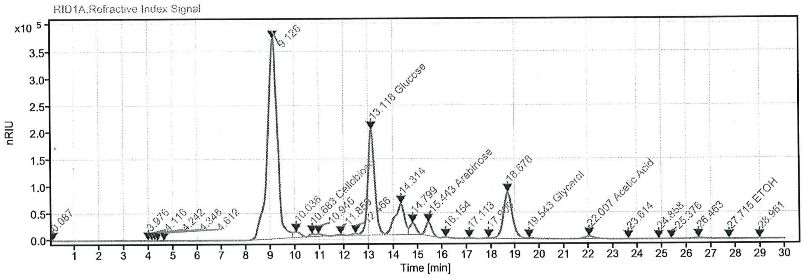
Figure A.24: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture content fermentation liquid sample 3.

Single Injection Report Agilent

Sample name:	CMLP +ENZ 2.1		
Data file:	CMLP +ENZ 2.1Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241121 191306.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-21 19:15:51+01:00
Inj. volume:	20.000	**Location:**	P2-F7
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	**RT [min]**	**RF**	**Area**	**Amount []**	**Concentration []**	**Group**
Cellobiose	10.68	-177790.327	69658.987	-0.392	-0.392	
Glucose	13.12	444344.338	3794093.469	8.539	8.539	
Arabinose	15.44	472095.510	452014.299	0.957	0.957	
Glycerol	19.54	364937.453	37879.362	0.104	0.104	
Acetic Acid	22.01	226290.766	145844.146	0.644	0.644	
ETOH	27.71	-366.356	9703.062	-26.485	-26.485	
			Sum	**-16.633**		

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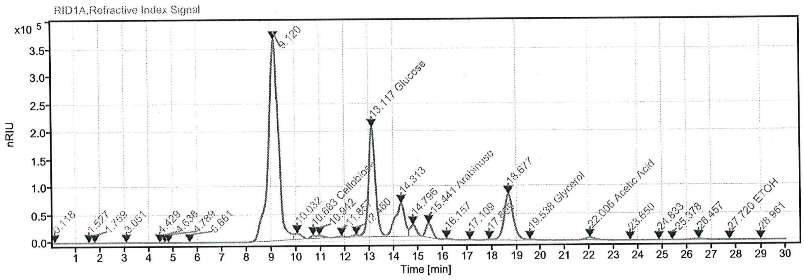
Figure A.25: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture content fermentation liquid sample 1.

Single Injection Report Agilent

Sample name:	CMLP +ENZ 2.2		
Data file:	CMLP +ENZ 2.2Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241121 194652.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-21 19:49:37+01:00
Inj. volume:	20.000	**Location:**	P2-F8
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Cellobiose	10.68	-182310.260	71067.740	-0.390	-0.390	
Glucose	13.12	444356.904	3855185.086	8.676	8.676	
Arabinose	15.44	472032.124	449369.357	0.952	0.952	
Glycerol	19.54	364822.667	37741.333	0.103	0.103	
Acetic Acid	22.00	226255.445	143869.186	0.636	0.636	
ETOH	27.72	-359.527	9552.921	-26.571	-26.571	
			Sum	**-16.593**		

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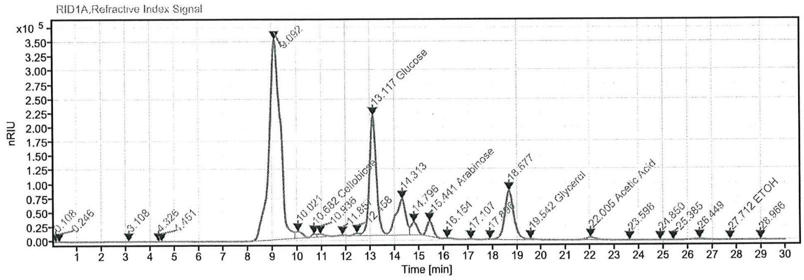
Figure A.26: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture content fermentation liquid sample 2.

Single Injection Report Agilent

Sample name:	CMLP +ENZ 2.3		
Data file:	CMLP +ENZ 2.3Sugars-AM-18102024.amxFREESUGARS-PM-21112024.pmx20241121 202040.dx	**Operator:**	SYSTEM
Instrument:	HPLC-1	**Injection date:**	2024-11-21 20:23:25+01:00
Inj. volume:	20.000	**Location:**	P2-F9
Acq. method:	Sugars-AM-18102024.amx	**Type:**	Sample
Processing method:	FREESUGARS-PM-21112024.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	None		



Signal: RID1A,Refractive Index Signal

Name	RT [min]	RF	Area	Amount []	Concentration []	Group
Cellobiose	10.68	-192054.443	74056.694	-0.386	-0.386	
Glucose	13.12	444408.007	4125264.249	9.283	9.283	
Arabinose	15.44	472490.207	469193.993	0.993	0.993	
Glycerol	19.54	363922.266	36689.692	0.101	0.101	
Acetic Acid	22.00	226165.276	139059.437	0.615	0.615	
ETOH	27.71	-351.580	9376.948	-26.671	-26.671	
			Sum	-16.065		

C:\CDSProjects\Default\Report Templates\Short_Quant_Estd.rdl [Rev. 1] Printed: 2024-11-22 12:13:41+01:00 Page 1 of 1

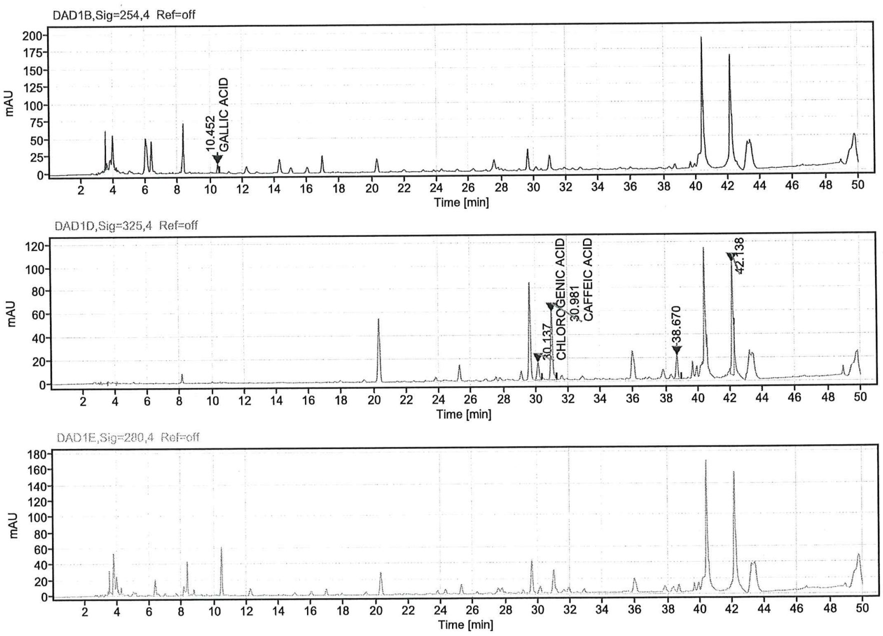
Figure A.27: Free Sugar Reading of Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture content fermentation liquid sample 3.

A.1.2 Polyphenol content Reports

Anton

Single Injection Report — Agilent Technologies

Data file:	C:\CDSProjects\Demo\Results\HCA\OTHERS\20250221-HCA-LMB-malthe.rslt\Anton BaselineHCA-LMB-AMX-2025.amxHCA-PMX-12022025.pmx20250221 161937.dx		
Sample name:	Anton Baseline		
Description:			
Sample amount:	0.000	**Sample type:**	Sample
Instrument:	HPLC_01	**Location:**	P2-a1
Injection date:	2025-02-21 16:20:34+01:00	**Injection:**	1 of 1
Acq. method:	HCA-LMB-AMX-2025.amx	**Injection volume:**	15.000
Analysis method:	*HCA-PMX-12022025.pmx	**Acq. operator:**	SYSTEM
Last changed:	2025-02-26 12:06:06+01:00		



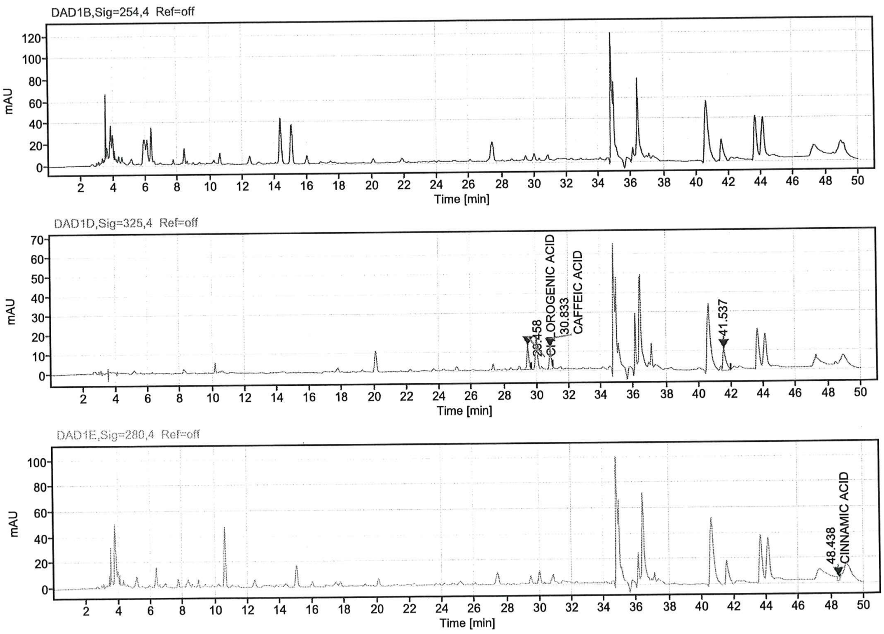
Sample Name	Name	RT	Area	Amount Unit	Concentration [mg/L]
Anton Baseline	GALLIC ACID	10.452	64.0400	2.484	2.4836
Anton Baseline	CHLOROGENIC ACID	30.137	136.4478	2.661	2.6611
Anton Baseline	CAFFEIC ACID	30.981	459.3948	5.560	5.5597

C:\CDSProjects\Demo\Report Templates\Kemi lbm.rdl [Rev. 5] Printed: 2025-02-26 14:38:31+01:00 Page 1 of 1

Figure A.28: The Polyphenol contents of the Baseline

Single Injection Report — Agilent Technologies

Data file:	C:\CDSProjects\Demo\Results\HCA\OTHERS\20250221-HCA-LMB-malthe.rslt\Anton Sali 75%HCA-LMB-AMX-2025.amxHCA-PMX-12022025.pmx20250221 171042.dx		
Sample name:	Anton Sali 75%		
Description:			
Sample amount:	0.000	**Sample type:**	Sample
Instrument:	HPLC_01	**Location:**	P2-A2
Injection date:	2025-02-21 17:11:39+01:00	**Injection:**	1 of 1
Acq. method:	HCA-LMB-AMX-2025.amx	**Injection volume:**	15.000
Analysis method:	*HCA-PMX-12022025.pmx	**Acq. operator:**	SYSTEM
Last changed:	2025-02-26 12:06:06+01:00		



Sample Name	Name	RT	Area	Amount Unit	Concentration [mg/L]
Anton Sali 75%	CHLOROGENIC ACID	29.458	94.1465	1.807	1.8068
Anton Sali 75%	CAFFEIC ACID	30.833	87.7615	1.236	1.2358
Anton Sali 75%	CINNAMIC ACID	48.438	30.1347	0.115	0.1153

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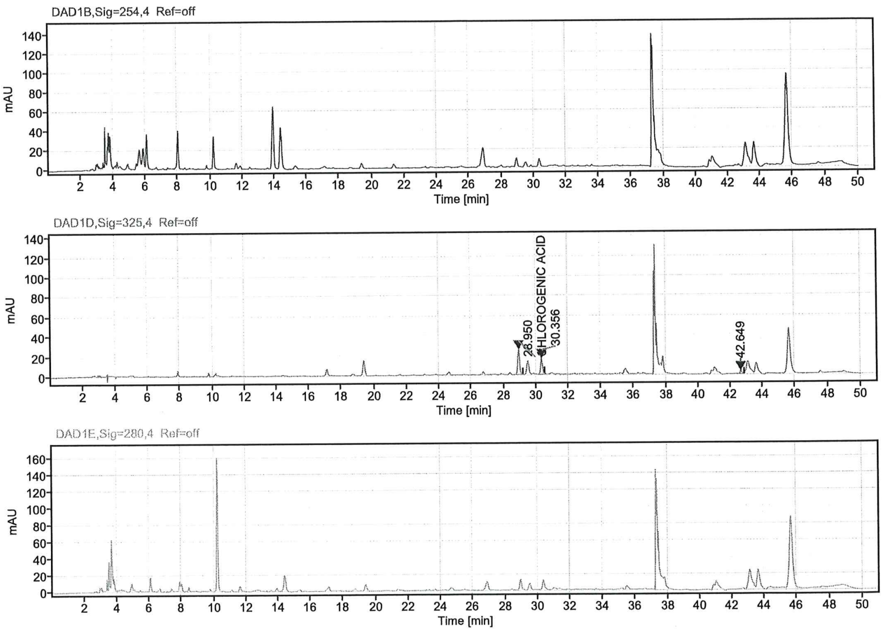
Figure A.29: The Polyphenol contents of *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 75% Moisture

Single Injection Report — Agilent Technologies

Data file:	C:\CDSProjects\Demo\Results\HCA\OTHERS\20250221-HCA-LMB-malthe.rslt\Anton Sali 85%HCA-LMB-AMX-2025.amxHCA-PMX-12022025.pmx20250221 180147.dx		
Sample name:	Anton Sali 85%		
Description:			
Sample amount:	0.000	**Sample type:**	Sample
Instrument:	HPLC_01	**Location:**	P2-A3
Injection date:	2025-02-21 18:02:44+01:00	**Injection:**	1 of 1
Acq. method:	HCA-LMB-AMX-2025.amx	**Injection volume:**	15.000
Analysis method:	*HCA-PMX-12022025.pmx	**Acq. operator:**	SYSTEM
Last changed:	2025-02-26 12:06:06+01:00		



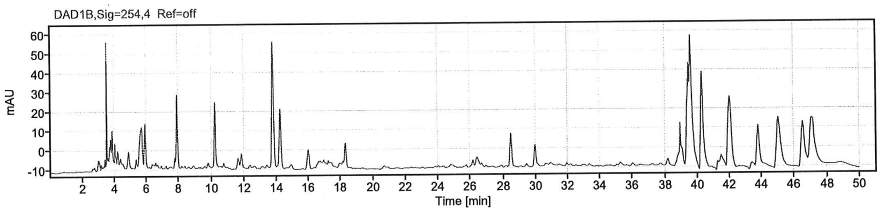
Sample Name	Name	RT	Area	Amount Unit	Concentration [mg/L]
Anton Sali 85%	CHLOROG ENIC ACID	28.950	196.7959	3.880	3.8798

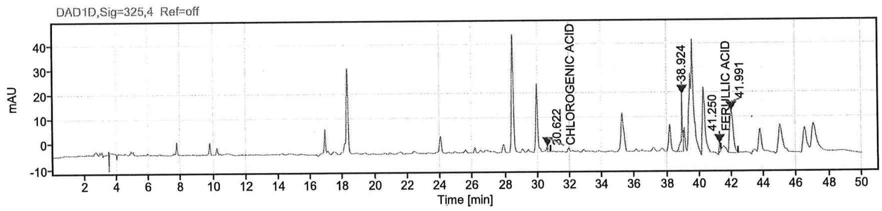
C:\CDSProjects\Demo\Report Templates\Kemi lbm.rdl [Rev. 5] Printed: 2025-02-26 14:38:38+01:00 Page 1 of 1

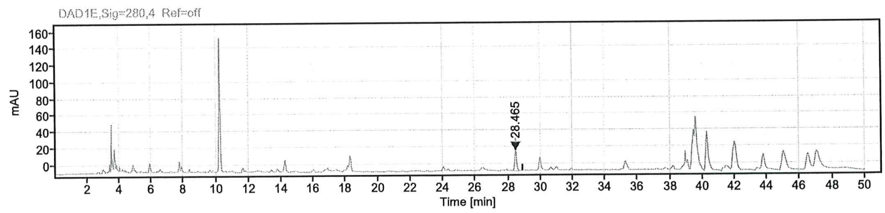
Figure A.30: The Polyphenol contents of the *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture

Single Injection Report | **Agilent Technologies**

Data file:	C:\CDSProjects\Demo\Results\HCA\OTHERS\20250221-HCA-LMB-malthe.rslt\Anton Sali PreHCA-LMB-AMX-2025.amxHCA-PMX-12022025.pmx20250221 185252.dx		
Sample name:	Anton Sali Pre		
Description:			
Sample amount:	0.000	**Sample type:**	Sample
Instrument:	HPLC_01	**Location:**	P2-A4
Injection date:	2025-02-21 18:53:48+01:00	**Injection:**	1 of 1
Acq. method:	HCA-LMB-AMX-2025.amx	**Injection volume:**	15.000
Analysis method:	*HCA-PMX-12022025.pmx	**Acq. operator:**	SYSTEM
Last changed:	2025-02-26 12:06:06+01:00		







Sample Name	Name	RT	Area	Amount Unit	Concentration [mg/L]
Anton Sali Pre	CHLOROG ENIC ACID	30.622	10.1600	0.111	0.1108
Anton Sali Pre	FERULLIC ACID	41.250	15.9108	0.383	0.3828

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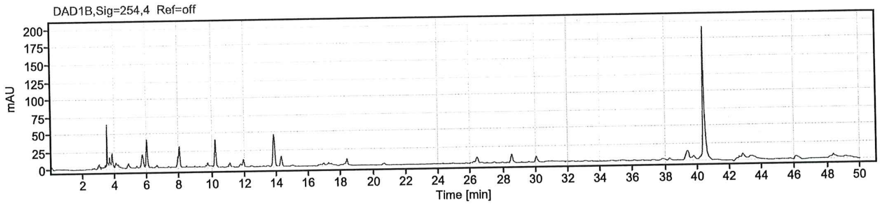
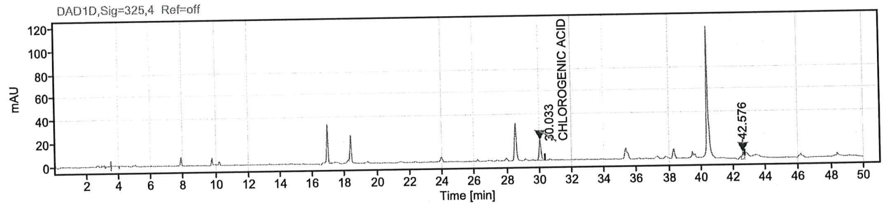
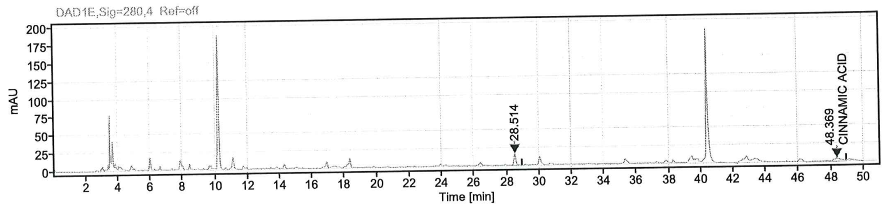
Figure A.31: The Polyphenol contents of the Thermal pretreated *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture

Single Injection Report — Agilent Technologies

Data file:	C:\CDSProjects\Demo\Results\HCA\OTHERS\20250221-HCA-LMB-malthe.rslt\Anton Sali Pre EnzHCA-LMB-AMX-2025.amxHCA-PMX-12022025.pmx20250221 203501.dx		
Sample name:	Anton Sali Pre Enz		
Description:			
Sample amount:	0.000	**Sample type:**	Sample
Instrument:	HPLC_01	**Location:**	P2-A6
Injection date:	2025-02-21 20:35:58+01:00	**Injection:**	1 of 1
Acq. method:	HCA-LMB-AMX-2025.amx	**Injection volume:**	15.000
Analysis method:	*HCA-PMX-12022025.pmx	**Acq. operator:**	SYSTEM
Last changed:	2025-02-26 12:06:06+01:00		







Sample Name	Name	RT	Area	Amount Unit	Concentration [mg/L]
Anton Sali Pre Enz	CHLOROG ENIC ACID	30.033	149.2149	2.919	2.9189
Anton Sali Pre Enz	CINNAMIC ACID	48.369	244.3705	1.826	1.8259

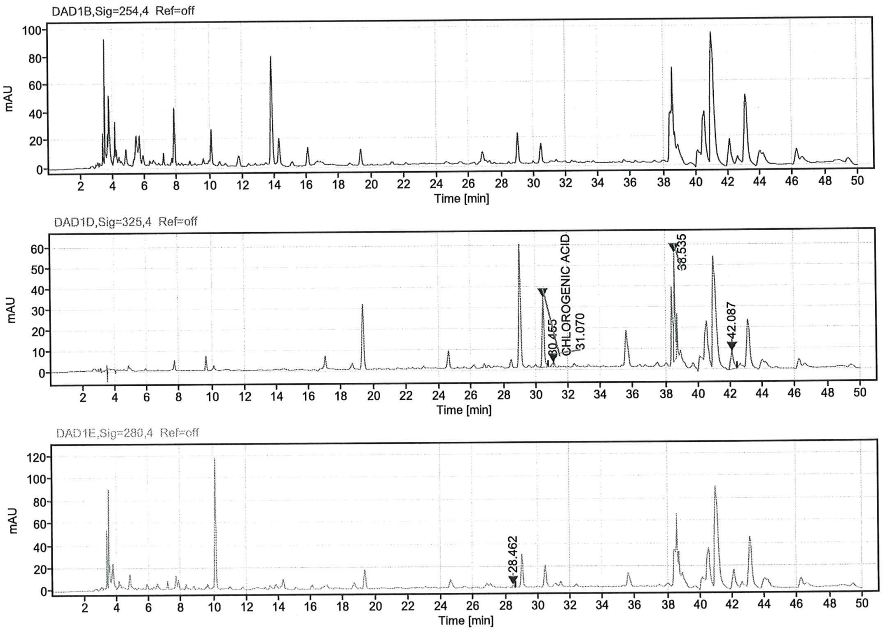
C:\CDSProjects\Demo\Report Templates\Kemi lbm.rdl [Rev. 5] — Printed: 2025-02-26 14:38:48+01:00 — Page 1 of 1

Figure A.32: The Polyphenol contents of the Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture

Single Injection Report

Agilent Technologies

Data file:	C:\CDSProjects\Demo\Results\HCA\OTHERS\20250221-HCA-LMB-malthe.rslt\Anton Plan PreHCA-LMB-AMX-2025.amxHCA-PMX-12022025.pmx20250221 194357.dx		
Sample name:	Anton Plan Pre		
Description:			
Sample amount:	0.000	**Sample type:**	Sample
Instrument:	HPLC_01	**Location:**	P2-A5
Injection date:	2025-02-21 19:44:53+01:00	**Injection:**	1 of 1
Acq. method:	HCA-LMB-AMX-2025.amx	**Injection volume:**	15.000
Analysis method:	*HCA-PMX-12022025.pmx	**Acq. operator:**	SYSTEM
Last changed:	2025-02-26 12:06:06+01:00		



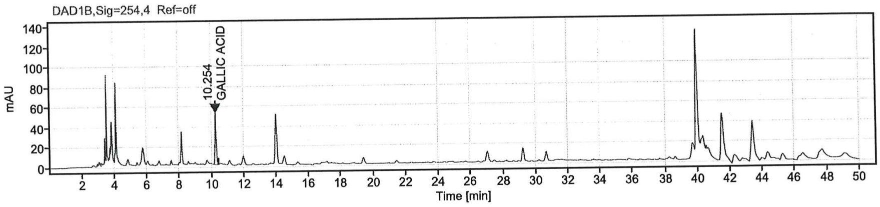
Sample Name	Name	RT	Area	Amount Unit	Concentration [mg/L]
Anton Plan Pre	CHLOROG ENIC ACID	30.455	271.3862	5.386	5.3861

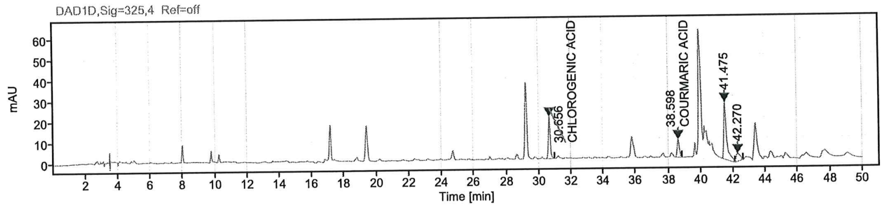
C:\CDSProjects\Demo\Report Templates\Kemi lbm.rdl [Rev. 5] Printed: 2025-02-26 14:38:45+01:00 Page 1 of 1

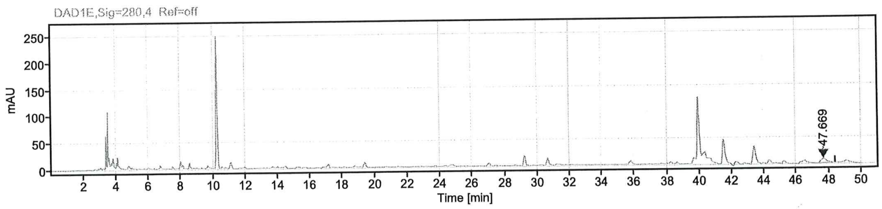
Figure A.33: The Polyphenol contents of the Thermal pretreated *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture

Single Injection Report | Agilent Technologies

Data file:	C:\CDSProjects\Demo\Results\HCA\OTHERS\20250221-HCA-LMB-malthe.rslt\Anton Plan Pre EnzHCA-LMB-AMX-2025.amxHCA-PMX-12022025.pmx20250221 212606.dx		
Sample name:	Anton Plan Pre Enz		
Description:			
Sample amount:	0.000	**Sample type:**	Sample
Instrument:	HPLC_01	**Location:**	P2-A7
Injection date:	2025-02-21 21:27:03+01:00	**Injection:**	1 of 1
Acq. method:	HCA-LMB-AMX-2025.amx	**Injection volume:**	15.000
Analysis method:	*HCA-PMX-12022025.pmx	**Acq. operator:**	SYSTEM
Last changed:	2025-02-26 12:06:06+01:00		







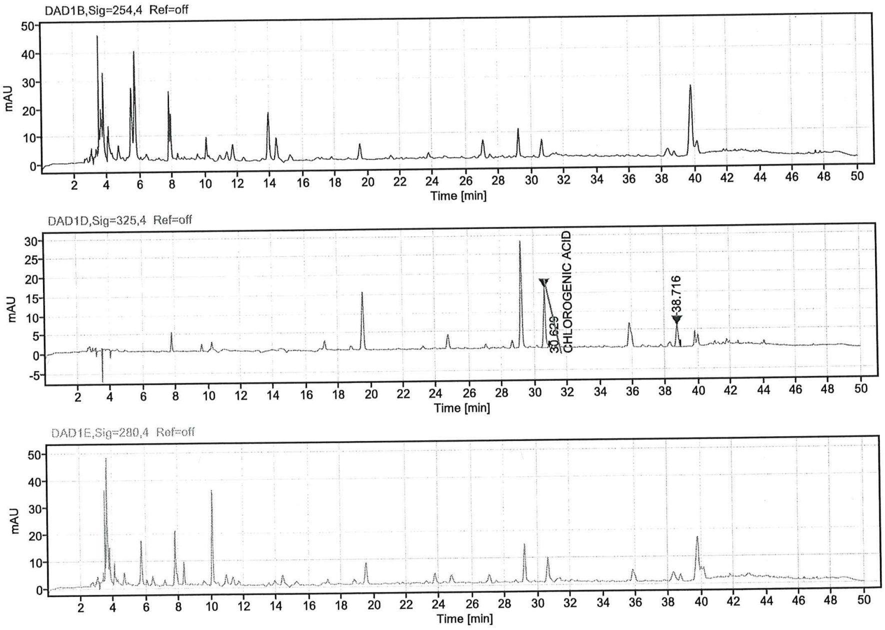
Sample Name	**Name**	**RT**	**Area**	**Amount**	**Unit**	**Concentration [mg/L]**
Anton Plan Pre Enz	GALLIC ACID	10.254	278.3165	8.949		8.9490
Anton Plan Pre Enz	CHLOROG ENIC ACID	30.656	162.4971	3.187		3.1871
Anton Plan Pre Enz	COURMAR IC ACID	38.598	75.4803	0.790		0.7903

C:\CDSProjects\Demo\Report Templates\Kemi lbm.rdl [Rev. 5] Printed: 2025-02-26 14:38:51+01:00 Page 1 of 1

Figure A.34: The Polyphenol contents of the Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture

Single Injection Report Agilent Technologies

Data file:	C:\CDSProjects\Demo\Results\HCA\OTHERS\20250221-HCA-LMB-malthe.rslt\Anton Sali FermenHCA-LMB-AMX-2025.amxHCA-PMX-12022025.pmx20250221 221711.dx		
Sample name:	Anton Sali Fermen		
Description:			
Sample amount:	0.000	**Sample type:**	Sample
Instrument:	HPLC_01	**Location:**	P2-A8
Injection date:	2025-02-21 22:18:09+01:00	**Injection:**	1 of 1
Acq. method:	HCA-LMB-AMX-2025.amx	**Injection volume:**	15.000
Analysis method:	*HCA-PMX-12022025.pmx	**Acq. operator:**	SYSTEM
Last changed:	2025-02-26 12:06:06+01:00		



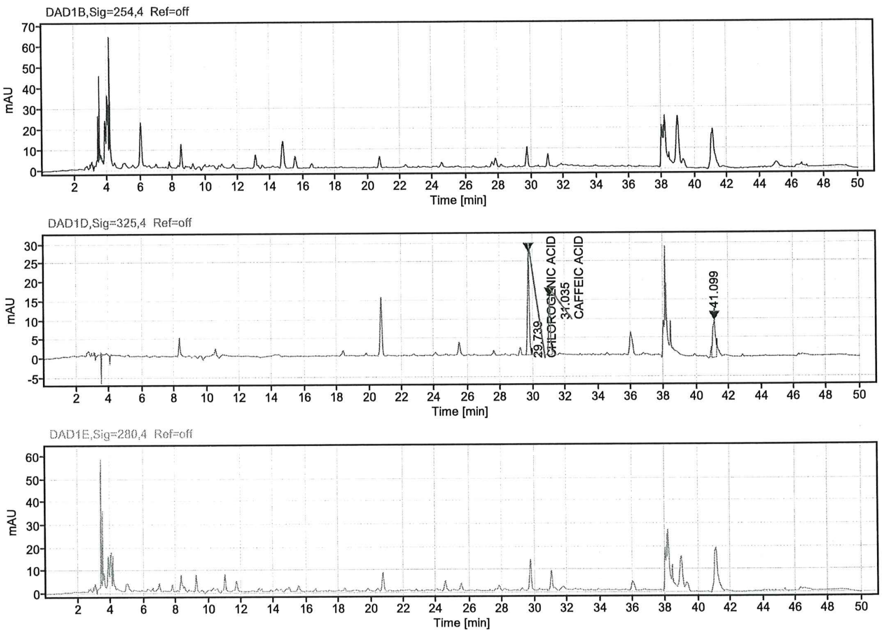
Sample Name	Name	RT	Area	Amount Unit	Concentration [mg/L]
Anton Sali Fermen	CHLOROG ENIC ACID	30.629	129.0050	2.511	2.5108

C:\CDSProjects\Demo\Report Templates\Kemi lbm.rdl [Rev. 5] Printed: 2025-02-26 14:38:53+01:00 Page 1 of 1

Figure A.35: The Polyphenol contents of the Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Salivarus* at 85% Moisture Fermentation Liquid

Single Injection Report — Agilent Technologies

Data file:	C:\CDSProjects\Demo\Results\HCA\OTHERS\20250221-HCA-LMB-malthe.rslt\Anton Plan FermenHCA-LMB-AMX-2025.amxHCA-PMX-12022025.pmx20250221 230817.dx		
Sample name:	Anton Plan Fermen		
Description:			
Sample amount:	0.000	**Sample type:**	Sample
Instrument:	HPLC_01	**Location:**	P2-A9
Injection date:	2025-02-21 23:09:14+01:00	**Injection:**	1 of 1
Acq. method:	HCA-LMB-AMX-2025.amx	**Injection volume:**	15.000
Analysis method:	*HCA-PMX-12022025.pmx	**Acq. operator:**	SYSTEM
Last changed:	2025-02-26 12:06:06+01:00		



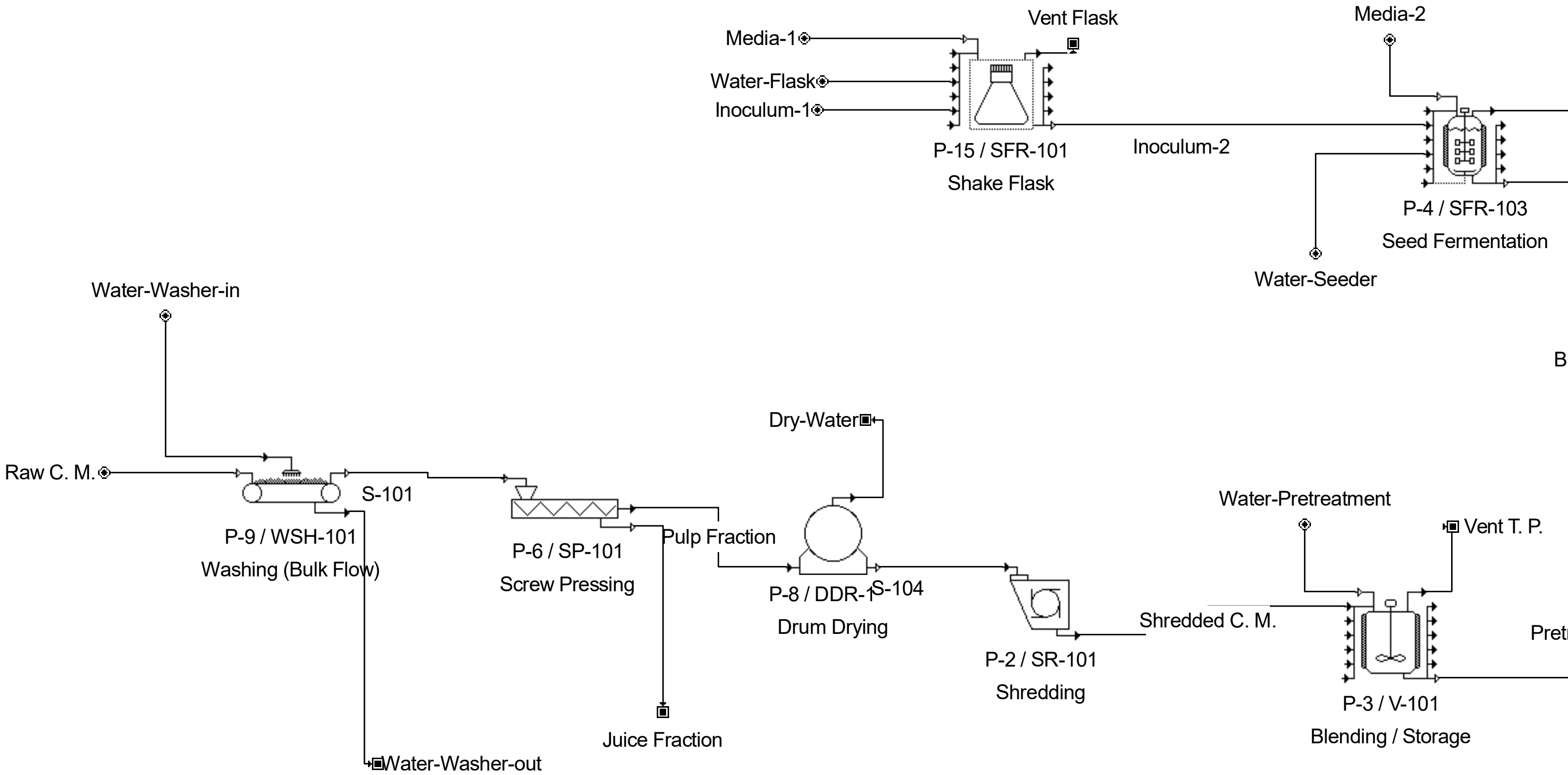
Sample Name	Name	RT	Area	Amount Unit	Concentration [mg/L]
Anton Plan Fermen	CHLOROG ENIC ACID	29.739	196.6891	3.878	3.8776
Anton Plan Fermen	CAFFEIC ACID	31.035	110.9201	1.505	1.5053

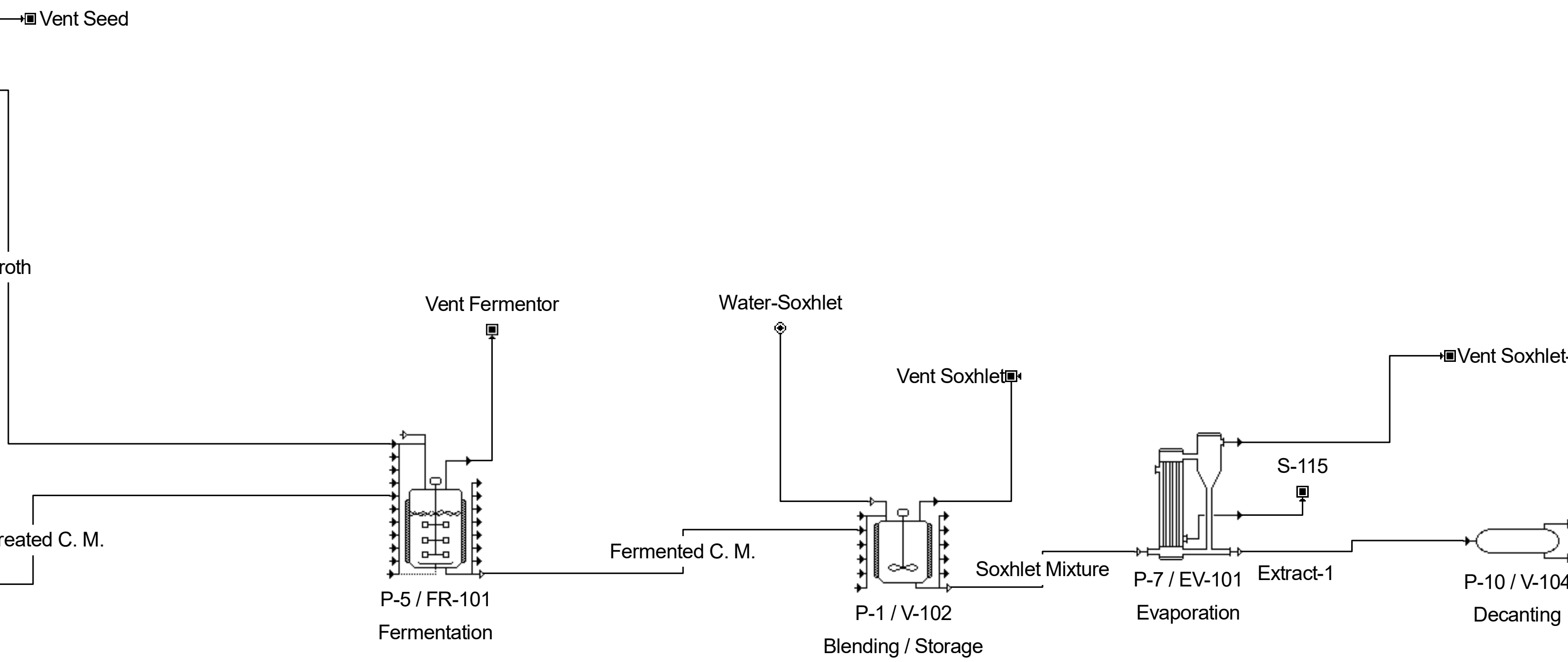
C:\CDSProjects\Demo\Report Templates\Kemi lbm.rdl [Rev. 5] — Printed: 2025-02-26 14:38:56+01:00 — Page 1 of 1

Figure A.36: The Polyphenol contents of the Thermal pretreated & Enzyme *Crithmum Maritimum* fermented with *Lactobacillus Plantarum* at 85% Moisture Fermentation Liquid

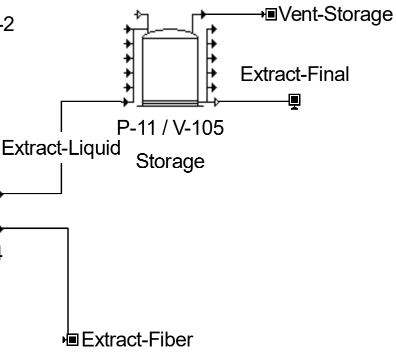
A.2 Appendix B SuperPro Designer model

A.2.1 Case 1: Fractionated Biomass Refinery


Media-1
Water-Flask
Inoculum-1
Vent Flask
P-15 / SFR-101
Shake Flask
Inoculum-2
Media-2
P-4 / SFR-103
Seed Fermentation
Water-Seeder
Water-Washer-in
Raw C. M.
S-101
P-9 / WSH-101
Washing (Bulk Flow)
Water-Washer-out
P-6 / SP-101
Screw Pressing
Pulp Fraction
Juice Fraction
Dry-Water
P-8 / DDR-1
S-104
Drum Drying
P-2 / SR-101
Shredding
Shredded C. M.
Water-Pretreatment
Vent T. P.
P-3 / V-101
Blending / Storage

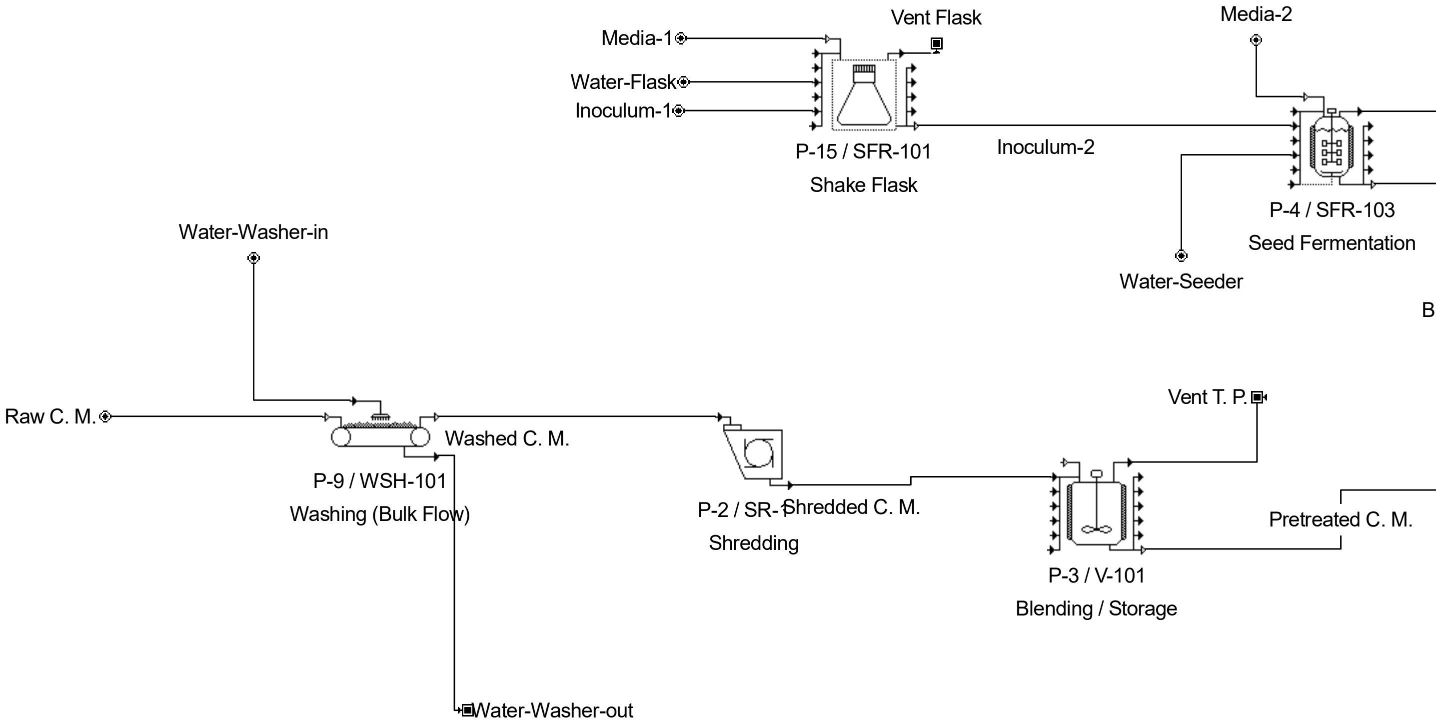


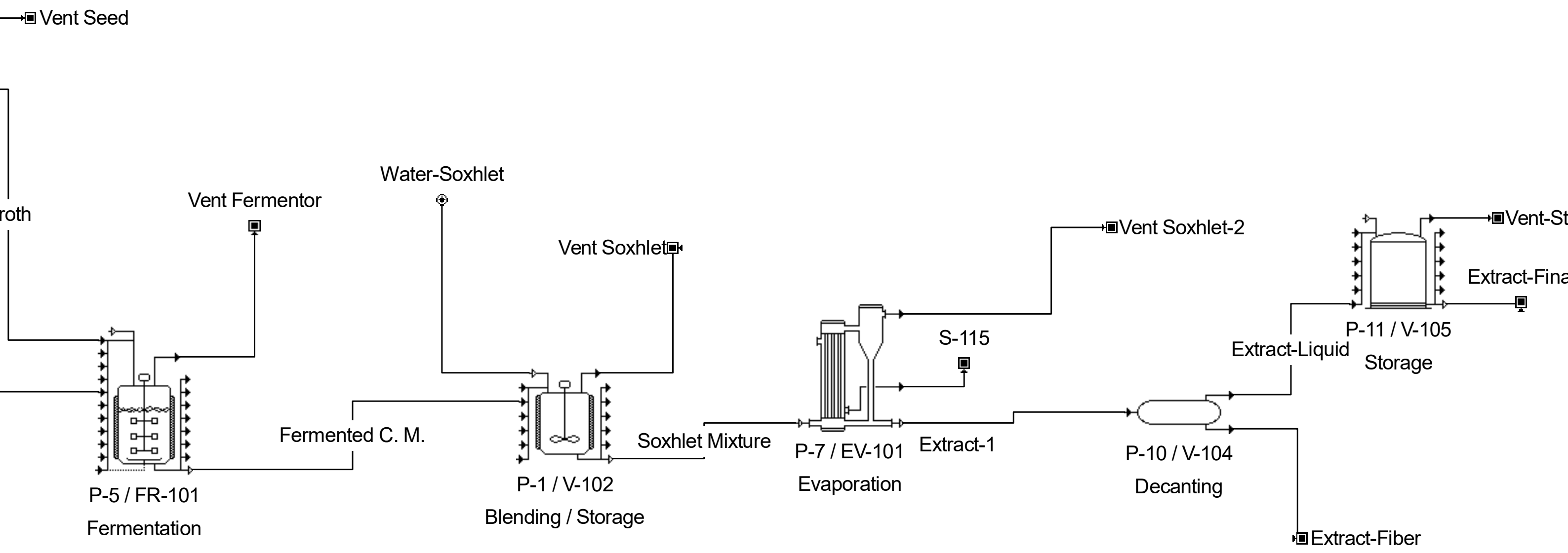
Vent Seed
roth
reated C. M.
Vent Fermentor
P-5 / FR-101
Fermentation
Fermented C. M.
Water-Soxhlet
Vent Soxhlet
P-1 / V-102
Blending / Storage
Soxhlet Mixture
P-7 / EV-101
Evaporation
S-115
Vent Soxhlet-
Extract-1
P-10 / V-104
Decanting



-2
Vent-Storage
Extract-Final
P-11 / V-105
Storage
Extract-Liquid
Extract-Fiber

A.2.2 Case 2: Whole Biomass Refinery


Vent Flask
Media-2
Media-1
Water-Flask
Inoculum-1
P-15 / SFR-101
Shake Flask
Inoculum-2
P-4 / SFR-103
Seed Fermentation
Water-Seeder
Water-Washer-in
Vent T. P.
Raw C. M.
Washed C. M.
P-9 / WSH-101
Washing (Bulk Flow)
P-2 / SR-1
Shredding
Shredded C. M.
Pretreated C. M.
P-3 / V-101
Blending / Storage
Water-Washer-out



Vent Seed
roth
Vent Fermentor
Water-Soxhlet
Vent Soxhlet
Vent Soxhlet-2
Vent-St
Extract-Fina
S-115
Extract-Liquid
P-11 / V-105
Storage
Fermented C. M.
Soxhlet Mixture
P-7 / EV-101
Evaporation
Extract-1
P-10 / V-104
Decanting
P-5 / FR-101
Fermentation
P-1 / V-102
Blending / Storage
Extract-Fiber

A.3 Appendix C Economic Evaluation Reports

EER	Page
Fractionated Biomass Input 0.1 t	110
Fractionated Biomass Input 1 t	122
Fractionated Biomass Input 2 t	134
Whole Biomass Input 0.1 t	146
Whole Biomass Input 1 t	157
Whole Biomass Input 2 t	168

Table A.1: Overview of the economic reports

Economic Evaluation Report

June 1, 2025

for Fractionated Critmum Maritimum Flowsheet 1

1. EXECUTIVE SUMMARY (2025 prices)

Total Capital Investment	12,048,000 €
Capital Investment Charged to This Project	12,048,000 €
Operating Cost	4,340,000 €/yr
Main Revenue	28,000 €/yr
Other Revenues	1,072 €/yr
Total Revenues	29,000 €/yr
Batch Size	5.00 kg MP
Cost Basis Annual Rate	530.15 kg MP/yr
Unit Production Cost	8,186.86 €/kg MP
Net Unit Production Cost	8,186.86 €/kg MP
Unit Production Revenue	55.37 €/kg MP
Gross Margin	- 14,685.51 %
Return On Investment	- 26.87 %
Payback Time	N/A
IRR (After Taxes)	N/A
NPV (at 7.0% Interest)	- 35,483,000 €

MP = Total Flow of Stream 'Extract-Final'

2. EQUIPMENT SPECIFICATION AND FOB COST (2025 prices)

Main Equipment				
Quantity/ Standby/ Staggered	**Name**	**Description**	**Unit Cost (€)**	**Cost (€)**
1 / 0 / 0	FR-101	Fermentor	454,000	454,000
		Vessel Volume = 88.95 L		
1 / 0 / 0	SFR-103	Seed Fermentor	454,000	454,000
		Vessel Volume = 8.26 L		
1 / 0 / 0	V-102	Blending Tank	162,000	162,000
		Vessel Volume = 553.37 L		
1 / 0 / 0	V-101	Blending Tank	135,000	135,000
		Vessel Volume = 71.61 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	101,000	101,000
		Mean Heat Transfer Area = 0.04 m2		
1 / 0 / 0	SR-101	Shredder	67,000	67,000
		Rated Throughput = 0.43 kg/h		
1 / 0 / 0	DDR-101	Drum Dryer	45,000	45,000
		Drum Area = 0.09 m2		
1 / 0 / 0	SP-101	Screw Press	39,000	39,000
		Throughput = 100.00 kg/h		
1 / 0 / 0	V-104	Decanter Tank	30,000	30,000
		Vessel Volume = 0.00 L		
1 / 0 / 0	V-105	Flat Bottom Tank	29,000	29,000
		Vessel Volume = 0.23 L		
		Unlisted Equipment		379,000
			TOTAL	1,895,000

3. FIXED CAPITAL ESTIMATE SUMMARY (2025 prices in €)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	1,895,000
2. Installation	687,000
3. Process Piping	663,000
4. Instrumentation	758,000
5. Insulation	57,000
6. Electrical	190,000
7. Buildings	853,000
8. Yard Improvement	284,000
9. Auxiliary Facilities	758,000
TPDC	6,145,000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	1,536,000
11. Construction	2,151,000
TPIC	3,687,000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	9,831,000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	492,000
13. Contingency	983,000
CFC = 12+13	1,475,000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	11,306,000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost (€/h)	Annual Amount (h)	Annual Cost (€)	%
Operator	50.47	36,491	1,841,640	100.00
TOTAL		36,491	1,841,640	100.00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Cell	0.00	0	kg	0	0.00
CIP - Caustic	0.04	14,164	kg	498	0.54
Crithmum Mariti	6.70	10,600	kg	71,052	77.34
Media	142.90	140	kg	19,994	21.76
Water	2.00	163	MT	325	0.35
TOTAL				91,869	100.00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2025 prices) - PROCESS SUMMARY

Consumable	Units Cost (€)	Annual Amount		Annual Cost (€)	%
4000 mL Shake Flask	1.79	11	item	19	100.00
TOTAL				19	100.00

7. WASTE TREATMENT/DISPOSAL COST (2025 prices) - PROCESS SUMMARY

Waste Category	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Solid Waste				0	0.00
Aqueous Liquid				245	100.00
P-4:CIP-1(Water flush)	3.66	26	MT	97	39.42
P-4:CIP-1(Caustic Flush)	3.66	14	MT	52	21.14
P-4:CIP-1(Final Water Flush)	3.66	26	MT	97	39.42
Organic Liquid				0	0.00
Emissions				0	0.00
TOTAL				245	100.00

8. UTILITIES COST (2025 prices) - PROCESS SUMMARY

Utility	Unit Cost (€)	Annual Amount	Ref. Units	Annual Cost (€)	%
Std Power	0.07	2,150	kW-h	157	18.55
Steam	8.78	64	MT	563	66.47
Chilled Water	0.29	434	MT	127	14.98
TOTAL				848	100.00

9. ANNUAL OPERATING COST (2025 prices) - PROCESS SUMMARY

Cost Item	€	%
Raw Materials	92,000	2.12
Labor-Dependent	1,842,000	42.43
Facility-Dependent	2,129,000	49.06
Laboratory/QC/QA	276,000	6.36
Consumables	0	0.00
Waste Treatment/Disposal	0	0.01
Utilities	1,000	0.02
Transportation	0	0.00
Miscellaneous	0	0.00
Advertising/Selling	0	0.00
Running Royalties	0	0.00
Failed Product Disposal	0	0.00
TOTAL	4,340,000	100.00

10. PROFITABILITY ANALYSIS (2025 prices)

A.	Direct Fixed Capital	11,306,000 €
B.	Working Capital	176,000 €
C.	Startup Cost	565,000 €
D.	Up-Front R&D	0 €
E.	Up-Front Royalties	0 €
F.	Total Investment (A+B+C+D+E)	12,048,000 €
G.	Investment Charged to This Project	12,048,000 €
H.	**Revenue/Savings Rates**	
	Extract-Fiber (Revenue)	1,037 kg /yr
	Extract-Final (Main Revenue)	530 kg /yr
	Juice Fraction (Revenue)	7,147 kg /yr
I.	**Revenue/Savings Price**	
	Extract-Fiber (Revenue)	0.80 €/kg
	Extract-Final (Main Revenue)	53.35 €/kg
	Juice Fraction (Revenue)	33.39 €/1000 kg
J.	**Revenues/Savings**	
	Extract-Fiber (Revenue)	834 €/yr
	Extract-Final (Main Revenue)	28,282 €/yr
	Juice Fraction (Revenue)	239 €/yr
1	Total Revenues	29,355 €/yr
2	Total Savings	0 €/yr
K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	4,340,000 €/yr
2	Net AOC (K1-J2)	4,340,000 €/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	8,186.86 €/kg MP
	Net Unit Production Cost	8,186.86 €/kg MP
	Unit Production Revenue	55.37 €/kg MP
M.	Gross Profit (J-K)	- 4,311,000 €/yr
N.	Taxes (25%)	0 €/yr
O.	Net Profit (M-N + Depreciation)	- 3,237,000 €/yr

Gross Margin	- 14,685.51 %
Return On Investment	- 26.87 %
Payback Time	N/A

MP = Total Flow of Stream 'Extract-Final'

Economic Evaluation Report

June 1, 2025

for Fractionated Critmum Maritimum Flowsheet 1 1 ton

1. EXECUTIVE SUMMARY (2025 prices)

Total Capital Investment	13,887,000 €
Capital Investment Charged to This Project	13,887,000 €
Operating Cost	5,656,000 €/yr
Main Revenue	289,000 €/yr
Other Revenues	10,790 €/yr
Total Revenues	300,000 €/yr
Batch Size	50.09 kg MP
Cost Basis Annual Rate	5,310 kg MP/yr
Unit Production Cost	1,065.27 €/kg MP
Net Unit Production Cost	1,065.27 €/kg MP
Unit Production Revenue	56.53 €/kg MP
Gross Margin	- 1,784.41 %
Return On Investment	- 29.70 %
Payback Time	N/A
IRR (After Taxes)	N/A
NPV (at 7.0% Interest)	- 43,559,000 €

MP = Total Flow of Stream 'Extract-Final'

2. EQUIPMENT SPECIFICATION AND FOB COST (2025 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost (€)	Cost (€)
1 / 0 / 0	FR-101	Fermentor	584,000	584,000
		Vessel Volume = 889.35 L		
1 / 0 / 0	SFR-103	Seed Fermentor	454,000	454,000
		Vessel Volume = 82.77 L		
1 / 0 / 0	V-102	Blending Tank	224,000	224,000
		Vessel Volume = 5537.70 L		
1 / 0 / 0	V-101	Blending Tank	168,000	168,000
		Vessel Volume = 716.10 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	101,000	101,000
		Mean Heat Transfer Area = 0.44 m2		
1 / 0 / 0	SR-101	Shredder	67,000	67,000
		Rated Throughput = 4.29 kg/h		
1 / 0 / 0	DDR-101	Drum Dryer	45,000	45,000
		Drum Area = 0.93 m2		
1 / 0 / 0	SP-101	Screw Press	39,000	39,000
		Throughput = 1000.00 kg/h		
1 / 0 / 0	V-104	Decanter Tank	30,000	30,000
		Vessel Volume = 0.01 L		
1 / 0 / 0	V-105	Flat Bottom Tank	29,000	29,000
		Vessel Volume = 2.33 L		
		Unlisted Equipment		435,000
			TOTAL	2,176,000

3. FIXED CAPITAL ESTIMATE SUMMARY (2025 prices in €)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	2,176,000
2. Installation	783,000
3. Process Piping	762,000
4. Instrumentation	871,000
5. Insulation	65,000
6. Electrical	218,000
7. Buildings	979,000
8. Yard Improvement	326,000
9. Auxiliary Facilities	871,000
TPDC	7,050,000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	1,763,000
11. Construction	2,468,000
TPIC	4,230,000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	11,280,000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	564,000
13. Contingency	1,128,000
CFC = 12+13	1,692,000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	12,972,000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost (€/h)	Annual Amount (h)	Annual Cost (€)	%
Operator	50.47	39,468	1,991,922	100.00
TOTAL		39,468	1,991,922	100.00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Cell	0.00	0	kg	0	0.00
CIP - Caustic	0.04	30,534	kg	1,074	0.12
Crithmum Mariti	6.70	106,000	kg	710,518	77.80
Media	142.90	1,401	kg	200,242	21.93
Water	2.00	686	MT	1,373	0.15
TOTAL				913,207	100.00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2025 prices) - PROCESS SUMMARY

Consumable	Units Cost (€)	Annual Amount		Annual Cost (€)	%
4000 mL Shake Flask	1.79	11	item	19	100.00
TOTAL				19	100.00

7. WASTE TREATMENT/DISPOSAL COST (2025 prices) - PROCESS SUMMARY

Waste Category	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Solid Waste				0	0.00
Aqueous Liquid				529	100.00
P-4:CIP-1(Water flush)	3.66	57	MT	208	39.39
P-4:CIP-1(Caustic Flush)	3.66	31	MT	112	21.12
P-4:CIP-1(Final Water Flush)	3.66	57	MT	208	39.39
Organic Liquid				0	0.00
Emissions				0	0.00
TOTAL				529	100.00

8. UTILITIES COST (2025 prices) - PROCESS SUMMARY

Utility	Unit Cost (€)	Annual Amount	Ref. Units	Annual Cost (€)	%
Std Power	0.07	21,391	kW-h	1,565	18.47
Steam	8.78	642	MT	5,639	66.58
Chilled Water	0.29	4,325	MT	1,265	14.94
TOTAL				8,469	100.00

9. ANNUAL OPERATING COST (2025 prices) - PROCESS SUMMARY

Cost Item	€	%
Raw Materials	913,000	16.14
Labor-Dependent	1,992,000	35.22
Facility-Dependent	2,443,000	43.20
Laboratory/QC/QA	299,000	5.28
Consumables	0	0.00
Waste Treatment/Disposal	1,000	0.01
Utilities	8,000	0.15
Transportation	0	0.00
Miscellaneous	0	0.00
Advertising/Selling	0	0.00
Running Royalties	0	0.00
Failed Product Disposal	0	0.00
TOTAL	5,656,000	100.00

10. PROFITABILITY ANALYSIS (2025 prices)

A.	Direct Fixed Capital	12,972,000 €
B.	Working Capital	266,000 €
C.	Startup Cost	649,000 €
D.	Up-Front R&D	0 €
E.	Up-Front Royalties	0 €
F.	Total Investment (A+B+C+D+E)	13,887,000 €
G.	Investment Charged to This Project	13,887,000 €

H.	**Revenue/Savings Rates**	
	Extract-Fiber (Revenue)	10,378 kg /yr
	Extract-Final (Main Revenue)	5,310 kg /yr
	Juice Fraction (Revenue)	71,470 kg /yr

I.	**Revenue/Savings Price**	
	Extract-Fiber (Revenue)	0.81 €/kg
	Extract-Final (Main Revenue)	54.50 €/kg
	Juice Fraction (Revenue)	33.39 €/1000 kg

J.	**Revenues/Savings**	
	Extract-Fiber (Revenue)	8,403 €/yr
	Extract-Final (Main Revenue)	289,380 €/yr
	Juice Fraction (Revenue)	2,386 €/yr
1	Total Revenues	300,169 €/yr
2	Total Savings	0 €/yr

K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	5,656,000 €/yr
2	Net AOC (K1-J2)	5,656,000 €/yr

L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	1,065.27 €/kg MP
	Net Unit Production Cost	1,065.27 €/kg MP
	Unit Production Revenue	56.53 €/kg MP

M.	Gross Profit (J-K)	- 5,357,000 €/yr
N.	Taxes (25%)	0 €/yr
O.	Net Profit (M-N + Depreciation)	- 4,124,000 €/yr

Gross Margin	- 1,784.41 %
Return On Investment	- 29.70 %
Payback Time	N/A

MP = Total Flow of Stream 'Extract-Final'

Economic Evaluation Report

June 1, 2025

for Fractionated Critmum Maritimum Flowsheet 1 2 ton

1. EXECUTIVE SUMMARY (2025 prices)

Total Capital Investment	15,017,000 €
Capital Investment Charged to This Project	15,017,000 €
Operating Cost	6,955,000 €/yr
Main Revenue	581,000 €/yr
Other Revenues	21,599 €/yr
Total Revenues	602,000 €/yr
Batch Size	100.21 kg MP
Cost Basis Annual Rate	10,622 kg MP/yr
Unit Production Cost	654.75 €/kg MP
Net Unit Production Cost	654.75 €/kg MP
Unit Production Revenue	56.71 €/kg MP
Gross Margin	- 1,054.59 %
Return On Investment	- 33.47 %
Payback Time	N/A
IRR (After Taxes)	N/A
NPV (at 7.0% Interest)	- 51,090,000 €

MP = Total Flow of Stream 'Extract-Final'

2. EQUIPMENT SPECIFICATION AND FOB COST (2025 prices)

Main Equipment				
Quantity/ Standby/ Staggered	Name	Description	Unit Cost (€)	Cost (€)
1 / 0 / 0	FR-101	Fermentor	643,000	643,000
		Vessel Volume = 1779.04 L		
1 / 0 / 0	SFR-103	Seed Fermentor	461,000	461,000
		Vessel Volume = 165.92 L		
1 / 0 / 0	V-102	Blending Tank	234,000	234,000
		Vessel Volume = 11076.24 L		
1 / 0 / 0	V-101	Blending Tank	185,000	185,000
		Vessel Volume = 1432.20 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	101,000	101,000
		Mean Heat Transfer Area = 0.88 m2		
1 / 0 / 0	SP-101	Screw Press	67,000	67,000
		Throughput = 2000.00 kg/h		
1 / 0 / 0	SR-101	Shredder	67,000	67,000
		Rated Throughput = 8.57 kg/h		
1 / 0 / 0	DDR-101	Drum Dryer	58,000	58,000
		Drum Area = 1.86 m2		
1 / 0 / 0	V-104	Decanter Tank	30,000	30,000
		Vessel Volume = 0.03 L		
1 / 0 / 0	V-105	Flat Bottom Tank	29,000	29,000
		Vessel Volume = 4.66 L		
		Unlisted Equipment		469,000
			TOTAL	2,344,000

3. FIXED CAPITAL ESTIMATE SUMMARY (2025 prices in €)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	2,344,000
2. Installation	834,000
3. Process Piping	820,000
4. Instrumentation	938,000
5. Insulation	70,000
6. Electrical	234,000
7. Buildings	1,055,000
8. Yard Improvement	352,000
9. Auxiliary Facilities	938,000
TPDC	7,584,000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	1,896,000
11. Construction	2,654,000
TPIC	4,550,000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	12,134,000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	607,000
13. Contingency	1,213,000
CFC = 12+13	1,820,000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	13,954,000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost (€/h)	Annual Amount (h)	Annual Cost (€)	%
Operator	50.47	42,777	2,158,913	100.00
TOTAL		42,777	2,158,913	100.00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Cell	0.00	0	kg	0	0.00
CIP - Caustic	0.04	38,500	kg	1,355	0.07
Crithmum Mariti	6.70	212,000	kg	1,421,036	77.81
Media	142.90	2,809	kg	401,393	21.98
Water	2.00	1,230	MT	2,461	0.13
TOTAL				1,826,245	100.00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2025 prices) - PROCESS SUMMARY

Consumable	Units Cost (€)	Annual Amount		Annual Cost (€)	%
4000 mL Shake Flask	1.79	11	item	19	100.00
TOTAL				19	100.00

7. WASTE TREATMENT/DISPOSAL COST (2025 prices) - PROCESS SUMMARY

Waste Category	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Solid Waste				0	0.00
Aqueous Liquid				667	100.00
P-4:CIP-1(Water flush)	3.66	72	MT	263	39.37
P-4:CIP-1(Caustic Flush)	3.66	39	MT	141	21.11
P-4:CIP-1(Final Water Flush)	3.66	72	MT	263	39.37
Organic Liquid				0	0.00
Emissions				0	0.00
TOTAL				667	100.00

8. UTILITIES COST (2025 prices) - PROCESS SUMMARY

Utility	Unit Cost (€)	Annual Amount	Ref. Units	Annual Cost (€)	%
Std Power	0.07	42,779	kW-h	3,129	18.47
Steam	8.78	1,285	MT	11,278	66.58
Chilled Water	0.29	8,651	MT	2,531	14.94
TOTAL				16,938	100.00

9. ANNUAL OPERATING COST (2025 prices) - PROCESS SUMMARY

Cost Item	€	%
Raw Materials	1,826,000	26.26
Labor-Dependent	2,159,000	31.04
Facility-Dependent	2,628,000	37.79
Laboratory/QC/QA	324,000	4.66
Consumables	0	0.00
Waste Treatment/Disposal	1,000	0.01
Utilities	17,000	0.24
Transportation	0	0.00
Miscellaneous	0	0.00
Advertising/Selling	0	0.00
Running Royalties	0	0.00
Failed Product Disposal	0	0.00
TOTAL	6,955,000	100.00

10. PROFITABILITY ANALYSIS (2025 prices)

A.	Direct Fixed Capital	13,954,000 €
B.	Working Capital	365,000 €
C.	Startup Cost	698,000 €
D.	Up-Front R&D	0 €
E.	Up-Front Royalties	0 €
F.	Total Investment (A+B+C+D+E)	15,017,000 €
G.	Investment Charged to This Project	15,017,000 €
H.	**Revenue/Savings Rates**	
	Extract-Fiber (Revenue)	20,756 kg /yr
	Extract-Final (Main Revenue)	10,622 kg /yr
	Juice Fraction (Revenue)	142,940 kg /yr
I.	**Revenue/Savings Price**	
	Extract-Fiber (Revenue)	0.81 €/kg
	Extract-Final (Main Revenue)	54.68 €/kg
	Juice Fraction (Revenue)	33.39 €/1000 kg
J.	**Revenues/Savings**	
	Extract-Fiber (Revenue)	16,827 €/yr
	Extract-Final (Main Revenue)	580,755 €/yr
	Juice Fraction (Revenue)	4,773 €/yr
1	Total Revenues	602,355 €/yr
2	Total Savings	0 €/yr
K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	6,955,000 €/yr
2	Net AOC (K1-J2)	6,955,000 €/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	654.75 €/kg MP
	Net Unit Production Cost	654.75 €/kg MP
	Unit Production Revenue	56.71 €/kg MP
M.	Gross Profit (J-K)	- 6,353,000 €/yr
N.	Taxes (25%)	0 €/yr
O.	Net Profit (M-N + Depreciation)	- 5,027,000 €/yr

Gross Margin	- 1,054.59 %
Return On Investment	- 33.47 %
Payback Time	N/A

MP = Total Flow of Stream 'Extract-Final'

Economic Evaluation Report

June 1, 2025

for Critmum Maritimum Flowsheet 1.2

1. EXECUTIVE SUMMARY (2025 prices)

Total Capital Investment	11,459,000 €
Capital Investment Charged to This Project	11,459,000 €
Operating Cost	4,195,000 €/yr
Main Revenue	544,000 €/yr
Other Revenues	6,285 €/yr
Total Revenues	551,000 €/yr
Batch Size	10.24 kg MP
Cost Basis Annual Rate	1,085 kg MP/yr
Unit Production Cost	3,864.95 €/kg MP
Net Unit Production Cost	3,864.95 €/kg MP
Unit Production Revenue	507.39 €/kg MP
Gross Margin	- 661.74 %
Return On Investment	- 22.89 %
Payback Time	N/A
IRR (After Taxes)	N/A
NPV (at 7.0% Interest)	- 30,550,000 €

MP = Total Flow of Stream 'Extract-Final'

2. EQUIPMENT SPECIFICATION AND FOB COST (2025 prices)

Main Equipment				
Quantity/ Standby/ Staggered	**Name**	**Description**	**Unit Cost (€)**	**Cost (€)**
1 / 0 / 0	FR-101	Fermentor	454,000	454,000
		Vessel Volume = 136.12 L		
1 / 0 / 0	SFR-103	Seed Fermentor	454,000	454,000
		Vessel Volume = 12.71 L		
1 / 0 / 0	V-102	Blending Tank	170,000	170,000
		Vessel Volume = 782.22 L		
1 / 0 / 0	V-101	Blending Tank	135,000	135,000
		Vessel Volume = 109.65 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	101,000	101,000
		Mean Heat Transfer Area = 0.06 m2		
1 / 0 / 0	SR-101	Shredder	67,000	67,000
		Rated Throughput = 4.17 kg/h		
1 / 0 / 0	V-104	Decanter Tank	30,000	30,000
		Vessel Volume = 0.00 L		
1 / 0 / 0	V-105	Flat Bottom Tank	29,000	29,000
		Vessel Volume = 0.45 L		
		Unlisted Equipment		360,000
			TOTAL	1,800,000

3. FIXED CAPITAL ESTIMATE SUMMARY (2025 prices in €)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	1,800,000
2. Installation	657,000
3. Process Piping	630,000
4. Instrumentation	720,000
5. Insulation	54,000
6. Electrical	180,000
7. Buildings	810,000
8. Yard Improvement	270,000
9. Auxiliary Facilities	720,000
TPDC	5,841,000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	1,460,000
11. Construction	2,044,000
TPIC	3,505,000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	9,346,000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	467,000
13. Contingency	935,000
CFC = 12+13	1,402,000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	10,748,000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost (€/h)	Annual Amount (h)	Annual Cost (€)	%
Operator	50.47	35,585	1,795,916	100.00
TOTAL		35,585	1,795,916	100.00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Cell	0.00	0	kg	0	0.00
CIP - Caustic	0.04	16,350	kg	575	0.56
Crithmum Mariti	6.70	10,600	kg	71,055	69.16
Media	142.90	215	kg	30,748	29.93
Water	2.00	183	MT	366	0.36
TOTAL				102,744	100.00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2025 prices) - PROCESS SUMMARY

Consumable	Units Cost (€)	Annual Amount		Annual Cost (€)	%
4000 mL Shake Flask	1.79	11	item	19	100.00
TOTAL				19	100.00

7. WASTE TREATMENT/DISPOSAL COST (2025 prices) - PROCESS SUMMARY

Waste Category	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Solid Waste				0	0.00
Aqueous Liquid				283	100.00
P-4:CIP-1(Water flush)	3.66	30	MT	112	39.42
P-4:CIP-1(Caustic Flush)	3.66	16	MT	60	21.13
P-4:CIP-1(Final Water Flush)	3.66	30	MT	112	39.42
Organic Liquid				0	0.00
Emissions				0	0.00
TOTAL				283	100.00

8. UTILITIES COST (2025 prices) - PROCESS SUMMARY

Utility	Unit Cost (€)	Annual Amount	Ref. Units	Annual Cost (€)	%
Std Power	0.07	3,837	kW-h	281	21.96
Steam	8.78	87	MT	763	59.72
Chilled Water	0.29	801	MT	234	18.33
TOTAL				1,278	100.00

9. ANNUAL OPERATING COST (2025 prices) - PROCESS SUMMARY

Cost Item	€	%
Raw Materials	103,000	2.45
Labor-Dependent	1,796,000	42.82
Facility-Dependent	2,025,000	48.28
Laboratory/QC/QA	269,000	6.42
Consumables	0	0.00
Waste Treatment/Disposal	0	0.01
Utilities	1,000	0.03
Transportation	0	0.00
Miscellaneous	0	0.00
Advertising/Selling	0	0.00
Running Royalties	0	0.00
Failed Product Disposal	0	0.00
TOTAL	4,195,000	100.00

10. PROFITABILITY ANALYSIS (2025 prices)

A.	Direct Fixed Capital	10,748,000 €
B.	Working Capital	173,000 €
C.	Startup Cost	537,000 €
D.	Up-Front R&D	0 €
E.	Up-Front Royalties	0 €
F.	Total Investment (A+B+C+D+E)	11,459,000 €
G.	Investment Charged to This Project	11,459,000 €

H.	**Revenue/Savings Rates**	
	Extract-Fiber (Revenue)	1,132 kg /yr
	Extract-Final (Main Revenue)	1,085 kg /yr

I.	**Revenue/Savings Price**	
	Extract-Fiber (Revenue)	5.55 €/kg
	Extract-Final (Main Revenue)	501.60 €/kg

J.	**Revenues/Savings**	
	Extract-Fiber (Revenue)	6,285 €/yr
	Extract-Final (Main Revenue)	544,373 €/yr
1	Total Revenues	550,658 €/yr
2	Total Savings	0 €/yr

K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	4,195,000 €/yr
2	Net AOC (K1-J2)	4,195,000 €/yr

L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	3,864.95 €/kg MP
	Net Unit Production Cost	3,864.95 €/kg MP
	Unit Production Revenue	507.39 €/kg MP

M.	Gross Profit (J-K)	- 3,644,000 €/yr
N.	Taxes (25%)	0 €/yr
O.	Net Profit (M-N + Depreciation)	- 2,623,000 €/yr

	Gross Margin	- 661.74 %
	Return On Investment	- 22.89 %
	Payback Time	N/A

MP = Total Flow of Stream 'Extract-Final'

Economic Evaluation Report

June 1, 2025

for Critmum Maritimum Flowsheet 1.2 1 ton

1. EXECUTIVE SUMMARY (2025 prices)

Total Capital Investment	13,694,000 €
Capital Investment Charged to This Project	13,694,000 €
Operating Cost	5,755,000 €/yr
Main Revenue	5,456,000 €/yr
Other Revenues	62,980 €/yr
Total Revenues	5,519,000 €/yr
Batch Size	102.50 kg MP
Cost Basis Annual Rate	10,865 kg MP/yr
Unit Production Cost	529.63 €/kg MP
Net Unit Production Cost	529.63 €/kg MP
Unit Production Revenue	507.97 €/kg MP
Gross Margin	- 4.26 %
Return On Investment	7.15 %
Payback Time	13.99 years
IRR (After Taxes)	N/A
NPV (at 7.0% Interest)	- 7,551,000 €

MP = Total Flow of Stream 'Extract-Final'

2. EQUIPMENT SPECIFICATION AND FOB COST (2025 prices)

Main Equipment				
Quantity/ Standby/ Staggered	**Name**	**Description**	**Unit Cost (€)**	**Cost (€)**
1 / 0 / 0	FR-101	Fermentor	620,000	620,000
		Vessel Volume = 1361.29 L		
1 / 0 / 0	SFR-103	Seed Fermentor	454,000	454,000
		Vessel Volume = 127.41 L		
1 / 0 / 0	V-102	Blending Tank	235,000	235,000
		Vessel Volume = 7826.45 L		
1 / 0 / 0	V-101	Blending Tank	178,000	178,000
		Vessel Volume = 1096.54 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	101,000	101,000
		Mean Heat Transfer Area = 0.62 m2		
1 / 0 / 0	SR-101	Shredder	67,000	67,000
		Rated Throughput = 41.67 kg/h		
1 / 0 / 0	V-104	Decanter Tank	30,000	30,000
		Vessel Volume = 0.01 L		
1 / 0 / 0	V-105	Flat Bottom Tank	29,000	29,000
		Vessel Volume = 4.51 L		
		Unlisted Equipment		429,000
			TOTAL	2,143,000

3. FIXED CAPITAL ESTIMATE SUMMARY (2025 prices in €)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	2,143,000
2. Installation	774,000
3. Process Piping	750,000
4. Instrumentation	857,000
5. Insulation	64,000
6. Electrical	214,000
7. Buildings	964,000
8. Yard Improvement	321,000
9. Auxiliary Facilities	857,000
TPDC	6,944,000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	1,736,000
11. Construction	2,430,000
TPIC	4,167,000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	11,111,000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	556,000
13. Contingency	1,111,000
CFC = 12+13	1,667,000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	12,777,000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost (€/h)	Annual Amount (h)	Annual Cost (€)	%
Operator	50.47	39,837	2,010,544	100.00
TOTAL		39,837	2,010,544	100.00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Cell	0.00	0	kg	0	0.00
CIP - Caustic	0.04	35,256	kg	1,240	0.12
Crithmum Mariti	6.70	106,000	kg	710,550	69.55
Media	142.90	2,157	kg	308,240	30.17
Water	2.00	826	MT	1,653	0.16
TOTAL				1,021,683	100.00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2025 prices) - PROCESS SUMMARY

Consumable	Units Cost (€)	Annual Amount		Annual Cost (€)	%
4000 mL Shake Flask	1.79	11	item	19	100.00
TOTAL				19	100.00

7. WASTE TREATMENT/DISPOSAL COST (2025 prices) - PROCESS SUMMARY

Waste Category	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Solid Waste				0	0.00
Aqueous Liquid				611	100.00
P-4:CIP-1(Water flush)	3.66	66	MT	241	39.38
P-4:CIP-1(Caustic Flush)	3.66	35	MT	129	21.11
P-4:CIP-1(Final Water Flush)	3.66	66	MT	241	39.38
Organic Liquid				0	0.00
Emissions				0	0.00
TOTAL				611	100.00

8. UTILITIES COST (2025 prices) - PROCESS SUMMARY

Utility	Unit Cost (€)	Annual Amount	Ref. Units	Annual Cost (€)	%
Std Power	0.07	38,266	kW-h	2,799	21.91
Steam	8.78	870	MT	7,638	59.79
Chilled Water	0.29	7,994	MT	2,339	18.31
TOTAL				12,775	100.00

9. ANNUAL OPERATING COST (2025 prices) - PROCESS SUMMARY

Cost Item	€	%
Raw Materials	1,022,000	17.75
Labor-Dependent	2,011,000	34.94
Facility-Dependent	2,407,000	41.83
Laboratory/QC/QA	302,000	5.24
Consumables	0	0.00
Waste Treatment/Disposal	1,000	0.01
Utilities	13,000	0.22
Transportation	0	0.00
Miscellaneous	0	0.00
Advertising/Selling	0	0.00
Running Royalties	0	0.00
Failed Product Disposal	0	0.00
TOTAL	5,755,000	100.00

10. PROFITABILITY ANALYSIS (2025 prices)

A.	Direct Fixed Capital	12,777,000 €
B.	Working Capital	278,000 €
C.	Startup Cost	639,000 €
D.	Up-Front R&D	0 €
E.	Up-Front Royalties	0 €
F.	Total Investment (A+B+C+D+E)	13,694,000 €
G.	Investment Charged to This Project	13,694,000 €
H.	**Revenue/Savings Rates**	
	Extract-Fiber (Revenue)	11,320 kg /yr
	Extract-Final (Main Revenue)	10,865 kg /yr
I.	**Revenue/Savings Price**	
	Extract-Fiber (Revenue)	5.56 €/kg
	Extract-Final (Main Revenue)	502.18 €/kg
J.	**Revenues/Savings**	
	Extract-Fiber (Revenue)	62,980 €/yr
	Extract-Final (Main Revenue)	5,456,407 €/yr
1	Total Revenues	5,519,387 €/yr
2	Total Savings	0 €/yr
K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	5,755,000 €/yr
2	Net AOC (K1-J2)	5,755,000 €/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	529.63 €/kg MP
	Net Unit Production Cost	529.63 €/kg MP
	Unit Production Revenue	507.97 €/kg MP
M.	Gross Profit (J-K)	- 236,000 €/yr
N.	Taxes (25%)	0 €/yr
O.	Net Profit (M-N + Depreciation)	979,000 €/yr
	Gross Margin	- 4.26 %
	Return On Investment	7.15 %
	Payback Time	13.99 years

MP = Total Flow of Stream 'Extract-Final'

Economic Evaluation Report

June 1, 2025

for Critmum Maritimum Flowsheet 1.2 2 ton

1. EXECUTIVE SUMMARY (2025 prices)

Total Capital Investment	14,852,000 €
Capital Investment Charged to This Project	14,852,000 €
Operating Cost	7,249,000 €/yr
Main Revenue	10,914,000 €/yr
Other Revenues	125,968 €/yr
Total Revenues	11,040,000 €/yr
Batch Size	205.02 kg MP
Cost Basis Annual Rate	21,732 kg MP/yr
Unit Production Cost	333.55 €/kg MP
Net Unit Production Cost	333.55 €/kg MP
Unit Production Revenue	507.99 €/kg MP
Gross Margin	34.34 %
Return On Investment	27.95 %
Payback Time	3.58 years
IRR (After Taxes)	19.10 %
NPV (at 7.0% Interest)	13,750,000 €

MP = Total Flow of Stream 'Extract-Final'

2. EQUIPMENT SPECIFICATION AND FOB COST (2025 prices)

Main Equipment				
Quantity/ Standby/ Staggered	**Name**	**Description**	**Unit Cost (€)**	**Cost (€)**
1 / 0 / 0	FR-101	Fermentor	683,000	683,000
		Vessel Volume = 2722.25 L		
1 / 0 / 0	SFR-103	Seed Fermentor	489,000	489,000
		Vessel Volume = 254.51 L		
1 / 0 / 0	V-102	Blending Tank	252,000	252,000
		Vessel Volume = 15652.98 L		
1 / 0 / 0	V-101	Blending Tank	196,000	196,000
		Vessel Volume = 2193.08 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	101,000	101,000
		Mean Heat Transfer Area = 1.24 m2		
1 / 0 / 0	SR-101	Shredder	68,000	68,000
		Rated Throughput = 83.33 kg/h		
1 / 0 / 0	V-104	Decanter Tank	30,000	30,000
		Vessel Volume = 0.04 L		
1 / 0 / 0	V-105	Flat Bottom Tank	29,000	29,000
		Vessel Volume = 9.01 L		
		Unlisted Equipment		462,000
			TOTAL	2,310,000

3. FIXED CAPITAL ESTIMATE SUMMARY (2025 prices in €)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	2,310,000
2. Installation	831,000
3. Process Piping	809,000
4. Instrumentation	924,000
5. Insulation	69,000
6. Electrical	231,000
7. Buildings	1,040,000
8. Yard Improvement	347,000
9. Auxiliary Facilities	924,000
TPDC	7,484,000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	1,871,000
11. Construction	2,619,000
TPIC	4,490,000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	11,974,000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	599,000
13. Contingency	1,197,000
CFC = 12+13	1,796,000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	13,770,000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost (€/h)	Annual Amount (h)	Annual Cost (€)	%
Operator	50.47	44,562	2,249,010	100.00
TOTAL		44,562	2,249,010	100.00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Cell	0.00	0	kg	0	0.00
CIP - Caustic	0.04	44,402	kg	1,562	0.08
Crithmum Mariti	6.70	212,000	kg	1,421,100	69.61
Media	142.90	4,309	kg	615,722	30.16
Water	2.00	1,497	MT	2,994	0.15
TOTAL				2,041,378	100.00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2025 prices) - PROCESS SUMMARY

Consumable	Units Cost (€)	Annual Amount		Annual Cost (€)	%
4000 mL Shake Flask	1.79	11	item	19	100.00
TOTAL				19	100.00

7. WASTE TREATMENT/DISPOSAL COST (2025 prices) - PROCESS SUMMARY

Waste Category	Unit Cost (€)	Annual Amount		Annual Cost (€)	%
Solid Waste				0	0.00
Aqueous Liquid				770	100.00
P-4:CIP-1(Water flush)	3.66	83	MT	303	39.35
P-4:CIP-1(Caustic Flush)	3.66	44	MT	162	21.10
P-4:CIP-1(Final Water Flush)	3.66	83	MT	303	39.35
Organic Liquid				0	0.00
Emissions				0	0.00
TOTAL				770	100.00

8. UTILITIES COST (2025 prices) - PROCESS SUMMARY

Utility	Unit Cost (€)	Annual Amount	Ref. Units	Annual Cost (€)	%
Std Power	0.07	76,511	kW-h	5,596	21.90
Steam	8.78	1,740	MT	15,275	59.79
Chilled Water	0.29	15,984	MT	4,676	18.30
TOTAL				25,548	100.00

9. ANNUAL OPERATING COST (2025 prices) - PROCESS SUMMARY

Cost Item	€	%
Raw Materials	2,041,000	28.16
Labor-Dependent	2,249,000	31.03
Facility-Dependent	2,595,000	35.79
Laboratory/QC/QA	337,000	4.65
Consumables	0	0.00
Waste Treatment/Disposal	1,000	0.01
Utilities	26,000	0.35
Transportation	0	0.00
Miscellaneous	0	0.00
Advertising/Selling	0	0.00
Running Royalties	0	0.00
Failed Product Disposal	0	0.00
TOTAL	7,249,000	100.00

10. PROFITABILITY ANALYSIS (2025 prices)

A.	Direct Fixed Capital	13,770,000 €
B.	Working Capital	394,000 €
C.	Startup Cost	689,000 €
D.	Up-Front R&D	0 €
E.	Up-Front Royalties	0 €
F.	Total Investment (A+B+C+D+E)	14,852,000 €
G.	Investment Charged to This Project	14,852,000 €
H.	**Revenue/Savings Rates**	
	Extract-Fiber (Revenue)	22,640 kg /yr
	Extract-Final (Main Revenue)	21,732 kg /yr
I.	**Revenue/Savings Price**	
	Extract-Fiber (Revenue)	5.56 €/kg
	Extract-Final (Main Revenue)	502.20 €/kg
J.	**Revenues/Savings**	
	Extract-Fiber (Revenue)	125,968 €/yr
	Extract-Final (Main Revenue)	10,913,545 €/yr
1	Total Revenues	11,039,513 €/yr
2	Total Savings	0 €/yr
K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	7,249,000 €/yr
2	Net AOC (K1-J2)	7,249,000 €/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	333.55 €/kg MP
	Net Unit Production Cost	333.55 €/kg MP
	Unit Production Revenue	507.99 €/kg MP
M.	Gross Profit (J-K)	3,791,000 €/yr
N.	Taxes (25%)	948,000 €/yr
O.	Net Profit (M-N + Depreciation)	4,151,000 €/yr
	Gross Margin	34.34 %
	Return On Investment	27.95 %
	Payback Time	3.58 years

MP = Total Flow of Stream 'Extract-Final'