Master’s Thesis

Title: techno-economic evaluation of polyphenols extraction process from grape seed

Master’s in Chemical Engineering

Sustainable technologies and biotechnologies for energy and material

(STEM)

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Acknowledgement

Current study has been carried out based on ongoing project in Caviro. The project has been defined for Research and Development (R&D) department in compliance with company's circular economy goals. Performing an internship for a short period of time during Covid-19 situation has resulted in some obstacles which have been tried to overcome. Working in such environment was a great opportunity which was provided by University of Bologna and specifically Dr. Lorenzo Bertin. On the other hand, I would like to thank Caviro and its R&D department and mainly Dr. Rosa Prati and Dr. Roberto Conti for providing such opportunity and trying to teach me how to work in a company. Working in such environment has given me confidence to start my career hopefully in near future.

Dedicated to my family and my love

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Abstract

Caviro is one of the main wine producers in Italy. Caviro has two main headquarters in Emilia-Romagna. The one which is known as production plant is based in Faenza, while the other one where the bottling and storing is mainly carried out is in Forli. Wine and by-products are produced in Faenza. Then, produced wine is sent to the plant in Forli for storing, aging in case of some wines, analogical testing, and bottling for selling. Extraction of polyphenols from grape seeds has been investigated for couple of years. According to company's goals and in compliance with circular economy goals of the company, the extraction process has been determined as a high added value by-product production.

During my internship in the company, I had the opportunity to carry out extraction in lab-scale and pilot scale. In lab-scale experiments, 1 L Pyrex bottles were used as extraction chambers. Based on literature review, liquid to solid ratio of 4.75 has been applied. 850 mL of 50% v/v ethanol solution as extraction solution. The extraction cycle consists of two stages. Each stage lasts for four hours. After the first stage, solution is measured transferred to another Pyrex bottle. According to the same L/S ratio, fresh seeds has been added to the bottle to conduct the second stage of the extraction. The obtained solution was distilled in vacuum condition (-1barg) to recover the ethanol and concentrate the product.

Exhausted seeds were washed to recover the adsorbed ethanol from seeds to optimize the process. By optimizing the process around 95% of inserted ethanol has been recovered. The washing solution has been used for 4 washing cycles to fulfil the recovery goals. By conducting the mass balance all obtained results were verified.

Keywords: Polyphenols. Extraction technologies. Circular economy. Optimization. Business plan preparation. Techno-economical evaluation

1. Introduction

1.1. Food and energy demand

Growing worldwide population has resulted in a rise in demand for food, fuel, and other various end-products (Maina et al., 2017). Food demand has led to a rise in agriculture sector (Foley et al., 2011). All of them being the reason for rising waste generation, generally waste and especially food waste (Ravindran & Jaiswal, 2016) management has gain importance for a sustainable production. To ensure food safety and food supply chain sustainability, food waste management techniques has been developed and enhanced toward minimizing waste disposal which causes various negative environmental impact.

1.2. Food waste generation and generation steps

Municipal waste generation is projected to increase to 1.42 kg per person per day in 2025 (The World Bank, 2017). The produced waste needs to be treated and valorised as much as possible. As a relevant example, Italian waste data indicates that Emilia Romagna has the second highest food and beverage waste generation in Italy (Demichelis et al., 2019). Which has made waste prevention and management policies to be applied effectively to reduce the environmental and economic burden of generated waste.

Globally, estimations show that 10-20 percent of plant-based wastes are produced during agriculture and post-harvesting stages; and 15-20 percent due to processing (FAO, 2011). Due to food production industry contribution on waste, currently policies are addressed to decrease the disposal amount. However, from the produced food waste small amount is addressed to valorisation and most of them are used as animal feed (Jin, Yang, et al., 2018). To step toward sustainable food production, first it worth to know what is defined as waste.

1.3. Food waste definition and defined as by-product

Food waste is defined as residuals of food industry with high organic compounds obtained during conversion from raw material to desired food to be consumed; Being undesirable in food industry defines them as waste in most European legislations (Commission Regulation (EEC) 442, 1975; Commission Regulation (EEC) 689, 1991).

However, nowadays the term food by product is used nowadays for most of residues which can lead to production of valuable products in market (Charis M. Galanakis, 2012). While, food waste is addressed to residues of biorefineries. In other words, Food waste is defined as non-recycled or unutilized products of food processing (Garcia-Gonzalez et al., 2016). The current approach toward wastes has narrowed the border between by-product and waste. What was defined as waste, is being defined as by-product.

1.4. Food waste amount and value

Globally, total food waste is estimated to be 1.6 G tons, while 1.3 G tons of it is recognised as edible (Imbert, 2017). Other data mainly considering European countries indicate that along the food supply chain, 30-40 percent of food is wasted (Charles et al., 2018) which corresponds to around one third of edible parts of food (FAO, 2011).

Food waste through consumer and retail stage is believed to be higher than waste generated in processing stage (Thyberg & Tonjes, 2016). A study by Kumar et al. in 2017 conducts that 42 percent of generated waste is household sourced and 39 percent has industrial origin. There are some policies to minimize, or separate such amount from their generation source, management of such wastes are harder than the waste generated in supply chain due to waste characteristics and various composition of food waste from different regions or even different municipalities. To better recognise their value, around 46 percent of disposed waste from household and food supply chain is reported to be organic (Campuzano & González-Martínez, 2016).

Around 90-100 million tons of waste has generated in food industry (FUSIONS, 2015) which was predicted to increase to 126 million tons by 2020 (European Commission, 2017) in European Union. From which the agricultural activities contribution is the highest. As an economic evaluation, investigations show that Italian food waste value is estimated around 8.5 billion euros annually(Waste Watcher, 2018).

Largest amount of plant-based waste is resulted from agricultural activities, second largest amount is related to beverage industry; grape pomace having a large portion of it contains lots of high added value compounds which makes grape pomace a good feedstock for biorefinery approach (Martinez et al., 2016; Yates et al., 2017).

Global grape wasted as biomass is around 5-13 M tons per year (Corbin et al., 2015; Massey, 2015) which is estimated to correspond to 20-30 percent of total mass of wine production (Zabaniotou et al., 2018). Having such large volume, has driven attention to optimizing the wineries plant. To have more statistical data, from 1000 kg of grape, 750 L wine and 120 kg grape pomace can be obtained in wineries (Jin, Neilson, et al., 2018; Oliveira & Duarte, 2016). Or in another study it has been observed that for production of 6 L of wine, one kilogram of pomace is produced (Mendes et al., 2013). As mentioned, such wastes are currently known as by-products due to their characteristics and potentialities.

1.5. Food and winery waste classification, characterization, and composition

During different stages in food industry, high BOD (biological oxygen demand) and COD (chemical oxygen demand) wastes are generated (Ravindran & Jaiswal, 2016). The compounds have growing market if correctly and efficiently recovered.

Food waste two main categories are named as plant based, which contributes to 63 percent (Pfaltzgraff et al., 2013), and animal-based waste; it can further sub-categorised to fruits and vegetables, cereals, oil crops, root and tubes, meat and its derivatives, fish and sea food, and dairy products (FAO, 2011; Charis M. Galanakis, 2012). Discussing each categories' characteristics is out of scope of this study, however, to have an insight toward the origins of current study, fruits and mainly grape based wastes are discussed to understand their capability of being used in valorising processes. As an insight, 11 percent of Italian food waste generated is corresponding to vegetable and fruit processing (Varzakas, 2012). Which shows the importance of prevention and managing strategies toward such category.

In another general approach, food supply chain waste has been categorised into a) food loss (lost during production phase), b) unavoidable food waste (lost during consumption), and c) avoidable food waste (edible amount which is lost) (Food and Agriculture Organization of the United Nations, n.d.). Having diverse composition, avoidable and unavoidable portions are still under investigation and require more attention from policy makers and governments. Besides, food loss has gained attention through recent decades.

Wine sector has large contribution toward food waste generated. Winery waste or by-products are reach in bioactive compounds like phenolics (Barba et al., 2016). It worth mentioning winery waste streams which are stalk, skin, marc, vine shoot, and seeds (Zheng et al., 2012). Through the text another stream known as Vinasse is mentioned which is defined as the waste or by-product stream of ethanol production.

Winery waste is categorised into two groups; solid, being 7.5 percent grape stalk, 45 percent pomace, 6 percent seeds, and various other solid wastes, and liquid which is mainly wastewater (Broome & Warner, 2008). There are numerous articles and studies regarding wastewater treatment of wineries. Caviro as a leading company in wine sector in Italy has investigated and applied a sufficient wastewater treatment plant serving its goals toward sustainability. Besides, there are ongoing projects toward solid waste portion to recover and valorise containing added value compounds.

Following scheme is a best description of wine industry waste production at each stage of the supply chain.

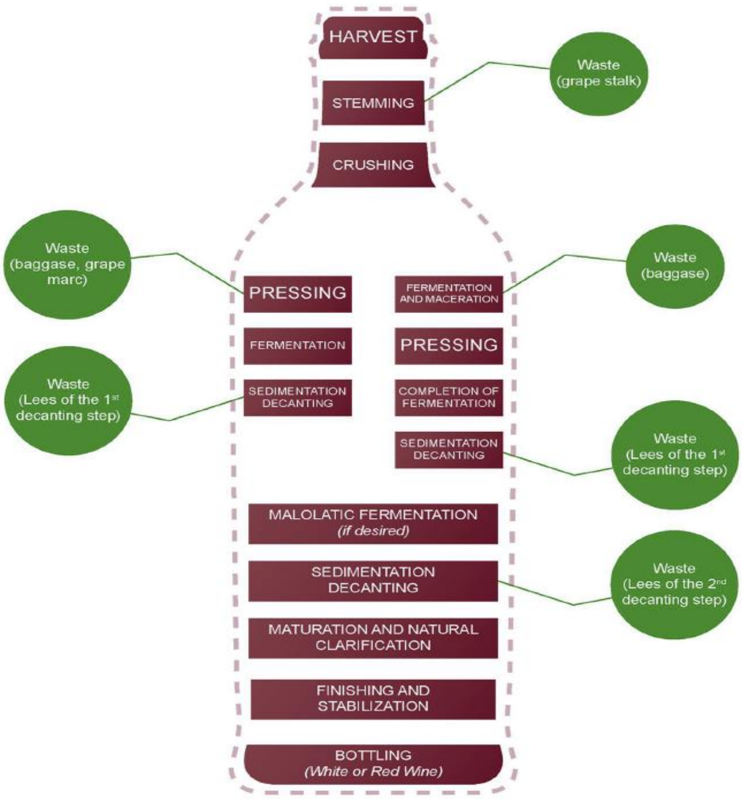


Fig.1 Overview of wastes and by-products generated during wine processing (Barba et al., 2016)

1.6. Food and winery waste hazards (environmental, social, economic)

Food waste has negative environmental impact due to resource depletion, green-house gas emission (FAO, 2013; Garnett, 2011; Lundqvist et al., 2008)(FAO,2013; Lundqvist et al., 2008; Garnet, 2011). Food waste has also negative social and economic impact due to food security and cost for consumers and supply chain, respectively (Buzby & Hyman, 2012; Parry et al., 2015; Venkat, 2012). Accumulation of food waste creates an ideal environment for growth of human health damaging organisms.

Vinification process, despite general view, has high environmental impact due to high amount of resource depletion (Oliveira & Duarte, 2016; Ruggieri et al., 2009). Winery waste and by-products can be hazardous if not correctly treated before disposal (Devesa-Rey et al., 2011). All these conventionally ignored impacts, has led to initiation of various studies toward waste streams potentialities to minimize disposed wastes and transfer residual streams from waste to by-product zone.

1.7. Food waste potentialities and utilization

Considering food supply chain side streams as waste is underestimation of their potentiality. Food waste can be considered as crude oil in the beginning of industrialization era. Which may seem an exaggeration, however, their wide range of potentiality can prove such approach. Food waste has the potentiality to produce biofuel, biohydrogen, and biomethane (Azadi et al., 2013; Das et al., 2012; Liguori et al., 2015; Parmar & Rupasinghe, 2013).

Waste is defined as a cheap resource for valuable components, because of available recovery techniques. Thanks to these technologies, recovery, recycle, and sustainability of high added-value compounds in food industry is assured (Charis M. Galanakis, 2012). By development of novel technologies, consideration of food waste as a renewable crude source of energy and material has become close to reality.

Food waste can be used as feed for biorefinery to produce bio-based added value products (Mirabella et al., 2014). Or digested anaerobically to produce biofuel, generally renewable energy carriers (Evangelisti et al., 2014; C. Zhang et al., 2014; R. Zhang et al., 2007). Or incinerated with energy recover (Grosso et al., 2010). Among which two later ones are considered as conventional approaches toward waste management.

Food waste being considered as feedstock for added value compounds production, has made their valorisation a good step toward sustainability of food industry (C. S. K. Lin et al., 2013). Recently, production of high added-value products is under investigation which reveals that their production can be 3.5 times more profitable than biofuel conversion (Tuck, 2012). Which has grown attention due to both environmental and economic beneficials. The following graph presents a schematic view of the idea.

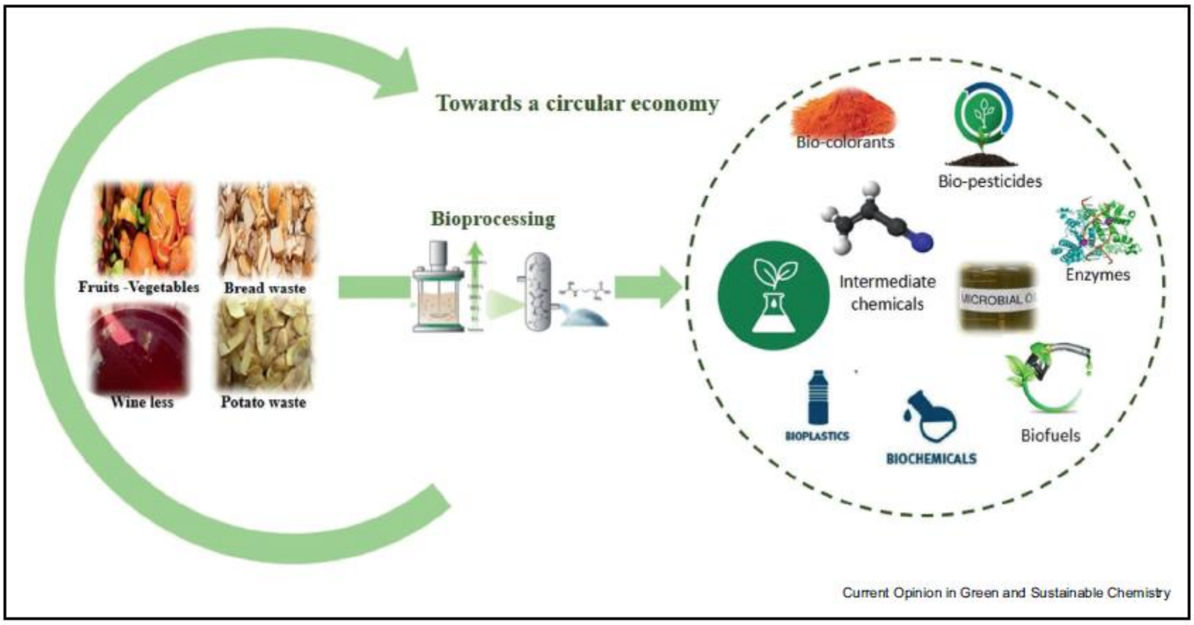


Fig.2 Food supply chain waste bioprocessing (Maina et al., 2017).

As a relevant application of waste management techniques, Grape pomace, skin, and wine lees are used as feedstock for dietary fibre, phenolic compounds, calcium tartrate, and enocyanin production (Charis M. Galanakis, 2012), conventionally. While, novel technologies have resulted in various compounds production.

Food waste is a key area in Circular Economy (CE) (European Commission, 2017) in which it is recognised as underutilized resource which should enter as feedstock to economy. Circular economy is gaining interest day by day and is known as a perfect substitute for current linear economy.

1.8. Traditional and conventional food and winery waste management

The most widely applied techniques on food waste are based on taking advantage of their energy potentiality and composting the rest. In addition, through some early studies, recovery of some compounds is industrialized based on optimizing the process of food production. As an example, Winery wastes are usually sent to energy industry, composting, and dumping (Ahmad et al., 2020). Wine lees having five percent volume by volume composition of total wine production (Alañón et al., 2011) are mostly disposed to wastewater treatment plant or used as animal feed (Maugenet, 1973). While, as more sustainable production, in some countries like Italy, wine waste was used to produce compost and distilled to produced spirits like grappa and tartaric acid (Dimou et al., 2015; Oliveira & Duarte, 2016); EU policies has reduced subsidies on distilleries to motivate using these by-products for production of higher added value products than distilling or composting them (Ncube et al., 2021).

Based on data relating to UK, LCA results show that AD (anaerobic digestion) followed by composting is environmentally more beneficial than landfilling with utilization of landfill gas, IVC (in vessel composting), and incineration with energy recovery and serves the goals of circular economy (Slorach et al., 2019). Since AD and composting both are in compliance with the goals of circular economy, had been considered as the most appropriate methods toward food waste management (Ravindran & Jaiswal, 2016).

With the help of novel technologies, policy makers have passed some laws and even reached global agreements toward global level waste managements. Food waste management, which has led to consideration of sustainable production and consumption, is known as fundamental requirement for sustainable development by Paris agreement in 2015 (Imbert, 2017).

1.9. Disadvantage of conventional waste treatment policies

Conventional approaches have proven to be insufficient to deal with the amount of food waste and even to some extent responsible for environmental problems faced in the world. Landfills and land-spreads can cause water and soil contamination by nutrients and free metals present in food industry waste, which can threaten human health(M. P. Zacharof & Lovitt, 2014).

Due to lots of negative impacts corresponding to conventional waste disposal such as landfilling, land spreading, or disposing to rivers, new approaches must be developed (Myrto Panagiota Zacharof, 2017). However, through studies, it has been observed that to ensure sustainability of food supply chain, combination of valorisation strategies should be applied. As an example, one study suggests that a single process or valorisation stage like AD and composting is not sufficient to recover all the potential high added value compounds (Tuck, 2012).

1.10. Policies and hierarchies toward waste management

There should be policies to prevent food waste; however, food waste is an inevitable large portion of nowadays world which is required to be dealt with (Thyberg & Tonjes, 2016). To satisfy such demand, policies have targeted production chain towards developing them into sustainable plants.

Prior to solving the problem, to have better indication and insight of the situation, European commission has classified waste management to three stages, pre-use: reduce; use: reduce, resynthesize, remanufacture, refurbish, repair, direct reuse, and repurpose; post-use: recover and recycle (Luttropp & Lagerstedt, 2006). The following scheme properly describes the EU priorities.

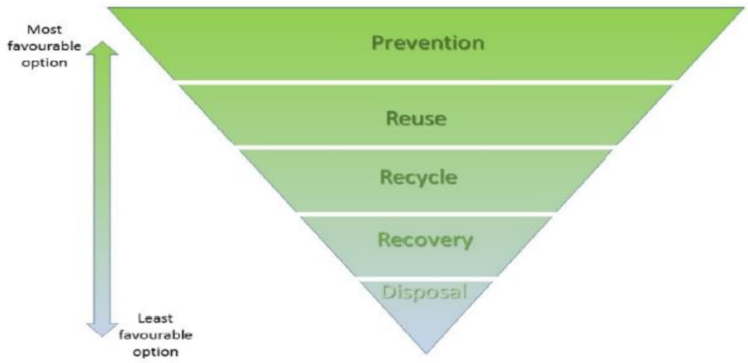


Fig.3 The European waste hierarchy(European Commission (DG ENV), 2010).

There are some regulations concerning novel waste management techniques. According to EC (European Commission) regulation 1493/1999 (EC, 1999) about wine industry, grape pomace after vinification should be sent to distillation to recover ethanol and produce tartrates (Tacchini et al., 2019).

1.11. Interest and demand toward novel approaches

Prevention and reuse policies are currently gaining interest toward waste reduction prior to digestion and landfill (Imbert, 2017). General idea behind novel food waste treatment strategies can be summarized as the waste and by-products of main process can become feedstock and raw material for a secondary process; and the waste from second can become raw material for the third and so on (Liguori et al., 2013).

According to current legislations and in compliance with circular economy model and bio-refinery concept, wastes are used as feedstock for production of energy and high added value compounds. All in all, the need for disposing waste in environmentally friendly ways and reuse, recycle, and recover them as much as possible is rising (The Biocomposite Centre, 2008).

1.12. CE initiation and starting point

Having negative environmental impact, landfills are losing interest; while biorefinery approach to produce high added value and nutritional products followed by biofuel and energy production is becoming more reliable and proven to have lower environmental impact and even having economic advantages (Thyberg & Tonjes, 2016).

Circular economy model has been developed by Chinese and European policy makers to lessen environmental impact of world economy (EU Commission, 2014; Murray et al., 2017). Despite the obstacles on the transition to CE, CE (Circular Economy) and bio-refinery approach have developed as a substitute for current linear economy which is based on 'take, make, and dispose' (Maina et al., 2017).

One of the major problems which is hoped to be overcome through introduction of novel technologies is price of biobased compounds. There are alternatives or bio-based products ready to be substituted by 90 percent of fossil-based products (Perimenis et al., 2011; Taylor, 2008), while lower price of fossil based products decreases feasibility of biobased substitutes production.

1.13. CE definition, requirement, criteria, and Rx approach

A general definition of CE is ‘closed materials cycle economy or resource circulated economy’ (Yang & Feng, 2008) . Which gives a good insight that the idea of circular economy is based on closing energy and material or generally flows of resources in a process (Geng & Doberstein, 2008; Haas et al., 2015). In details, circular economy is an economic method toward integration of economic growth and environmental sustainability (Park et al., 2010) through Rx approach. 3R being reducing, reusing, and recovering (Kirchherr et al., 2017), 4R being reuse, recycle, repair, and remanufacture (Stahel, 2016), and 5R which is based on repair, reuse, remanufacture, refurbish, and recycle are introduced as Rx approaches to satisfy current and future generation resource demand (Prieto-Sandoval et al., 2018).

CE through literature has been classified in three scales, micro (companies or manufactures), meso (eco-industrial parks), and macro (city, region, nation, and finally globe) (Kirchherr et al., 2017; Yuan et al., 2006). As mentioned earlier, production chain is targeted as first barrier to overcome and manage to reach sustainability of food sector.

To satisfy circular economy goals, eco-innovation, which is well described in Fig.4, is required to close the life cycle loop; where eco-innovation is defined as ‘the production, application, or exploitation of a good, service, production process, organizational structure, or management or business method that is novel to the firm or user which results, throughout its life cycle, in a reduction of environmental risk, pollution, and relevant alternatives’ (Kemp & Pearson, 2007).

Biorefinery application requires large investment; so, it should be applied on a wide range and not on a specific bioconversion. Mentioned criteria is crucial in term of making bio-refinery approach economically and to some extent environmentally feasible (Myrto Panagiota Zacharof, 2017).

Shifting from linear production to circular production has environmental benefits; however, the change requires local willingness, new market, competitive production, and above all development of new technologies and processes (Ncube et al., 2021).

The project under investigation places in micro scale since Caviro is a winery; the project seems feasible due to the company’s environmental management maturity and operating cost reduction due to the project application. These two factors are main parameters ensuring feasibility of proposed project in compliance with CE approach (Stahel, 2016).

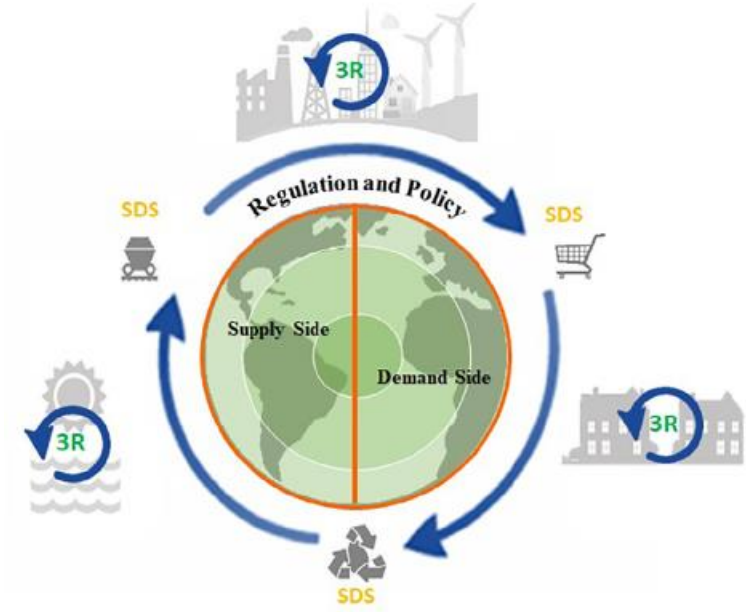


Fig.4 Eco-innovation determinants towards CE (Prieto-Sandoval et al., 2018).

1.14. Bio-Economy, Bio-refinery approach serving CE

Bioeconomy is defined as opportunities and possibilities of converting renewable source of food and in general biological resources into high added value products and energy (Ravindran & Jaiswal, 2016). The term is defined as biotechnological applications and production of bio-products are gaining interest.

Optimization of the energy and material flow of an existing process leads to bio-refinery approach (Octave & Thomas, 2009). Bio-refinery concept has been imitated from petroleum refinery, where in biorefinery different technologies have been utilized to recover products from food waste (Jin, Yang, et al., 2018).

Biorefinery concept can be applied in three phases or generations (Kamm & Kamm, 2004) 1) processing one feedstock to a target product; 2) applying novel technologies to valorise by-products of process to high added value compounds; 3) applying technologies which are able to convert different feedstock to corresponding products are high added value compounds; the flexibility and economic feasibility rises as the approach goes forward (Jin, Yang, et al., 2018).

Applying bio-refinery approach results in extraction of high value-added compounds like polyphenols, in compliance with European regulations about food waste management and circular economy, which are used as food additives to improve social health level (Barba et al., 2016).

All the mentioned factors have led to analysis of bio-refinery approach and corresponding circular economy to discover their advantages and disadvantages. The assessment of novel approach have been conducted to ensure surpassing of advantages against drawbacks. A general view on biorefinery concept is provided as follow.

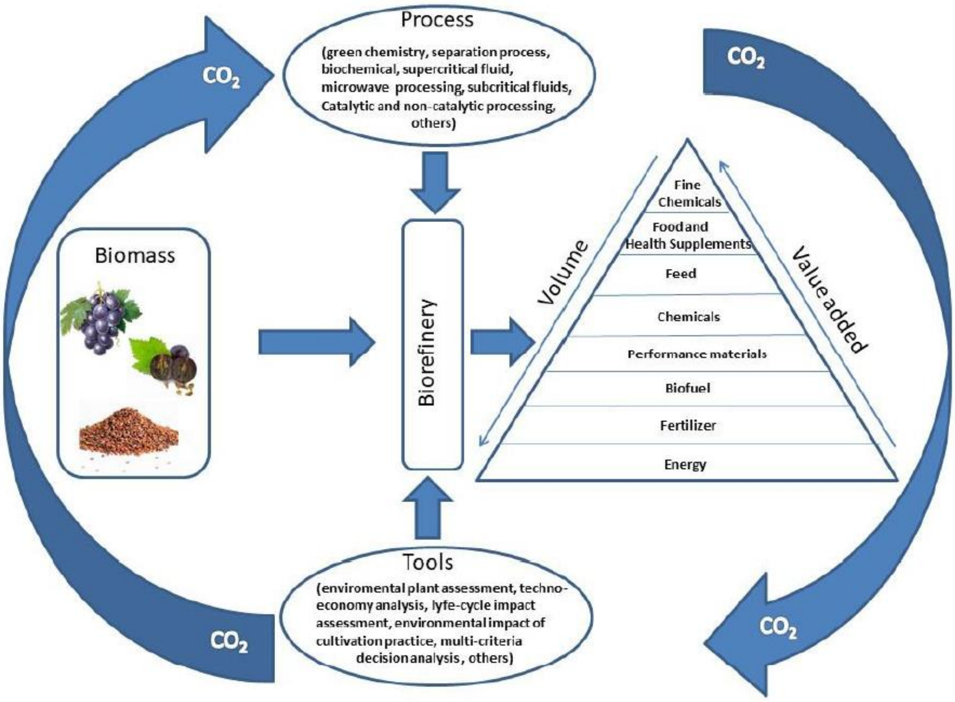


Fig.5 The bio-refinery concept (Lucarini et al., 2018).

1.15. CE, bioeconomy, biorefinery aim, advantage, and market

Recovering added value compounds from food supply chain industry and especially beverage industry can cause large economic benefits due to being a very cheap feedstock (M. P. Zacharof et al., 2014). Besides, in 2016, 1300 tons of CO_2 equivalents were produced which could be reduced by exploiting winery wastes and by-products in biorefinery concept following circular economy aim to produce high added value compounds (Bevilacqua et al., 2017). Statistically, environmental and economic advantage of such approach has made the change toward circular economy inevitable.

European bioeconomy has around 2 trillion euros as turnover and 22 million employees (European Commission, 2012). According to international organizations, green economy based on circular bioeconomy approach can lead to 15-60 million jobs in next twenty years (ILO (International Labour Organization), 2012). It has been suggested that by 2025 every euro invested in this field can generate 10 euros (European Commission, 2012). CE combined with bio-refinery proposed to have a good perspective toward renovated competitiveness, economic gain, increase in job opportunities, organizational, social, and technical innovations (European Commission, 2014).

CE as restorative and regenerative approach aims at reducing dependency on fossil based raw material and satisfy Europe such demand by internal potentialities through waste management (Ellen Mac Arthur Foundation, 2015). It has been argued that application of CE has the potentiality to reduce raw material requirement of European industry by 14-24 percent by 2030 (Venkata Mohan et al., 2016). In addition, CE application is assumed to reduce European industry expenses by 600 billion euros per year (Scarlat et al., 2015), producing 500,000 job opportunities, and reducing CO_2 emission by around 400 million tons(Maina et al., 2017).

Advantages of biorefinery concept can be listed as follow (Jin, Yang, et al., 2018):

1. Lowering waste generation of process by almost fully consumption of original feedstock.
2. Increase in plant revenue by addition of new by-product with growing market.
3. Positive synergy impact of novel technologies.
4. Creation of new job opportunities by addition of new operating battery limits.
5. A great step toward energy self-efficiency of plant.

Along social benefits, due to reducing pollution, bioeconomy and CE result in increment of public health (Bourguignon, 2017).

According to (Kretschmer et al., 2013; J. Lin et al., 2011) obstacles on the transition path from linear economy to circular bioeconomy are:

1. High volume and high level of variation in composition of food waste.
2. Low coordination of waste generators throughout the food supply chain.
3. Storage, since food waste is highly active which leads to decomposition and degradation.
4. Some bio-based products cost more than their fossil-based alternatives.
5. Economic viability of plants and technologies.

Following figure derived from literature mentions all positives and negatives points of biorefinery approach applied on food waste and forestry residues.

Strengths	Weaknesses
❑ Offers the possibility to turn waste streams into valuable resources, and improve sustainability of agriculture and food production; ❑ Potential for 'green' jobs and economic activity if sustainability concerns addressed; ❑ Many conversion technologies have been developed and Europe is believed to hold a strong position in biorefinery research; ❑ The sector is believed to have great potential, one highlight being the potential to produce simultaneously both bio-based chemicals and energy in biorefineries; ❑ Bio-based plastics with strong development potential identified.	❑ Commercial demonstration of technologies lags behind, inter alia due to high costs, financing constraints and a current lack of demand-pull effect; ❑ Sustainability risks exist even for an industry based on wastes and residues, given prevailing existing uses and environmental functions; ❑ Availability of sufficient biomass constrained by logistical, technical, economic and environmental factors, and seasonality; ❑ Wastes and residues tend to be bulky, low value per tonne, heterogeneous and diffuse; their processing in biorefineries therefore tends to be expensive, putting them at a cost disadvantage.
Opportunities	**Threats**
❑ The sector's significant potential to create jobs and economic growth makes it an attractive target for decision making in times of economic downturns; ❑ The on-going revision of EU biofuel policy in an attempt to mitigate ILUC by moving towards biofuels from wastes and residues may provide a stimulus to the wider biorefinery sector; ❑ The Bioeconomy Communication as a high-level policy initiative with the potential to stimulate decision making by industry and European and national policy makers; ❑ The Biobased PPP could develop into a promising initiative helping inter alia bring about large-scale demonstration; ❑ Private sector initiatives to move towards bio-based sourcing (notably in the food packaging industry).	❑ Policy determination to reduce wastes in the food chain which should increase future raw material costs; ❑ The current political focus on bioenergy and biofuels (promoted through renewable energy targets) puts bio-based material uses at competitive disadvantage; ❑ The lack of sustainability criteria for biomaterials (or even for solid biomass energy) in light of the on-going discussion on conventional biofuels may undermine trust in the sector; ❑ The lack of technical standards for bio-based products may complicate market penetration; ❑ Lack of public awareness as regards bio-based products; ❑ The oil price (and development of unconventional fossil sources) is an important determinant of the profitability of many bio-based operations but its development is outside of the sector's control.

Fig.6 Main strength, weaknesses, opportunities, and threats on way toward utilization of food waste as feedstock for production of material and energy (Kretschmer et al., 2013).

1.16. 2G biorefinery approach, Italy application, food bioprocessing

There are some biorefinery applications and their data to be referred in the literature. The available data corresponds to application of the first and second generation biorefineries. Provided two examples ensure feasibility and benefits of novel approaches.

A study by (Martinez et al., 2016)Martinez et al., 2016 proposes polyphenols extraction from grape pomace utilizing supercritical CO_2 extraction technique followed by feeding leftovers to acidogenic digestion to produce PHA, and finally sending residues to anaerobic methanogenic digestion to produce biogas.

Application of AD and thermal valorisation as 2nd generation biorefinery approach on Italian biowaste, having high carbon content (higher than 40%) and high C/N ratio(10-30), has been conducted, which illustrates that AD is proven to have less environmental impact along higher valorisation (Demichelis et al., 2019).

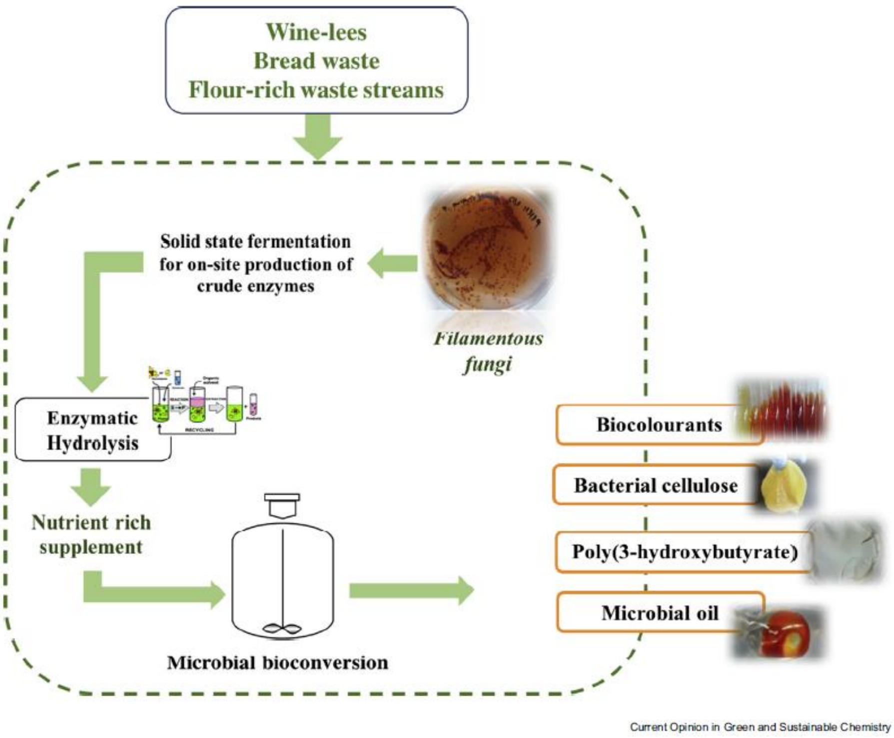


Fig.7 Example of bioprocessing for waste valorisation (Maina et al., 2017).

It has been already discussed that CE and biorefinery approach is the future of current economy and especially food industry. Through food industry, the industry with large economic and environmental contributions is wine industry. It worth introducing generally the grape market, its characteristics, and potentialities.

1.17. CAVIRO

The so far provided information highlights the future approach toward food and food waste of world economy. Italy is a leading wine producer and second exporter contributing to around 20 percent of global wine market (Nomisma Wine Monitor on OIV data; Nomisma Wine monitor on GTI data). Being the leading company in Italy in wine production, Caviro plays an important role in Italian and world's wine and more generally food industry. Introducing Caviro as a just wine producer would be underestimation. To better understand the company, it would be beneficial to get to know company potentialities, policies, and developments. Caviro's R&D department is highly active toward investigating existing potentialities in the group and production line and studying novel technologies to exploit such potentialities toward development of the company.

First, to better understand Caviro's impact on Italian economy, it would be suggested to introduce its potentialities. The company exports wine to over 70 countries, leading to 64 million euros as turnover in 2018 (Caviro, 2019). Italian market corresponds to 74 percent of Caviro sales (Caviro, 2019). The company's revenue can be classified as 71% from wine, 18% from alcohol, must, tartaric acid, and 9-11% from energy, environment sustainability branch. The total revenue of company in 2018 was 330 million euros (Caviro, 2019). All in all, Caviro contains high potentiality for application of CE and biorefinery approach.

Caviro through the last decades has invested on changing approach toward circular economy, which has resulted in producing added value products and lowering environmental impact, Caviro has become a leading group through its subsideries (Caviro Extra and Enomondo). Therefore, the company is the first producer of alcohol and biomethane based on production volume. In addition, 99 percent of generated waste is used energy source (Caviro, 2019). As a result, it disposes only 0.66 percent of generated waste which has led production of 560,000 tons per year of processed agro-industrial waste annually (Caviro, 2019). Through application of CE approach, Caviro has also reached first place in wastewater treatment.

Through energy production from waste, the company has reach 114,000 MWh self-produced thermal energy and 109,000 MWh self-produced electricity, satisfying 40,000 families' demand (Caviro, 2019).

Through producing alcohol, tartaric acid, and other added value products from must, the group has successfully fulfilled its goals toward circular economy. However, for full sustainability and fully application of CE and bio-economy concept, Caviro has launched various projects to recover value-added products from its waste streams before sending to AD stage. Through economical evaluations and market value, interest has been gained by bioactive compounds recovery, especially polyphenols. Polyphenols contain high potentiality and even have negative environmental impact if disposed inappropriately.

1.18. Grape Market

Grape is the second largest fruit crop cultivated globally; from which 80 percent is used to produce wine(Oreopoulou & Russ, 2007). Globally, *vitis vinifera* is the most cultivated grape for wine production (Barba et al., 2016). Wine industry was estimated to have 11 billion euros market (Zion Market Research, 2017). In such market, Spain, China, Italy, Turkey, and France are responsible for around half of world's wine production in 2019 (Ahmad et al., 2020). Obviously, grape market has large contributions on world economy and the amount of food waste.

Global wine production in 2013 was 281 million tons (Da Ros et al., 2014); With 27 million tons of grape production, Europe plays an important role in 50 million tons of global grape production (Scoma et al., 2016). For which Italy, France, Spain contributed for 46 percent of it (Barba et al., 2016). Italy is the leading country in wine production (OIV, 2019) with production of 54.8 million hL, corresponding to 19 percent of global production (Ncube et al., 2021).

Corresponding waste generated in grape market are namely stalk, grape pomace (50% skin, 25% seed, 25% stem), wine lees, and wastewater. Such food waste in the industry is highly potential feedstock for biorefinery concept (Jin, Yang, et al., 2018). To take advantage of such potentiality, generated waste, characteristics are required to be discussed.

1.19. Grape base waste generation and their classification and characteristics

Generally, winery wastes are classified as compounds with high levels of chemical oxygen demand (Barba et al., 2016). Among which grape pomace corresponds to 25% (w/w) of processed grape (Sirohi et al., 2020). Grape pomace containing mainly skin and seeds as solid, which contribute to around 75 and 28 percent of total solid waste, respectively (Brenes et al., 2016). Further detailed data required for ensuring the waste potentiality.

Grape pomace contains sugar, pigments, phenolic compounds, tartaric acid, fibre which are widely used as food additives. Besides, grape seeds have linoleic acid, omega-6 fatty acids 17%, and phenolics around 6 percent (Ciuta et al., 2011).

M. Tacchini et al. study in 2018 investigated grape samples from Caviro, indicates that white grape pomace is rich in flavonols, while red grape pomace highlighted presence of anthocyanins, and grape seeds are mainly characterized by procyanidins.

The provided data already justifies the utilization of grape pomace as feedstock for biorefinery approach, however further investigation through their amount, composition, and bioactivity is required.

1.20. Winery waste and by-products usage, amount, and composition

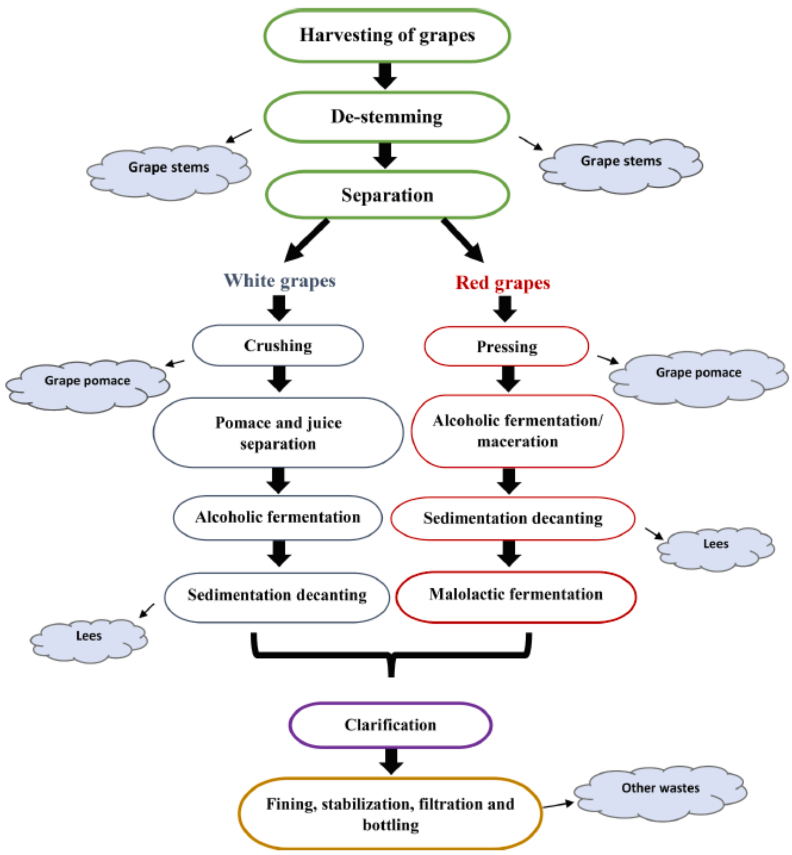


Fig.8 By-products of wineries (Sirohi et al., 2020).

Before discussing available compounds in grape pomace, it worth mentioning conventional usage of the grape pomace and other grape based wastes. Grape pomace containing grape skin can be a good source for animal feed (Arvanitoyannis et al., 2006). Grape stalk contributing to large portion of grape based wastes (5 tons per hectare per year) are rich in lignocellulosic compounds which are used for composting and soil fertilisers (Barrantes Leiva et al., 2014; Begalli et al., 2009; Nerantzis & Tataridis, 2006). Grape seeds correspond to 30 percent of wet pomace can be used to extract oil from them. While, grape stems contributing to 7 percent of grape raw matter, are usually used as animal feed or landfilled (Ahmad et al., 2020).

There are traditionally known as waste streams like lees which are rich in microorganisms, tartrate, and ethanol(Pérez-Bibbins et al., 2015). Ethanol and tartrate recovery have been successfully put in operation by Caviro as a step toward circular economy.

However, according to mentioned potentialities, the current waste management is not sufficient to exploit the potential of each present compound in the grape pomace. These insufficient techniques rise attention toward biorefinery application on grape pomace (Myrto Panagiota Zacharof, 2017).

1.21. Feasibility of valorisation process

Recovery of compounds from waste streams should meet market capacity, safety, and standards for human consumption; otherwise, it will not be feasible (Charis M. Galanakis, 2012).Feasibility parameters are the most important ones toward industrializing a proposed process. According to Research and Development department of Caviro, the current project has satisfied the feasibility parameters. It would be out of scope of this thesis to discuss the matter, since the main goal is to propose a techno-economic evaluation of polyphenol extraction from grape seed.

1.22. Extraction of high added value compounds

Extraction stage is the most important stage in valorising food waste; through which there are numerous technologies proposed and studied in literature (Charis M. Galanakis, 2012). In the same study Galakanis proposes a general 5 recovery stages, being: 1) macroscopic matrix pre-treatment, 2) molecule separation, 3) molecule extraction, 4) purification, 5) product formation. A good

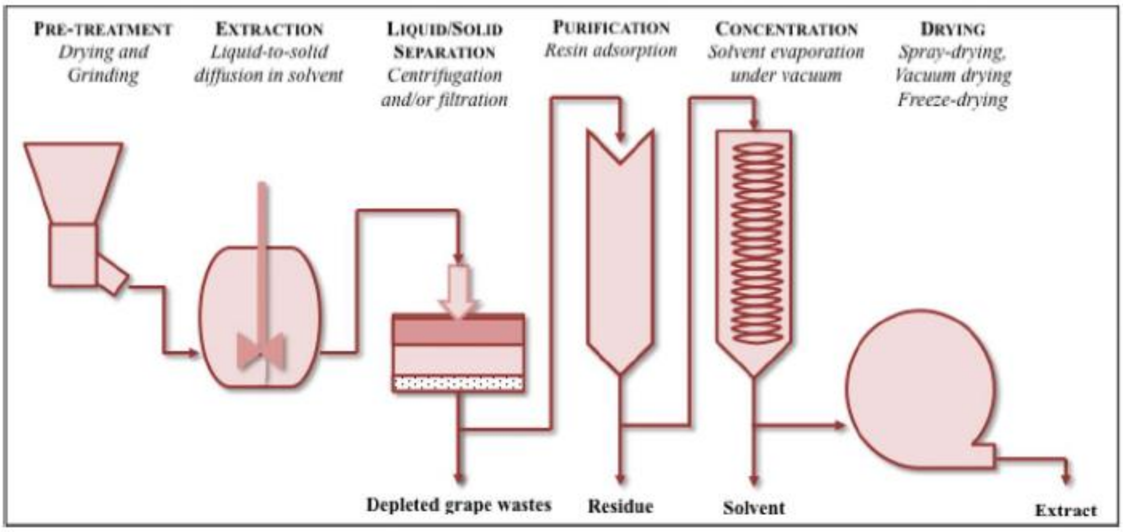


Fig.9 Conventional extraction (Yammine et al., 2018).

In general, food waste streams contain potentiality toward production of biofuel, enzyme, biogas, bio-chemical compounds, and biohydrogen through biorefinery approach(Octave & Thomas, 2009).

1.23. Grape seed bioactive compounds, composition, amount, …

From different components of grape pomace, seeds correspond to 40-50 percent of total solid waste generated in wineries (Sirohi et al., 2020). In addition, through research conducted on grape pomace, grape seeds are rich in bioactive compounds, followed by skin and pulp, results presented in table.2 are in compliance with such declaration (Chandra & Ramalingam, 2011; Pastrana-Bonilla et al., 2003). Therefore, grape seeds have been targeted for recovery of their added value compounds.

Grape seeds contain numerous high added value compounds from which the ones with considerable composition are oil (13-19%), protein (11%), non-digestible hydrocarbons (80%, mainly cellulose and pectin), significant amounts of phenolic compounds (flavonoids, procyanidins, and resveratrol), sugar and minerals (Brenes et al., 2016).

Among the present compounds in grape seeds, polyphenols, classified in Fig.9, have the highest economic value, which has resulted in increasing attention toward extraction of them. Besides, Phenolic compounds are covalently bound to complex polysaccharides present in cell wall (Sirohi et al., 2020) which leads to difficulties in extracting them. In detail, grape seeds are great source of oil and phenolic compounds; after defatting, residual seeds contain: 6.5 percent of moisture, 11 percent protein, 5.7 percent ash, 46 percent acid insoluble lignin, 1.4 percent acid soluble lignin, 8.1 percent extractives in water, and 5.3 percent extractives in ethanol (Prado et al., 2014). Grape seeds also contain vitamin E and its derivatives (Hong et al., 2009).

On the other hand, 60-70 percent of extractible polyphenols from grape pomace are present in seeds (Yilmaz & Toledo, 2006), 30-35 percent in skin, and a small portion (around 10%) in the pulp. Seeds' polyphenol weight composition is between 5-8 percent (Sirohi et al., 2020).

Further investigations on seeds residuals of polyphenol extraction indicates that de-phenolised residues of phenolic compounds extraction are good source for Polyhydroxyalkanoates (PHAs) production (Martinez et al., 2016). To complete the biorefinery approach, further studies must be carried out on the latter subject.

The study (Tuck, 2012) has reported composition of grape seeds under investigation as follow:

Compound	Composition in percentage
Moisture	25-40
Polysaccharide	36-46
Organic acid	2-7
Oils, fatty acids	13-20
Phenolic compounds	4-6

Table.1 Grape seed composition (Tuck, 2012).

It worth mentioning that bioactivity and concentration of bioactive compounds are different among grape types and even the same grape type from different regions; it has been proven that bioactivity and concentration are highly dependent on grape variety, location, fertilization conditions, soil, and harvest period(Brenes et al., 2016).

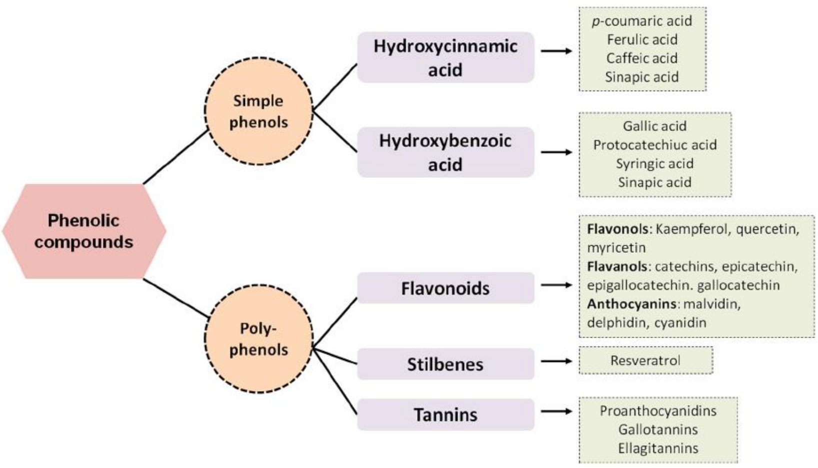


Fig.10 Phenolic compounds classification (Sirohi et al., 2020).

1.24. Polyphenol extraction justification, EU policy, polyphenol negative environmental impact

Application of anaerobic digestion on winery waste have shown some restrictions due to presence of polyphenols and copper (Melamane et al., 2007). Biologically, high concentration of phenolic compounds leads to acidic conditions (low PH), and due to their antioxidant activity, they prevent biological degradation; these two characteristics of phenolics make them environmentally hazardous (Kalli et al., 2018). In addition, Due to antimicrobial potential of polyphenols, their separation from grape pomace prior to entering AD improves fermentation process (Lucarini et al., 2018). While, by recovering them from grape pomace, not only environmental contribution can be prevented; but also it can lead to positive effect on environment and contribute to economic and health benefits.

According to definitions provided earlier in compliance with European Commission classifications, polyphenol recovery from grape seeds can be classified by as reuse or direct reuse (Thierry et al., 1995) for production of a by-product aiming at lowering waste generation. Choosing polyphenols as desired compound to be extracted, its characteristics and potential market needs further research.

1.25. Polyphenol characterisation, classification, amount, and potentialities

Phenolic compounds are present in vacuoles of plants and lipo-protein bilayers (Agati et al., 2012). Grape seeds contain a wide range of compounds with high added value potentialities, which are nutraceuticals like phenolic compounds (gallic acid, hydroxybenzoic and cinnamic acid derivatives, quercetin, kaempferol, monomeric flaval-3-ols, i.e. (+)-catechin, (+)-epicatechin, gallo-catechin and epicatechin 3-O-gallate, procyanidin dimers, trimers, and more highly polymerized procyanidins) (Xu et al., 2010), unsaturated fatty acids (Durante et al., 2017; Garavaglia et al., 2016; Shinagawa et al., 2018), vitamin E, carotenoids, and phytosterols (Giannini et al., 2016).

Phenolic compounds are effective agents against ultraviolet radiation, pathogens, and environmental stress (Koubaa et al., 2015). Besides, phenolic compounds are well soluble in hydro-alcoholic mixtures (C. M. Galanakis et al., 2013; Tsakona et al., 2012) which is exploited characteristic in extraction process. Along phenolic compounds contribution to wine colour, through various studies their medical usage has been investigated (Manach et al., 2004; Vilkhu et al., 2008).

Polyphenols which are present in seeds are proven to have biological potentialities such as antiallergic, anti-inflammatory, anticancer, antiaging, antimicrobial and antifungal (presented in Fig.10), antioxidant, insulinotropic, anti-lipotropic, cardio protective, and vasodilatory impacts (Haminiuk et al., 2012). As an indication the antibacterial and antifungal activity of such compounds are listed as follow:

	Antibacterial (MIC μg/mL)	Antifungal (EC_{50} μg/mL)	
	Pseudomonas syringae pv. *syringae*	*Sclerotinia minor*	*Sclerotinia sclerotiorum*
VCR UAE	>1000	n.d.	n.d.
VCR NAV	>1000	n.d.	n.d.
VCB UAE	>1000	n.d.	n.d.
VCB NAV	>1000	n.d.	n.d.
VIN UAE	>1000	>15	>15
VIN NAV	>1000	>15	>15
Positive Control	125	<0.5	<0.5

Table.2 Antibacterial and antifungal activity of grape pomace extracts, from red grapes (VCR), white grapes (VCB), and grape seeds (VIN) (Tacchini et al., 2019).

	Pulp	Skin	Seeds
Tannins	Trace	100–500	1000–6000
Anthocyans	0	500–3000	0
Phenolic acids	20–170	50–200	0

Table.3 Mean concentration of phenolic compounds (Enological Chemistry, 2012).

Having antioxidant, antiviral, antimicrobial, and anti-inflammatory properties, polyphenols are used as animal feed, feedstock or additive for cosmetics, pharmaceuticals, food , fertilizer, biomass, and biofuel industries (Sirohi et al., 2020).

Polyphenols have shown capability of fighting against development of chronic diseases like diabetes, cardiovascular, carcinogenic, neurodegenerative diseases(Georgiev et al., 2014; Iriti & Varoni, 2014; Kalli et al., 2018; Nassiri-Asl & Hosseinzadeh, 2016); through their antioxidant activity, which is proven to be more effective than vitamin C and E, and β-carotene (Kalli et al., 2018).

Through clinical tests, grape seed polyphenols effect on insulin secretion and glucose level have been tested and proved that such polyphenols have insulinotropic effect which makes them effective toward diabetes treatment (De Groote et al., 2012; Sapwarobol et al., 2012).

In addition, polyphenols extracted from grape seed have anti-cancer potential which has been studied in various articles through various mechanisms (Apostolou et al., 2013; Dinicola et al., 2014; Hamza et al., 2018). Grape seed origin polyphenols also have been proven to have positive effect interfering neurodegenerative diseases like Alzheimer through its antioxidant characteristic (Ma et al., 2014).

1.26. Polyphenol recovery benefits, and its usage

Grape seeds are proven to have health beneficial impacts through numerous articles, for which the starting point is the antioxidant activity of existing compounds (Agarwal et al., 2007; Castrillejo et al., 2011; Chou et al., 2010; Engelbrecht et al., 2007; Erdemli et al., 2017; García-Lomillo et al., 2014; Kar et al., 2009; Kaur et al., 2008; Keser et al., 2013; Montagut et al., 2010; Romani et al., 2006; Sano et al., 2007; Souza et al., 2014).

Further research has revealed that capsulated grape seeds' extracts in nanovesicles have shown contribution through intestine disorder treatment due to their antioxidant activity (Manca et al., 2020). There is also evidence indicating polyphenols anti-depressant activity (Rabiei et al., 2017).

Besides, grape seed extracts contain anti-aging compounds whose effect has been tested clinically by American Dermatology (Letawe et al., 1998). Gallic acid as an example of such compounds has been proven to be effective toward inactivating aging enzymes of skin (Wittenauer et al., 2015).

On the other hand, grape pomace extracts and generally winery by-products are highly bioactive; which allows inhibition of food born bacteria(Friedman, 2014). According to such potentiality, grape pomace extracts contribution toward food safety is under further investigation (Kalli et al., 2018).

All characteristics and parameters discussed by now, indicate that recovery of polyphenols from grape seed will contribute to environmental, economic, and even health benefits. However, before fully justifying the process feasibility, the most important industrial factor should be discussed.

1.27. Extraction techniques, shift to novel technologies, solvent choice

Conventional extraction processes were based on solvent extraction and increasing mass transfer by temperature increment; while novel approaches like PEF (pulsed electric field), ultrasound assisted, high pressure extraction techniques (Charis M. Galanakis, 2013) aim at enhancing mass transfer without temperature increase which may lead to degradation or decomposition of desired compounds (Barba et al., 2016). To ensure the perfect extraction, solvent needs to be chosen.

Being cheap, renewable, present in wineries (Yammine et al., 2018) and having GRAS (Generally Recognised As Safe) status according to FDA (Food and Drug Administration) makes ethanol a preferable solvent for extraction of food waste derived phenolic compounds (Charis M. Galanakis, 2012). While, extraction with hydrotropic solvents has resulted in lower antioxidant capacity when optimized to ensure high total polyphenol concentration in extract (Rajha, Chacar, et al., 2015).

1.28. Novel extraction methods and their goal

Due to limitations of conventional extraction techniques, various studies have suggested novel technologies for extraction of sensitive compounds from food waste. Novel technologies goals are reduction in solvent consumption, increase extraction yield, decrease extraction time, lower energy consumption, and higher extract quality (Chemat et al., 2015; Li et al., 2012; Rombaut et al., 2014).

Novel extraction methods mentioned in (Charis M. Galanakis, 2012) are as follow:

1. Microwave assisted extraction: increase of solubility through temperature increase (Banožić et al., 2020).
2. Super critical fluids (CO_2) extraction: highly selective
3. Subcritical fluid (CO_2, water) extraction (J. Zhang et al., 2020)
4. Ultrasound assisted extraction: Ultrasound waves accelerate extraction by creating cavities which enhances heat and mass transfer. It also increases efficiency by lowering extraction time (Nayak et al., 2018).
5. Electrically induced extraction (PEF and HVED): non thermal approach
6. High pressure processing (HPP) extraction (Filipe et al., 2020; Pereira et al., 2019)

7. Enzyme assisted extraction (EAE) (Stambuk et al., 2016; Tomaz et al., 2016).
8. Cold plasma extraction mostly used as pre-treatment (Bao, 2020).
9. Steam distillation (DIS) (Tacchini et al., 2019).

A brief description on each technology has been provided to make their evaluation easier. To find the best technology among mentioned strategies a comparison is also required.

Supercritical fluid (CO_2) extraction (SFE) of polyphenols from grape pomace experiments in lab and pilot scale followed by filtration steps and finally feeding the residues to AD has been conducted (Martinez et al., 2016).

Subcritical fluid (CO_2) extraction (SbFE) of polyphenols from white grape seeds at around 35-55^{o}C with ethanol and methanol as organic modifier has resulted in improvement in gallic acid, catechin, and epicatechin extraction (Palma & Taylor, 1999).

Ultrasound assisted extraction (UAE) contributes to cavity formation due to ultrasound waves; which lead to microjets creation destructing cell walls to permeate intracellular compounds (Vinatoru, 2001). Ultrasound assisted extraction performed by (Da Porto et al., 2013)Da Porto shows similar results compared to Soxhlet extraction (14g GAE/100 g DM).

Pulsed electric field (PEF) assisted extraction takes advantage of transmembrane potential; above a certain threshold of which intracellular permeability increases (Knorr et al., 2001; Zimmermann, 1986). Low intensity of electric field has resulted in increment of total polyphenols in the juice; whereas, increasing electric field does not necessarily increase total polyphenols concentration (Balasa et al., 2006). However, electric field results in cell collapse and solubilization of intracellular components (Rajha, Boussetta, et al., 2015).

Grape seed extraction with HVED (high voltage electric discharge) with supplementary ethanol extraction has resulted in polyphenol extraction equal to 9 g GAE/100g of DM (dry matter (N. Boussetta et al., 2013).

High pressure processing (HPP) extraction of grape seed and skin results in 10.8 g GAE/100g DM of polyphenols extracted from seeds and 3.4 g GAE/100g DM from skin (Casazza et al., 2010, 2012).

Enzymes like cellulase, β-glucosidase, xylanase, β-glucanase, pectinase break the polymeric chain of polysaccharides which results in releasing bounded compounds like polyphenols (Kumar et al., 2017). Enzyme assisted extraction (EAE) has resulted in extraction of mostly monomers and dimers of polyphenols due to acidic condition (Mattivi et al., 2009).

1.29. Pros and Cons of various technologies and comparison among them

As mentioned earlier, each strategy utilizes a specific characteristic of the extraction media to enhance mass transfer. Comparing different technologies and getting to know each technology's disadvantages, are helpful toward choosing the best technology serving our goals.

Based on mass transfer rules, grinding should increase mass transfer, which potentialities and drawbacks are well explained in table.4. However, conventional grinding leads to temperature increment in plant matrix causing degradation of desired components (Khanal et al., 2010); in addition, because of small particles, difficulties have been observed in filtration and purification stages (Yammine et al., 2018).

High pressure and high temperature extraction methods are not favoured due to high operating cost and thermal sensitivity of desired bioactive products (Barba et al., 2016). Therefore, exploiting other potentialities are more favoured.

As a novel approach, MAE contributes to the highest amount of extracted polyphenols (Bittar et al., 2013), while the operating cost of such technology is higher than UAE. Still MAE is industrially attractive and discussed through number of articles (Diaz-Ortiz et al., 2007; J. Mason et al., 2011; Li et al., 2012; Tatke & Jaiswal, 2011).

SbFE of polyphenols from white grape seeds show considerable amounts of contaminants in the extract, and it requires high pressure (Barba et al., 2016). Similarly, application of HVED results indicate cell rupture and extraction of intracellular compounds (mainly proteins) (Nadia Boussetta & Vorobiev, 2014). SFE and steam distillation (DIS) approaches contribute to high amount of fatty acid extraction (Tacchini et al., 2019). Contaminations made lead to post-extraction difficulties in filtration stages and decreasing the shelf life of the end-product.

Low solvent consumption, dramatic decrease in residence time (Da Porto et al., 2013), low operating temperature, and low operating and maintenance cost highlights ultrasound assisted extraction as a greener and cheaper recovery method than the others. (Roselló-Soto et al., 2015) Although, achieving high protein and polyphenol concentration higher operating cost than HVED and PEF has been reported (Rajha et al., 2014). Besides, long exposure to Ultrasound waves may result in degradation of polyphenols, especially anthocyanins (Pingret et al., 2013).

Another comparison conducted by M. Tacchini et al (Tacchini et al., 2019), through their study has resulted in following results. Which indicates that UAE extraction yield is higher than Naviglio technology, while polyphenol content and their activity are slightly lower. However, industrially UAE is more favoured.

Sample	Extraction methods	Extraction yield (%)	Total phenolic content (milligram gallic acid per gram of dried extract)	Antioxidant activity –DPPH – IC50 (µg/mL)
VCR	UAE	25.13 ± 3.71	189.11 ± 5.95	10.99 ± 1.74
	NAV	10.42 ± 2.38	159.58 ± 1.57	15.31 ± 4.95
VCB	UAE	27.59 ± 1.68	116.44 ± 3.49	22.44 ± 2.30
	NAV	13.41 ± 6.25	106.11 ± 5.46	20.82 ± 3.72
VIN	UAE	11.61 ± 4.39	446.72 ± 22.16	5.44 ± 0.40
	NAV	9.52 ± 0.76	506.24 ± 55.91	4.30 ± 0.31

Table 4. Comparison among extracts obtained by ultrasound assisted (UAE) and Naviglio technology extraction (NAV) from red grape pomace (VCR), white grape pomace (VCB), and grape seed (VIN). (Tacchini et al., 2019).

There are some doubts about PEF effect on intracellular interactions, extracted compounds themselves, their bioavailability, and bioactivity (Cholet et al., 2014). Also de-condensation of tannins has been observed utilizing PEF (Delsart et al., 2014). Application of such technique industrially needs further investigation through extraction mechanism, bioavailability, and bioactivity of extracts (Yammine et al., 2018).

	Drying/Grinding	PEF assisted extraction	HVED assisted extraction	US assisted extraction
Advantages	Easy implementation – High extraction efficiency	– Low energy requirements – Low processing costs – High selectivity, particularly for anthocyanins	– Low energy requirements – High extraction efficiency	Easy implementation Easy-operating High extraction efficiency – High adaptability of the US devices to the different raw material
Drawbacks	– Overheating of the plant matrix – Possible degradation of the bio-components – High energy consumption – Increased difficulties during the filtration and purification steps – Poor selectivity	– Poor adaptability of the PEF apparatuses (generator & treatment cells) to the different raw material – High investment cost	– Possible degradation of bio-compounds – Treatment in batch – Limited lifetime of electrodes – Increased difficulties during the filtration and purification steps – Poor selectivity	– Possible degradation of bio-compounds – Erosion of the transducers

Table 5. Advantages and disadvantages of conventional and novel approaches toward extraction enhancement. (Yammine et al., 2018).

Application of EAE requires control over PH, enzyme dosage, temperature, bioavailability of enzyme, and extraction time (Stambuk et al., 2016). They make EAE a less attractive approach than UAE.

The comparisons indicate that UAE is more reliable and easier to be applied in existing plant. Also, the technology is less complicated and results in higher yield than most extraction methods. All these factors make UAE economically favoured toward extraction of polyphenols from grape seeds.

On the other hand, the comparison has been made based on basics of each technology and in lab or pilot scale. It worth mentioning some applications of mentioned technologies.

1.30. Novel extraction techniques application

Pinot Meunier grape seeds (red grape) are used for polyphenol extraction with operation conditions being, 600 exponential pulse in water (L/S=5), 20 kV/cm, 320 kJ/kg; effective treatment time is 6 ms at 50^{o}C; extraction for one hour with 50^{o} ethanol as solvent. The results show a reduction of residence time by a factor of 2 (N. Boussetta et al., 2012).

PEF application is studied during alcoholic fermentation to increase tannins extraction by 34 percent (Delsart et al., 2014). While, in case of fermented grape pomace the recovery of anthocyanins was enhanced (Barba et al., 2015).

Subcritical fluid extraction (SbFE) of polyphenols from white grape seeds has been optimised by Palma and Taylor (1999) (Palma & Taylor, 1999). Extraction of polyphenols using the same approach and with using ethanol as co-solvent has resulted in selective extraction according to solvent capacity (Murga et al., 2000).

Supercritical fluid (CO_2) extraction (SFE) of polyphenols from grape pomace experiments in lab and pilot scale followed by filtration steps and finally feeding the residues to AD has been conducted (Martinez et al., 2016).

High pressure and high temperature extraction of polyphenols from grape pomace at around 150^{o}C has resulted in the highest amount of total phenolic compounds (Casazza et al., 2012). As an application example, accelerated solvent extraction by pressurized water is mostly used for recovery of compounds present in grape skin (Stavikova et al., 2011; Vergara-Salinas et al., 2013).

UAE results in higher total polyphenol content than SFE and DIS, the highest amount extracted from grape seeds (Tacchini et al., 2019).

1.31. Extraction parameters and their optimization

Comparing three approaches investigated in the study toward applying biorefinery concept on grape pomace, the path through which grape seed oil (GSO), polyphenols (GSKP), and biochar (GB) are produced results in higher NPV, IRR, and lower payback period than GSO+GSKP and GSO; while GSO+GSKP shows better results than GSO. Biochar contribution on operating cost is considerable due to energy recovery. Through sensitivity analysis, it has been observed that grape pomace total polyphenol content, biochar, polyphenol selling price, and plant capacity have main impacts on NPV (Jin et al., 2021).

(Tacchini et al., 2019)M. Tacchini et al, 2018 article has obtained samples from Caviro which has made its results interesting for the ongoing project. Through their study, optimisation of extraction solution composition has been done by comparing red and white grape pomace and seeds, through varying ethanol percentage from pure ethanol to pure water range. The measurement of polyphenol content in each stream has been done by HPTLC (high performance thin layer chromatography). Which reveals that 50^{o} ethanol solution best fits our goal.

Another important parameter in extraction is solid/liquid ratio which should be determined based on experiments. Various solid-liquid ratios have been tested for the application in the range of 0.1-0.25 g/ml. The optimal results which correspond to highest efficiency was obtained around 0.2 (Shi et al., 2003).

For a sufficient extraction yield, pre-treatment of sample, solvent/sample ratio, type of solvent, particle size, time and temperature of extraction should be considered (Spigno et al., 2007; Yilmaz & Toledo, 2006).

1.32. Polyphenol extraction, purification, and concentration step

Extraction process are usually followed by concentration, purification, and formulation. The same approach should be conducted after extraction of polyphenols from grape seeds. Since there are some particles and seeds' residuals in the solution. Also, for optimizing the process ethanol recovery is required. Besides, there are other solubilised compounds reducing the shelf life which should be recovered from the stream. In one study, after extraction with ethanol solution, seeds residues have been removed and the solution has undergone a purification stage through addition of 95⁰ ethanol solution to precipitate impurities (Jin et al., 2021).

Besides, concentration of extracted solution can be done by vacuum distillation or as a new approach, membrane process. 250 Da membrane has led to concentration of polyphenols up to 6.3 times the initial concentration (Díaz-Reinoso et al., 2009).

Novel extraction technologies are mostly studied in lab or pilot scale, whereas for industrial production, equipment and operation conditions analysis are required (Barba et al., 2016). The ongoing project aims at filling such gap toward industrial application of UAE and its techno-economic evaluation.

1.33. Techno-economic evaluation for scale up in application

Techno-economic analysis evaluates the technical performance and economic values of proposed process scheme (Jin et al., 2021). Techno-economic assessments are essential prior to scaling up and commercialization of proposed technology. It gives indications of capital investment, operating cost, profitability, revenue, and future research and development of the plant (Jin, Yang, et al., 2018).

Internal rate of return (IRR) is defined as discount rate which results to zero NPV after tax (Humbrid et al., 2011) or plant profit at time value money, and yield of investment (Kwan et al., 2015). Which is an indicator widely used for economic assessment. As an example, (Jin et al., 2021) study uses NPV, IRR, and pay-back period as economic indicators.

1.33.1. Prices and indicators

It worth mentioning the market price of extractible compounds from grape seeds. Grape seed oil = 4 USD_{2019}/kg, polyphenols = 20 USD_{2019}/kg, biochar = 2.47 USD_{2019}/kg (Jin et al., 2021). These prices are essential for a reasonable economic assessment.

1.33.2. Environmental, social, and safety evaluations

Environmental assessment of biorefinery approach as was predicted has been proven through GHG emission, air and water pollution, resource depletion through cradle to gate life cycle assessment (A. S. Nizami et al., 2017).

Social assessment considering land ownership, local stewardship of common property resources and labour rights can be done to ensure social aspects of the proposed biorefinery procedure (A.-S. Nizami et al., 2016). Being a battery limit plant makes such evaluation a little bit irrelevant for our case.

2. Objective

The ultimate goal of our study is meeting circular economy perspectives of CAVIRO in compliance with European commission. To meet this goal, CAVIRO has implemented numerous strategies to satisfy the aim of circular economy. The most important or general strategy which has been implemented is waste reducing approach. This approach has led to waste valorisation, reusing, repairing, recycling of material. By reducing the disposed waste, production would go toward circularity rather than linearity.

Through analysis, grape seeds have the highest capacity for valorisation. Compounds which have the highest added value are polyphenols. Polyphenols need to be extracted and processed so they could be used in other sectors or as additives in wineries. The main goal is to evaluate different methods of extraction and comparing them with conventional extraction method present in the pilot plant. Comparison has been conducted among numerous methods via technical properties. The options have been narrowed down to 2 extraction options and then opportunities for filtration, purification and formulation have been considered.

Technical evaluation has been introduced in the introduction where the microwave assisted extraction, supercritical fluid extraction, ultrasound assisted extraction and conventional extraction have been involved, the results narrowed down the options for our extraction to ultrasound assisted extraction and conventional extraction. Technical comparison will be further discussed in our study based on literature and observations.

The most interesting part of our study is economic evaluation of the results of technical comparison. Since conventional extraction equipment is present in the plant, economic evaluation of it would be easy and ready, while for the remaining options detailed data from suppliers are required. Since, the objective is to provide a business plan for a proposed option, CAPEX, OPEX, NPV, and ROI has been evaluated and used for comparison and as indicator for the options.

The proposed process requires to be optimized to increase efficiency. An early stage study has been conducted on the ongoing process in pilot plant. Ethanol recovery has been determined as a variable which requires optimization. The optimization has been done first, numerically, then by experiments in lab scale and has been proposed as a verified optimization to be performed in upcoming productions in pilot plant.

By having all the assessments and evaluations, a well-designed business plan in compliance with the company's circular economy goals is projected and prepared in the current study. The provided business plan is accompanied by time schedule (cronoprogram) to give better indication for scaling up and reaching industrial scale production.

3. Material and Methods

The production in lab and pilot scale is considered batch. Since the operation conditions like temperature, pressure, and more importantly product quality can be easily controlled and monitored. In addition, since the production is considered seasonal production, batch mode would be the best choice. Besides, cleaning and sterilization would be more feasible in pilot plant production. Besides, considering 100 days of production in pilot plant would meet company's needs currently.

3.1. Extractors

Based on technical properties and feasibility of the different technologies present for extraction, number of extraction chambers are introduced. However, as compared in the introduction section, the sufficient, economic, and simple apparatus for extraction are conventional stirred tank (ST) and ultrasound assisted extraction chamber proposed by the Russian company. Extractors which are currently used in pilot plant are tanks with 500 L capacity, equipped by a side propeller as mixer, and a jacket. There are 4 tanks to increase the production and have a better efficiency.

Fig. 11 Stirred tank extraction chambers.

3.2. Filtration and seed separation

There are two stages of separation. One between the first and second stage of extraction in conventional mode, one after the second stage of extraction, and a micro filtration which should be implemented to avoid contamination of the single stage vacuum distillation unit. The first two are the filters which separate the seeds. Filter bags have been used in pilot plant with some modifications for seed separation. However, for higher production and automatic production, decanter separators have been considered and asked for data from suppliers. The data will be presented in corresponding section.

For the last stage of separation, filter with smaller pore size is required to separate the seed residuals and skin which is present in the feedstock. These contaminants are better to be separated to ensure quality of product and longer life of the single stage distillation unit. For such purpose, currently the same filter bag with the subsequent manual filter and grid is currently used in pilot plant and it is projected to use a centrifuge from the same company to have an automatic production.

3.3. Single stage distillation unit (ethanol recovery)

The unit consists of a vacuum pump, a still, a shell and tube condenser, a double pipe reboiler, a recirculation pump, an extra condenser for non-condensable fraction, and a washing system. The unit has the capacity of 100 L/h. The unit has been provided by REDA. The vacuum pump provides the pressure of -1 barg which is required to evaporate the solution at around 25-30 ^{o}C with respect to the change of composition by evaporation of ethanol. Besides, a still is present where the solution is evaporated by the heat provided by an external double pipe heat exchanger. On the top, there is a shell and tube condenser where the evaporated solution which is mostly ethanol and water is condensed and stored in a tank. The non-condensable part is condensed through a small exchanger with lower operating temperature. All condensed streams are stored in the same tank. The recirculation pump ensures the efficient heat exchange from double pipe and efficient evaporation. In addition, a washing system with corresponding pipes has implemented to wash the whole unit at anytime required.

Fig. 12 Single stage distillation unit (Dealcolatore)

3.4. Purification

For purification stage a spray dryer is used to produce a powder from the extract which is the bottom product of the single stage distillation unit. The quality of extract intended is around

20-25 ^{o}Bx. Spray dryer supplier is a French company which has suggested the mentioned quality for better performance of the spray dryer. The spray dryer is necessary for providing longer shelf life for the extracted polyphenols.

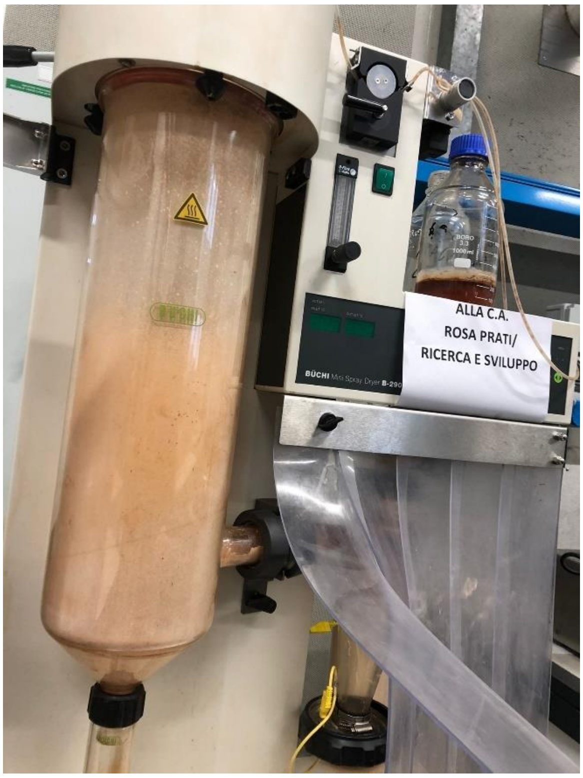


Fig. 13 Spray dryer

3.5. Solution for extraction

In both ultrasound assisted and conventional extraction, the solution required for extraction is the same. The solution is water and ethanol with 50% v/v of ethanol content. The solution is provided by mixing ethanol present in the production plant and delivered to pilot plant in containers with 1000L capacity with purity of 96% v/v and demineralized water existing in the plant as utility.

3.6. Grape Seeds

Seeds are provided from the wine production plant. The seeds are the residuals of the white wine production which were disposed or fed into Anaerobic Digestion unit. Seeds are delivered in bags to the pilot plant. It has been projected to have industrial scale production, a screw feeding line is required.

3.7. Electricity

Electricity is required as utility to operate pumps, boiler providing the heat required for extraction chamber jackets, vacuum pump, and agitators. Electricity required for production has been evaluated based on each equipment's requirement.

3.8. Water

Water is used in the whole process as heating and cooling media. Water flow is heated in an external boiler to provide heat in extraction chamber's jacket and double pipe reboiler of the single stage distillation unit. Water is also provided as cooling media for condenser.

3.9. Pump

During pilot scale production, a small portable pump is used for transferring ethanol to extraction tanks, transferring solution from first tank to the second one, and transferring the solution to reservoir tank of the single stage distillation unit.

3.10. Agitator

For an efficient extraction, mixing of the seeds and extraction solution is required. For such purpose, an automatic agitator with fixed rotation speed is connected to a shaft which has a propeller at its end. Propeller has been determined, to have both axial and radial mixing. However, in some cases mixing has shown inefficiency due to the distance of propeller from the bottom of the tank.

3.11. Jacket

It has been determined to operate the extraction stage at around 40^{o}C. For such purpose around each extraction vessel an external heating jacket has been assembled. All jackets are connected to the boiler. The outlet of the boiler is connected to 4 automatic valves corresponding to 4 tanks. These 4 automatic valves are connected to a control panel, where the temperature is set to 40^{o}C

3.12. Reservoir tanks

Two tanks are used as reservoir. One for collecting the single stage distillation unit feed with the capacity of 1000 L. Another with the same capacity for collecting the alcohol and water solution which is the top product of the distillation unit. There is a spare tank for collecting the residual of the top product with capacity of around 400 L. There are other two tanks for collecting the bottom product which is the final product in pilot plant.

3.13. Product specification

The bottom product is tested in laboratory with brix measurement machine. Distillation is stopped when the quality reaches 20-25^{o}Bx. The degree is crucial for efficient use of the spray dryer. However, for other usage, higher degree of brix can be reached.

3.14. CAPEX, OPEX, NPV, ROI

Technical evaluation is based on literature and laboratory. While, for economic assessment, there are well accepted indicators for economic evaluation. CAPEX which is capital expenditure or capital expense is the money an organization or corporate entity spends to buy, maintain, or improve its fixed assets, such as buildings, vehicles, equipment, or land. While the land cost and equipment cost which are the same in the options are better to be neglected for better comparison.

OPEX, which stands for operating expenses or expenditure, refers to the costs incurred by your business via the production of goods and services. It can include a broad range of expenses, including materials, labour, machinery, packaging, shipping materials, and so on. Which in our case is based on labour and utility mostly which is based on different equipment used in different options.

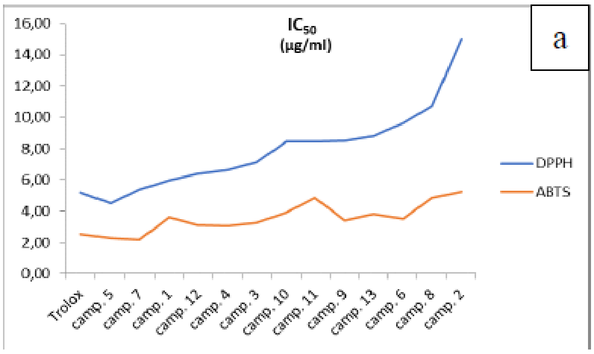
In finance, the net present value (NPV) or net present worth applies to a series of cash flows occurring at different times. The present value of a cash flow depends on the interval of time between now and the cash flow. It also depends on the discount rate. NPV accounts for the time value of the money.

Return of investment (ROI) is a performance measure used to evaluate the efficiency or profitability of an investment or compare the efficiency of a number of different investments. ROI tries to directly measure the amount of return on a particular investment, relative to the investment's cost.

4. Selling the product and product quality

Although obtained polyphenols are planned to be used as wine additive inside the company, the product should be compared with the polyphenols present in the market. The quality comparison has been carried out among the polyphenol extracts produced from different seeds and in different time of the year. To eliminate time gap and shelf-life factors, all produced extracts were fed to spray dryer right after production. So, products in the shape of powder have been sent to a laboratory outside the company to perform DPPH, ABTS, and FRAP tests to compare their bioactivity and H-NMR to compare their polyphenol content and sugar content among themselves and with other polyphenols present in the market.

The bioactivity has been measured as antioxidant activity and expressed in IC_{50} (ABTS and DPPH tests) and in mMol of Trolox per gram of dried extract (DPPH, ABTS, and FRAP tests). Although through the conducted tests there are some deviations, the goal is to compare the products with the best products in the market.



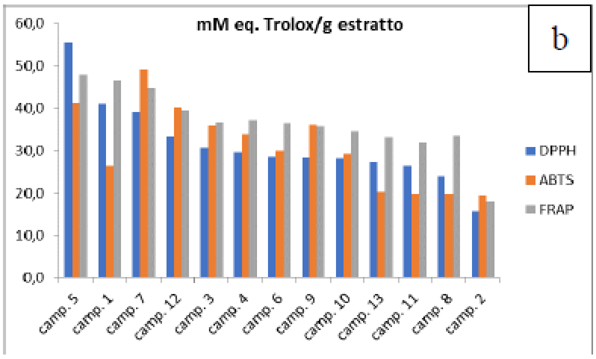
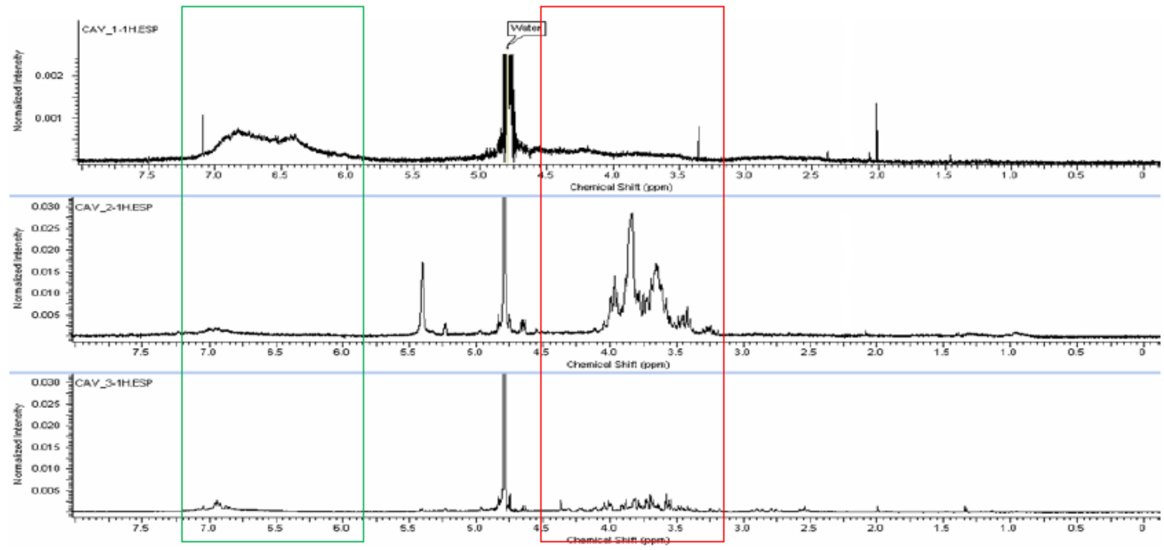
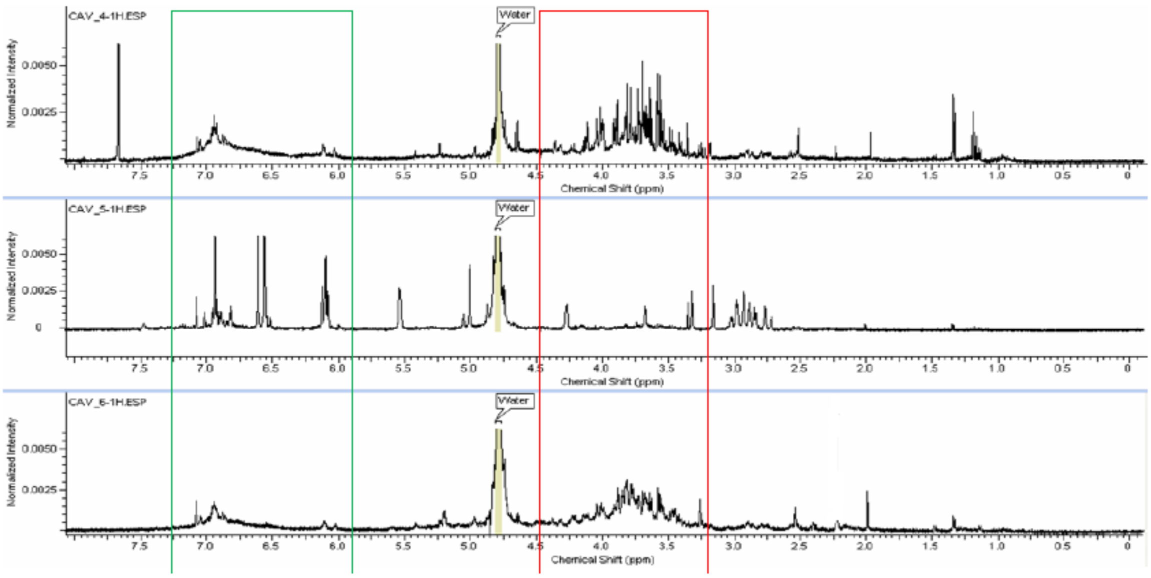


Fig. 14 Trend of antioxidant activity of samples 1-13 in order from most active to least active, expressed as IC50 (Fig. a) and mMol of Trolox / g of dry extract (Fig. b)

Above figure illustrates the antioxidant activity of our products and the ones present in the market. Among the samples, samples numbered from 9 to 13 are our products obtained in Caviro. Sample 9-11 are obtained in lab scale from different grape seeds, sample 12 obtained in pilot plant with conventional extraction, and sample 13 obtained in pilot plant with an existing ultrasound assisted extraction chamber. Besides, samples 4 and 5 are one of the best in the market. The results of the three tests show slightly different results regarding the order of activity of the samples and this could depend on the different specificity of the three assays. In any case, all tests agree in indicating samples 5, 7 and 12 as the most active, while samples 11, 8 and 2 as those that express the least antioxidant activity.

On the other hand, product obtained in the pilot plant shows compatibility with the best product in the market. Therefore, polyphenols production in pilot plant seems to have comparable results with the market. Sample 12 produced in pilot plant with perfect conditions shows good and compatible results in all assays. For further comparison and to know about the polyphenol and sugar content of the products, proton NMR spectra of the samples are as follow.





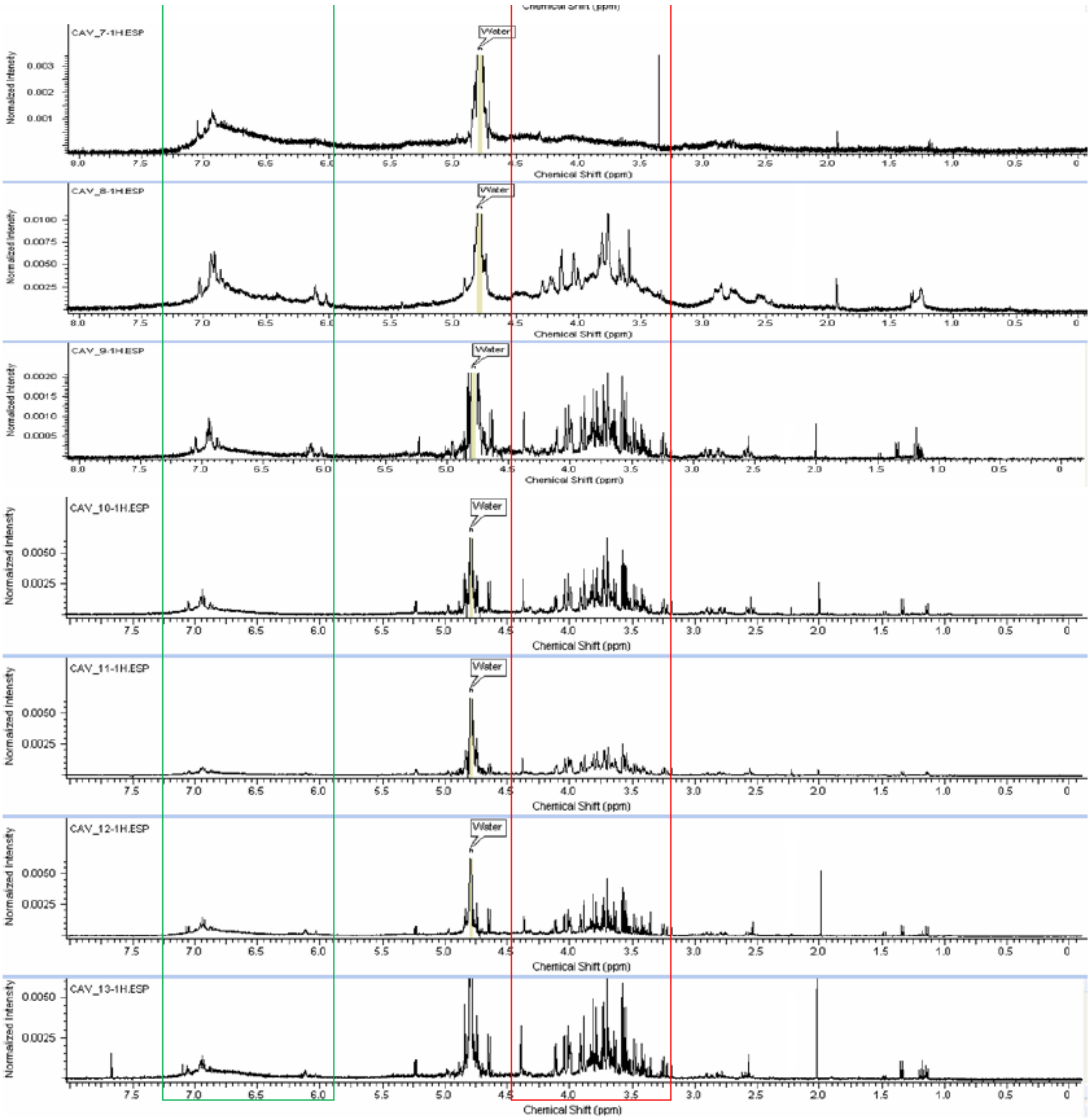


Fig. 15 1H-NMR spectra of the analysed samples; the polyphenol zone is highlighted in green, while the sugar zone is in red.

Samples 1, 5 and 7 (Fig. 15), which alternate in the first places in terms of bioactivity in the three tests, do not show a detectable presence of sugars. We reserve the right to repeat the analysis of samples 1 and 7 at a higher concentration to bring out the signals in the area of polyphenols. Analyses of the remaining samples revealed different relationships molars between sugars and polyphenols, on which the differences in activity could depend antioxidant, and hard-to-interpret signals in the area between 6 and 7 ppm that they could be attributable to procyanidin polymers naturally present in grape seeds. Particularly interesting is sample 5 which shows, both in the DPPH and ABTS tests, an activity higher (=> IC50 lower) than that of the positive control (Trolox; Fig. 14a) and a fairly simple NMR spectrum (Fig. 16), indicating, in the region between 5 and 7 ppm, proton signals of flavanolic rings typical of procyanidins. These signals could give indications on the degree of polymerization of the molecules present in the solution, but this information needs a deeper look.

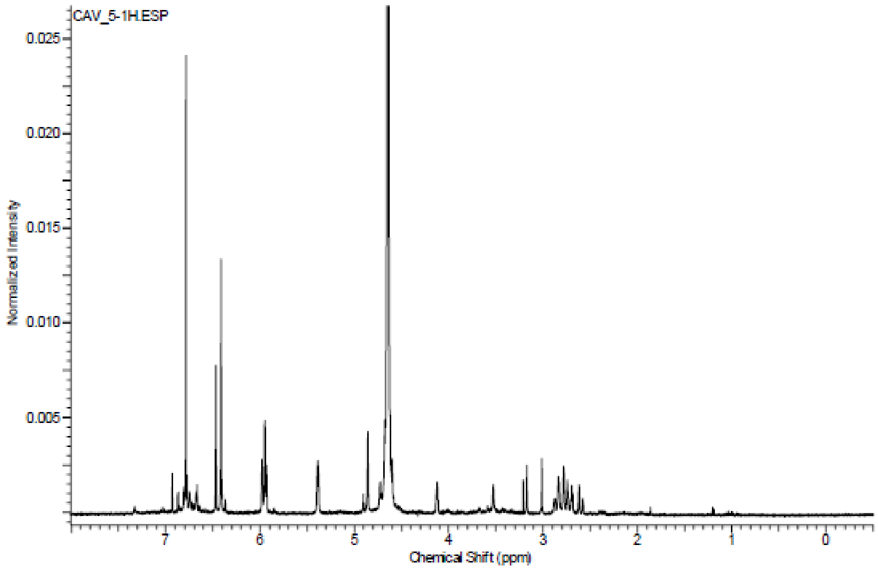


Fig. 16. 1H-NMR spectrum of the sample 5.

5. Set of experiments and their objectives

5.1. Lab-scale experiments

Extraction of polyphenols from grape seeds has been investigated for couple of years. According to company's goals and in compliance with circular economy goals of the company, the extraction process has been determined as a high added value by-product production. Since extracted polyphenols can be used to improve wine quality, the production is aimed at fulfilling internal need and then scaling up for extra production which can be sold.

During my internship, I have carried out extraction in lab scale and pilot scale. In lab scale experiments, extraction was carried out in 1L Pyrex bottles. For extraction an ethanol solution was provided with the volume of 850 mL with ethanol concentration of 50% v/v. To respect previous experiments and according to literature research, to have a sufficient polyphenol extraction, liquid solid ratio of around 5 fits extraction goals. According to literature review which has been presented in the introduction, L/S ratio of 4.75 has been considered for our case. So, in each Pyrex bottle, 170 g of seeds has been added. Pyrex bottles have been put in a chamber were the temperature and agitation speed could be controlled. The optimal extraction temperature has been determined as 40^{o}C and rotation speed equal to 200 rpm. Agitation was performed by a surface were the pyrex bottles were placed on it and fixed by jackets which were responsible for heat transfer. Extraction process was conducted under mentioned conditions for 4 hours.

After four hours, bottles were taken out. Then, the solution was separated from exhausted seeds and the volume was measured. Then the solution was placed in a new bottle with fresh seeds. The volumed was decreased due to solution adsorption on seeds. Fresh seeds were added based on the same liquid to solid ratio as the first extraction stage. The extraction process like the first stage has been carried out in 40^{o}C and with agitation speed of 200 rpm for four hours. After 4 hours same measurements have been carried out.

Since there were not equipment for measuring the polyphenol concentration, samples have been sent to corresponding laboratories outside the company to measure the polyphenol concentration. Measurements show that around 11% of recovery could be achieved which was in compliance with the literature review.

1st Extraction							
	seeds(g)	Solvent (1:1ethanol/water) (mL)	Liquid/solid ratio (mL/g)	Ethanol (mL)	Ethanol 96^{o} (mL)	time (h)	Temperature (C)
Pyrex 1	180	850	4.72	425	442.71	4	40 (38)
Pyrex 2	180	850	4.72	425	442.71	4	40(38)
Pyrex 3	180	850	4.72	425	442.71	4	40(38)
Pyrex 4	180	850	4.72	425	442.71	4	40(38)
Pyrex 5	180	850	4.72	425	442.71	4	40(38)
Pyrex 6	180	850	4.72	425	442.71	4	40(38)

2nd Extraction							
seeds(g)	Solvent(mL)	Liquid/solid ratio mL/g	Ethanol (v/v%)	Ethanol (mL)	Ethanol 99^o (mL)	time (h)	Temperature (C)
148.2353	700	4.72	41.5	290.5	293.43	4	40(38)
148.2353	700	4.72	41.5	290.5	293.43	4	40(38)
137.6471	650	4.72	41.5	269.75	272.47	4	40(38)
148.2353	700	4.72	41.5	290.5	293.43	4	40(38)
148.2353	700	4.72	41.5	290.5	293.43	4	40(38)
148.2353	700	4.72	41.5	290.5	293.43	4	40(38)

Table 6. Lab scale extraction experimental data

5.2. Pilot plant experiments

According to each tank capacity, 80 kg of seeds has been determined for extraction. Liquid to solid ratio of 4.75 has resulted in preparation of 380 L of aqueous ethanol solution of 50% v/v. For preparation of extraction solution, the available ethanol had concentration of 96% v/v. So, a simple mass balance has been carried out as follow:

$$V = \frac{380 * 50\%}{96\%}$$

So, V= 198 L. 198 L of ethanol (96^o) has been poured into the tank using a pump provided in pilot plant from the reservoir of ethanol with capacity of 1000 L. To reach our goal, 182 L of demineralized water has been added.

Agitator was started. Then 80 kg of white grape seeds has been added manually to the tank. The highest possible accuracy has been applied. When the seeds were inside the tank, water flow through the jacket has been started to ensure 40 ^oC condition of extraction. It should be mentioned that the set point was set to 38 ^oC to account for temperature rise due to friction caused by agitation. The tank has been carefully closed and extraction had been performed for four hours.

The mentioned procedure was carried out for two tanks (1 and 3). While the other two tanks were empty.

In the end of four-hour extraction, the extraction solution has been poured out from tanks no. 1 and 2 to tanks 3 and 4, respectively. The bottom outlet of each tank was opened and connected to a pump which allowed transfer of solution to the next tank. To avoid seed transfer, a grid was designed in the bottom end of each tank. While the wetted seeds have remained in the tank.

When the solvent was transferred completely, a new set of seeds have been added. Reasonably, solvent volume decreased to 310L. To perform the second stage of extraction with the same liquid to solid ratio 65 kg of seeds have been manually added. The procedure was the same as the first stage. Which means 4 hours agitated extraction.

5.2.1. Seeds discharge from tanks

In pilot plant seed discharge was carried out manually. However, an efficient system for seed discharge must be designed to make them ready for the next extraction cycle, each extraction cycle consists of two stages. While discharging the seeds, an interesting phenomenon has been detected. A layer of cake was formed between the layers of seeds remained in the tank after transferring the extraction solution. The cake mainly consists of skin, cell debris, and proteins. Cake formation should be avoided to recover more solvent after first extraction. In addition, it reminds that agitational mixing along with friction of seeds to each other may have provided the adequate shear stress to enhance collapse of cells present in the outer layer of seeds.

Fig. 17 Cake formation after solution discharge in ST extraction chamber

The cake which can be seen in the figure is responsible for inefficient discharge of the solution from the tank. Cake height depends on the type of the seeds provided for extraction. Since the solution is discharged from the bottom outlet, applied suction makes the cake layer rough enough to block the solution pathway. Such phenomena should be avoided. There are various techniques to avoid such phenomena like more efficient mixing with two impellers and lower rotation speed, increasing shaft length, and discharging with mixer turned on. From possibilities, discharging with mixer turned on has been tested. However, since the impeller is located with a long distance from the bottom and the cake is formed beneath the impeller, cake formation was not prevented as expected. Besides, other possibilities require modifications to the existing system which is not favoured by the company. Therefore, a practical solution to the existing process was needed. Based on literature one way to prevent cake formation would be aeration to destroy the structure of the cake as it forms. A test has been conducted under supervision of R&D department manager, using pressurized air and applying it by a pipe inside the tank as discharging the solution faces difficulties. It would be a temporary solution and requires further studies which could not be carried out during internship.

Fig. 18 cell debris and cake formation potentiality observed in lab scale experiments.

After another four hours, the extraction solution with the same procedure of transfer, has been transferred to a reservoir tank. From each cycle 275 L of solution has been obtained. The difference between inputted solvent and the obtained was assumed to be present in the seeds leftovers or evaporated. Further experiment has been carried out to evaluate the evaporated and adsorbed amounts. Since two cycles have been carried out, totally 550 L of solution were sent to reservoir tank.

5.2.2. De-alcoholizer

The one being utilized in pilot plant is DVR-100. Operating at vacuum conditions, DVR-100 is able to distil the introduced solution. Having capacity of 100 L/h, the process is expected to last for 6 hours. The solution has been filtered manually with a filter having pore size of 10 microns before entering the reservoir designed to feed RVD-100.

To start the process, first checks have been conducted to be sure every part was sealed and closed. When every part was checked, the vacuum pump was started accordingly to reach vacuum condition throughout the equipment. The pressure provided by vacuum pump was around -1 barg. Having vacuum pressure, the feeding line valve was opened. The vacuum pressure caused feeding of the still. When was finished, corresponding valve was closed. Then pumps of the equipment which were responsible for hot and cold-water circulation has been turned on. From Perry's chemical engineering handbook, table 2-214 and 2-305, it has been decided to set the set point of cold water at around 5-7 ^{0}C while for reboiler the setpoint was varying through distillation from 14 ^oC to 30 ^oC. After around 6 hours of distillation, 80 L of concentrated extract with the specification of 50 ^{o} brix along with 470 L of aqueous ethanol solution with 55.5% v/v have been obtained. From which the following results have been obtained. The most important one is reaching a solution containing 20 percent of total polyphenol.

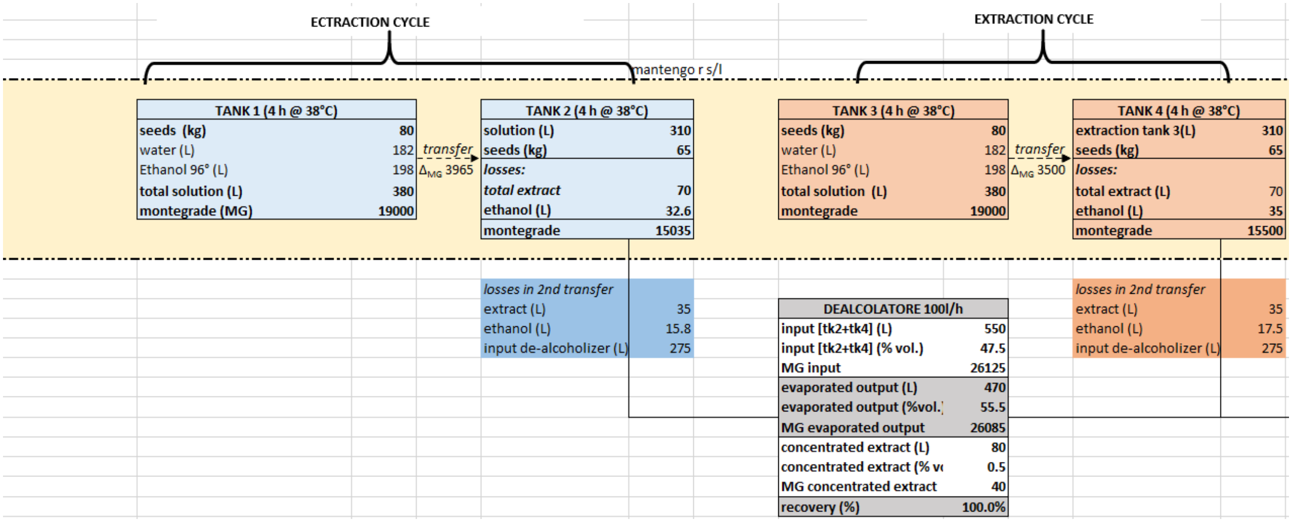


Fig. 19 Pilot plant extraction process scheme

5.3. *Pilot Plant PFD & stream specification*

5.3.1. *PFD*

For drawing the PFD, VISIO software has been utilized. In the scheme, 4 stirring tanks are indicated as they are present in the pilot plant. According to experiments carried out in the pilot plant, First, cycle is carried out using tank number 1 and 3, while for second cycle, tank no.2 and 4 are utilized. Stirring tanks are heated up to 40 ^oC using a jacket supplied by hot water. Filtration steps are shown currently as cross flow filtration stage, but further information is not present. Since, type of filter has not been determined yet.

The de-alcoholizer has been illustrated as a set of equipment such as a tank as still, double pipe exchanger as a reboiler and a total condenser system (reflux ratio is zero).

5.3.2. *Stream Specifications*

According to the experiments carried out, stream specifications have been indicated. However, there are some new elements, like density which has been obtained from literature (‘https://www.sciencedirect.com/science/article/pii/S0021961407000900’). There is ethanol content of slurry which is just an indication of the filtration feed, and it does not mean ethanol content of solution has decreased. All material balances are visible. To have an indication of total timing; tube sizing or flowrates which are feasible are required.

5.3.3. Ethanol Content of wet seeds

This value has been obtained by distilling seeds after extraction in experiment which has been carried out on 28-01-2021. In that experiment we could evaluate amount of ethanol present in the seeds after extraction in mL/g.

5.3.4. Polyphenol Content

To evaluate polyphenol content, from experience and literature it has been taken that, seeds have 8.5% w/w PF. But it should be noted that the whole amount can not be extracted. According to literature and the results obtained from previous pilot plant extractions, 90% of polyphenol extraction yield would be a good approximation and close to reality.

Through provided data, in the second stage extraction the yield is lower than first one. However, increasing the process efficiency with two stage extraction is more important. It can be calculated that first stage extraction recovers around 90 percent of polyphenol content in seeds, while the second stage extracts around 75 percent.

5.3.5. Densities

Densities of the solution and pure ethanol have been obtained from the literature source indicated previously. About other densities they are a simple division of mass by volume.

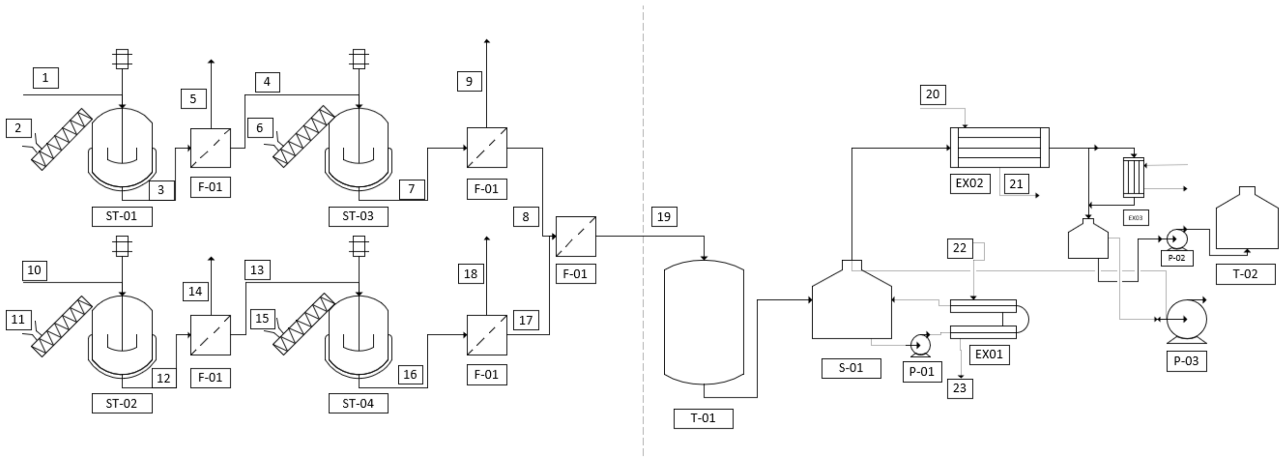


Fig. 20 Process flow diagram of extraction process in pilot plant

	First extraction					Second extraction				
	1	2	3	4		5	6	7	8	
Content	Extraction solution	dry seeds	slurry (seed+sol)	solution	Evaporation	wet seeds	dry seeds	slurry (seed+sol)	solution	Evaporation
Temperature (C)	40	40	40	40	40	40	40	40	40	40
Pressure (bar)	1	1	1	1	1	1	1	1	1	1
Amount (L)	380	141	521	310	15.04	211	114	424	275	0.77495
Amount (kg)	307.8	80	387.8	251.1	11.73432	136.7	65	316.1	222.75	0.604461
PF content (% w/w)	0	8.5	1.75	2.46	0.00	0.448	8.5	3.90	5.01	0.00
Ethanol content (% v/v)	50	0	36.48	48.5	100	11.67	0	35.42	47.5	100
Density (kg/L)	0.81	0.568	0.74	0.81	0.78	0.64834335	0.568	0.74	0.81	0.78

First extraction						Second extraction						
9	10	11	12	13		14	15	16	17		18	19
wet seeds	Extraction solution	dry seeds	slurry (seed+sol)	solution	Evaporation	wet seeds	dry seeds	slurry (seed+sol)	solution	Evaporation	wet seeds	clean solution
40	40	40	40	40	40	40	40	40	40	40	40	40
1	1	1	1	1	1	1	1	1	1	1	1	1
149	380	141	521	310	15.044	211	114	424	275	0.77495	149	550
93.35	307.8	80	387.8	251.1	11.73432	136.7	65	316.1	222.75	0.604461	93.35	445.5
1.25	0	8.5	1.75	2.46	0.00	0.448	8.5	3.90	5.01	0.00	1.25	5.01
12.68	50	0	36.48	48.5	100	11.67	0	35.42	47.5	100	12.68	47.5
0.62467955	0.81	0.568	0.74	0.81	0.78	0.64834335	0.568	0.74	0.81	0.78	0.62467955	0.81

Table 7. Stream specification of PFD

5.4. *Ethanol recovery*

Although the process is in its early stage, it needs some optimization to enhance the efficiency of the production and lower its burden. Being in the pilot stage, it would make more sense to optimize existing production. To this aim, the whole process has been studied to search for potentialities for improvement. By looking at raw materials, it had been observed that the most expensive and environmentally hazardous material is ethanol. Since the process is planned to satisfy circular economy goals, any possible waste and hazardous material should be taken care of. Through mass balances and lab scale experiments, it has been seen that a large portion of ethanol is adsorbed by seeds, which plays an important role in ethanol loss.

To justify mentioned optimization, wet seeds ethanol content should be evaluated. Since recovering ethanol from treated seeds would enhance the efficiency of the process, following calculations have been done.

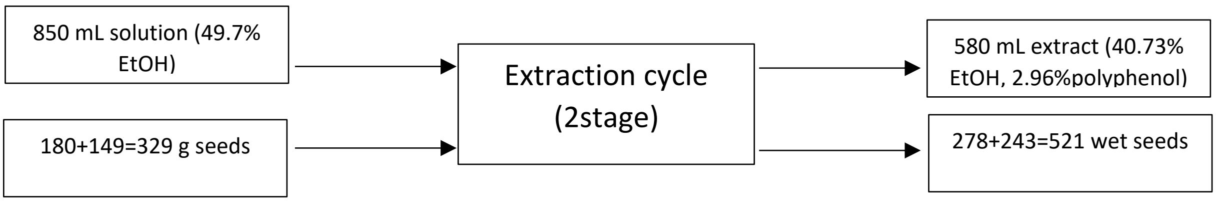


Fig. 21 Lab-scale extraction flowchart

All measurements have been done during one of extraction experiments in lab scale. The percentage of polyphenols in extract has been obtained from previous experimental data which has been conducted in collaborative laboratory.

Assumptions:

- 10 percent of inserted ethanol is evaporated in extraction.
- Extraction of other compounds are negligible
- No compression
- $P_{polyphenols}$ = 1.2 g/mL

Polyphenols extracted from dry seeds:

$$2.96*580=17.17 \text{ mL}$$

$$17.17 \text{ mL}*1.2 = 20.6 \text{ g polyphenols}$$

Mass balance for seeds:

$$M_{out} - M_{in} = M_{absorbed}$$

$$M_{wet} + M_{polyphenols} - M_{dry\ seeds} = M_{solution}$$

So,

$$M_{solution} = 212 \text{ g (absorbed solution in seeds)}$$

Ethanol:

$$V_{in} - V_{out} - V_{evaporated} = V_{absorbed}$$

$$422 - 236.23 - 42.2 = 143.57 \text{ mL}$$

Water:

$$V_{in} - V_{out} = V_{absorbed}$$

$$428 - 326.54 = 101.46 \text{ mL}$$

So, volume of the absorbed solution is 245 mL. while, its mass is 212.6 g. Therefore the density can be calculated.

$$\rho = \frac{212.6\ g}{245\ mL} = 867 * 10^{-3} \text{ g/mL} = 0.867 \text{ g/mL}$$

$\rho_{water} = 0.997$ g/mL

$\rho_{EtOH} = 0.789$ g/mL

So, based on assumptions, ethanol content of the mixture can be calculated.

$$0.789 . x + 0.997 . (1-x) = 0.867$$

So, x = 62.5%. Therefore, ethanol content of the absorbed solution is around 62.5% v/v. Although some simplifications have been done, the value is so close to experimental value, Experimental values are around 65% which means washing treated seeds to recover adsorbed ethanol would be feasible theoretically.

5.5. Ethanol loss experiments

To understand the amount of ethanol, which is lost at each stage of process, the whole process has been carried out once again in the laboratory. The process has been carried out the same as previous times. Besides, some measurements have been carried out in between. To perform such measurements, two set of experiments have been carried out. The idea has initiated from the point that extreme ethanol smell has been observed while discharging the seeds from extraction chambers.

Studying the whole process in the lab-scale and pilot scale, it has been seen that there are two potential resources for ethanol losses. One is evaporation, which is because of evaporation during transferring the solution. Evaporation amount in lab scale is believed to be more than pilot scale due to measurements which have been done in lab scale. Although such amount could not be evaluated in either of the scales, there is another source which can be measured easily and that is the amount adsorbed to the seeds.

Based on such theory, I have organized two set of experiments under supervision of R&D department managers. Since ethanol is adsorbed on seeds based on our theory, for recovering such ethanol, exhausted seeds demand washing with water. From previous experiments conducted in pilot plant, it had been suggested by my supervisor that around 11-14% v/v ethanol solution would result from washing the seeds which would be useless for our process. While, to improve my idea I have suggested cyclic washing. The idea was based on infinite mixing of ethanol and water. To prove feasibility of such idea, find the equilibrium point for washing, and efficient time for washing 6 Pyrex bottles for extraction like previous experiments were prepared. After finishing the extractions, with the same L/S ratio (4.75) water has been added to the first Pyrex bottle. All bottles were numbered from one to six. Bottles have been divided to two groups. Each bottle was used for first and second stage extractions. Bottles numbered from one to three have been undergone washing for 2 hours; while the others for 1 hour.

One hour and two hours have been considered based on the time gaps between each extraction and time schedule of the process. Time scheduling the process was one of the novel steps I have learnt during my internship. Based on which every single instruction to operators can be given. In addition, since the production is batch mode, time scheduling gives us daily, monthly, and annual production.

To evaluate the amount of ethanol recovered by washing, after each washing, a single stage distillation was carried out. For each distillation, 50 mL sample was taken and distilled. Top product after a couple of minutes after boiling starts was reached to 50 mL and then put in the alcohol meter machine. The reason behind performing the distillation was preventing contamination of the machine. Although such experiment was time consuming, it worth performing it. Feasibility of the process was proven; equilibrium point could be guessed around 28-30% v/v ethanol concentration in washing solution. And washing time was determined to be efficient around 1 hour for first stages of washing and 2 hours for second stage washing. Detailed data and results are presented in corresponding sections.

1st Wash (water)							
Washing solution volume in input (mL)	EtOH% in output (%v/v)	Output volume (mL)	Recovered Ethanol (mL)	Recovered Ethanol 99° (mL)	recovered ethanol percentage	Approximation on the amount recovered without samplingù	Washing time (h)
800	6.76	790	53.404	53.943434	12.6	53.404	2
740	13.02	710	92.442	93.375758	9.2	95.0797027	2
660	16.7	630	105.21	106.27273	3.0	106.1772727	2
800	6.11	790	48.269	48.756566	11.4	48.269	1
740	12.33	710	87.543	88.427273	9.3	90.19664865	1
660	18.2	645	117.39	118.57576	7.1	119.6511364	1

2nd Wash (water)						
Washing solution volume in input (mL)	EtOH% in output (%v/v)	Output volume (mL)	Recovered Ethanol (mL)	Recovered Ethanol of this sample (mL)	Washing time (h)	Percentage of recovered ethanol
630	21.96	620	136.152	84.346	2	7.0
570	25.9	550	142.45	45.336	2	1.5
500	26.64	490	130.536	0.854	2	-2.8
595	21.9	580	127.02	57.899	1	2.3
530	26.26	515	135.239	47.493	1	1.9
465	28.95	450	130.275	24.883	1	-1.2

Table8. Results of cyclic washing of exhausted seeds after extraction in first experiment.

	Input ethanol percentage	1st wash recovery	2nd wash recovery	Dealcoholizer recovery	Lost
Pyrex 1	100	12.6	7	62.7	17.7
Pyrex 2	100	9.2	1.5	61.1	28.2
Pyrex 3	100	3.0	1	57.6	38.4
		24.8			
Pyrex 4	100	11.4	2.3	59.7	26.6
Pyrex 5	100	9.3	1.9	62.7	26.1
Pyrex 6	100	7.1	1	60.6	31.3

Table 9. Ethanol mass balance in percentage in first experiment

Based on first experiment results, second experiment has been conducted to develop the mass balance corresponding to ethanol. To this aim, just three Pyrex bottles were prepared. Unlike the first experiment, even before and after each extraction stage, ethanol content was measured. It should be mentioned that to avoid sampling error, first Pyrex bottle was used just for measuring the ethanol content of extraction solutions and seeds. Obtained data are reported as follow.

1st Extraction							
	seeds(g)	Solvent (1:1ethanol/water) (mL)	Liquid/solid ratio (mL/g)	Composition of ethanol in solution	Ethanol (mL)	time (h)	Temperature (C)
Pyrex 1	180	850	4.72	49.73	422.705	4	40 (38)
Pyrex 2	180	850	4.72	49.73	422.705	4	40(38)
Pyrex 3	180	850	4.72	49.73	422.705	4	40(38)

2nd Extraction							
seeds(g)	Solvent (mL)	Liquid/solid ratio mL/g	Ethanol (mL)	time (h)	Temperature (C)	Extract ethanol percentage	Extract volume (mL)
148.24	700	4.72	290.5	4	40(38)	44	580
149.29	705	4.72	292.575	4	40(38)	40.73	580
150.35	710	4.72	294.65	4	40(38)	39.45	600

Table 10. Results of extraction in second experiment

1st Wash (water)							
Washing solution volume in input (mL)	EtOH% in output (%v/v)	Output volume (mL)	Recovered Ethanol (mL)	Recovered Ethanol 99° (mL)	recovered ethanol percentage	wet seed (g)	Washing time (h)
670	6.76	790	53.404	53.94343434	1335.1	228	1
800	6.06	780	47.268	47.74545455	-153.4	278	1
740	16.4	705	115.62	116.7878788	1708.8	278	1

2nd Wash (water)						
Washing solution volume in input (mL)	EtOH% in output (%v/v)	Output volume (mL)	Recovered Ethanol (mL)	wet seed (g)	Washing time (h)	Comment
630	21.96	620	136.152	193	2	Used for sampling
780	11.03	740	81.622	243	2	Start
705	19.43	670	130.181	243	2	

Table 11. Results of cyclic washing after extraction in second experiment

Ethanol input	Ethanol in seeds after 1st ex	Ethanol in seeds after 2nd ex	Evaporated ethanol (mL)	Ethanol in extract (output)	Ethanol in seeds after first wash	Ethanol in seeds after 2nd wash	Ethanol in washing solution	Ethanol loss	ethanol loss percentage
422	50.10	49.329	60.03	262.55	16.3736	16.0782	81.622	32.4518	7.69
422	50.10	49.329	60.03	262.55	21.944	23.21	130.181	45.154	10.7

Table 12. Ethanol mass balance in cyclic washing in second experiment

However, if mass balance is based on ethanol content of seeds after extraction, it has been seen that the evaporation amount was higher than the evaluated value. The evaporation loss is due to transferring liquid from dish to dish and sampling and lab scale measurements.

	Inputs seeds	wet seeds after 1st extraction	Ethanol content after extraction	Ethanol content after first wash	Percentage of the total	set seed after 2nd extraction	Ethanol content after second wash	Percentage of the total
Pyrex1	Sampling							
Pyrex2	180	278	18.02	5.9	3.886729858	243	6.63	3.817748815
Pyrex3	180	278	18	8.01	5.276729858	243	9.61	5.533720379

Table 13. Ethanol mass balance in cyclic washing in second experiment based on inserted ethanol

The optimization goal we were seeking was to reduce ethanol loss to less than 5 percent. Based on mass balance resulted from experiments such goal could be reached. However, infinite mixing of ethanol and water is applicable to binary mixtures. In our case Since polyphenols are present, such assumption is not completely true. In the sense of using same washing solution to wash other seeds, such assumption is good.

6. Result and discussion

6.1. Washing cycles

According to the experiment, 4 washing cycles will result in having seeds with ethanol content of 5%. This amount is acceptable. Since after first wash, the ethanol content of the seeds is 4%. All these percentages are with respect to the input ethanol. So, it has been confirmed that same washing solution can be used to wash seeds obtained from 4 extraction stages, or better to say 2 cycles (each cycle 2 stages). Same washing solution has been used for washing seeds in the third cycle but there was a dramatic decrease in ethanol recovery. Although the ethanol content of the washing solution was getting higher, but the amount of recovered ethanol made this step inefficient.

Based on this result, 4 washing cycles has been determined for the process.

6.2. Washing time

It has been observed that for adequate washing, one hour is efficient for seeds obtained from first stage of extraction and two hours for second stage. I have been proven by the first experiment. So, from this result, it would be reasonable to assume 1 hour washing for first stage seeds and 2 hours for second stage seeds.

6.3. Liquid volume in washing

It has been seen that starting by 800 mL water for washing, after 4 washing cycles, we obtain 670 mL of washing solution with 19.4 % v/v ethanol.

6.4. Ethanol lost by evaporation

From mass balance of experiment, in the best-case scenario, we will lose 14.7 percent of inserted ethanol due to evaporation. This amount needs to be recovered. By a shell and tube heat exchanger this amount can be recovered. By assuming that, following calculations has been done.

$$670*19.4/100 = 129.98$$

$$\text{Total ethanol: } 129.98 + 60 + 60 = 250 \text{ mL ethanol}$$

Since 14.7 % loss has been assumed for each cycle of extraction and then washing obtained seeds.

$$\text{Total volume: } 670 + 120 = 790 \text{ mL}$$

By these assumptions we can get into the result that, we obtain 790 mL of solution at 31.6 % v/v ethanol.

- ❖ This result has been obtained under condition of recovering the evaporated ethanol.

6.5. Applying the result in the pilot plant

Parameters kept constant

L/S = 800/180 = 4.44 washing

L/S = 850 / 180 = 4.72 for extraction

14% evaporation of ethanol in the process

Same washing solution absorption in seeds.

To reach our goal, 400 L of extraction solution (50% v/v ethanol) has been used.

So, 85 kg of seeds has been extracted should be extracted by the solution. Which is close to our pilot plant experience (80 kg)

14% of 200 L of ethanol is evaporated

$$14\% * 200 = 28 \text{ L}$$

378 L of demineralized water is required based on our assumption.

$$800/180 * 85 = 378 \text{ L}$$

To have the same absorption, in lab scale we have lost 130 mL (800 – 670) we take the proportion and we get to,

$$(130/800) * 378 = 61.425 \text{ L}$$

So, after 2 washing cycles we would have, 317 L of washing solution at 19.43 % v/v ethanol

Total ethanol: 317*19.43/100 + 28 +28 = 117.6 L

Total volume: 317 + 56 = 373 L

So, we would have 373 L of solution at 31.36 % v/v ethanol

This amount of ethanol inside a solution is high but still there is no place in the process where we can make use of it. However, we know that at the end of dealcoholization of our extract we obtain a solution with 57 % v/v ethanol. It should be examined that whether it is possible to put washing solution in dealcoholizer and obtain a solution which can be mixed with recovered ethanol solution. The final mixture can be used as an extraction solution if it reaches 50 % v/v ethanol.

According to ASPEN HYSYS simulation, it would be possible to reach higher concentration by the height of equipment we own. It means there is no thermodynamic difficulty on the way. Which was expected from experience.

Since we have a solution with 57 % v/v ethanol, we need to know what percentage of ethanol content in the washing solution would be adequate to have a final mixture of 50^{o} ethanol.

According to implemented chronoprogram, after 3 extraction cycles, we need to feed fresh solvent since we have 189 L of 57° ethanol. There is the point where our washing solution can help.

From single stage distillation we know:

$$< Xd >= \frac{S^0X^0 - SXs}{D}$$

<Xd> is average concentration of compound in distillate.

S^0 is the initial volume in still

X^0 is the initial concentration in the still

S final volume

X final concentration

Since we want to recover ethanol, we can neglect the ethanol concentration in the still at final stage. It is reasonable since ethanol is more volatile than other compounds.

So,

$$S^0X^0 = <Xd>\ D$$

$$<Xd> * D = 373*31.36/100$$

On the other hand, we want to obtain a final solution of 50° ethanol by mixing obtained solution and recovered solution after extraction.

From mass balance we have,

$$189 * 57/100 + <Xd>*D = 50*(D+189)/100$$

We know that second term is equal to 116.97.

So,

$$D= 260.4\ L \quad \text{as a result, } <Xd> = 44.9\ \%$$

This indicates that distillation starts with 373 L and finishes when 113 L of solution, which must be mostly water remains in the still.

With our current dealcoholizer we need 2 hours and 36 minutes to reach the desired concentration.

Finally, we get a solution, V = 449 L at 50% v/v ethanol. Which is ideal to start a new extraction.

6.6. Business plan preparation

By having all data required, business plan preparation for justifying economical aspects of project can be carried out. To this goal, 100 operating days per year has been assumed as a reference unit of production plant. Justification of the assumption is based on vinification process duration in each year and producing polyphenols for the following year. In other words, seeds are obtained during vinification process from September to February. Polyphenols are required in September of following year, in which by considering maintenance times and holidays, 100 days of operation looks reasonable in this early stage.

Having four tanks for extraction, extraction cycle time can be assumed 4 hours for treatment of 145 kg of seeds. Theoretically, seeds contain 85 g polyphenols per kilogram. From experiments, the amount extracted in each cycle is around 65 g total PF/kg of seeds. Liquid to solid ratio applied in extraction is 5. In business plan considerations, a slight scaling has been carried out with respect to the previous experiment in pilot plant. All these considerations, based on the PFD provided, resulted in following data:

BUSINESS PLAN OF PLANT WITH 4 EXTRACTION TANKS			
(80+65 KG PER CYCLE WITH 380L EACH) 2 CYCLES			
GRAPE SEEDS AND EXTRACTION YIELD			
G PFT/KG OF GRAPE SEED (THEORETICALLY)	85	g pft/kg	min 50g/kg – max 100g/kg
G PFT/KG OF GRAPE SEED (EXTRACTED)	65	g pft/kg	From experimental data
EXTRACTION YIELD	76%	%p/p	From experimental data
%SS OF THE CONCENTRATED LIQUID EXTRACT	20%	%p/p	From experimental data
EXTRACTION CYCLE			
GRAPE SEED PER CYCLE	145	kg	
NUMBER OF EXTRACTIONS (1ST AND 2ND WITH SAME SOLUTION)	2	Steps	
EXTRACTION TIME	9	hr	
EXTRACTION CYCLES WITH 4 TANKS	2	cycles	
CALCULATIONS			
TOTAL TREATED GRAPE SEEDS (2CYCLES)	290	kg	
WORKING HOURS PER DAY (3 SHIFTS)	24	hr	
DAILY TREATED GRAPE SEEDS	773	kg/day	
ANNUAL TREATED GRAPE SEEDS (100 DAYS/YEAR)	77	ton/yr	
CONCENTRATED LIQUID EXTRACT	25.13	ton/yr	
DRIED EXTRACT BASED ON YIELD	5.0	ton/yr	
OPERATIVE DAYS PER YEAR	100	days	

Table 14. business plan of the pilot plant extraction process

Extraction solution per each cycle has been assumed to be 400 L based on determined liquid to solid ratio. Based on the experiments, the amount of solution absorbed in the seeds and its ethanol content has been considered. All data are summarized as follow:

EXTRACTION SOLUTION (WATER AND ETHANOL)			
WATER X 2 CYCLES	364	L	
ETHANOL X 2 CYCLES	396	L	
SOAKED X 2 CYCLES	210	L	Goes to washing stages

LOST ETHANOL IN EACH CYCLE	105	L	96% v/v ethanol
ANNUAL ETHANOL LOST (100 OPERATION DAYS PER YEAR)	28000	L	

Table 15. Extraction solution consumption

Based on the data achieved in pilot plant experiments, the corresponding values for concentrated extract per cycle, per day, and per year can be calculated along with amount of ethanol recovered through the process. For assessment, 10 L extracted solution loss has been assumed deliberately to ensure the losses may occur during transferring the liquid from extraction chambers to reservoir of de-alcoholizer. De-alcoholization timing has been assumed 3.5 hours per cycle. The amount of concentrated extract assumed to be 50 L per cycle. All the data are reported as follow:

EFFICIENCY OF CONCENTRATING THE PRODUCT		
EXTRACT OBTAINED FROM 2 CYCLES OF EXTRACTION	540	L
CONCENTRATION CYCLE TIME (100 L/H CAPACITY)	5.4	h/cycle
REQUIRED TIME FOR DISCHARGING AND CLEANING (160L OF CONCENTRATED EXTRACT)	2	h
AMOUNT OF CONCENTRATED EXTRACT FROM 540L OF EXTRACTION SOLUTION	80	L
TOTAL PRODUCT CONCENTRATION TIME	7.4	h
CONCENTRATED PRODUCT FROM 4 EXTRACTIONS AT 50 ^{o}BX	160	L
EXTRACT OBTAINED IN 24 HOURS AT 50 ^{o}BX	519	L
RECOVERED ETHANOL SOLUTION AT AROUND 55% V/V ETHANOL CONTENT	455	L
RECOVERED ETHANOL (96% V/V ETHANOL)	261	L
OBTAINED EXTRACT IN 100 DAYS (CONSIDERING 8 DAYS FOR CLEANING AND ACCUMULATION)	47.74	ton/yr

Table 16. Efficiency of extract concentration process

Based on a previous single stage washing of seeds, and assuming availability of recovering the evaporated ethanol, following data has been achieved. It should be noted that, mentioned experiment is not in correspondence with the experiment indicated in tests and analysis section. In other words, the evaporation loss considered in here corresponds to extraction stage. However, it gives a good indication of ethanol amount which can be recovered throughout the process. 90 percent has been assumed for condensing ethanol efficiency which even can be assumed in washing system. Following data has been considered for further evaluations:

WASHING	
OBTAINED SOLUTION IN 2 CYCLES OF EXTRACTION	560
AVERAGE ETHANOL CONTENT	14
ETHANOL	82
TIME SCHEDULE OF THE PROCESS SHOULD BE CHECKED TO PERFORM CONCENTRATION IN SINGLE STAGE DISTILLATION UNIT	
RECOVERY OF THE EVAPORATED ETHANOL (96% V/V ETHANOL)	
PER EACH EXTRACTION CYCLE	15.5
IN 24 HOURS FOR ALL CYCLES	82

CONSIDERING 90% EFFICIENCY	74

Table 17. Recovery of ethanol

Based on the utility consumption of pilot plant, utility cost per cycle and per year can be evaluated. The consumption rate of each equipment is indicated. While for ethanol 10 percent of loss has been determined; considering that evaporated ethanol is recovered.

Another important factor in evaluation of utility cost is operating time of each equipment, since the process is in batch mode. 22 hours of extraction a day would be good assumption considering feeding and discharge. While assuming 3 cycles of 5.5 hours for concentration stage would be a reasonable assumption. On the other hand, the boiler providing hot water for jackets operates 6 hours a day experimentally. Data are reported as follow:

EXTRACTION UTILITY COST		
EXTRACTION CHAMBER NOMINAL POWER	0.75	kW
EXTRACTION CHAMBER CONSUMED POWER	0.56	kW
WORKING HOURS	22	h/d
ENERGY COST	0.09	€/kWh
ANNUAL ENERGY CONSUMPTION	222	€/yr
BOILER		
NOMINAL POWER	14.4	kW
REAL CONSUMED POWER	10.8	kW
WORKING HOURS	6	h/d
ENERGY COST	0.09	€/kWh
ANNUAL ENERGY CONSUMPTION	1750	€/yr
SINGLE STAGE DISTILLATION UNIT		
NOMINAL POWER	25	kW
REAL CONSUMED POWER	18.75	kW
WORKING HOURS	16.2	h/d
ENERGY COST	0.09	€/kWh
ANNUAL ENERGY CONSUMPTION	2750	€/yr
REAGENT AND UTILITY COST		
TOTAL ETHANOL AMOUNT	105.6	L
ETHANOL (96 %V/V) 10% LOST (RECOVERED FROM TREATED GRAPE SEEDS)	10.6	L
RECOVERED ETHANOL FROM SINGLE STAGE DISTILLATION	70	L
CONDENSED EVAPORATED ETHANOL DURING PROCESS	7.5	L
ADSORBED ETHANOL IN SEEDS (CANNOT BE RECOVERED BY WASHING)	29	L
WATER	97	m^3
WATER COST	0.1	€/ m^3
ETHANOL COST	0.6	€/L
ANNUAL WATER CONSUMPTION	10	€/yr
ANNUAL ETHANOL CONSUMPTION	6350	€/yr
TOTAL UTILITY COST	11,000	€/yr
LABOUR COST (3 WORKERS PER SHIFT, TWO IN OPERATION ONE FOR SUPPORT) FOR 100 DAYS	65,500	€/yr

Table 18. Experimental utility cost based on pilot plant experiments

Purchasing all the required equipment, the equipment costs are as follow:

CAPITAL COST		
SARBATOI MACERAZIONE	29,400	€
C.E.T. SNC DI CARMELLINI	4,300	€
ENOLOGICA SIPPI S.R.L.	2,157	€
ENOVENETA SPA	1,480	€
ADEGUAMENTO CAPANNONE	30,000	€

SCARICO AUTOMATICO + REC CONDENSE	50,000	€
MANCATI RICAVI ALCOOL IN GIACENZA	17,183	€
TOTAL	134,520	€

Table 19. Capital cost of the implemented in pilot plant

By having total equipment cost, fixed capital cost of plant can be evaluated utilizing Miller's method.

6.6.1. Miller's method

Miller's method has been utilized to calculate fixed capital cost of the plant. Miller's method account for 4 plant areas:

- Battery limits (B/L)
- Storage and handling
- Utilities
- Services

Fixed capital cost of Battery limit (B/L) area is calculated based on main equipment cost with applying appropriate factors.

An important thing to be considered is that, Miller's method corresponds to USD_{1958} however the total equipment cost we have obtained above is in euros. So, it is required to convert euros to USD and then by appropriate CEPCI value converting the price to USD_{1958}. $CEPCI_{1958}$ is 99.7 while the most recent available CEPCI value is the one for late 2019 $CEPCI_{2019}$ = 619 which can still give a good indication.

By summing all equipment costs, total equipment cost has been calculated. It worth mentioning that 7 main equipment has been considered.

$$\text{Total equipment cost} = 134{,}520\ €_{2019}$$

While each euro worth 1.19 USD_{2019},

$$\text{Total equipment cost} = 160{,}100\ USD_{2019}$$

By applying CEPCI factors for 2019 and 1958, main equipment cost in 1958 has been calculated as follow:

$$\text{USD (1958)} = \text{USD (2019)} * \frac{CEPCI(1958)}{CEPCI(2019)}$$

$$160100 * \frac{99.7}{619} = 25{,}800\ USD_{1958}$$

The obtained value is main plant item (MPI). Since we are in early flowsheet stage Miscellaneous unlisted items (MUE) is 10-20 percent of MPI in which 15 percent of MPI has been considered for MUE. Summing MPI and MUE results in Basic equipment cost for which all factors are going to be applied.

$$MPI = 25800\ \$_{1968}$$

$$MUE = 3900\ \$_{1968}$$

$$\text{Basic equipment cost} = 29{,}700\ \$_{1968}$$

Factors which should be applied to base equipment cost are presented in slide 10, Economic Indicators slides. MPI has been divided by number of main equipment to determine which set of factors should be considered.

$$MPI/7 = 4240\ \$_{1968}$$

Which is in the range of 3000-5000. So, factors presented in 3000-5000 has been applied. In all sections, average value of the range proposed, has been implemented. All these factors are applied to base equipment cost except the ones mentioned in their own section.

6.6.2. Miller's method factors

Field Erection of basic equipment: Using stainless steel 316 L as material of construction, high percentage of equipment requiring high field labor has been assumed since we are operating in batch mode and dealing with food industry. An average value of 19% has been considered.

Equipment foundation and structural supports: Average for predominance of alloy has been considered, 5.5 percent.

Piping includes ductwork exclude insulation: Average for liquids and solids has been chosen, 18.5% percent.

Insulation of equipment only: Average for chemical plants would be the best option to choose, 3.8 percent.

Insulation of piping only: Average for chemical plants,10 percent.

All electrical except building lighting and instrumentation: The most appropriate option would be plants with solids, 18.75 percent.

Instrumentation: Solids present in the plant is chosen, 17.5 percent.

Miscellaneous, include site preparation, painting and other items not accounted for above: 4 percent.

Buildings-Architectural & structural (excludes bldg. services) : Open air plants with minor buildings has been considered because our battery is part of a bigger outdoor plant, 20 percent.

Building services: Consists of compressed air for general service, electric lighting, sprinklers, plumbing, heating, ventilation with and without air conditioning. All these factors have been accounted as normal condition which result in 55 percent of building value.

B/L area cost can be calculated by summing factored items and basic equipment cost.

Storage and handling area cost, utilities area cost, and services area cost can be calculated based on B/L area cost using ranges and approaches presented in slide 15, Economic indicators slides. Average values of the range presented in the table has been utilized.

Obtained total value is sum of B/L, S&H, U, and S which can be indicated as total direct cost.

By applying factors presented in the slide 17, Economic Indicators slides, indirect contingency cost can be calculated which all result in fixed capital cost of the plant section under analysis.

So, calculating fixed capital of the plant and then converting the amount to USD_{2019}, following by another conversion to euros gives the Fixed Capital Cost of plant in current time.

$$CFC = 560,000\ €$$

For evaluation of working capital cost, detailed information are needed. Being in early stage of assessment, gives the right to use 15% of fixed capital cost as an assumption of working capital cost. So,

$$CWC = 84,000\ €$$

Having a battery limit under investigation, land cost is not relevant for our evaluation. So, Total capital cost indicated as CAPEX can be calculated by summing CFC and CWC.

$$CAPEX = 745,000\ €$$

6.6.3. OPEX

To evaluate operating cost, one year has been determined as reference unit. Utility consumption and cost has been calculated based on data available from pilot plant. Besides, 3 shifts have been considered for operation each day. Each shift is considered to be carried out by 3 people. So, operating labor cost has been calculated based on average wage in the company for operators. For raw materials, since the only raw material present is seeds and seeds are themselves by-products of winery process, they can be neglected. However, to have a good and complete business plan, the revenue earned by the company in case of selling the required amount of seeds, has been considered as raw material price for the intended process. The values are reported as follow:

$$C_{raw\ material} = 31,000\ €/yr$$

$$C_{labor} = 66,000\ €/yr$$

$$C_{Utilities} = 11,000\ €/yr$$

Having all the data, operating cost or OPEX can be calculated by summing them. So,

$$OPEX = 232,000\ €/yr$$

CAPEX and OPEX give good indications of the expenditures. However, to provide a justification for feasibility of the intended project, more adequate indicators for business analysis are needed. To this aim, net present value (NPV) and return of investment (ROI) have been evaluated.

6.6.4. Net Present Value (NPV)

To evaluate NPV, some data are required, first of all, the selling price of our product should be determined. As an assumption based on market data, 16 €/kg of concentrated extract containing 20 % polyphenols have been considered. According to business plan scheme, production rate is considered 25 tons per year. So, Annual revenue and net cash income can be calculated as follow:

$$A_S = 402{,}000 \text{ €/yr}$$

While annual production cost excluding provision for plant depreciation is:

$$A_{TE} = 232{,}000 \text{ €/yr}$$

By subtracting production cost from the revenue annual cash income is calculated.

$$A_{CI} = A_S - A_{TE}$$

$$A_{CI} = 170{,}000 \text{ €/yr}$$

To evaluate net annual cash income, tax contribution needs to be considered. For the evaluation of annual tax amount (A_{IT}) , following formula has been used. Where tax fraction rate (t) has been assumed 25 percent, which is common for industries. Besides, annual tax allowance (A_D) has been considered 12600 €/yr. By subtracting the obtained amount from annual cash income, net annual cash income (A_{NCI}) is obtained.

$$A_{IT} = (A_{CI} - A_D) * t$$

$$A_{IT} = 40{,}000 \text{ €/yr}$$

$$A_{NCI} = A_{CI} - A_{IT}$$

$$A_{NCI} = 130{,}000 \text{ €/yr}$$

All required data are calculated except for annual expenditure of capital. It can be evaluated as a lost amount in time zero, or as an annual expenditure by dividing total capital cost by plant lifetime. So, plant life time should be determined. 10 years of lifetime has been considered. Which is a common lifetime among chemical plants. For further simplification, second approach toward total capital cost contribution has been considered for the aim of this project. So, total capital cost (CAPEX) has been divided by 10 and the result was considered as annual expenditure of capital (A_{TC}). By subtracting the result from net annual cash income, net annual cash flow after tax (A_{CF}) is calculated.

$$A_{TC} = \text{CAPEX}/10 = 64{,}500 \text{ €/yr}$$

$$A_{CF} = A_{NCI} - A_{TC}$$

$$A_{CF} = 66{,}000 \text{ €/yr}$$

By calculating net annual cash flow, net present value (NPV) of the plant can be calculated, accordingly. An important factor for correct evaluation of NPV is the amount of disinvestment at the end of plant lifespan. Commonly, 10 percent of total capital investment (CAPEX) would be good assumption for early stage assessments. It worth mentioning that this value should be added to the NPV which has been evaluated according to following formula.

$$NPV = \sum_{t=0}^{n} \frac{(net\ annual\ cash\ flow)t}{(1+i)^\wedge t}$$

Variable 'i' in the formula corresponds to discount rate. This value has been assumed to be 15 percent for the project purpose.

So,

NPV = 395,000 €

The obtained valued by far justifies development of the plant. To have another business indicator, return of investment have been evaluated for further analysis by business professionals.

Return of investment (ROI) has been calculated by considering A_{CF} with respect to capital investment and disinvestment.

$$ROI = \frac{(10 *\ \mathrm{ACF} + \mathrm{Disinvestment} - \mathrm{CAPEX})}{CAPEX} * 100$$

ROI = 12.38%

APPLICATION OF MILLER'S METHOD		
TOTAL MILLER'S FACTOR	131.375	% of total equipment cost
MUE	20,200	€
BATTERY LIMIT AREA COST	358,000	€
STORAGE AND HANDLING AREA (4% OF B/L)	14,500	€
UTILITY AREA (10% OF B/L)	35,800	€
SERVICE AREA (4% OF B/L+S&H+U)	16,300	€
TOTAL DIRECT COST	424,400	€
INDIRECT COSTS (20% OF INDIRECT)	85,000	€
DIRECT + INDIRECT COSTS	509,200	€
CONTINGENCY (10% OF DIRECT + INDIRECT)	51,000	€
FIXED CAPITAL COST (CFC)	560,200	€
WORKING CAPITAL COST (CWC) (15% OF CFC)	84,000	€
CAPEX	644,000	€
BUSINESS ANALYSIS OR ECONOMIC ASSESSMENT		
UTILITY COST	11,000	€/yr
OPERATING LABOUR COST	65,500	€/yr
RAW MATERIAL (GRAPE SEEDS)	30,800	€/yr
OPEX	232,500	€/yr
ANNUAL REVENUE & NET CASH INCOME		
ANNUAL REVENUE OF SELLING THE PRODUCT	402,000	€
ANNUAL PRODUCTION COST EXCLUDING PROVISION FOR PLANT DEPRECIATION	232,500	€
ANNUAL CASH INCOME	170,000	€
ANNUAL AMOUNT OF TAX	39,000	€
NET ANNUAL CASH INCOME	130,000	€
ANNUAL EXPENDITURE OF TOTAL CAPITAL COST	64,500	€
NET ANNUAL CASH FLOW AFTER TAX (A_{CF})	66,000	€
NET PRESENT VALUE (NPV)	395,000	€
RETURN OF INVESTMENT (ROI)	12.4	%

Table 20. Economic evaluation of production process in pilot plant

All these values justify development of the intended plant based on equipment in pilot plant. However, filtration, formulation, and seed washing stages have not been considered in this early-stage evaluation. However, having implemented the business, when equipment data are provided, same procedure can be undergone for comparison and justification of the different options.

6.7. Improvement

One important factor of bioactive compounds production, as mentioned previously, is shelf life. For increasing the shelf life. The product needs formulation stage which is intended to be carried out with a spray drying system. The offer proposed to the company by REUS is a spray dryer which requires electricity as utility. So, utility cost, along with equipment cost should be considered. Although the equipment requires 15 m^3/h compressed air at 6 bar, since the requirement can be easily met by utility lines of plant, it has been neglected. The following table reports all available data.

EQUIPMENT	SPRAY DRYER	
PRODUCTION RATE	2.5 kg/h	kg/h
COST OF EQUIPMENT	187,150 €	€
ELECTRICITY CONSUMPTION	16.8 kW	kW
OPERATING HOURS PER DAY	24 h	h
UTILITY COST PER YEAR	3600 €	€

Table 21. proposed spray dryer specifications

Spray drying is carried out after concentration. According to de-alcoholizer production rate and intake of proposed equipment, operating hours and production rate of spray dryer has been evaluated. However, spray dryer requires maintenance timing, too. All in all, being in early stage of design, operating 24 hours a day can be assumed with having in mind the maintenance fact for future detailed analysis.

Carrying out the same business plan evaluation procedure, by consideration of 115 €/kg of powder as selling price, All following values and indicators have been evaluated.

	AMOUNT		COMMENT
CAPEX	1,333,000	€	Different miller's factors from previous ones
OPEX	325,000	€/yr	
ANNUAL REVENUE OF SELLING PRODUCT	690,000	€/yr	
ANNUAL PRODUCTION COST	325,000	€/yr	
ANNUAL CASH INCOME	365,000	€/yr	
ANNUAL AMOUNT OF TAX	88,000	€/yr	
NET ANNUAL CASH INCOME	277,000	€/yr	
ANNUAL EXPENDITURE OF CAPEX	133,000	€/yr	
NET ANNUAL CASH FLOW	144,000	€/yr	
NPV	855,000	€	
ROI	17.9 %	%	

Table 22. Economic assessment of conventional extraction followed by spray dryer

Note: 16 €/kg of liquid extract has been determined as selling price. Considering the fact that 4 kg of liquid extract is required for 1 kg of powder, the equivalent price would be 64€. However, for having a feasible evaluation the mentioned price is inevitable.

It would be the most expensive part of plant. In first sight, it may look economically unjustifiable. However, it should be considered that, the selling price of powder is much higher than liquid extract. So, the proposed procedure of business plan can be carried out again for purified product.

Adding one new equipment will cause changes in the miller's method factors and will contribute to changes of utility cost. All these changes will influence CAPEX, OPEX, NPV, and ROI. All changes are reported in the following table.

APPLICATION OF MILLER'S METHOD		
MUE	48,250	
TOTAL MILLER'S METHOD FACTORS	100.15	% of total equipment cost
BATTERY LIMIT AREA COST (B/L)	740,400	€
STORAGE AND HANDLING AREA (4% OF B/L)	30,000	€
UTILITY AREA (10% OF B/L)	74,000	€
SERVICE AREA (4% OF B/L+S&H+U)	33,800	€
TOTAL DIRECT COSTS	877,800	€
INDIRECT COSTS (20% OF INDIRECT COSTS)	175,500	€
DIRECT + INDIRECT COSTS	1,053,000	€
CONTINGENCY (10% OF DIRECT + INDIRECT)	105,300	€
FIXED CAPITAL COST (CFC)	1,158,700	€
WORKING CAPITAL COST (CWC) (15% OF CFC)	173,800	€
CAPEX	1,332,500	€
BUSINESS ANALYSIS OR ECONOMIC ASSESSMENT		
UTILITY COST	14,700	€/yr
OPERATING LABOUR COST	65,500	€/yr
RAW MATERIAL (GRAPE SEEDS)	30,700	€/yr
OPEX	324,700	€/yr
ANNUAL REVENUE & NET CASH INCOME		
ANNUAL REVENUE OF SELLING PRODUCT	690,000	€
ANNUAL PRODUCTION COST EXCLUDING PROVISION FOR PLANT DEPRECIATION	324,700	€
ANNUAL CASH INCOME	365,200	€
ANNUAL AMOUNT OF TAX	88,000	€
NET ANNUAL CASH INCOME	277,000	€
ANNUAL EXPENDITURE OF TOTAL CAPITAL COST	133,200	€
NET ANNUAL CASH FLOW AFTER TAX (A_{CF})	143,800	€
NET PRESENT VALUE (NPV)	855,000	€
RETURN OF INVESTMENT (ROI)	17.93	%
ADDITION OF SPRAY DRYER		
EQUIPMENT COST	187,000	€
NOMINAL POWER CONSUMPTION	24	kW
POWER CONSUMPTION	16.8	kW
OPERATING HOURS PER DAY	24	h
ELECTRICITY COST	0.09	€/kWh
ANNUAL CONSUMPTION	3600	€/yr

Table 23. Economic assessment of extraction process followed by spray dryer

6.8. Process options

Verifying the feasibility of the project in pilot plant, industrial scale production can be investigated. Previous investigations regarding industrial production have resulted in recognition of some novel technologies mainly ultrasonic assisted extraction to enhance the extraction efficiency and yield. Further investigation through downstream of the process had resulted in some possibilities regarding filtration and formulation stages. In this study the ultimate goal is to prepare a comparison among different options by application of the proposed business plan. Preliminarily, each equipment has been discussed based on the requirements the offers received from various suppliers to meet goal. Then different combination of introduced equipment have been provided to be evaluated by the business plan model.

6.8.1. Extraction chamber options

Reasonably, the first option in industrial scale extraction would be conventional extraction with the tanks present in pilot plant. Furthermore, since ultrasonic assisted extraction has been proven to be more efficient option, two offers have been received from two different suppliers. One option is ultrasound assisted stirred tank (US-ST) offered by REUS. While the other one is an ultrasound extraction chamber offered by Applied Acoustics Centre LTD.

6.8.1.1. Ultrasound assisted extraction chamber

Ultrasound assisted stirring tanks proposed by REUS are supplied with ultrasound transmitter plates on the bottom section of the stirring tank. The scheme of the tank explains the shape. Major differences between US-ST and conventional ones, are Conical shape of the bottom and the plates which are installed on this section. The production rate of the proposed equipment shows improvement. However, it should be further discussed. All specifications of the equipment and its price is presented as follow.

6.8.1.2. Ultrasound extraction chamber

The equipment has a semi-donut angled shape. It applied the ultrasound waves by 24 ultrasonic plates assembled on outer surfaces. It conducts mixing with a circulation circuit powered by an external pump. Which is delivered along the main equipment. It has been considered that ultrasonic equipment reduces the extraction time from 4 hours at each stage to 1 hour. Which in first sight appeals to be a significant increase in plant efficiency. To perform the business plan analysis, same approach as before has been considered. To have a better comparison with the conventional approach, two extraction chambers has been considered to perform 2 stages of each cycle. However, utilizing the new equipment may even result in elimination of the second stage. To have better insight, further tests and analysis are required. For now, we consider two extraction chambers with following specifications.

EQUIPMENT	US REACTOR + CIRCULATION + SEED SEPARATION
PRICE	150,000 €
INSTALLED POWER	13.2 kW

Table 24. proposed Ultrasound assisted extractor

Their effect on CAPEX and OPEX can be evaluated based on provided data. Treated seed per cycle, liquid-solid ratio, and selling price are assumed to be the same as conventional plant. As mentioned earlier, extraction timed is considered to be reduced by a factor of 4. So, utilizing 2 equipment to meet 2 extraction cycle requirements, will result in having 2 hours as extraction time for preparation of de-alcoholizer feed. All data corresponding to business plan are indicated as follow. Like conventional plant business plan extraction chambers are assumed as one equipment for CAPEX evaluation using Miller's method.

APPLICATION OF MILLER'S METHOD		
TOTAL MILLER'S FACTOR	83.65	% of total equipment cost
MUE	64,000	€
BATTERY LIMIT AREA COST	901,000	€
STORAGE AND HANDLING AREA (4% OF B/L)	36,000	€
UTILITY AREA (10% OF B/L)	90,000	€
SERVICE AREA (4% OF B/L+S&H+U)	41,000	€
TOTAL DIRECT COST	1,068,000	€
INDIRECT COSTS (20% OF INDIRECT)	213,600	€
DIRECT + INDIRECT COSTS	1,282,000	€
CONTINGENCY (10% OF DIRECT + INDIRECT)	128,000	€
FIXED CAPITAL COST (CFC)	1,410,000	€
WORKING CAPITAL COST (CWC) (15% OF CFC)	211,500	€
CAPEX	1,621,000	€
BUSINESS ANALYSIS OR ECONOMIC ASSESSMENT		
UTILITY COST	23,000	€/yr
OPERATING LABOUR COST	65,500	€/yr
RAW MATERIAL (GRAPE SEEDS)	69,200	€/yr
OPEX	417,000	€/yr
ANNUAL REVENUE & NET CASH INCOME		
ANNUAL REVENUE OF SELLING THE PRODUCT	904,800	€
ANNUAL PRODUCTION COST EXCLUDING PROVISION FOR PLANT DEPRECIATION	417,000	€
ANNUAL CASH INCOME	488,000	€
ANNUAL AMOUNT OF TAX	119,000	€
NET ANNUAL CASH INCOME	369,000	€
ANNUAL EXPENDITURE OF TOTAL CAPITAL COST	162,000	€
NET ANNUAL CASH FLOW AFTER TAX (A_{CF})	207,000	€
NET PRESENT VALUE (NPV)	1,201,000	€
RETURN OF INVESTMENT (ROI)	37.65	%

Table 25. Economic assessment of ultrasound assisted extraction

An early-stage comparison indicates that using Ultrasound chamber for extraction, results in considerable increment of net present value and return of investment. Although more detailed data are required for more accurate evaluation, being in early stage scaling up analysis, the new equipment appeals to be a better option than the conventional stirred tanks.

Application of business plan on the ultrasound system with spray dryer to produce powder does not contribute to reasonable evaluation. Since it has been seen that the spray dryer operates 24 hours a day to satisfy the concentrated extract production rate. However, in the novel option the production rate has increased (more than double). So, the current proposal of spray dryer is not sufficient for our application. So, a new offer should be received for the evaluation. Just to have an indication, it can be assumed that spray dryer operates 200 days a year. With this assumption following data has been achieved, considering 115 €/kg as selling price.

APPLICATION OF MILLER'S METHOD		
TOTAL MILLER'S FACTOR	54.65	% of total equipment cost
MUE	112,000	€
BATTERY LIMIT AREA COST	1,327,000	€
STORAGE AND HANDLING AREA (4% OF B/L)	53,000	€
UTILITY AREA (10% OF B/L)	132,700	€
SERVICE AREA (4% OF B/L+S&H+U)	60,500	€
TOTAL DIRECT COST	1,574,000	€
INDIRECT COSTS (20% OF INDIRECT)	315,000	€
DIRECT + INDIRECT COSTS	1,888,000	€
CONTINGENCY (10% OF DIRECT + INDIRECT)	189,000	€
FIXED CAPITAL COST (CFC)	2,077,000	€
WORKING CAPITAL COST (CWC) (15% OF CFC)	312,000	€
CAPEX	2,389,000	€
BUSINESS ANALYSIS OR ECONOMIC ASSESSMENT		
UTILITY COST	30,200	€/yr
OPERATING LABOUR COST	65,500	€/yr
RAW MATERIAL (GRAPE SEEDS)	69,300	€/yr
OPEX	523,500	€/yr
ANNUAL REVENUE & NET CASH INCOME		
ANNUAL REVENUE OF SELLING THE PRODUCT	1,380,000	€
ANNUAL PRODUCTION COST EXCLUDING PROVISION FOR PLANT DEPRECIATION	523,000	€
ANNUAL CASH INCOME	856,000	€
ANNUAL AMOUNT OF TAX	211,000	€
NET ANNUAL CASH INCOME	645,000	€
ANNUAL EXPENDITURE OF TOTAL CAPITAL COST	239,000	€
NET ANNUAL CASH FLOW AFTER TAX (A_{CF})	407,000	€
NET PRESENT VALUE (NPV)	2,280,000	€
RETURN OF INVESTMENT (ROI)	80,22	%

Table 26. Economic assessment of ultrasound assisted extraction followed by spray dryer

Being a comparative assessment, obtained value of ROI has been calculated and presented as above. It would not be reasonable value. Therefore, selling price of the product could be decreased. By decreasing the selling price of dried extract (powder) to 100 €/kg, ROI reaches reasonable amount which would be 23.71%, while the NPV would be 1.6 million euros.

After conducting all experiments and optimizations, based on the obtained results, polyphenol production by ultrasound assisted extraction chamber would be justified. However due to European regulations about exporting food products outside European Union, grape seeds could not be exported to Russia for conducting test-runs. All economic assessments were conducted based on literature data about the efficiency of the ultrasound assisted extraction. Since provided grape seeds in Caviro have shown some difficulties like cake formation and discharging the slurry, having the test-run data is essential for further investigation of such process option.

Although the process seems profitable by using ultrasound assisted extraction, conventional extraction which is under operation in pilot plant meets the company's goal. Polyphenol extraction from grape seed as mentioned in corresponding section is designed to meet circular economy goals of the company. Besides, the process is considered as waste treatment before anaerobic digestion for the company. In addition, due to company policies currently it has been suggested to use the pilot plant for polyphenols production. However, comparing different options both technically and economically, would be a proposal for future considerations and modifications.

6.9. Washing stage

Washing stage considerations mentioned earlier, have a considerable contribution to the utility cost. According to previous assumptions-based business plan, ethanol loss has been assumed as 10 percent in total. According to calculations performed on washing stage, this loss can be reduced to 5 percent in total. Since nearly 75 percent of the annual utility cost is caused by ethanol loss, reducing it by a factor of 2 looks interesting for our process. Experiments regarding washing stage have been carried out in lab scale. However, further pilot plant tests are required for approval of such system. Since for washing treated seeds a simple stirred tank reactor like the ones in pilot plant is sufficient, CAPEX contribution has been neglected at this stage. Although for further analysis in the future, having detailed data of assembled equipment, a better evaluation can be performed. All in all, applied washing system contribution to NPV and ROI are presented as follow for conventional system with and without spray dryer, and ultrasonic system.

ST EXTRACTION WITHOUT SPRAY DRYER	WITHOUT WASHING	WITH WASHING
NPV	395,426 €	409,497 €
ROI	12.38%	16.74%
ST EXTRACTION WITH SPRAY DRYER		
NPV	855,044 €	869,115 €
ROI	17.93%	20%
US EXTRACTION WITHOUT SPRAY DRYER		
NPV	1,200,978 €	1,232,638 €
ROI	37,65%	41.54%
US EXTRACTION WITH SPRAY DRYER		
NPV	2,216,415 €	2,279,735 €
ROI	74.94%	80%

Table 27. Comparison among process options with and without washing stage

All the presented results indicate a considerable contribution. It also worth mentioning that 5 percent of total ethanol consumption is adsorbed to seeds and could not be recovered by simple washing. Which makes this 5 percent inevitable. Therefore, the recovery rate achieved by proposed washing stage is the highest achievable rate of ethanol recovery.

6.10. Filtration

After extraction seeds should be separated from the solution. Along seeds, small particles mainly grape skin, and cell debris should be eliminated from the solution to avoid contamination in the concentration stage. To this aim, as indicated in PFD of the process, a filtration stage is required. With a 10-micron filtration this requirement can be satisfied. Due to high solid-liquid ratio and high particle size, decanter centrifuges should be used. Options which should be considered are as follow:

1. Sifter offered by Habrotek
2. Decanter centrifuge offered by flotwegg
3. Rotary vacuum filter offered by JX filtration

The only data available at this point is about the sifter offered by Habrotek. Other offers do not have detailed data about price and power consumption at this point. The price and power consumption of the equipment offered by Habrotek are as follow:

EQUIPMENT	HABROTEK SIFTER
PRICE (EQUIPMENT + CONTROL PANEL)	19,480 €
INSTALLED POWER	2.2 kW

Table 28. Proposed sifter specifications

The contribution of the equipment on NPV and ROI of the option with US extraction without spray dryer shows following results.

APPLICATION OF MILLER'S METHOD		
TOTAL MILLER'S FACTOR	100,15	% of total equipment cost
MUE	67,000	€
BATTERY LIMIT AREA COST	1,027,000	€
STORAGE AND HANDLING AREA (4% OF B/L)	41,000	€
UTILITY AREA (10% OF B/L)	103,000	€
SERVICE AREA (4% OF B/L+S&H+U)	47,000	€
TOTAL DIRECT COST	1,217,000	€
INDIRECT COSTS (20% OF INDIRECT)	243,000	€
DIRECT + INDIRECT COSTS	1,460,000	€
CONTINGENCY (10% OF DIRECT + INDIRECT)	146,000	€
FIXED CAPITAL COST (CFC)	1,607,000	€
WORKING CAPITAL COST (CWC) (15% OF CFC)	241,000	€
CAPEX	1,848,000	€
BUSINESS ANALYSIS OR ECONOMIC ASSESSMENT		
UTILITY COST	23,500	€/yr
OPERATING LABOUR COST	65,500	€/yr
RAW MATERIAL (GRAPE SEEDS)	70,000	€/yr
OPEX	446,000	€/yr
ANNUAL REVENUE & NET CASH INCOME		
ANNUAL REVENUE OF SELLING THE PRODUCT	905,000	€
ANNUAL PRODUCTION COST EXCLUDING PROVISION FOR PLANT DEPRECIATION	446,000	€
ANNUAL CASH INCOME	458,000	€
ANNUAL AMOUNT OF TAX	111,000	€
NET ANNUAL CASH INCOME	347,000	€
ANNUAL EXPENDITURE OF TOTAL CAPITAL COST	185,000	€
NET ANNUAL CASH FLOW AFTER TAX (A_{CF})	162,000	€
NET PRESENT VALUE (NPV)	998,000	€
RETURN OF INVESTMENT (ROI)	-2.29	%

Table 29. Economic assessment of extraction process with a proposed separation apparatus

As it can be seen adding this equipment has resulted in a decline on both indicators. Which was predicted, since adding an equipment results in increment in CAPEX. By applying the washing system, we can overcome this decrease. While even we can sell the product at higher price. Since the maximum price that we can sell it is 20€, by increasing selling price to 17 €/kg of concentrated extract, ROI of 20.66% and NPV of 1.2 million euros could be achieved.

7. Conclusion

Waste is getting meaningless day by day, since it has been proven that in any industry, disposed waste could be used as feedstock in another sector. Therefore, waste has been defined as by-product. Based on circular economy goals, any waste generated in food industry has been studied to exploit its potentialities. As a result, the disposed food waste has been minimised. While high added value products and energy have been produced from such by-products.

Polyphenols have been proven to have health benefits along their usage as wine additive to improve its quality. Health impacts are all based on polyphenols antioxidant activity. In addition, even their presence has shown negative impact on anaerobic digestion performance. Extracting such compound from grape seeds could also have economic advantage along improving anaerobic digestion efficiency.

Although polyphenols extraction has been well studied in the literature, their industrial production requires optimization and economic evaluation. Based on economic evaluation their production could be justified. Introducing novel technologies has resulted increased efficiency in the production. In addition, optimization of the process has resulted in more profitability and better efficiency of the process.

The proposed process is a waste treatment after all. Therefore, usually company policies are in the way to have highest efficiency with lowest possible investment. Since polyphenols production could be considered as seasonal production, the production achieved in pilot plant currently meets company's needs. Although the production is conventional, extraction with novel technologies could be considered as proposal for future modifications if the company would like to scale up the production.

Comparing novel technologies technically have narrowed down the possible approaches into conventional and ultrasound assisted extractions. Developing such production approaches have been studied. Conventional extraction has been implemented in pilot scale. While ultrasound assisted extraction still requires more studies to better understand the efficiency and operation of proposed apparatus. However, through inquiry from the supplier and some hypothesis based on literature, economic evaluation of such production has been carried out as well.

On the other hand, the product can be obtained in two possible modes being concentrated liquid extract and dried powder. Considering required equipment, corresponding economic evaluations have been carried out. Based on available data, extraction with ultrasound assisted extractor has shown higher production cost, while by producing more than conventional extraction, by selling more product it would be a better option for scaling up to industrial scale production. Besides, increasing shelf life of product by adding spray dryer have increased the price of product to a level which based on company's goal would not be feasible. However, by considering industrial production, both approaches can be applied to produce immediate needs by just concentrating. While a portion in powder mode. In addition, washing stage has been proven to contribute to economically and technically better results.

Along with the obtained results and calculations, some further studies have carried out on the product. Although, polyphenols are considered as high added value compound produced from grape seeds which are considered as by-product (conventionally waste) of wineries, the obtained extract has shown considerable amount of sugar. Based on literature review and some negotiations in the company with other companies, produced polysaccharides have proposed to be separated and sold. Sugar as by-product can be obtained by sequential ultrafiltration and nanofiltration to separate polyphenols and polysaccharides with reasonable efficiency.

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