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Conversion of Problematic Winery Waste into Valuable Substrate for Baker's Yeast Production and Solid Biofuel: A Circular Economy Approach[§]

Running head: Conversion of Winery Waste into Value

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SUMMARY

Research background. Wine production, regarded as a major sector in food industry, is often associated with the utilization of large amount of resources. Furthermore, wine making produces high quantity of grape pomace that is generally used for low value applications such as fertilizer and animal feed. The present research aims at exploring the possibility of improving the overall sustainability of traditional winemaking.

Experimental approach. A zero waste process was developed. It includes the production of white wine and the substantial valorisation of grape pomace, which is transformed into solid biofuel, tartaric acid and concentrated grape extract as a feedstock for industrial baker's yeast production.

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Results and conclusions. We estimate that a substantial renewable energy surplus of approx. 3 MJ/kg processed grapes could be achieved during this transformation. The suitability of grape extract as a potential substrate for industrial baker's yeast production was assessed and the feasibility of its partial replacement of molasses (up to 30%) was demonstrated.

Novelty and scientific contribution. We present a circular economy approach to the conversion of winery biowaste into high-value resources such as feedstock and solid biofuel.

Keywords: zero waste transformation; grape pomace; solid biofuel; feedstock; baker's yeast; circular economy

INTRODUCTION

Grape (*Vitis vinifera*) is one of the most important crops and it is cultivated all over the world. This fruit is consumed both as fresh and as processed product (mainly wine) (*1*). In the year 2021, the world production of wine was estimated at 260 millions of hectolitres (*2*). Italy, France and Spain account for 53 % of the world wine production (Fig. S1) (*3*,*4*). Consequently, wine production is regarded as one of the major food industries but is associated with the utilization of large number of resources (water, organic and inorganic fertilizers). Wine production produces high amounts of grape pomace which is generally used for low value applications such as fertilization and animal feed.

The production of white and red grape wine usually begins with the crushing of mature grapes. For white wines, the crushed grapes are pressed to separate the juice, which is clarified and then fermented. For the production of red wine after crushing, a maceration is carried out before the pressing. The fermentation is usually stopped by the separation of the yeast and the addition of sulphites (*5*). Grape pomace accounts for about 20−25 % (the percentages given in the text are meant as mass percentages, except when indicated as volume percentage) of the weight of the grapes crushed in wine making and consists mainly of peels (skins), seeds and stems (*6*). Its composition varies depending on grape variety, method of processing, environmental conditions and the ratio of skin:seeds:stem (*7*). However, main constituents are water (60−80 %), cellulose (10−20 %), pectin (8−10 %), sugars (6−8 %) and lipids and proteins (2−4 %) (*8*). The share of organic matter of grape pomace is high, varying between 26 and 42 %.

Increasing agro-industrial activities and industrial manufactures have a huge impact on the environment, energy demand, carbon dioxide emissions and climate change. Therefore, the society and industrial manufactures are heading towards integrated sustainability (*9-13*). In order to contribute to a sustainability transformation, waste from different industries must serve as raw materials for new products and applications in the frame of a circular economy aiming at "zero waste" society.

Over the last decades, the utilization of grape pomace has been quite inefficient. Only 3 % of grape pomace produced is being reused for animal feed, Grappa or as waste-based compost. Due to the above-mentioned high organic content, this by-product currently represents a growing environmental issue. Furthermore, the discharge of grape pomace on landfills rises problems related to the pollution of surface and ground water, foul odour, potential disease spreading by flies and pests, as well as oxygen depletion in soil and ground waters by tannins and other compounds (*14*).

Due to the increasing development of sustainable agriculture as well as consumers´ demand for the use of natural over synthetic products, the efforts of the utilization of grape pomace are now heading in the direction of production of (*15*):

- Food additives
- Nutraceuticals
- Ingredients of functional foods/dietary supplements
- Medical remedies
- Fertilizers
- Animal feed
- Antimicrobial components
- Cosmetics
- Biomass for biofuels

In 2018, approx. 7 million tons of grape pomace was subject to disposal (*16*). It is important to note that winemaking is carried out through practices with hundreds of years of tradition. In this context, the application of new technological solutions designed to reduce the amount of biowaste as well as keep the quality of the end product often requires changes in mentality. In Germany, the fertilizer legislation was recently updated with special requirements for the application of pomace in viticulture including specific analysis of total nitrogen, as well as the regulation of land field surface area that can be fertilized with this biowaste. Furthermore, the storage of grape pomace should not exceed a maximum period of 6 months. Any application of fertilizer must be in accordance with the principles of good professional practice (*17*).

The present research aims at exploring the possibility of introducing the integrated sustainability concept into traditional winemaking by turning a problematic residual material to valuable resources. This is precisely the high sugar content of grape pomace (up to 26.34 g/100g white grape pomaces and 8.91 g/100 g in red grape pomaces (*18*)) that makes the disposal of this fraction critical. Grape pomace undergoes spontaneous fermentation very soon after the pressing leading to a complex mixture of different organic metabolites. The key concept of this work is the simultaneous stabilization of grape pomace and solid/liquid sugar extraction as a carbon source for

industrial fermentations. Sugar is then recovered as an aqueous solution and it is important to note that sugar isolation is avoided because of its challenging economic feasibility.

For this purpose, the main by-product of wine production, grape pomace, was assessed as a feedstock for baker´s yeast production, which belongs to one of the most important industrial bioprocesses. Raw materials used in this large-scale production process are significant contributors to the cost of this low-value high volume product (*9,18*). Molasses is used as a main raw material in the industrial baker's yeast production. In this research, in order to produce high quality baker's yeast that has similar characteristics to the commercial yeast, the mixture of beet and sugar cane molasses was used. Molasses was only partly replaced by grape pomace (up to 30 %) in order to be able to simulate the industrial parameters. Given the available amount of grape pomace and the demand for molasses, a higher fraction would not be realistic. Molasses is not only a carbon source for the yeast, but brings several other macro- and micro-nutrients to the fermentation process. Thus, in order to avoid nutritional imbalance due to the different composition of grape pomace, the fraction was limited. In this regard, the new solution to be introduced must be both ecological and economical sustainable and in terms of product quality.

In this work, white wine was produced and the resulting grape pomace was further processed into solid biofuel (pellets), concentrated grape extract and tartaric acid (largely used as acidification agent, antioxidant, taste enhancer and chelating agent (*20*)).

MATERIALS AND METHODS

Production of Riesling wine

84.9 kg of mature grapes (Riesling Mandelberg), kindly provided by Michael Schneider Winery (Boppard, Germany), were washed and pressed in a hydraulic press (hydraulic press 20 L, Speidel, Ofterdingen, Germany) (Fig. 1). 50.4 kg of grape juice and 32.5 kg of grape pomace (in further text regarded as grape pomace 1) were obtained after pressing. Subsequently, the grape juice was supplemented with 10 g of mineral nutrient (VitaFerm® Ultra F3, Erbslöh, Geisenheim, Germany) and inoculated with 10 g of yeast (Oenoferm® Freddo, Erbslöh, Geisenheim, Germany) according to the producer´s instruction. The fermentation was carried out at 15 °C for 35 days and stopped by adding potassium metabisulfite (100 mg/L). 45.2 kg of wine were produced (5.2 kg of CO_2 were released) and further filtrated in a depth filtration apparatus (Edelstahl Weinfilter SF 10 P, Motorgeräte Fischer GmbH, Lahr, Germany) using sheet filters (20x20 FZ 20; Zambelli, Vicenza, Italy) resulting in 32.9 kg of Riesling wine with an alcohol content of 13.3 % vol.

Production of grape extract and pellets

32.5 kg of grape pomace 1 were mixed with 25 kg of deionized water, heated up to a temperature of 90 °C for 20 minutes under constant stirring, cooled down to 35 °C and pressed (hydraulic press 20 L, Speidel, Ofterdingen, Germany) resulting in 18.8 kg of grape pomace 2 and 28.7 kg of extract. Grape pomace 2 was dried at 60 °C for 24 hours (vacuum drying oven Heraeus Instruments; Hanau Germany) and then used as raw material for pellet production (EcoWorxx Pelletmaker PM22E; Raddestorf, Germany). Moisture content of the pellets was determined at 105 °C until constant weight. Durability was determined according to the standard ISO 17831-1:2015(en) (*21*). The net calorific value of the pellets was determined according to the standard ISO 18125:2017(en) (*22*).

The extract was concentrated by evaporation in a scraped surface evaporator (Labor- und Prozesstechnik GmbH; Ilmenau, Germany) at a pressure of 134 mbar (approximate boiling temperature was 51.5 °C) and a rotation speed of 260 rpm to obtain 4.5 kg concentrated extract. The separation of crystalized tartaric acid was achieved by centrifugation in a Sorvall RC-5B Plus Superspeed Centrifuge (Thermo Fisher Scientific; Waltham, Massachusetts, USA) for 10 minutes at 4,000 rpm resulting in 0.103 kg of tartaric acid and 3.8 kg of concentrated grape extract (cGE in Fig. 1).

The cGE was then stored at 4 °C and its microbial stability was checked twice a week by adding few drops of cGE on dip slides (VWR International GmbH, Darmstadt, Germany), which were then incubated at 35 °C (WTC Binder dry oven incubator, type 33115099003100; Tuttlingen, Germany) over a period of three months. Furthermore, the Brix value of the extract was measured twice a week by means of a refractometer (Kern ORA 3SA Refraktometer Analog, Kern & Sohn GmbH; Balingen, Germany).

Preparation of media and fed-batch fermentation

The fed-batch fermentation process involved in baker's yeast production is usually initiated by inoculating a medium free of C-source. The addition of C- and N-source according to dosage profiles begins immediately after the inoculation. In this work, fed-batch fermentations were performed with the media (C-Sources) presented in Table 1. The reference medium, molasses, was prepared as a mixture of 93 % of beet and 7 % of sugar cane molasses and diluted with deionized water (Brix value was 45). Ammonia 10 % vol (Bernd Kraft GmbH, Duisburg; Germany) was dosed separately as a N-source and the pH value was adjusted with 25 % sulphuric acid (Merck KgaA, Darmstadt; Germany).

A 1-L bioreactor (Sartorius, Biostat® Qplus; Göttingen, Germany) was filled with 0.5 L water and 2.7 g monoammonium phosphate (MAP) (Sigma-Aldrich; St. Louis, Missouri, USA) and autoclaved at 121 °C for 20 minutes (Autoclave Tuttnauer 5075 ELV, Biomedis Laborservice GmbH; Gießen, Germany). MAP is the phosphorus source and a part of the nitrogen source (together with

ammonia and nitrogen from molasses). Molasses and cGE were supplemented with a trace element solution (1 ml $CuSO_4$-/$FeCl_3$-solution/kg of medium (3.52 g/L of $CuSO_4$ x 5 H_2O and 72 g/L $FeCl_3$ x 6 H_2O)) and 300 µL of antifoam and autoclaved at 121 °C for 20 minutes. Molasses and cGE were sterilised in separate flasks, except for the fermentation F6, where molasses and cGE were sterilised as a mixture. Directly after the sterilization, the media were placed on a shaker (IKA Shaker® KS 250; Staufen, Germany) at 150 rpm, aerated with air (2 slpm) for 1 hour with the aim to strip out volatile, potentially inhibitory compounds such as volatile fatty acids, SO_2, NO_x. The media is then supplemented with vitamin stock:

1. 180 µL biotin solution (1 g/L of biotin and 1 g/L of $NaHCO_3$)
2. 0.5 mL thiamine/pyridoxine-solution (10 g/L thiamine mononitrate and 30 g/L pyridoxine hydrochloride)
3. 3 mL pantothenic acid-solution (180 g/L calcium pantothenate).

Medium´s weight lost due to water evaporation during the sterilization and aeration was adjusted with sterilized water.

Dosage profiles for molasses or molasses-cGE mixture and ammonia and aeration profile are scaled down from the large-scale fermentation process. The profile of the fermentation with 100% molasses mixture is presented in Fig S2. The dosing profiles of the other fermentations were very similar. The pH during medium sterilisation potentially has an effect on the stability of the sugar contained in molasses and cGE, respectively. In case of sugar decomposition that would lead to a loss of biomass yield and formation of complex reaction products that could potentially inhibit yeast vitality and performance. After inoculation (15 to 22 g of yeast, depending on the solid matter content of the corresponding inoculate taken directly from the industry), the media (molasses or molasses-cGE mixture and ammonia) were then gradually added according to the dosage profiles determined by the company's procedure. After approximately 20 h of fermentation, the bioreactor was harvested and the yeast cake was separated from the vinasse by means of vacuum filtration.

Yeast characterisation

Dry matter content was determined at 140 °C for 2 h (Memmert drying cabinet, Schwabach, Germany). Protein content was determined by the Dumas method (*23*) at 950 °C. Fermentative capacity was determined throughout the measurement of CO_2 production in stainless steel vessels, in which pressure increase was continuously measured and recorded. The corresponding method and equipment were specially designed by Uniferm GmbH & Co. The fermentative capacity was determined in a standard dough (containing salted water and flour) and sweet dough (containing salted water, flour, butter and sugar) directly after the fermentation.

RESULTS AND DISCUSSION

Zero waste transformation and renewable energy generation

The mass balance of this process is presented in Fig. 2. 50.4 kg of grape juice (24° Brix) and 32.5 kg of grape pomace (grape pomace 1 in Fig. 1) were obtained from 84.9 kg of grapes resulting in a juice yield of 59 % (Fig. 2). This value is lower than typical yields achieved in industrial winemaking (70−75 %) and may result from the pressure limitation of our pilot scale press. The fermentation of the grape juice yielded in 44.1 kg of white wine. After solid-liquid extraction, concentration and centrifugation, 3.8 kg of cGE were obtained with a Brix value of 58 (44.8 g/kg of processed grapes). The produced grape pellets had a moisture content of 9.3 %, an acceptable durability index of 92.3 %, which is higher than that found in literature (*24*) and a net calorific value of 18.7 MJ/kg (20.6 MJ/kg based on dry matter), which is very similar to the value reported for wood pellets (*25*).

Furthermore, the transformation of 1 kg of grapes generates 4140.9 kJ of energy in form of solid biofuel (0.221 kg pellets) (Fig. 3). This process requires 278.3 kJ for the evaporation of 0.118 kg of water at 60 °C (evaporation enthalpy: 2358.5 kJ/kg water (*26*)) during the drying of grape pomace 2 and 679,8 kJ for the evaporation of 0.285 kg water at 51.5 °C (evaporation enthalpy: 2385.1 kJ/kg water (*26*) during the concentration of the grape extract. According to this heat balance, these drying steps have the major contribution to the total energy demand of this process. Overall, we estimated that a substantial renewable energy surplus of approx. 3 MJ/kg processed grapes might be achieved during this transformation. Additionally, the vapour generated in this process can be condensed and reused for extraction and cleaning purposes.

Quality of the produced cGE

Over a period of three months, no colour change nor microbial contamination of cGE was observed (Fig. S3) and the Brix value remained unchanged. These observations suggest the possible long-term storage of this medium, which is a highly desirable characteristic in an industrial setting. All fractions (wine, biofuel and cGE) are very valuable products and zero waste manufacturing could be achieved.

Fed-batch fermentations

As already mentioned, laboratory fermentation technique developed at Uniferm GmbH & Co. has been optimized over the years and the process is a scale down of the industrial fermentation process (*11*). Media containing concentrated grape extract (Table 1) were prepared in different ways in order to assess the potential of this alternative substrate. The sterilisation of molasses and cGE was performed separately as well as together as a mixture. It was found that the sterilisation did not affect the concentrated grape extract in a harmful way, only the change of colour due to the Maillard

reactions occurred (*27*). The highest amount of yeast was harvested on the media supplemented with 10 % of cGE. The amount produced on reference media and the media supplemented with 30 % of cGE are the same.

To evaluate the quality of the produced baker´s yeast, dry matter, protein content and fermentative capacity were measured. The results shown are the average value of the experiments (from Table 1: F1-F3 corresponds to 0 % of CGE in C source, F4-F6 corresponds to 10 % of CGE in C source, F7-F11 corresponds to 30 % of CGE in C source). Fig. 4a shows the results of dry matter content of yeast grown on molasses and media containing molasses and cGE. Dry matter of yeast grown on molasses and cGE is higher than the one grown on only molasses. Dry matter content of yeast produced on all media (including the reference ones containing only molasses) is according to the literature (*28,29*). High quality baker´s yeast was produced on media partly replaced with concentrated grape extract, which not only lowers the need for the volatile priced molasses (*12*) but also qualifies grape extract as an alternative substrate in this industrial production.

Another quality parameter that was measured is baker´s yeast protein content (Fig. 4b). There is a very slight difference in the protein content measured in the yeast grown on reference media than in the yeast grown in media supplemented with cGE. Considering that the pH value plays an important role, the media containing only molasses was prepared with the optimal pH value for the growth (*30*). In other media, different pH values were tested. It seems that the measured protein content was higher for the yeast that was grown in the media supplemented with cGE prepared at lower pH value (Table 1). Furthermore, it is known that the nitrogen starvation can cause decrease in the protein content of the yeast cells (*31*), so it could also explain the lower protein content of the produced yeast. In this study one ammonia profile has been used (Fig. S2), but other dosage profiles were tested in previous studies. However, fresh baker´s yeast contains 40.6–58.0 % of proteins (*32*), which indicates that the yeast grown on media containing both molasses and cGE is of high quality.

One of the most important parameter of industrial baker´s yeast is fermentative capacity: the capability to produce carbon dioxide upon its introduction into dough (*33*). In this research fermentative capacity was measured in two different doughs, standard and sweet dough (Figs. 4c and 4d). Overall, the yeast grown on reference media exhibited higher fermentative capacity on both doughs (155 mbar in standard and 224 mbar for sweet dough). However, baker´s yeast grown on media supplemented with 10 % cGE was almost the same for standard dough (153 mbar) and lower for sweet dough (209 mbar). When more cGE media was used in the fermentation, the fermentative capacity is lower (146 mbar for standard, 203 mbar for sweet dough). The yeast produced with up to 30 % share of cGE can be regarded as good quality yeast. However, the negative trend indicates that higher shares will probably affect the quality in a negative way.

The results of the investigating maltose-fermenting baker´s yeast strains show poor fermentative activity on high sugar doughs. This may be related to the co-location of the MAL loci and the invertase (SUC) genes at the telomeres of yeast. As a result, yeast strains that have duplications of the MAL loci may also have duplications of the invertase (SUC) genes, which results in overexpression of the invertase enzyme. Furthermore, it is also known that intrinsic osmotolerance can also be a limiting factor in successful fermentation of yeasts in high sugar environments. The possible explanation of why the yeast´s leavening capacity decreases with the increasing of cGPE is the influence of the cGPE medium on the expression of MAL loci and invertase genes and the invertase production (*34*).

In previous research, grape juice was investigated for baker´s yeast growth. Mahmood et al. (*35*) reported the maximum yeast biomass growth of 41 g/L. Furthermore, Curto *et al.* (*36*) reported that the yields of baker´s yeast on grape marc were lower than those obtained in industrial production. However, yeast quality (particularly for fibre, ash and digestibility) were similar to a commercial product produced on molasses. The production of the biomass of *Saccharomyces cerevisiae* was investigated on the medium using date extract as the only carbon source. The cultivation of baker´s yeast in Erlenmeyer flasks yielded 40 g/L (*37*). Agro-industrial residue of apple pomace in the production of baker´s yeast has also been investigated. Bhushan *et al.* (*38*) reported that the yield obtained with apple pomace extract was 96 % to that of expected theoretical yield and thus, qualified as an alternative to molasses. Furthermore, Joshi et al. (*39*) reported that supplementing the apple pomace extract with growth stimulators is not required for baker´s yeast fermentation. Lisičar Vukušić *et al.* (*13*) also reported the potential of apple pomace to be employed as a carbon source for the growth of baker´s yeast. Slightly lower biomass yield was obtained in the medium containing a mixture of apple pomace and molasses than in the medium containing only molasses. However, yeast produced on alternative substrate showed a bit higher fermentative activity than the one produced on molasses.

Circular economy concept

Main output of this research is a circular economy concept that allows for turning grape pomace into a source of valuable products such as solid biofuel and a feedstock for baker's yeast production (Fig. 5 and Fig. 6) (*40*,*41*).

CONCLUSIONS

This research show how agro-industrial waste can be fully valorised. In this regard, high-quality yeast was produced on media, which was partly replaced with the cGE. Further investigation is needed in order to determine the optimal ratio of molasses and cGE as well as the optimal growth

conditions for baker´s yeast. Furthermore, it was estimated that renewable energy excess of approx. 3 MJ/kg processed grapes may be achieved during this conversion. The value of the grape pomace has been improved through a transformation based on a zero waste bioprocess.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary materials are available at: www.ftb.com.hr.

AUTHORS' CONTRIBUTION

All authors contributed to this research. The idea for the experiments came from S. Barbe and M. Mösche. T. Millenautzki and A. B. Saaid participated in the wine production and the further preparation of the concentrated grape extract. L. Müller gathered the raw material and set up the apparatus for the production of grape extract. L. Clavijo and A. M. Saaid produced the pellets and performed its analyses. J. Lisičar Vukušić, L. Reichert, J. Hof and M. Mösche participated in preforming the fed-batch fermentations and characterisation of yeast. J. Lisičar Vukušić, T. Millenautzki and S. Barbe researched the literature, analysed the data and wrote the article. M. Mösche and S. Barbe participated in the conception of the work, data analysis and interpretation, drafting the article and critical revision. All authors approved the final version of the manuscript.

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Table 1. Composition of media used in baker´s yeast cultivation (F: fermentation, cGE: concentrated grape extract)

Medium	Molasses/%	cGE/%]	pH
F1	100	0	5.3
F2	100	0	5.3
F3	100	0	5.3
F4	90	10	5.0
F5	90	10	5.3
F6	90	10	5.3
F7	70	30	4.5
F8	70	30	5.0
F9	70	30	5.3
F10	70	30	5.3
F11	70	30	5.3

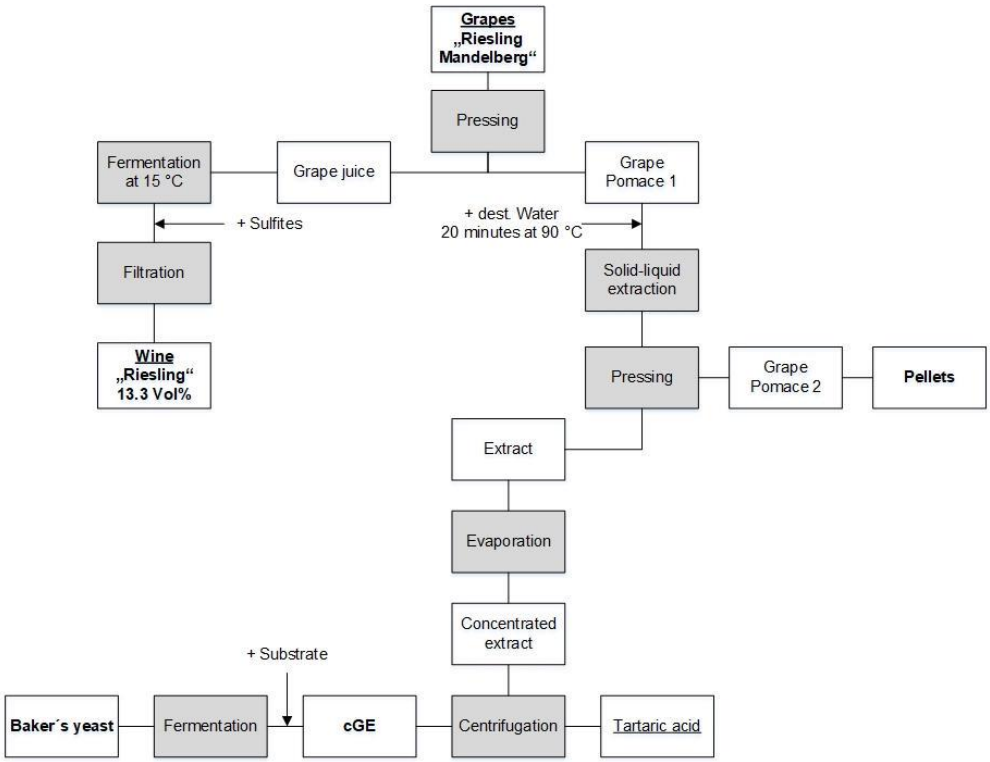

Fig. 1. Scheme of grape processing in this research (cGE: concentrated grape extract)

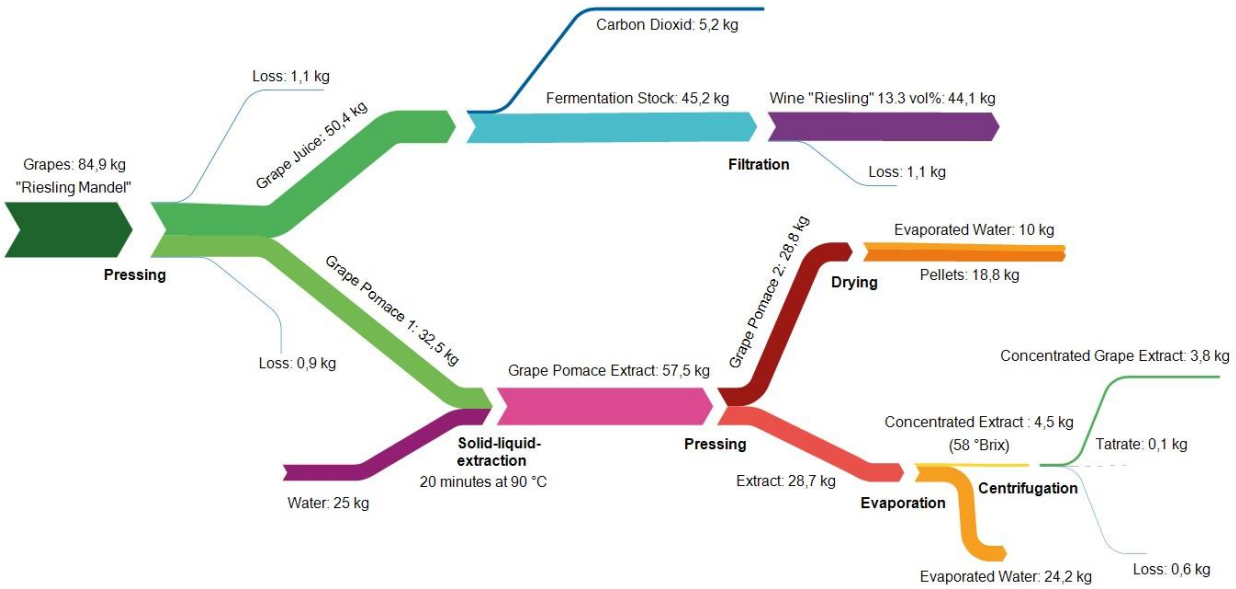

Fig. 2. Overall mass balance during the transformation of grapes into white wine, pellets and concentrated grape extract

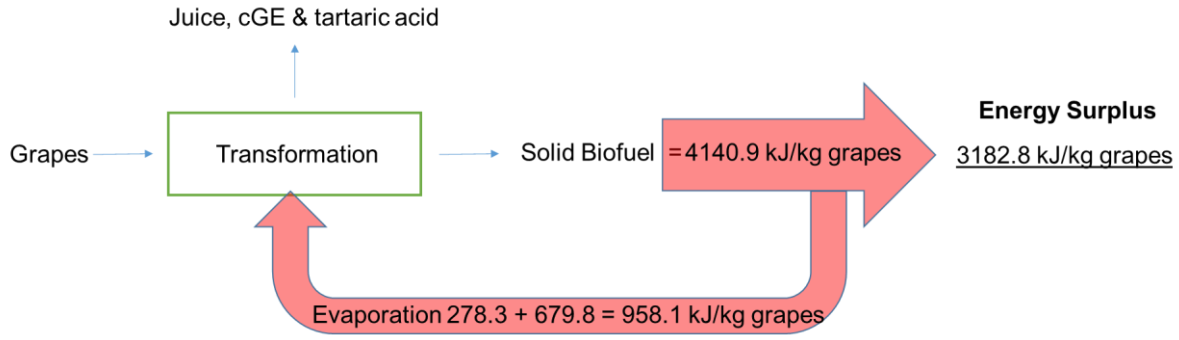

Fig. 3. Overall balance during the transformation of grapes into juice, cGE and tartaric acid

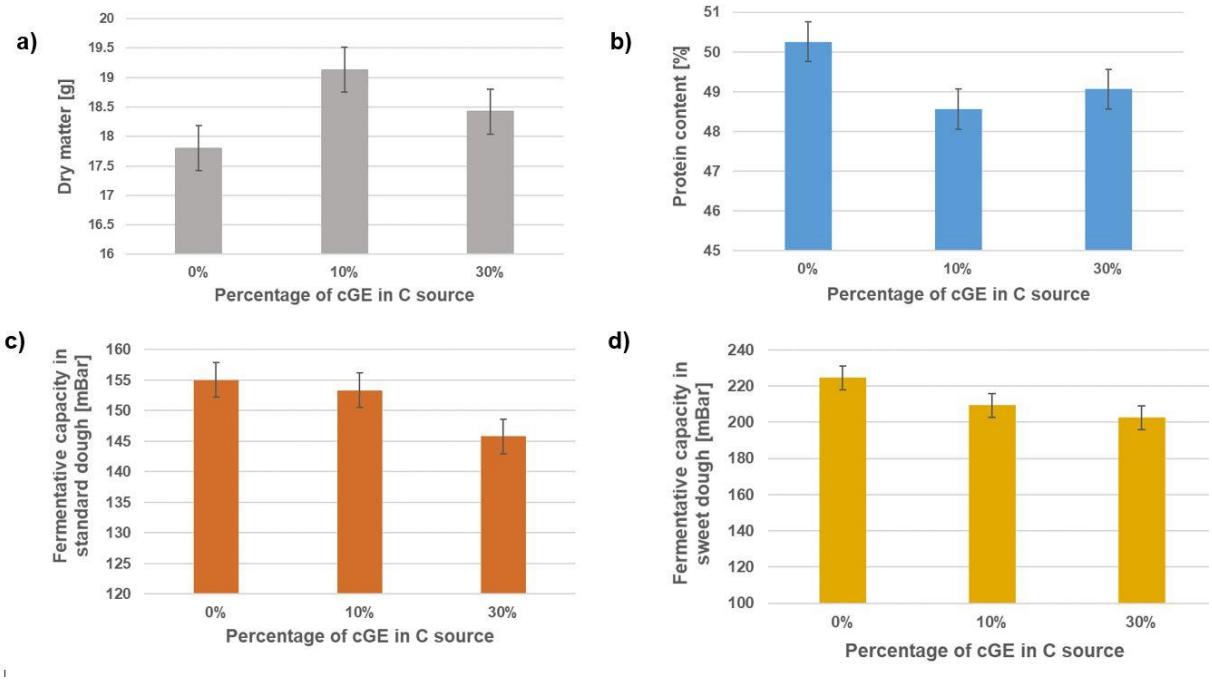

Fig. 4. Results of the baker´s yeast growth on different media containing molasses and concentrated grape extract: a) dry mater content of baker´s yeast, b) protein content of baker´s yeast, c) fermentative capacity of baker´s yeast tested in standard dough, d) fermentative capacity of baker´s yeast tested in sweet dough

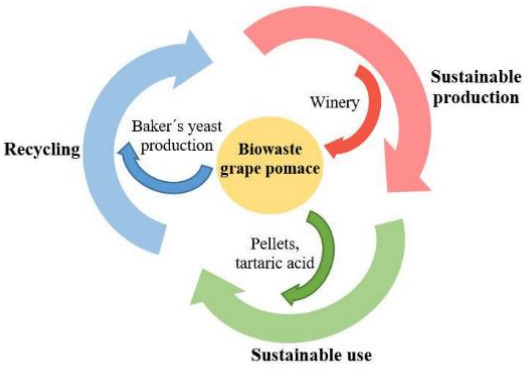

Fig. 5. Circular economy concept of turning winery waste into valuable substrate (adapted from (*40*)

The use of biomass as a source of energy is at the bottom of the value pyramid (Fig. 6), meaning it can be employed for energy production only after higher value uses are satisfied. That is why this research opens very interesting opportunities for wine makers trying to valorise winery waste and make the production more sustainable

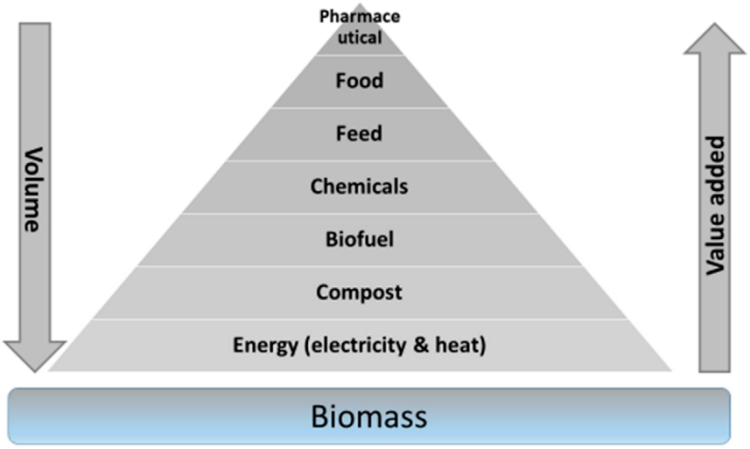

Fig. 6. Biomass value pyramid (*41*)

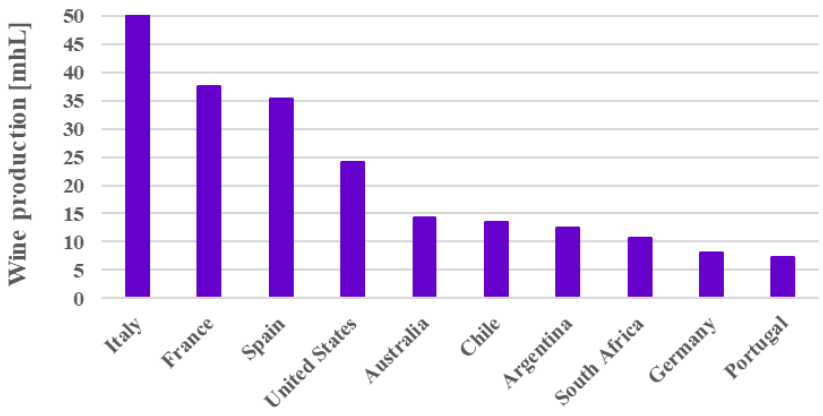

Fig. S1. Wine production worldwide in 2021 (in million hectolitres) (*4*)

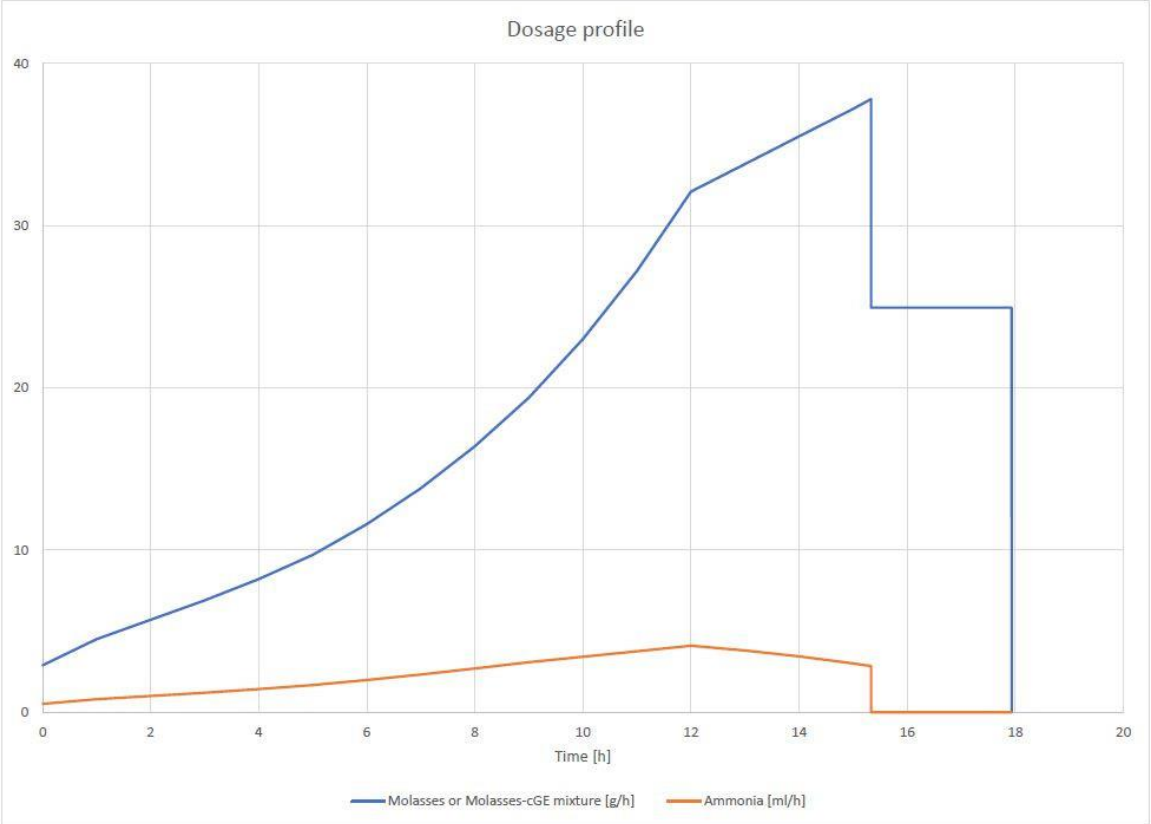

Fig. S2. Dosage profile of substrates during the fed-batch fermentation

Fig. S3. Concentrated grape extract and uncontaminated dip slides