Environmental Engineering and Management Journal
October 2021, Vol. 20, No. 10, 1661-1671
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

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FROM WASTE TO RESOURCE: BIOWAFER PROJECT

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Abstract

Even though the problem of resource depletion of our planet is getting increasingly worse, most of the input of agro-food industry is discarded, causing expensive management and disposal issues. According to the Circular Economy principles, these problems can be faced by giving more value to wastage, turning it into inputs to innovative supply chains: the biorefineries. The BIOWAFER project addresses those issues investigating how agri-food by-products consistently produced in Emilia-Romagna Region (Italy) can be assigned to consecutive biorefinery processes integrated in order to obtain high-added value molecules for cosmetics and pharmaceuticals. Among all food chains considered, tomato, wine and cheese industries were chosen for waste valorisation. In order to overcome the seasonal waste production, a detailed analysis of storage and stabilization of the by-products was performed. *Streptococcus zooepidemicus* was chosen to obtain hyaluronic acid through waste fermentation process. The biorefinery was integrated with waste bioconversion by *Hermetia illucens* larvae followed by sustainable chemical extraction processes of potential antioxidants, lipids and peptides. Finally, the residues of all the above-mentioned phases will be used for pyro-gasification, to obtain biochar and syngas for agronomic and energy purposes respectively, allowing the closure of the supply chains in a circular way. Many other circular approaches are presented in literature, but, in our knowledge, no one use fermentation coupled with larvae rearing for waste reduction and conversion. Despite some challenges in scaling-up the proposed biorefinery, the project aims to overcome a linear production system by making the supply chains increasingly circular.

Key words: agri-food chain, biochar, biorefinery, circular economy, *Hermetia illucens*

Received: April, 2020; Revised final: August, 2021; Accepted: September, 2021; Published in final edited form: October, 2021

1. Introduction

The continuous population growth will bring the global population to 9 billion in 2050 (PRB, 2000) further increasing the exploitation of planetary resources (up to 100 Gt raw material consumed in 2030) (OECD, 2012). In this scenario, any recovery that prevents a resource from leaving the economic circle can have a positive impact. Therefore, all the organic wastes can be seen as precursors of functional materials, allowing to limit the exploitation of non-renewable raw materials. This possibility also becomes crucial in view of what was proposed in 2015 by the United Nations in the document "Transforming our world: the 2030 Agenda for Sustainable Development" that incorporates the seventeen Sustainable Development Goals (United Nations General Assembly, 2015). The 12th goal aims to guarantee sustainable production and consumption models suggesting to "do more and better with less",

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increasing the benefits in terms of well-being taken from economic activities, reducing the use of resources, degradation and pollution in the entire production cycle, thus improving the quality of life and prosperity of future generations. In this context, biorefinery systems in which biomasses are converted into a wide spectrum of high added-value products, play a key role. One of the major barrier hindering the development of biorefinery based processes is related to the seasonal nature and the annual variability of the biomass supply that may disfavour the use of several types of feedstocks due to logistic problems and economic unsustainability (Giuliano et al., 2016). To date, waste and by-products of the agri-food industry are often considered wastes and treated consequently, increasing production costs and producing impacts on the environment. As reported in the european FUSION final report project (Scherhaufer et al. 2015), an estimation of landifill and inciniration process of food waste produces a global warming potential of 0.76 and 0.05 kg CO_2eq/kg food waste treated, respectively. In the last two decades, interests in the biorefinery concept is increased exponentially as estimated by google scholar number of pubblications per year since the 2000 using the keyword “biorefinery”.

In literature, several examples of food waste biorefinery are presented, aiming the valorization through extraction of high-value molecules or conversion into biomassess (De Corato et al., 2018) (Teigiserova et al., 2019) (Mohan et al., 2016a; 2016b) (Philippini et al., 2020). Among all possible available scenarios, the use of bacteria and algae are the most explored with insect representing a new possible approach in bioconversions of waste into a high lipid and protein content biomass (Rajendran et al., 2018) (Wang et al., 2017a; 2017b). Insects are known to be capable of digest organic waste producing larvae capable of reducing waste at high rates. Among all the insects adopted in these bioconversions, black soldier fly dipters (*Hermetia illucens*), due to their high waste reduction rates (40-60%) (Mentari et al., 2020), rapresent a good candidate for biorefinery purposes. Moreover, black soldier fly are known to be rich in fat (20-40%) (Chia et al., 2020) and proteins (30-45%) (Chia et al., 2020): while proteins (in terms of amminoacid composition) are not affected by the rearing substrate, fat content (in terms of saturated and unsaturated fatty acid composition) instead is higly dependent on the rearing substrate, with high energy content (carbohydrates) affecting the lipid composition, in particular lauric acid (C12) abundance (Spranghers et al., 2017).

BIOWAFER project research is aimed at identifying how to develop an integrated and sustainable biorefinery for the valorisation of waste from agro-industries in the Emilia-Romagna region. Thereby the present paper explores how it is possible obtaining products with high added value, for the cosmetic and pharmaceutical industries considering the waste and the by-products of the agri-food chains as precursors of basic chemicals and functional materials, allowing to limit the exploitation of non-renewable raw materials. In this paper, a discussion about the development of an integrated bio-refinery system is presented trough a case study in which different techniques and residual biomassess have been integrated in order to overcome the limits of traditional bio-refinery plants.

2. Material and methods

To develop an integrated biorefinery, a part of literature review was carried out for both the techniques and the most representative supply chains of the region and their residual biomasses production. The most promising techniques were selected for obtaining molecules with high added value and an attempt was made to implement them by adopting a circular biorefinery approach. An integration of different techniques has been proposed in the case study, in order to maximise the recovery of active substances while reducing the final disposal of residues.

2. 1. Case study

The biowafer case study is presented in Fig.1. Three agri-food farms have been selected in order to provide residual biomasses. Selected residual biomasses, supplied continuously throughout the year, even if dissimilar in different seasons, will be enhanced by adopting a circular approach of biorefinery.

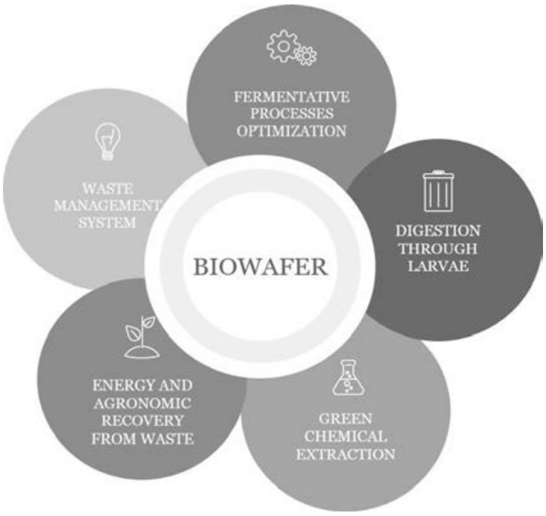

Fig. 1. BIOWAFER project

Through consequential and integrated processes, the input materials of the different phases are enhanced without producing further by-products and waste. Each upstream residue becomes a substrate for the respective downstream phases, and each of the different phases leads to different chemical products with high added value of interest for the cosmetic and pharmaceutical sector.

The techniques used in the biorefinery process include fermentation by selected bacterial strains to obtain hyaluronic acid, polygalacturonans, plant keratin, and lactobionic acid from an adequate mixture of sugars and proteins. The proposed methodology represents an alternative to the extraction of compounds from animal substrates, with significant economic and ethical benefts. Selected by-products will be digested by dipteran larvae, to obtain high added value biomass. In order to maximise the valorisation of by-products, also fermentation residues will be digested by dipteran larvae, to obtain other compounds of interest, including bioactive peptides and polyunsaturated fatty acids. Chemical extraction using conventional and green techniques will allow to extract different molecules, mainly antioxidants. The residues of all the above-mentioned phases will be used for pyro-gasification, to obtain biochar and syngas for agronomic and energy purposes respectively.

The production of energy and the return of biochar as soil improver for agricultural production allow closure of the supply chain in a circular way. In comparison to other circual biorefineries, the use of fermentation coupled with larvae rearing for waste reduction and conversion is a novelty. Despite in this project scaling-up process is not investigated, potential challenges will be taken into account.

2.1.1. Chemical analysis

The residual biomasses have been characterized according the following methodologies (Table 1). For chemical extraction from residual biomasses and from larvae the following methodologies have been used:

- for the extraction of lycopene from tomato peels a solvent ultrasound assisted extraction at 40°C with acetone, ethyl acetate and 2-methyltetrahydrofuran (MTF) has been performed with a liquid solid ratio of 30 mL/g of residue starting from the method reported in (Zuorro, 2020) with some modifications. Lycopene was quantified via a spectrophotometer, reading the absorbance at 503 nm in n-exane;
- for the polyphenol extraction from grape residues a solvent ultrasound assisted extraction at room temperature with ethanol and acetone at different concentration in water was selected considering a solvent/residue ratio of 20 mL/g based on Vatai and coworkers work (Vatai et al., 2008) with some modifications. Polyphenols were quantified via a spectrophotometer, reading at 760 nm using Folin Ciocalteu method;
- moisture of residues was evaluated gravimetrically after incubation at 105°C until a constant weight;
- lipid quantification was carried out in n-exane (or other solvents as indicated in the result and discussion section) gravimetrically after Soxhlet extraction;
- a rapid lipid extraction using MTF was selected for fat recovery from larvae as presented in (Smets et al., 2021). Briefly, 3 g of fresh larvae is mixed with 8 g of MTF and 9 g of bi-distilled water in a centrifuge tube and immersed in a water bath at 45°C for 30 minutes, then centrifuged at 4000 rpm for 15 min in a pre-heated centrifuge. After recovery of the organic phase, other 8 g of solvent are added reaping the extraction steps other 2 times in order to increase the recovery. The extract is then separated from the solvent via a rotary evaporator;
- the characterization of extracted lipids was carried out via a gas-chromatographic method after ester derivatisation in order to observe the main fatty acids profile;
- to extract proteins, one step protein extraction protocol was used. Briefly, defatted insect pellet was treated with 40 mL of 1 M NaOH in water bath at 40 °C for 1 h. The supernatant was neutralized, centrifuged for 15 min at 4000 rpm and then recovered and stored at -20 °C for further uses (Caligiani et al., 2018). Protein content was evaluated via Kjeldhal method for total nitrogen quantification considering 6.25 as a conversion factor. In parallel, bromelain enzyme was used in order to extract protein content. Briefly, defatted insect pellet was treated with 3% bromelain in 0.05 M phosphate buffer pH 8 for 13h at 50 °C followed by a 90 °C treatment in order to deactivate the enzyme. The solution was centrifuged for 15 min at 4000 rpm, the supernatant recovered and stored at -20 °C for further uses (Firmansyah and Yusuf Abduh, 2019);

Table 1. Methods of analysis used for each parameter

Parameters	***Methods***
pH	M.D. agricultural policies 13/09/99 met.III,1 (potentiometric)
Residue at 105°	M.D. agricultural policies 13/09/99 met. II,2 (gravimetric)
TOC	M.D. agricultural policies 13/09/99 met.VII.3 (Walkley)
Nitrogen	M.D. agricultural policies 13/9/99 met.XIV.2 (Kjeldahl)
Fiber	wende U.S. AOAC (15°ED)
Saccharose	UV method
Glucose	UV method
Fructose	UV method
Citric acid	UNI EN 1137 1997
D-lactic acid	UV method
L-lactic acid	UV method
Acetic acid	UV method

– both protein hydrolysates were fractionated in content < 1 kDa, >10 kDa and between these two values and characterized in terms of nitrogen content via Kjeldhal method.

3. Results and discussion

3.1. Selection of the supply chains

The agri-food sector is relevant for the economy of the Emilia-Romagna Region. Given the intensity of local agricultural and agri-food production, the gross specific availability of residual biomasses (mass of dry matter per km^2) is among the highest in Italy (about 80 tons year DM/km^2) (ANPA, 2001).

Emilia Romagna is confirmed as the most productive region in tomato cultivation. Emilia Romagna is the region with the largest proportion of land cultivated with industrial tomatoes, amounting to 25,833 hectares (ISTAT, 2020), which represents almost 68% of the production in the northern area (Emilia Romagna, Veneto, Piemonte and Lombardia) (ISTAT, 2020). The industrial processing of tomatoes generates a significant by-product, of which 10-30% consists of peels and seeds (Beninati et al., 2019). However, the partially dehydrated peels and seeds have a low calorific value. Tomato peels are rich in polyphenol compounds with antioxidant activity, namely carotenes and carotenoids with a protein and lipid content of 13.3% and 6%, respectively (Navarro-González et al., 2011). Among these, the most relevant is lycopene, a molecule known for fruits red-orange typical color.

Lycopene is a liposoluble molecule, mainly known for its antioxidant activity (i.e. biological properties in reducing free radicals) and in protecting the skin from photo-ageing damage; moreover, clinical studies demonstrate a possible correletion as anticancer, antidiabetic, cardioprotective, antinflammatory (Imran et al., 2020). Thus, the recovery of lycopene and other active substances from tomato processing residues is therefore of prime importance, for use in several fields, including pharmaceutical and cosmetic industries.

Moreover, Emilia Romagna produces 6,611,490 hl of wines being the third region for wine production in Italy (ISTAT, 2020). Wine processing residues account for 20% of the dried matter of harvested grapes (Beninati et al., 2019). The reform of the Common Market Organisation in the wine sector entails the progressive reduction, until disappearance, of distillation. It is therefore essential to create a new integrated, sustainable and standardised system which will contribute not only to the problem of disposing of this organic waste, but also to its industrial valorisation. Wine residues are known for being rich in polyphenols, in particular anthocyanins, with high antioxidant properties and as inhibitor for skin ageing (Matos et al. 2019). Like lycopene for tomato peels, anthocyanins also pose some correlation in disease prevention, in particular as anticancer, antidiabetic and ant obesity (Khoo et al., 2017). Thus, the extraction of anthocyanin molecules is of high interest for pharmaceutical and cosmetic industry.

The 11.62% of the Grana Padano PDO cheese (the largest PDO production in Europe), is produced in Emilia Romagna (CLAL, 2021). Whey is one of the main residues of the dairy industry, which produces up to 6 litres per kilo of cheese (PEFCR, 2018). Its traditional use as pig feed is no longer sufficient for the total utilisation of this by-product, and disposal by other means is either expensive or environmentally problematic. One of the most important properties of whey is its high value protein content, known to possess antimicrobial, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial, and chelating agent activity (Marshall 2004).

Another interesting by-product of dairy sector, in particular for cured cheeses known in the sector as “washed rind”, is the residue of treatment with water, salt and micro-organisms used for ageing the cheese. This residue, indicated as “morge” in France, is not well studied but promising considering hits potential fat and protein content, as well as antimicrobial activity. Fat are well known to be a potential raw material for soaps and creams in cosmetic (Ahmad and Ahsan, 2020), while proteins (and in particular peptides) are largely studied in cosmetology applications for skin and hair treatment (Secchi, 2008).

3.2. Collection, storage, stabilization, chemical-physical characterization of by-products

The seasonality and the amounts available for each supply chain under study is reported in the Table 2 according to the productions of the participating partner companies.

For each residual biomass under study chemical-physical was carried out in order to select, the most suitable storage method . First, standard storage methods were examined and subsequently alternative storage methods were identified: freezing (-20 °C), drying (65 °C) and freeze-drying processes. Other preservation methods based on pH, radiation or addition of chemical agents have not been investigated because no evidence has been found in literature.

The tests were performed in triplicate on all matrices for each parameter. For each parameter the most reliable one has been selected and the corresponding protocol has been defined concerning both the preparation of the sample according to the physical state of the biomass (fresh, frozen, dried, freeze-dried) and the extraction efficiency.

The best stabilization method fro tomato peels is freeze-drying: it allows to maintain the physical characteristics unaltered with respect to freezing and drying (Table 3), and is the most effective also in terms of mass reduction, (about 60%). Freezing and drying are slower processes that deteriorate the product.

Table 2 Annual availability for tomato peels, grape pomace, grape dregs and whey
(Data according to the productions of the partner companies involved in the project)

Residual Biomasses	***Tomato peels***	***Pomace***	***Dregs***	***Whey***	***“Morge”***
Seasonality	August September	September October November	All over of the year	All over of the year	All over of the year
Quantity	1.500 tons/year	15.000 tons/year	3.500 tons/year	40 tons/day	6 kg/day

Table 1 Chemical-physical characteristics of tomato peels in different physical states

Parameters	***U.M.***	***Fresh***	***Freezing 20 °C***	***Drying 65 °C***	***Freeze-dried***
pH		4.46	4.52	4.79	4.69
Residue at 105° C	%	35.41	37.9	94.84	96.52
Nitrogen	N% D.M.	3.02	3.42	0.35	0.27
TOC	% D.M.	56.40	60.22	59.01	63.64
Saccharose	g/kg	2.52	1.03	0.95	2.58
Glucose	g/kg	0.5	0.47	0.10	0.11
Fructose	g/kg	0.12	0.05	0.02	0.14
Citric acid	g/kg	0.14	0.02	0.01	0.15
L-lactic acid	g/kg	1.06	0.61	0.53	1.15
D-lactic acid	g/kg	1.92	0.76	0.70	1.9
Acetic acid	g/kg	2.44	0.84	0.75	2.03
Fiber	% weight	49.32	58.32	52.4	50.22

The pomace, if not used fresh, develops a severe degradation process, so the ideal method of preservation was freezing (Table 4).

Table 2. Chemical-physical characteristics of pomace in different physical states

Parameters	***U.M.***	***Fresh pomace***	***Freezing pomace***
pH		3.67	3.74
Residue at 105° C	%	37.38	36.05
Nitrogen	% N D.M.	0.48	0.3
TOC	%	51.74	51.45
Saccharose	g/kg	0.15	0.18
Glucose	g/kg	0.06	1.58
Fructose	g/kg	0.09	1.73
Citric acid	g/kg	0.07	0.09
L-lactic acid	g/kg	0.12	0.84
D-lactic acid	g/kg	0.04	0.34
Fiber	%	33.47	38.39

The dregs do not require any special care for storage. It can be stored in a container in a dry place, for this reasonfreezing and freeze-drying processes were not applied, The drying method could be effective in keeping the residual biomasses stable over the year (Table 5). Whey can be used fresh or frozen, in fact dry-freezing and drying degrade its chemical characteristics. While the best storage method for “morge” is dry delivery.Results of chemical-physical characterization of whey and “morge” are reported in Table 6-7.

3.3. Optimization of fermentation processes

Potential biotechnological sources for the products of interest were identified and analyzed, in particular focused on: hyaluronic acid and bioactive peptides. Biotechnology has been a tool in food processing and food production for centuries.

Table 3. Chemical-physical characteristics of dregs in different physical states

Parameters	***U.M.***	***Fresh dregs***	***Dry dregs***
pH		3.26	3.53
Residue at 105° C	%	40.23	58.45
Nitrogen	% N D.M.	0.12	0.21
TOC	%	47.72	48.51
Glucose	g/kg	1.82	2.11
Fructose	g/kg	0.53	0.47
Citric acid	g/kg	0.29	0.13
L-lactic acid	g/kg	1.20	0.60
D-lactic acid	g/kg	0.20	0.01
Acetic acid	g/kg	0.10	<0.01
Fiber	% weight	26.39	28.42

Table 4. Chemical-physical characteristics of whey

Parameters	***U.M.***	***Whey***
pH		4.15
Residue at 105° C	%	6.96
Nitrogen	N% D.M.	1.99
TOC	mg/l	32.01
Glucose	g/l	0.29
Lactose	g/l	56.16
Galactose	g/l	1.25
Glycerol	g/l	0.05

Table 7. Chemical-physical characterization of “morge”

Parameters	***U.M.***	***“Morge”***
pH		4.89
Residuo 105°C	%	89.61
azoto	N % D.M.	0.36
TOC	mg/l	52.45
fibra	% peso	2.99

Recently it has become essential in reutilizing food residues from different food supply chains. The exploitation of living organisms, or parts of living organisms, can be a cost-effective solution to the food waste problem. Several examples are available from the conventional biotechnologies, with no need to produce transgenic organisms for improving the processes: a) digestion of residual biomass to obtain biogas; b) fermentation of residual biomass to obtain biofuels, e.g. bioethanol; c) improvement of production of biodiesel or other biofuels from plant biomass; d) biomass used as cheap substrate for production of high-value materials; e) designed fermentation processes to obtain specific high-value chemicals; f) extraction of useful substances from residual biomass.

Hyaluronic acid is one of the possible products with high added value that can be obtained from waste and by-products of the agri-food supply chains. It is a polymer of a subunit composed of D-glucuronic acid and N-acetyl-D-glucosamine (Necas et al., 2008), ranging in weight between 103 to 107 Da. Applications of hyaluronic acid concern medical treatments of joints, eye lubrification, skin texture improvement, tissue reparation and scar formation, nutraceutical products and drug delivery (Fallacara et al., 2018; Huang and Chen, 2019). Being a component of vertebrate tissues, particularly in the extracellular matrix of connective tissues, this substance has been traditionally extracted from animal sources, such as vitreous of bovines or rooster combs. Even though this can be considered a way of reusing animal waste from food supply chains, the process is not sustainable and generates risks for the presence of immunogenic compounds and pathogens. Enzymatic synthesis is possible, with enzymes taken from microorganisms, but it requires high quality reagents.

Bioactive peptides are short aminoacidic chains, from 2 to 40 amino acids in length, which derive from mature proteins through partial proteolysis. Bioactive peptides are among the beneficial features of fermented foods, produced by bacteria, yeasts and fungi, with potential activities as immune system modulators, antioxidants, opioid mimics, cholesterol reduction and antihypertensive action (Pavlicevic et al., 2020). Plant materials from tomato and grape, as well as milk, cheese and whey, contain hundreds of proteins which can be used as starting materials (Maestri et al., 2019).

Growth conditions of strains of microorganisms which can grow on biomass and produce hyaluronic acid (Liu et al., 2011) have been optimized. *Streptococcus zooepidemicus*, is being studied for the possibility of using as a growth substrate residues from the supply chains of industrial tomato, milk and dairy, wine production (Amado et al., 2017). All the selectied residual biomasses containsugars and other molecules which can be substrates for the growth of the bacterium and for the synthesis of hyaluronic acid. The fermentation process is affected by pH, temperature, aeration and stirring, with a moderate stirring favoring the formation of high molecular weight polymers. Interesting possibilities for using plant cell cultures in hyaluronic acid production can also be considered (Rakkhumkaew et al., 2013).

3.4. Waste and by-products digestion by insect larvae

Selected residual biomasses were subjected to digestion through *Hermetia illucens* (Diptera: Stratiomyidae) larvae in order to obtain other interesting compounds for cosmetic and pharmaceutical applications. In the second phase of the project, also residue of the fermentation will be subjected to digestion through *Hermetia illucens* larvae in order to obtain other interesting compounds for cosmetic applications, such as bioactive peptides (Firmansyah and Yusuf Abduh, 2019), polyunsaturated fatty acids - which can be found into the larvae at a percentage of 7% (Smets et al., 2020), and antioxidants peptides (Zhu et al., 2020).

The *Hermetia illucens* larvae used in this study were obtained from the mass rearing of the Department of Sustainable Crop Production (Di.Pro.Ve.S) of the Università Cattolica del Sacro Cuore (Piacenza, Italy).

The rearing was set up in an air-conditioned room (28 ± 2 °C e 50 ± 5% R.H.). In the room, three sections have been set up, each dedicated to a separate development stage of the insect. Adults were reared in a wooden frame holding a mosquito net cage which allowed air and humidity exchange. To provide the required brightness for couplings (200 mol $\cdot$ m$^{2}\cdot$ s^{-1} according to Sheppard et al., 2002), a panel of LED lamp was placed above the cage. The photoperiod was 16 hours of light and 8 hours of darkness. Adults were fed with a solution of water and sugar, placed in a dedicated box. Some supports were placed inside the cage to increase the available area for adult activity and to stimulate their "lekking behaviour", their mating behavior (Tingle et al., 1975). Oviposition took place in the grooves of specific supports, placed in a box also containing decomposing material to attract females. Oviposition supports were replaced every two or three days: supports containing eggs were placed into small trays with hydrated chicken feed, to feed the newborn larvae. This substrate was used due to its availability in the market, its high protein content and its high palatability, ensuring them a balanced and quick development. Tray covers had a small ventilation area, in order to maintain inside the trays, the relative humidity needed to obtain a high percentage in hatched eggs. After approximately seven days, newborn larvae were transferred in open boxes and they were fed with hydrated chicken feed until the 50% of larvae became prepupae. Upon reaching this stage, they stop feeding and then they become pupae. The tanks were then covered with aerated lids, to easily collect emerging adults and to transfer them into the mating cage.

Larvae were reared on different substrates: hydrated chicken feed (control sample), tomato peels, pomace and dregs from the wine chain. Ad hoc tests were set up to obtain information about *Hermetia illucens* larval growth and mortality on each substrate in order to evaluate their suitability for mass larval production. To extract lipid and protein components and to study their antioxidant, antimicrobial activities, *Hermetia illucens* larvae were collected just before reaching the prepupae stage. The larval weight and mortality at 7, 10 and 14 days from the start were measured and every thesis was replicated four times. Larval growth on unaltered and shredded residual biomasses were evaluated in laboratory scale, omitting in this study industrial scaling-up. It worth to mention that industrial larvae rearing require space in order to allow optimal substrate conversion. The results are reported in Fig. 2.

Initially, larvae had homogeneous weight. After 7, 10 and 14 days the control larvae weight was higher than that of the residual biomasses, both shredded and unaltered ones, even if the differences mitigate after 10 and 14 days. The larvae fed with residual shredded biomasses showed a higher growth compared to the ones fed with unaltered residual biomasses, both at 7 and 10 days, but, after 14 days, there were no differences in larval weight between the two substrates. Considering the percentages, the differences in the growth are more immediate to be evaluated (Fig. 3).

The average mortality measured during the 14 days of the experiment was 0% in control and in shredded residual biomasses samples, instead it was 3.26% in the unaltered residual biomasses. In conclusion, the performance after 7 days is higher if residual biomasses are shredded. Otherwise, if the larvae are reared for 14 days, there is no significant difference between the two types of residual biomasses.

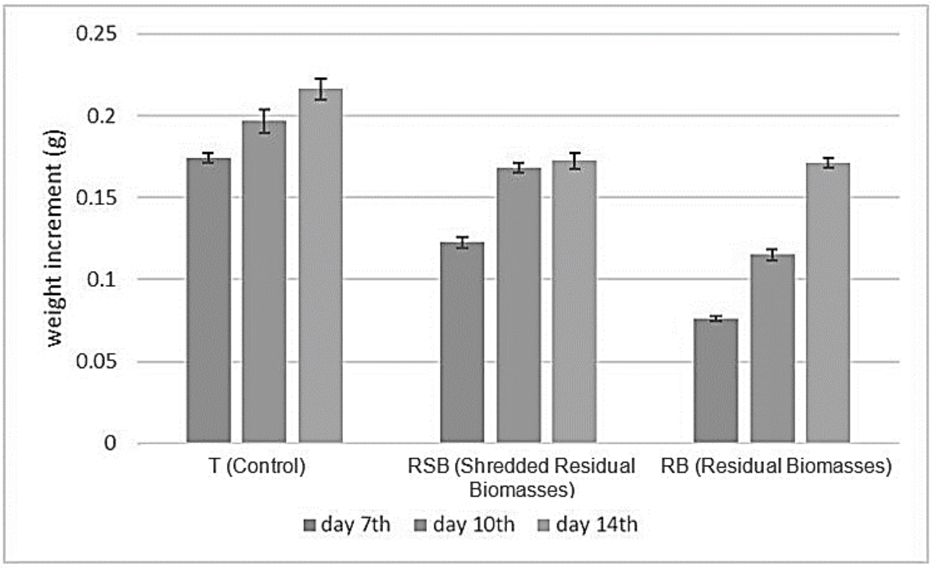

Fig. 2. Larval weight increase in control test, residual shredded biomasses test and unaltered residual biomasses test

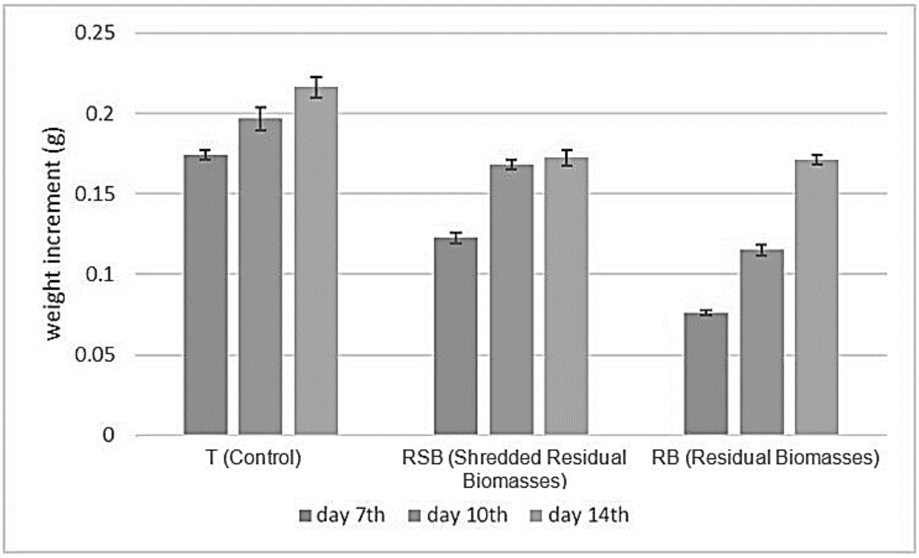

Fig. 3. Larval weight increase in control test, residual shredded biomasses test and unaltered residual biomasses test [%]

3.5. Development of eco-friendly extraction techniques for the chemical extraction of molecules of cosmetic interest

Insects provide a valid alternative to some of cosmetic ingredients, due to their high value biomass, whit high value protein and fat content (Tzompa-Sosa et al., 2014). Amongst these insects *Hermetia illucens* larvae represent a valid option as resource for cosmetic ingredients, since their fat profile is similar to natural oils that are already used in cosmetics (Verheyen et al., 2018), and since the protein and enzyme content show a high antioxidant activity (Firmansyah and Yusuf Abduh, 2019). Literature studies state that insect biomass composition varies according to species, life stage and its diet (Finke and Oonincx, 2014). In the case of *Hermetia illucens*, during larval stage, lipid content increases up to 40% and is strictly dependent on the lipid and carbohydrates content in the feeding substrate (Devic and Fahmi, 2014; Spranghers et al., 2017).

Extraction of molecules of interests from the selected residual biomasses respecting the concept of “green chemistry” is of vital importance in order to obtain a sustainable biorefinery. In order to achieve this goal, solvent extraction was performed taking into account the origins (fossil or renewable resource), boiling point and yield of extraction of the solvents. Among the solvents, MTF was selected because comes from renewable feedstock and has low toxicity on humans and environment; moreover, is considered a good solvent for fat and carotenoids extractions (Sicaire et al., 2014).

Moreover, in order to increase the yield of extraction, ultra sound assisted solvent extraction was considered among other recent technologies known to be enhancers in extraction processes.

Solvent selection for lycopene extraction led to choose for MTF in terms of yields of lycopene extracted, while solvent selection and concentration for polyphenol extraction from dregs show a high yield using 75% acetone or 50% ethanol (data not shown). Fat content of tomato peels, grape dregs and cheese "morge" resulted in 3.5%, 1.6% and 43.0% of dry matter, respectively. Protein content of tomato peels, grape dregs and cheese "morge" resulted in 15.2%, 18.1% and 47.0% of dry matter, respectively. Results show that cheese "morge" has the highest potential to be reach in lipids and proteins, while tomato peels and grape dregs a high protein content. Despite the obvious recovery of high valued molecules like carotenoids and anthocyanins, all the residues of the supply chains studied are a resource in terms of protein and peptides to be used in cosmetics formulation.

The analysis of larvae fed on tomato peels, showed a lipid content in the Soxhlet extraction with petroleum ether of 24% of dry matter (against a 21% of dry matter for group control larvae fed on chicken feed) and in the extraction with MTF of 40% of dry matter (against a 23% of dry matter for group control larvae), showing a highest yield of extraction with MTF in comparison to petroleum ether. The ultrasonic assisted extraction showed a high yield of extraction (98%), indicating the validity of the extraction method compared with the Soxhlet extraction. Soxhlet extraction has the advantage to repeat extraction process in cycles, using a limited amount of solvent that is recycled in each cycle through evaporation and recondensation.

Unfortunately, Soxhlet extraction require to operate for several hours and relatively high energy consumption to heat the solvent to his boiling point and, moreover, require a large amount of tap water for the recondensation process or energy consumption in case of high boiling point solvents. Ultrasonic assisted extraction, instead, is a valid alternative in terms of modern extraction technique, making the extraction process greener and more suitable in a circular economy approach. The characterization of lipid content from larvae revealed a content of fatty acids in the range C10 - C20 with lauric acid representing the major component among them, followed by palmitic, oleic, myristic and linoleic acids. Lauric acid has been demonstrate to have potent antibacterial and antiviral activities (Anzaku et al., 2017) and could also been easily converted into monolaurin, showing potent preservative proprieties (Ushakova et al., 2016). Verheyen and co-workers (Verheyen et al., 2018) compare *Hermetia illucens* larvae oils to other natural oils suggesting its substitution in skin cosmetic formulations. In this process is recommended to remove phospholipids and unsuitable free fatty acids, as well as to improve its color and odor characteristics.

Preliminary results on the characterization of fractionated protein hydrolysates show a higher content of the < 1kDa fraction and a lower content of the > 10kDa fraction in the enzyme hydrolysate in comparison with the other extract.

3.6. Energy and agronomic recovery of residues

The real closure of a circular economy approach can be obtained only when all the material is recycled or upcycled, for a new utilization, or used in energy production at the very end. Combustion is not an option in this case, because it destroys materials and liberates emissions, while producing some forms of energy.

In the present case study, pyrolysis, or pyrogasification, is the method for the final disposal of all the residues of the previous phases. In fact, every kind of organic material can be a feedstock for pyrolysis, as such without treatments, or else in mixtures with wood and dry biomass. The pyrolysis process is a controlled combustion at fixed temperatures (300-750°C) and low oxygen concentration, and therefore it does not incinerate the feedstock. This process always generates energy in the form of steam and heat, a syngas containing CO, CO_2, CH_4 and H_2, and a bio-oil which can be used as fuels. Most of the carbon contained in the feedstock however is preserved after pyrolysis in the form of “biochar”, in which carbon is stabilized in organic form (Joseph and Lehmann, 2009).

Biochar has a “nanostructure” with extended surfaces and cavities, deriving from the structure of the feedstock which is mostly preserved (Marmiroli et al., 2018). This extended surface is reactive due to chemical functionalization and reactive groups, and this makes the biochar an interesting material with useful properties. According to EU legislation, biochar from plant origin can be applied as a soil amendment or improver, also in organic agriculture. Biochar improves soil properties such as water retention and texture, increases the pH of acidic soils, modifies the mobility of nutrients, contributes to sequestration of organic and inorganic contaminants, and stimulates the growth of microorganisms. As a final result, it improves the growth of plants. Pyrolysis is therefore considered as a desirable process in closing the cycle in agricultural context, because the carbon contained in the residual biomass is returned to the soil as amendment, in a stable form which is not subjected to further degradation and without emissions of CO_2.

A series of guidelines are available to evaluate the biochar production and its quality (EBC, 2020; IBI, 2015). The European Biochar Certificate in particular, has been developed to become the voluntary European industrial standard ensuring a sustainable biochar production and low hazard use in agronomic systems.

Biochar will therefore represent the final product of the residual biomass of tomato, dairy and wine supply chains under study in the BIOWAFER project.

4. Conclusions

BIOWAFER will make the availability of waste and by-products of the agri-food sector in Emilia Romagna known, identifying their potential for exploitation. The project therefore exists in the context of a circular economy, producing new products with high added value (like lycopene, polyphenols, anthocyanins, lipids and proteins/peptides) and proposing alternative solutions to the traditional disposal of the residual biomasses of agri-food chains.

Thanks to a biorefinery approach that envisages consecutive and integrated processes, the valorization of these residual biomasses, provides an alternative path of qualification leading to high added value products, of potential interest for the cosmetic and pharmaceutical industries.

The use of eco-friendly extraction processes allows to reach the goal of eco-friendly biorefinery with: a) a total reduction of residues in a circular way; b) obtaining molecules of interest required by the market because they are natural and of plant origin; c) reduction of synthetic molecules; d) use of an eco-friendly solvent like MTF with higher yields of extraction. Many other circular approaches are presented in literature, but, in our knowledge, no one use fermentation coupled with larvae nursery for waste reduction and conversion.

However, one of the most challenge aspects in scaling larvae rearing on residual wastes is the space requirement: larvae in fact tend to rear on the surface of the substrate because of the lack of oxygen in the lower layers. Another important aspect to be evaluated is the potential impact factor of the rearing process of larvae on the environment. Moreover, it is crucial to address the possibility that larvae reared on fermentation residue could lead to a lower mass content (in term of lipid content) biomass due to the possible lack of carbohydrates from the rearing substrate.

The biochar obtained from pyro-gasification of the residues of all the project phases (fermentation residue, larvae nutrition substrate with larvae manure, larvae residue after defatting and hydrolysis process), which can be used for agronomic purposes, will instead contribute to increasing soil quality, by increasing agricultural yield, reducing the use of synthetic fertilizers, thus improving soil structure and its water retention capacity, with obvious positive effects also in terms of water saving. Biochar sequesters carbon in a stable form, helping stakeholders and farmers to develop more sustainable production models, which can offset other greenhouse gas-producing activities.

In future steps we will study other molecules of potential interest obtainable from the same matrices and integration with other supply chains will be evaluated. Furthermore, the economic and environmental sustainability of what is proposed will be studied as these approaches can become widely used only if truly sustainable.

Acknowledgements

This work was done in the framework of the project "BIOWAFER" co-financed by 2014-2020 POR FESR, Emilia-Romagna Region, Italy - www.biowafer.org

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