A Biocascade approach towards the recovery of high-value natural products from biowaste: State-of-art and future trends

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Abstract

In a circular economy, products are made from renewable resources and the waste streams generated during production are either reused, recycled or recovered. The Biocascade methodology considers bio-waste as a resource that can be exploited to produce high-value products such as pharmaceuticals, food ingredients and nutrients; and low-value products such as feed, energy or soil conditioners. The Biocascade principle ensures optimal biomass exploitation by following a hierarchy from high-to-low value, where the waste from one process is the starting material for the next. Biowaste from plant origin is a very suitable resource for applying the Biocascade methodology, both in terms of worldwide production and of variety of components. In this review, the biowaste from sour cherry wine, ornamental kalanchoe plants and red clover feed production, have been examined for processing using a Biocascade approach. Starting from the biowaste characterization, the most relevant components have been identified highlighting their potential uses. The extraction methodology is then discussed in terms of solvent used, operating conditions and yield. Based on the information retrieved from literature, different process flowsheets have been proposed to maximize the use of the biowaste following the Biocascade perspective and targeting zero-waste generation.

Keywords: Biocascade, sour cherry pomace, kalanchoe leaves, red clover, biowaste

Statement of novelty

Very often the study of the bio-waste valorization is performed based on different competences specialized in a particular field. It is common to find detailed component characterization and analysis of different extraction procedures but there is a lack in defining technologies that are able to translate laboratory procedures to a more profitable scale. In this review work the three different aspects named target component identification and quantification, extraction, and flowsheet

synthesis are discussed though the selection of relevant works from the literature. This work widens the scope of the Biocascade methodology to encompass different streams. In particular, sour cherry pomace, kalanchoe leave and red clover grass have been considered as possible matrices to apply a biocascade approach. A thorough study of the literature available is culminated by a proposed valorization strategy for each of the target biomasses. The case studies presented highlight the versatility of the methodology and extend the horizons for the application of the Biocascade methodology in different biomass and bio-waste types across different sectors.

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1. Introduction

"Never waste or throw away anything that can be turned to account" [1].

This is one of the advices that can be found in the series of books inspired by the works of Mrs. Isabelle Beeton in the 19th century. Those books contained rules and suggestions on the management of the food within a household kitchen. Nowadays, this very same concept can be applied in the field of bio-waste management, which is one of the major issues able to influence our society at different levels: environment, health and economics.

The environment is affected due to the improper bio-waste management. In fact, the strategy to eventually dispose the bio-waste in the landfills led to the uncontrolled production of methane, which is one of the most harmful greenhouse gases [2-4]. According to RedCorn et al., in the US, food waste alone contributes for $3.4x10^7$ t of carbon dioxide equivalent emissions [5]. Moreover, leakage of undesirable substances from the waste can lead to the spoiling of natural resources such as water and soil [4, 6] and can cause problems for the human health [7]. Another undesirable strategy to manage the problem of bio-waste is incineration. The high content of water and the potential in generating toxic compounds like dioxins ~~[3, 8],~~ make~~s~~ incineration unsuitable for bio-waste treating [3, 8].

Together with the environmental and health problems, bio-waste management also affects the economics in the form of the costs related to the disposal [4]. Again, RedCorn et al. point out that in the US, the disposal fees related to food waste amount to 1.9 billion dollars [5].

It is in this context that specific regulations have been defined to mitigate the problem of bio-waste management. First, to prevent the uncontrolled discharge in the landfills and the reduction of biodegradable waste, the Landfill Directive (1999/3/EC) was issued in the European Union [9]. The EU Waste Framework Directive has defined a waste management hierarchy, which applies also to bio-waste, consisting of five points: (i) prevention, (ii) preparing for reuse, (iii) recycling, (iv) other recovery, and (v) disposal [10]. As it is possible to notice, the first action is to prevent the generation of bio-waste. This is followed by the reuse, for example to feed the animals. Third step

in the hierarchy deals with the processing of the bio-waste to produce high-value components, followed in the fourth step by the use of bio-waste for the generation of biofuels and energy by, for example, anaerobic digestion and the production of a high-quality soil amendment through composting. Last possible actions are landfilling or incineration for definitive disposal.

With reference to point (iii) in the aforementioned hierarchy, it is only in the last decades that bio-waste has been recognized as a source of high-value components that can be recovered in order to produce pharmaceutical ingredients, nutraceuticals, functional foods and food ingredients [3, 4, 6-9, 11-16]. The last two elements become even more relevant taking into account that the public attention to food properties in terms of healthiness, naturality and quality is an important factor in modern times [7]. In particular, this field is strongly associated with the concept of Circular Economy (CE), which has been promoted in the EU and several other countries starting from 1990s [17, 18]. The traditional linear economy based on raw materials manufacturing – selling – discarding as waste leads to significant losses along the value chain and it is proven to be unsustainable at different levels, i.e., economic, environmental and social. According to the Ellen MacArthur Foundation "*The concept (of Circular Economy) is characterized, more than defined, as an economy that is restorative and regenerative by design and aims to keep products, components, and materials, at their highest utility and value at all time, distinguishing between technical and biological cycles. It is conceived as a continuous positive development cycle that preserves and enhances natural capital, optimises resource yields, and minimizes system risks by managing finite stocks and renewable flows*" [19]. Then, by definition, within the circular economy "*waste does not exist, and is designed out by intention*". This means that, ideally, the CE promotes an economy based on the reutilization and the recycling of all the available assets, leading to a notable reduction of the use of finite resources. This discussion might sound very optimistic and right now the economy, as a matter of fact, is still far to achieve this objective. At the same time, it is fundamental to start this transition from a linear to a circular economy. One approach to manage bio-waste that is inspired by the CE is the so-called Biocascade. Following the approach proposed by Brar et al. [20]

"Biocascading, makes use of entire plants and not just easily extractable sugars in order to produce a variety of possible chemicals". Extending this concept, it is considered that every part of the bio-waste is exploited in order to obtain a valuable product [21, 22]. Along the production chain, what is classified as the waste of one process becomes the feedstock of the subsequent process. This approach potentially ensures the complete utilization of a bio-waste and the recovery of the aforementioned high value components. After the recovery, if further bio-waste is present, it should be ideally used for clean energy production by means of anaerobic digestion, i.e., a state-of-art biological process used for the production of biogas.

It is now important to frame the bio-waste in terms of legislation to better understand the kind of biomasses involved. With reference to the Directive 2008/98/EC – Article 3 (4) of the European Parliament and of the Council, bio-waste is defined as "*biodegradable garden and park waste, food and kitchen waste from households, restaurants, caterers and retail premises and comparable waste from food processing plants*" [10]. This general definition includes the waste derived from food and beverages industries and from agro-industrial processing. The same document provides two other important definitions, i.e., the definition of "waste" and "by-product". Waste is defined in Article 3(1) as "*any substance or object which the holder discards or intends or is required to discard"*. On the other hand, in Article 5 "*a substance or object, resulting from a production process, the primary aim of that item, may be regarded as not being waste referred to in point (1) of Article 3 but as being a by-product only if the following conditions are met: (a) further use of the substance or object is certain… "*. In the light of these two definitions, and taking into account the concept of circular economy, the objective is to shift the bio-waste into a by-product by processing it to recover high value components, i.e., making a further use of it. Typically, bio-waste can be classified in two macro-categories, i.e. animal and plant derived bio-waste. Each category has its target components, as animal derived waste is mainly a source of proteins and lipids, while plant derived waste contains mainly carbohydrates, phenols, oils, phytosterols and fibers [12]. Plant derived wastes are in general the most investigated biomasses since they are produced in high

amounts [12, 14, 15]. Among this category of waste, up to now the research has been focused mainly on cereals, olive crops, apple pomace and orange pomace [12]. The scope of this review paper is to analyze the exploitation of different alternative biomasses classified as bio-waste from different production processes for the recovery of high value components using a biocascade approach. When a productive process involving biological raw material is considered, two main kinds of treatment can be recognized: whole-stream and component-oriented [23]. In the first case, the bio-waste is subjected to limited pre-treatment (only if necessary) and can be used in applications such as the energy generation by anaerobic digestion. In the second case, which is the one considered in this work, the bio-waste is fractionated in different streams, each of them pre-treated (if necessary) and processed to recover specific components. Only after the recovery, if no other products can be obtained, the waste can be processed as a whole-stream. In particular, three different bio-waste were selected:

1. Sour cherry pomace (food industry): source of polyphenols and fatty acids;
2. Kalanchoe leaves (gardening industry): source of flavonoids;
3. Red clover (animal feed industry): source of plant-based proteins and phytoestrogens;

For each of them, the target components, their application and the available recovery techniques are reported and a possible scheme to apply the biocascade principle is proposed. Common aspect to all the process schemes proposed will be that each of the final streams exiting the plant is a product. No waste is present in the proposed processes, in agreement with the biocascade and the circular economy concepts. Each of the biomasses will be described separately for what concerns:

- Bioactive compounds to be recovered;
- Extraction methods;
- Implementation within a biocascade perspective.

2. Sour Cherry

Sour cherry (*Prunus cerasus* L.) is an acidic fruit typically used for the industrial production of juice, jam, marmalade, liquor and wine. The sour cherry world production was estimated to 1,529,000 tons in 2018 [24]. Opposite to its sweet "relative", the sweet cherry (*Prunus avium* L.), it is not consumed as it is, due to its acid taste and large extent of its mass can be expected to end as a by-product or bio-waste from industrial food processing.

Different parts can be distinguished in the cherry: skin, flesh, seed (shell + kernel) and stem. The most important part for the production is represented by the flesh, which is usually pressed to obtain the concentrated liquid fraction of the product. After processing, a high amount of waste is left, from here on referred to as "sour cherry pomace". This bio-waste is a mixture of different parts of the cherry fruit that are typically a residue of the production process and is composed mainly by (i) skins and flesh leftovers, (ii) seeds and (iii) stems. These three sources contain different bioactive compounds that can be recovered with appropriate extraction methods.

2.1 Sour cherry skins and flesh leftovers

2.1.1 Recoverable components

Sour cherry is widely recognized as a potential source of valuable products such as polyphenols, mainly phenolic acids and flavonoids [25-34]. These compounds are known for their health-benefit properties such as antioxidant, anti-inflammatory, anticancer, and antidiabetic activities [26, 28-31, 34-41]. Moreover, among flavonoids, anthocyanins stand out due to their commercial application as natural pigments. In fact, they can be used as food additives and can become fundamental constituents in the development of functional foods. According to different authors, the various components that can be recovered from this bio-waste are mainly concentrated in the skin of the cherries, and becomes then a source to be exploited [26, 29, 30, 32, 41, 42]. Table 1 reports a list of the different compounds identified in the literature together with the correspondent quantification.

Table 1 List of different compounds found in the cherry skins and flesh leftovers extracts (fresh: fresh weight, dry: dry weight)

Component	Value	Reference
Phenolic acids		
Caffeic acid	-	[31, 33]
Chlorogenic acid	305±42 μg/100 g_{fresh}	[26]
Coumaric acid	7.5±0.3 μg/100 g_{fresh}	[26]
Ferulic acid	17±1 μg/100 g_{fresh}	[26, 31]
Neochlorogenic acid	0.23±0.01 mg/g_{dry}	[31]
Protocatechuic acid	1261±189 μg/100 g_{fresh}	[26]
p-Hydroxybenzoic acid	19±1 μg/100 g_{fresh}	[26]
p-Coumaric acid	-	[31, 33]
Vanillic acid	47±2 μg/100 g_{fresh}	[26, 33]
Flavanols		
Catechin	0.22±0.02 mg/ g_{dry}	[31]
(+)-Catechin	63±8 μg/100 g_{fresh}	[26, 33]
(-)-Epicatechin	46±6 μg/100 g_{fresh}	[26, 33]
Flavonols		
Quercetin	-	[33]
Quercetin-3-O-glucoside	4.4±1 μg/100 g_{fresh}	[26]
Kaempferol	22.6±6 μg/100 g_{fresh}	[26]
Kaempferol-3-O-rhamno-glucoside	2.1±1.6 μg/100 g_{fresh}	[26]
Anthocyanins		
Cyanidin-3-O-glucoside	23±5 μg/100 g_{fresh}	[26, 29, 31]
Cyanidin-3-O-glucosyl-rutinoside	0.19±0.02 mg/ g_{dry}	[29, 31]
Cyanidin-3-rutinoside	-	[29, 31]
Cyanidin-3-sophoroside	-	[29, 31]
Flavanones		
Naringenin	-	[33]

The presence of polyphenolic compounds justifies the antioxidant activity of the extracts. Moreover, the presence of anthocyanins promotes the use of the extracts in cosmetics, drinks and nutraceuticals as a source of natural colorants and antioxidants [26]. According to Yilmaz et al., sour cherry pomace can be considered as source of food additives thanks to the high content of bio-active compounds, pointing out that in Turkey alone, 600 kg anthocyanins per year could be potentially produced from this bio-waste [31].

2.1.2 Recovery methods and performance

Different factors affect the recovery process: (i) the waste source, (ii) the industrial processing, (iii) the solvent used, (iv) the type of extraction process and (v) the operating conditions. The first one is related to the characteristics of the starting fruit and its composition, while the second one influences mainly the amount of components that can be actually recovered. Therefore, points (i) and (ii) influence mainly the feedstock for the recovery process, whereas points (iii), (iv) and (v) are directly connected to the recovery process itself. Table 2 reports an overview of the different works dealing with the extraction of phenolic compounds from sour cherry skins and flesh leftovers according to the biomass origin and the source of the waste. Then, the extraction method (solvent and operating conditions) and the performance of the process are reported in Table 3.

Table 2 Overview of the published works according to the biomass origin and the source of the waste

Biomass origin	Source of the waste	Reference
Portugal	Liquor industry	[26]
Serbia	Laboratory production	[28, 30]
Poland	Laboratory production	[29]
Turkey	Fruit juice industry	[31]
Turkey	Fruit juice pilot plant	[32]
Denmark	Cherry liqueur production	[33]
	Cherry wine production	[43]

Table 2 shows that, as already mentioned in the introduction, the use of sour cherry at an industrial level ranges from the production of liqueurs to the production of juices and wines. Together with the origin of the cherries, this factor can influence the composition of the extracts. It must be noticed that in some experimental works, the pomace was produced directly in the laboratory after pressing of the cherries.

Table 3 Overview of the published works according to extraction method (SFE: supercritical fluid extraction, HPSE: high pressure solvent extraction, Sub-FE: subcritical fluid extraction), solvent used, operating conditions (T: temperature, P: pressure) and performance (GAE: gallic acid equivalent, extr: extract, EC3G: cyanidin 3-glucoside equivalent, cyn-3-rut: cyanidin-3-rutinoside equivalent)

Extraction Process	Solvent Composition	T [°C]	P [MPa]	Solid/Solvent	Time	Yield	Total Phenolic Content	Total Anthocyanins	Refs.
Decoction	H_2O	100	-	1:10	15 min	18±2 %	289±5 mg GAE/100 g_{fresh}	1.6±0.9 mg EC3G/100 g_{fresh}	[26]
Maceration	H_2O	25	-	1:10	24 h	6.8±0.4 %	31±1 mg GAE/100 g_{fresh}	1.8±0.3 mg EC3G/100 g_{fresh}	[26]
Shaking	EtOH	-	-	1:5 g/mL 1:2.5 g/mL	60 min 30 min	-	-	-	[28, 30]
SFE	CO_2-EtOH	35	10	1:0.8 g/g	15 min 60 min	-	227 mg GAE/100 g_{fresh}	56.95 mg EC3G/100 g_{fresh}	[29]
Maceration	EtOH	-	-	2:1 g/g	-	-	49.2 mg GAE/100 g_{fresh}	10.04 mg EC3G/100 g_{fresh}	[29]
Maceration	1% HCl-MeOH	-	-	-	-	-	477.1 mg GAE/100 g_{fresh}	98.72 mg EC3G/100 g_{fresh}	[29]
Shaking	51% EtOH-H_2O (0.01% HCl)	75	-	1:12 g/mL	100 min	-	14.23±0.38 mg GAE/g_{dry}	0.41±0.02 mg EC3R/g_{dry}	[31]
HPSE	EtOH	60	176-193	0.06-0.07:1 g/mL	25 min	-	3.8 mg GAE/g_{fresh}	-	[32]
Sub-FE	CO_2-EtOH	50.6-54.4	54.8-59	0.2:1 g/g	40 min	-	0.6 mg GAE/g_{fresh}	-	[32]
Maceration	MeOH	-	-	-	24 h	-	3.52 mg GAE/g_{fresh}	-	[32]
Maceration	EtOH	-	-	-	24 h	-	2.92 mg GAE/g_{fresh}	-	[32]
Shaking	H_2O	20	-	-	20 min	-	13.7±2.0 mg GAE/L	-	[33]
Shaking	MeOH	20	-	-	20 min	-	21.3±4.5 mg GAE/L_{extr}	-	[33]
Shaking	EtOH	20	-	-	20 min	-	17.4±1.2 mg GAE/L_{extr}	-	[33]
Shaking	Acetone	20	-	-	20 min	-	16.3±3.3 mg GAE/L_{extr}	-	[33]
Shaking	2-Propanol	20	-	-	20 min	-	16.3±3.3 mg GAE/L_{extr}	-	[33]
Shaking	70% MeOH-H_2O	20	-	-	20 min	-	36.5±2.3 mg GAE/L_{extr}	-	[33]
Shaking	70% EtOH-H_2O	20	-	-	20 min	-	37.1±9.6 mg GAE/L_{extr}	-	[33]
Shaking	70% Acetone-H_2O	20	-	-	20 min	-	92.8±15.5 mg GAE/L_{extr}	-	[33]
Shaking	70% 2-Propanol-H_2O	20	-	-	20 min	-	39.9±0.5 mg GAE/L_{extr}	-	[33]
Stirring	50 mM Citric acid-H_2O	50	0.101325	1:2 g/g	12 h	-	82.1±2.9 mg/kg	48.6±0.9 mg EC3G/kg_{fresh}	[43]
Enzyme assisted	50 mM Citric acid-H_2O	50	0.101325	1:2 g/g	12 h	-	69.5-82.1 mg/kg	31.6-37.0 mg EC3G/kg_{fresh}	[43]

As it is possible to observe in Table 3, polar solvents are required for the extraction of the target phenolic compounds, i.e., phenolic acids and anthocyanins. In fact, even when a non-polar solvent like CO_2 is used, the addition of a polar co-solvent is necessary. Only Muchagato Mauricio et al. and Rødtjer et al. used pure water for the extraction [26, 33]. This case would be ideal for the recovery process because in this case the extract could be directly used in food applications. Unfortunately, the extraction yields using pure water are normally lower. Enzymatic degradation of the plant material has been proposed as an alternative to increase extraction yields in water [44]. Roda-Serrat et al. (2019) reported that enzymatic treatment of sour cherry pomace with cellulase and pectinase did not increase the extraction yield of anthocyanins and phenolic acids, but increased in both cases the filtration efficiency in downstream processing by ultrafiltration [43]. Ethanol is also considered a valid food grade solvent due to its low toxicity and its numerous applications in the food industry [28, 30, 32]. Among the other organic solvents, methanol is mostly used to perform exhaustive extractions for comparison with other solvents and techniques. Rødtjer et al., who made an analysis with different solvents to evaluate the limit concentration between the antioxidative and prooxidative activity of the extracts, tested also other organic solvents such as Acetone and 2-Propanol [33].

On the other hand, pressure, temperature, solid/solvent ratio and time are the operating parameters used to optimize the process. In general, conventional extraction and maceration are the most solvent- and time-consuming processes. As it is expected, an extraction conducted at high pressure [32], high temperature [26] or in supercritical conditions [29, 32] decreases the extraction time, though contemporarily they are energy consuming processes. When conventional solvent extraction is used, only Muchagato Mauricio et al. and Woźniak et al. reported simple maceration of the solid in the solvent [26, 29], while in all the other cases a mild shaking process (200-900 rpm) is usually adopted. Typically, the comparison between the efficiency of the various techniques is done in terms of extraction yield, total phenolic content and total anthocyanins in the extracts. Moreover, the total antioxidant capacity is generally taken into account to evaluate the quality of the extracts, using a wide range of techniques [29]. From the performance point of view, it emerges a wide variety of

results. Since the different research works report results using different units, only the ones with the same units will be considered in the comparison. The highest amount of extracted phenolics were reported using the exhaustive extraction with MeOH in the work of Woźniak et al. [29], both in terms of total phenolics (477.1 mg GAE/100 g of pomace) and total anthocyanins (98.72 mg EC3G/100 g of pomace). Even though methanol is an effective extraction solvent for this application, this method is not recommended in a possible food application, due to its toxicity. In fact, MeOH is usually adopted only as benchmark, in order to compare it with other more sustainable solutions. Considering more eco-friendly processes, the highest total phenolic content is reported by Adil et al. using high-pressure extraction with ethanol (380 mg GAE/100 g sample) [32], followed by the decoction in boiling water by Muchagato Mauricio et al. (289 mg GAE/100 g fresh biomass) [26]. Both solutions can be considered environmentally sustainable, but a further economic analysis for what concerns the energy costs would be needed. For this reason, Muchagato Mauricio et al. suggest the maceration in water at 25°C as best solution from an economic point of view [26]. Slightly lower results, but still comparable, were obtained by Woźniak et al. by means of supercritical CO_2 extraction with ethanol as co-solvent, who performed an optimization for this method in terms of total phenolic content (227 mg GAE/100 g of pomace) [29]. On the other hand, for what concerns the total anthocyanins, the only comparison possible is the one between Muchagato Mauricio et al. and Woźniak et al. [26, 29]. In particular, the latter (56.95 mg EC3G/100 g using supercritical CO_2) reports an amount of total anthocyanins more than 30 times higher than the former (1.8 mg EC3G/100 g). This significant difference might be explained by the different origin of the pomaces examined. While the first one comes from a liqueur producer, the latter was obtained in laboratory after fruit pressing. Probably, in the second case, the majority of the anthocyanins remained in the skins, justifying the extreme difference in these results. As a general consideration, all the papers confirm the antioxidant activity of the extracts, evaluated by means of different techniques such as the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and the FRAP (Ferric Reducing Antioxidant Power) assays.

2.1.3 Downstream processing

From a biocascade perspective, the loop on the recovery of components from cherry skins and flesh leftovers can be closed only after a reliable method for the preservation of the recovered components is employed. In fact, as reported by different authors, phenolic compounds, and particularly the anthocyanins, are subjected to degradation [26, 28, 30, 31, 34, 45]. One technique that is currently available is the encapsulation in proteins by means of freeze drying. In particular, Tumbas Šaponjac et al. characterized the encapsulation of cherry skins extracts in whey and soy proteins for six weeks storage, finding a good preservation of the antioxidant and color parameters in the encapsulates [28], while Oancea et al. studied only the anthocyanins encapsulation in a mixture of whey and acacia gum [46]. Also, the first research group analyzed the addition of the same encapsulates in cookies, as a substitute of 10-15 % of wheat flour [30, 34]. This process represents a valid and sustainable option for the employment of sour cherry byproducts in food applications. Contemporarily, Mauricio et al. analyzed the residue of the extraction in terms of carbohydrates (82 %), fat (11 %), protein (6%) and ash (1%), finding that this can be further valorized as energy source, again closing the loop on the cherry skins and flesh leftovers [26]. Carvalho et al. reported purification of anthocyanins from red cabbage by adsorption in chitosan films obtained from shrimp waste, thus opening the options for the use of naturally coloured chitosan in different fields [47].

2.2 Sour cherry stones

2.2.1 Recoverable components

The stones are another source for the recovery of high-value components from the sour cherry bio-waste. In particular, similarly to other fruits, essential oils can be extracted from the kernels of sour cherry stones, which are contained inside the shells. The oil content in the kernels has been reported to be between 17 – 30 % fresh weight, measured as the total oil yield obtained using different extraction methods (Table 6). Sour cherry kernel oil is a known source of fatty acids [48-54], which are important components in the human diet. Contemporarily, tocopherols and tocotrienols

(tocochromanols), i.e. vitamin E-active components and strong liposoluble antioxidants, have been reported in different works [48, 49, 51, 53, 54]. Furthermore, several authors also reported phenolic compounds [51, 53] and sterols [48, 49]. Korlesky et al. detected also the presence of TAGs (triglycerides) and DAGs (diglycerides) [48], while Górnaś et al. and Yilmaz et al. reported the presence of carotenoids [49, 51].

The different components than can be recovered from the sour cherry kernels oil, together with the correspondent recovery range, are resumed in Table 4.

Table 4 List of different compounds found in the cherry kernel oil

Component	Value	Reference
Fatty acids [%]		
Myristic (C14:0)	0.07-0.5	[48, 52]
Palmitic (C16:0)	4-11	[48-54]
Palmitoleic (C16:1)	0.33-0.63	[48, 49]
Stearic (C18:0)	1.33-3.45	[48, 49, 51, 54]
Oleic (C18:1)	42.9-63.9	[48-54]
Vaccenic (C18:1)	1	[54]
Linoleic (C18:2)	27-46.06	[48-54]
α-Eleostearic (α-ESA C18:3)	5.72-15.76	[48, 49]
Linolenic (α-C18:3)	0.1-5.7	[48-54]
Arachidic (C20:0)	0.1-1.38	[48, 49, 52, 54]
Gadoleic (C20:1)	0.41-0.67	[48, 49]
Behenic (C22:0)	0.2	[48]
Lignoceric (C24:0)	0.21	[48]
Tocopherols [ppm]		
α-Tocopherol	4.7-385	[48, 49, 51, 54]
β-Tocopherol	0.4-25	[49, 53, 54]
γ-Tocopherol	197.2-1333	[48, 49, 51, 54]
δ-Tocopherol	15.1-182	[48, 49, 51]
Tocotrienols [ppm]		
α-Tocotrienol	21.5-22	[49, 54]
γ-Tocotrienol	4	[49]
Phytosterols [ppm]		
Campesterol	159-416	[48, 49]
Cholesterol	169	[49]
Citrostadienol	55	[49]
Gramisterol	125	[49]
Stigmasterol	7.2	[48]
β-Sitosterol	3610-8528	[48, 49]
γ-Sitosterol	4000-5000	[53]
Δ5-Avenasterol	782	[49]
Δ7-Avenasterol	64	[49]
Δ7-Stigmasterol	112	[49]

24-Methylene-sterol	310	[49]
Others [ppm]		
Plastochromanol-8	1200	[53]
Squalene	1028.1-1200	[49, 53]
Vitamin E	<1000	[53]
β-Carotene	10.03	[51]
1-Monoolein	3000-4000	[53]

With regards to the fatty acids composition, it emerges from Table 4 that sour cherry kernel oil is mostly rich in monounsatured fatty acids in the form of oleic acid (42.9-63.9 %) and polyunsaturated fatty acids in the form of linoleic (27-46.06 %), linolenic (0.12-5.7 %) and α-eleostearic (5.72-15.76 %) acids. Linoleic and linolenic acids are classified as ω-6 and ω-3 fatty acids and they are called "essential" because they cannot be synthesized by the human organism and must be taken through the diet [55]. They have an important role as constituents of the cell membrane and they provide protection against cardiovascular diseases. Furthermore, α-eleostearic acid has been found to have beneficial properties against cancer cells [48]. Saturated fatty acids are mainly represented by palmitic (4-11 %) and stearic acids (1.33-3.45 %). The presence of tocochromanols, i.e., tocopherols and tocotrienols, which are important antioxidants both in the human diet and in the food production, have been reported by different authors with very high ranges [48, 49, 51, 54]. Among the tocopherols, α-tocopherol (4.7-385 ppm) and γ-tocopherol (197.2-1333 ppm) are the main representative of this category of compounds in the sour cherry kernel extracts. The high variation can be attributed to the different origins of the cherry kernels. For what concerns the tocotrienols, almost the same amount of α-tocotrienol (21.5-22 ppm) was found by Matthäus et al. and Górnaś et al. [49, 54], and the latter also reports 4 ppm of γ-Tocotrienol. Numerous components belonging to the group of phytosterols were identified by Górnaś et al. [49]. Among them, the most abundant is β-sitosterol (3610-8528 ppm), which was also reported by Korlesky et al. [48]. According to the European Food Safety Authority (EFSA), the consumption of phytosterols is important in the lowering of the LDL-cholesterol levels [56].

2.2.2 Recovery methods and performance

As already mentioned in the previous Section for skin and flesh leftovers, in the case of the kernels the origin can also influence the quality and the composition of the recovered extracts, as shown in Table 5. For example, Górnaś et al. analyzed the effect of six different sour cherry cultivars widely grown in the Baltic Countries and Russia, finding different composition values for the same components and using the same extraction method [48]. The other factors that affect the recovery of high value components from the kernel extracts are the extraction method, the solvent employed and the operating conditions that are reported together with the performance in Table 6.

Table 5 Overview of the published works according to the origin and the source of the waste

Origin	Source of the waste	Reference
Utah	Fruit juice industry	[48]
Latvia	Laboratory production	[49]
Turkey	Local vendor production	[50]
Turkey	Fruit juice industry	[51]
Romania	Local vendor production	[52]
Hungary	Fruit processing industry	[53]
Turkey	Laboratory production	[54]

Table 6 Overview of the published works according to extraction method (SFE: supercritical fluid extraction), solvent used, operating conditions (T: temperature, P: pressure) and performance (fresh: evaluated with reference to the fresh weight, dry: evaluated with reference to the dry weight, SFA: total saturated fatty acids, MFA: total monounsaturated fatty acids, PFA: total polyunsaturated fatty acids)

Extraction Process	Solvent Composition	T [°C]	P [MPa]	Solid/Solvent	Time	Oil Yield	SFA	MFA	PFA	Tocochromanols	Sterols	Carotenoids	Reference
Soxhlet	Hexane	-	-	-	24 h	30.9%$_{fresh}$ (kernels) 6.9 % (seeds)	12%	48.7 %	39.3 %	525.2 ppm	3776.2 ppm	-	[48]
Ultrasound	Hexane	-	-	1:5 g/mL	5min/extr	17.5-37.1%$_{dry}$	9.4-11.7%	26-46.1%	44-62.3%	118.2±0.5 – 163.6±4.2 mg/100 g oils	313.6±7.8 – 1041.3±15.2 mg/100 g oil	0.51±0.02 – 1.75±0.06 mg/100 g oil	[49]
Maceration	Petroleum ether	-	-	-	-	30.11%$_{fresh}$	6.08%	44.2 %	49.25 %	-	-	-	[50]
Orbital shaker	n-Hexane	-	-	1:2.5 g/mL	15 min/extr	17±0.59 %$_{fresh}$ (kernel)	7.56±0.2 %	46.8±0.16%	45.64±0.19 %	428.62 ±2.67 mg/L (tocopherols)	-	8.47±0.2 mg/L (β-carotene)	[51]
Orbital shaker	3% EtOH-Hexane	-	-	1:2.5 g/mL	15 min/extr	17±0.59 %$_{fresh}$ (kernel)	7.01±0.23%	47.94±1.33%	45.03±1.52 %	376.56±3.59 mg/L (tocoph)	-	10.03±0.27 mg/L (β-carotene)	[51]
SFE	CO_2	60	30	4:1 g/g/min	2 h	17±0.59 %$_{fresh}$ (kernel)	8.57±0.2%	44.99±1.38%	46.64±1.11 %	312.15±3.04 mg/L (tocoph)	-	5.65±0.21 mg/L (β-carotene)	[51]
SFE	3%EtOH-CO_2	60	30	4:1 g/g/min	2 h	17±0.59%$_{fresh}$ (kernel)	7.33±0.12%	45.54±1.33%	47.12±1.72 %	381.68±2.23 mg/L (tocoph)	-	6±0.11 mg/L (β-carotene)	[51]
Soxhlet	Petroleum ether	-	-	-	4 h	22.5%$_{fresh}$ (kernel)	18.8%	42.9%	38.2%	-	-	-	[52]
Soxhlet	Hexane	-	-	-	-	32-36%$_{fresh}$	3-4%	50-53%	35-38%	-	-	-	[53]
Twisselmann	Petroleum ether	-	-	1:35 g/mL	6 h	50.8±0.4%$_{dry}$	6.9%	64.9±0.3%	27.1±0.1 %	240.2±1.4 ppm			[54]

From the analysis of Table 6 it can be noticed that, since the main objective of the extraction is the recovery of oils, non-polar solvents are necessary. n-Hexane and petroleum ether are two solvents that ensure an efficient oil recovery, but at the same they are extremely toxic compounds that would not fit in food-related applications. The only attempt found to use an eco-friendly process is the one made by Yilmaz et al. [51], who used supercritical carbon dioxide, which is recognized for its low toxicity and the easy solvent removal, despite the drawback of the high costs for operation. Yilmaz et al. tested also the addition of ethanol to n-Hexane and SFE-CO_2 and they were able to recover more phenolic compounds than the correspondent case with the pure non-polar solvent. The same holds for Bak et al., who extracted different phenolic compounds after oil recovery from the exhaust kernels using methanol as solvent [53].

Different parameters are used to evaluate the efficiency of the extraction and the quality of the extracts in the case of the cherry kernels: oil yield, total fatty acids (usually divide in saturated, monounsaturated, polyunsaturated fatty acids), total tocopherols, total sterols and total carotenoids.

As reported in Table 6, the highest value of oil yield is 50.8% by Matthäus et al. [54], followed by Górnaś et al. who reported a value of 37.1 % [49]. In the other works, the average range is 17-30%. This substantial difference might be explained by the fact that both Matthäus et al. and Górnaś et al. used stones from fresh (not processed) cherries, while the cherries analyzed from the other authors were a waste from fruit processing. This could be a further demonstration that both origin and processing methods have an influence on the composition and the quality of the extracts. On the other hand, the influence of the geographical location, cultivation year, growing conditions, harvest time and post-harvest handling cannot be overlooked. In general, the fatty acids are homogenously distributed between mono- and polyunsatured fatty acids, both averagely accounting for ca. 45% of the oil, while saturated fatty acids always constitute the minor fraction. For what concerns the total tocochromanols (tocopherols+tocotrienols), Górnaś et al. found a maximum value of 1636 ppm [49],

which is almost seven times higher than the value found by Matthäus et al. [54]. Considering the total tocopherols only, maximum values of 525.2 ppm and 428.62 ppm were found by and Korlesky et al. and Yilmaz et al., respectively [48, 51]. Considering that both the authors performed a pure n-Hexane extraction, the difference in the values might be related to the different origin and processing of the cherry stones. In relation to the sterols content, Górnaś et al. [49] found almost three times the amount reported by Korlesky et al. [48]. Finally, Yilmaz et al. [51] obtained a maximum concentration of 10.03 ppm of β-carotene adding 3% EtOH to n-Hexane, value that is in line with the 17.5 ppm of total carotenoids reported by Górnaś et al. [49].

Up to now, the literature has been mostly focused on the characterization of the extracted oils to assess the potential of sour cherry stones as a source of high value components, highlighting the presence of different compounds important for the human health. At this point further optimization of the supercritical extraction method or the development of new eco-friendly techniques is necessary for the eventual commercialization of the extracts and their application in the food or cosmetic industry. At the same time, the effect of the sour cherry seeds extracts in medicine applications have been studied by several authors [57-59].

2.2.3 Downstream processing

The by-product of the extraction of sour cherry kernels is represented by the exhaust kernels and the shells, which are typically not considered in the extraction process. Several authors report the effective use of this further waste for the production of activated carbon [60-63]. Activated carbon from sour cherry stones can be used as adsorbent in different applications for the removal of hazardous contaminants from waste gases and wastewater [61]. Table 7 reports a small overview of the processes used and the contaminants removed from wastewater in three different applications of sour cherry stones used to produce activated carbon.

Table 7 Overview of different methods for the production of activated from sour cherry stones

Process	Contaminants removed	Reference
Thermochemical conversion with H_3PO_4 (500°C)	Pb^{2+}, Cd^{2+}, Ni^{2+}	[60]
Chemical treatment with different concentrations of H_2SO_4	Cr(VI)	[61]
Chemical activation with zinc chloride (700°C)	Yellow 18	[62, 63]

The production of activated carbon after the extraction of high value components from the kernels can represent the final block of the biocascade loop for what concerns the sour cherry stones.

2.3 Sour cherry stems

The last source of high value components that can be extracted from the sour cherry waste is represented by the stems, for which very few works are present in the literature. In particular, Svarc-Gajic et al. have been the first to analyze the possibility to extract high value compounds from the sour cherry stems using subcritical water (150 °C, 20 bar, 30 min) as solvent [27, 64]. From the analysis of the extracts, they detected the presence of different alcohols, fatty acids and organic acids. Moreover, they reported good antioxidant activity of the extracts and demonstrated that they exhibit anticancer properties [27, 64]. These results might be the opening of a further pattern for the total exploitation of the sour cherry by-products.

2.4 Biocascade plant for sour cherry pomace processing

The recovery of high value components from sour cherry pomace can be achieved by a combination of different processes. In this section a possible plant configuration is proposed in agreement with the Biocascade philosophy and reported in Fig. 1.

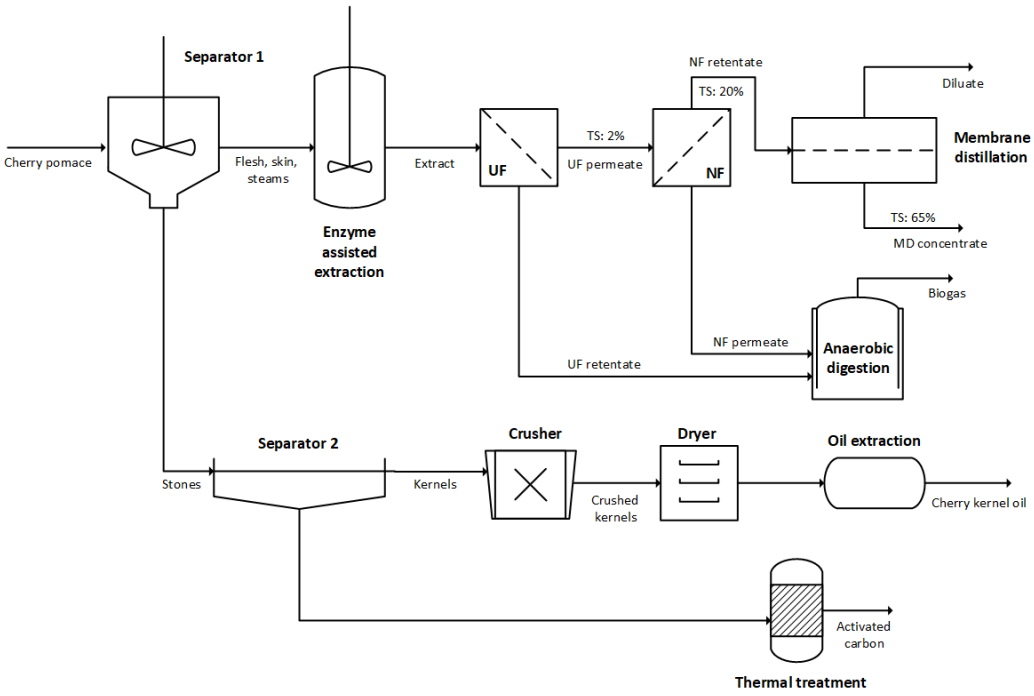

Fig. 1 Biocascade approach for sour cherry pomace

The first step consists in the separation of the pomace into two main streams, one containing flesh, skins and stems (FSS) and the other containing the stones (ST). The two streams are the feed to the two main sections of the plant, i.e., production of phenolic acids and anthocyanins (stream FSS) and production of essential oils and activated carbon (ST). Flesh, skins and stems are sent to a stirred extraction tank, where with the aid of plant cell-wall degrading enzymes the phenolic compounds are transferred from the solid to the liquid solution. After the extraction, further purification and concentration of the components of interest is needed. This can be done using two membrane processes in series, one performing ultrafiltration (UF) and the second one performing nanofiltration (NF). The residue obtained from the two membranes is sent to an anaerobic digestion unit for biogas production. The last purification step, which increases notably the total solid content in the extract can be done using a membrane distillation process.

For what concerns the stones, a second unit (SEPARATOR 2) is needed to separate the shells from the kernels. While the first are directly sent to thermal treatment for the production of activated carbon, the latter are processed to produce the sour cherry kernel oil. First, they are crushed to increase

the specific surface area (CRUSHER) and dried under a stream of hot air (DRYER) to reduce the amount of moisture. Next, the kernels are sent to an oil extraction unit based either on organic solvent extraction using hexane, or preferably based on supercritical fluid extraction using CO_2. After the extraction, the exhaust kernels are sent to thermal treatment together with the shells, while the oil is collected.

3. *Kalanchoe* leaves

Kalanchoe is a genus including a significant number of succulent plants belonging to the family of Crassulaceae. Their flowers, characterized by a large variety of colors [65], are typically used as ornamental plants [66]. In the flower business, the leaves of the plants are discarded and represent a waste. In order to have an estimation of the amount of waste generated, it is worth to mention that Denmark alone accounts for nearly one fourth of the European production of about 200 million plants per year [67].

Kalanchoe leaves are known in different parts of the world for their wide variety of beneficial properties in folk medicine. In fact, up to now they have shown to possess anti-inflammatory [66, 68-74], anti-leishmanial [69, 74-77] anti-hypersensitive activities [76, 78, 79], and to have refrigerant, depurative, disinfectant and hemostatic effect [66]. Furthermore, they have been successfully used in the treating of ulcers [70, 71, 80, 81], rheumatism [71, 80, 81], infections [71, 75, 81-84], fever [82, 83, 85], skin diseases [79, 82, 83, 86], pulmonary infections [80], genitourinary disorders [75, 79], psychiatric disorders [79], insect bites [71, 84], diuresis [84] and jaundice [74, 79]. Most of the literature about *Kalanchoe* leaves extraction deals with the characterization of the extracts, together with the evaluation of different biological activities [66, 68, 70, 71, 73-76, 79-82, 84-86]. Furthermore, different works tested the extracts for a number of medical applications, such as the reparation of kidney damage in lupus nephritis mice [78], the dissolution and growth inhibition of CaOx crystals leading to nephrolithiasis [87], the evaluation of the gastroprotective activity in acute gastric lesions models [70], the cytotoxicity against various

tumor cell lines [71], the inhibition of the local effects induced by snake venom [72] and the effectiveness in relaxing myometrial strips [77].

3.1 Recoverable components

Numerous works have been focused on the extraction of high value components from the leaves of different species of *Kalanchoe*. The main compounds of interest that can be recovered from this biomass are flavonoids, known for their anti-inflammatory and antioxidant activities and whose presence justifies the abovementioned natural properties of the plant. Table 8 provides an overview of the different flavonoids that have been qualitatively identified in the different *Kalanchoe* species.

Table 8 List of different compounds identified in the different *Kalanchoe* species leaves extracts

Component	Reference
K. pinnata	
3',4'-dimethoxy quercetin	[80]
3,5,7,3',5'-pentahydroxyflavone	[84]
4',5'-dihydroxy-3',8-dimethoxyflavone 7-O-β-D-glucopyranoside	[76]
Acacetin 7-O-α-rhamnopyranosyl-(1→6)-β-D-glucopyranoside	[79]
Diosmetin 7-O-α-rhamnopyranosyl-(1→6)-β-D-glucopyranoside	[79]
Eupafolin-*O*-deoxy-hexoside-*O*-pentoside	[68, 70, 72]
Isorhamnetin hexose pentose	[74]
Kaempferol	[68, 70, 72]
Kaempferol 3-O-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside	[76, 79, 86]
Kaempferol 3-O-β-D-xylopyranosyl-(1→2)- α-L-rhamnopyranoside	[79]
Kaempferol 3-O-rutinoside	[74]
Kaempferol-*O*-deoxy-hexoside-*O*-hexoside	[72]
Kaempferol-*O*-deoxy-hexoside-*O*-pentoside	[68, 70, 72]
Myricetin 3-O-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside	[79]
Myricetin 3-O-α-rhamnopyranoside (myricitrin)	[79]
Patuletin-*O*-deoxy-hexoside-*O*-hexoside	[68, 70, 72]
Quercetin (3,3,4,5-7-pentahydroxyflavone)	[85]
Quercetin 3-O-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside	[69, 74, 76, 79, 80, 86]
Quercetin 3-O-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside 7-O-β-D-glucopyranoside	[79]
Quercetin 3-O-α-rhamnopyranoside (quercitrin)	
Quercetin 3-O-α-L-rhamnoside	[75, 79, 86]
Quercetin-*O*-deoxy-hexoside-*O*-hexoside	[80]
Quercetin-*O*-deoxy-hexoside-*O*-pentoside	[72]
Quercetin-*O*-hexoside	[68, 70, 72]

Quercetin glucose rhamnose	[68, 70, 72]
Quercetin glucose arabinose rhamnose	[74]
	[74]
K. brasiliensis	
Eupafolin-di-*O*-acetyl-deoxy-hexoside	[68, 70, 72]
Eupafolin-di-*O*-deoxy-hexoside	[68, 70, 72]
Eupafolin-*O*-hexoside-di-*O*-deoxy-hexoside	[68, 70, 72]
Eupafolin-*O*-hexoside-*O*-deoxy-hexoside	[72]
Eupafolin-*O*-deoxy-hexoside-*O*-acetyl-deoxy-hexoside	[68, 70, 72]
Eupafolin-O-rhamnoside-O-acetyl-rhamnoside	[72]
Patuletin 3-O-α-L-rhamnopyranosil-7-O-α-rhamnopyranoside	[73]
Patuletin-di-*O*-acetyl-deoxy-hexoside	[68, 70]
Patuletin-di-*O*-deoxy-hexoside	[72]
Patuletin-O-acetyl-deoxy-hexoside-O-di-acetyl-deoxy-hexoside	[72]
Patuletin-*O*-deoxy-hexoside	[68, 70, 72]
Patuletin-*O*-deoxy-hexoside-*O*-acetyl-deoxy-hexoside	[68, 70, 72]
Patuletin-*O*-deoxy-hexoside-*O*-acetyl-rhamnoside	[72]
Patuletin-*O*-hexoside-di-*O*-deoxy-hexoside	[68, 70, 72]
Patuletin-O-hexoside-O-acetyl-deoxy-hexoside-O-deoxy-hexoside	[72]
Patuletin-*O*-hexoside-*O*-deoxy-hexoside	[68, 70, 72]
Quercetin-*O*-hexoside-*O*-deoxy-hexoside	[72]
K. prolifera	
Kaempferol	[82]
Kaempferol 3-O-α-L-rhamnoside	[82]
Kaempferol 3-O-β-D-glucopyranoside	[82]
Quercetin	[82]
Quercetin 3-O-rutinoside	[82]
Quercetin 3-O-sophoroside	[82]
Quercetin 3-O-β-D-glucopyranoside	[82]
K. tomentosa	
Kaempferol	[83]
Kaempferol-3-O-α-L-rhamnoside	[83]
Kaempferol-3-O-β-D-glucoside (astragalin)	[83]
K. gastonis-bonnieri	
6-C-β-D-glucopyranoside-8-C-β-D-glucopyranoside	[75]
Quercetin 3-O-α-L-rhamnopyranoside-7-O-β-D-glucopyranosil-(1→3)-α-L-rhamnopyranoside	[75]

As reported in Table 8, over 40 different flavonoids can be found in the leaves of *Kalanchoe* species. Kaempferol and quercetin derivatives are common in most of the species studied, with the exception of *K. brasiliensis*, which was found to contain eupafolin and patuletin derivatives. Due to the large number of components, quantification of flavonoids is shown later in Table 10 as total

flavonoid content (TFC). Phenolic compounds have also been found in the extracts [66, 71, 72, 79]. The presence of all these compounds corroborates the beneficial activities of the extracts.

3.2 Recovery methods and performance

In a manner similar to the previous biomasses analyzed, different factors such as the origin and the species analyzed, together with the solvent used and extraction method influence the quality of the extracts. Table 9 reports an overview of different works dealing with the extraction of flavonoids from the leaves of *Kalanchoe* species, classified according to the species and the origin, while Table 10 shows the classification based on the extraction method (solvent and operating conditions) and the performance of the process.

Table 9 Different species and origin of Kalanchoe leaves

Species	Origin	Reference
Kalanchoe pinnata	Brazil	[68-70, 72, 76, 77,
	Indonesia	86]
	India	[78, 80]
	Poland	[66, 87, 88]
	Germany	[71]
	Malaysia	[79]
	Ghana	[85]
	Bangladesh	[81]
	French Guyana	[84]
		[74]
Kalanchoe brasiliensis	Brazil	[68, 70, 72, 73]
Kalanchoe daigremontiana	Poland	[71]
	Switzerland	[79]
Kalanchoe milloti	Poland	[71]
Kalanchoe nyikae	Poland	[71]
Kalanchoe prolifera	Indonesia	[82]
Kalanchoe tomentosa	Indonesia	[83]
Kalanchoe gastonis-bonnieri	Brazil	[75]
Kalanchoe integra	Ghana	[81]

From the analysis of Table 9 it is highlighted that, among the 9 different species included in the list, *Kalanchoe Pinnata* is by far the most popular and diffused, and it can be found in all the continents. The second and third most studied species are *Kalanchoe Brasiliensis* and *Kalanchoe*

Daigremontiana, while only specific works consider the other species (*K. milloti, K. nyikae, K. prolifera, K. tomentosa, K. gastonis-bonnieri, K. integra*).

Table 10 Overview of the published works according to extraction method (SFE: supercritical fluid extraction), solvent used, operating conditions (T: temperature, P: pressure) and performance (TPC: Total phenolic content, TFC: Total flavonoid content, fresh: evaluated with reference to the fresh weight, dry: evaluated with reference to the dry weight, GAE: gallic acid equivalent, querc: quercetin)

Extraction Process	Solvent Composition	T [°C]	P [MPa]	Solid/Solvent	Time	Species	Extract yield	TPC	TFC	Refs
Turbo extraction in an industrial blender	H_2O	-	-	1:1 (w/v)	5 min	*K. brasiliensis*	4.9%$_{fresh}$ w/w	-	-	[68]
						K. pinnata	7.7%$_{fresh}$ w/w	-	-	
Pressing + Maceration	H_2O	-	-	-	-	*K. pinnata*	1.95 %$_{fresh}$ w/w	-	-	[78]
Trituration in a blend	H_2O	40	-	1:5 (w/v)	18 min	*K. pinnata*	38.7 %$_{dry}$ w/w	-	-	[69]
Soxhlet	50% MeOH-H_2O	-	-	-	72 h	*K. pinnata*	5.52 %$_{dry}$ w/w			[87]
Soxhlet	Chloroform + MeOH	-	-	-	8 h	*K. pinnata*	-	-	-	[66]
Maceration	MeOH	-	-	-	-	*K. pinnata*	-	-	-	[80]
Accelerated solvent extraction	70% EtOH-H_2O	40	10	3-7:1 g/mL	10 min	*K. daigremontiana*	-	124.68 μg/g$_{dry}$	-	[71]
Maceration	70-96% EtOH-H_2O	-	-	3-100 g/30-400 mL	-	*K. daigremontiana*	-	39.22 μg/g$_{dry}$	-	[71]
						K. Pinnata	-	79.11 μg/g$_{dry}$	-	
						K. milloti	-	48.04 μg/ g$_{dry}$	-	
						K. nyikae	-	9.61 μg/ g$_{dry}$	-	
Maceration	MeOH	Room T	-	20 kg/50 L	3 days	*K. tomentosa*	1.8 %$_{fresh}$ w/w			[83]
Blending	H_2O	-	-	-	-	*K. gastonis-bonnieri*	1.47 %$_{fresh}$ w/w			[75]
Blending	H_2O	-	-	-	-	*K. pinnata*	-	242 mg/g	32 mg/g	[81]
						K. integra	-	340 mg/g	42 mg/g	
Blending	MeOH	-	-	-	-	*K. pinnata*	-	-	108 mg/g	[81]
						K. integra	-	315 mg/g	178 mg/g	
Maceration	H_2O	50	-	-	-	*K. pinnata*	3%$_{fresh}$ w/w	-	1 mg$_{querc}$/g$_{dry}$	[76]
Stirring + Sonication	MeOH	-	-	9:100 g/mL	2 h + 20 min	*K. pinnata*	-	-	-	[79]
Maceration	50% EtOH-H_2O	-	-	-	24,48,72h	*K. pinnata*	-	-	-	[85]
Maceration with occasional shaking and stirring	MeOH	Room T	-	450 g/2 L	15 days	*K. pinnata*	-	1.49 g GAE/g$_{dry,extr}$	-	[84]
Pressing in a roller mill	-	-	-	-	-	*K. pinnata*	5.8 %$_{fresh}$ w/w	-	-	[77]
Hot aqueous extraction	H_2O	50	-	20 % (w/v)	30 min	*K. pinnata*	-	-	3.348 %$_{dry}$ w/w	[86]
Pressurized liquid extraction	Ethyl Acetate	40	-	-	5 min	*K. pinnata*	3%$_{fresh}$ w/w	-	-	[74]
Maceration	MeOH	-	-	-	-	*K. pinnata*	8 %$_{fresh}$ w/w	-	-	[74]

For what concerns the extraction solvents (Table 10), since the target components are flavonoids, polar solvents are involved. Water and methanol are largely used as solvents, followed by ethanol. El Abdellaoui et al. investigated also the use of ethyl acetate [74]. In the perspective of the development of a clean process for the recovery of flavonoids from *Kalanchoe* leaves, the large use of water provides a good indication. With regards to the extraction method, different processes can be observed in Table 10, ranging from the simple maceration [76, 80, 82, 83, 85] and simple blending [75, 81] to more sophisticated and energy-consuming methods such as Soxhlet extraction [66, 87], accelerated solvent extraction [71] or pressurized liquid extraction [74]. It is worth to notice that in the case of *Kalanchoe* leaves a solvent-free method based on pressing can be also employed [77, 78]. The outcome of Table 10 is that for this biomass there are different methods available for the development of a clean and low energy-consuming process at a large scale.
The comparison between the different processes and solvent involved is generally performed in terms of extract yield, total phenolic content (TPC) or total flavonoid content (TFC). Among the results for *Kalanchoe pinnata* when the extract yield referred to the fresh weight of the leaves, the highest result was obtained with water as solvent by de Araújo et al. with 7.7 % w/w FW [68], while dos Santos Nascimento et al. obtained the lowest yield using again water as solvent with 3 % w/w FW [75]. Since the plant source and the solvent are the same, the difference might be associated with the different extraction method, i.e., turbo extraction and maceration by de Araújo et al. and dos Santos Nascimento et al., respectively. In the middle, it can be highlighted the yield obtained by Wachter et al. with 5.8 % w/w FW by means of pressing [77]. When the dry weight of the leaves was used as basis, the results range between 38.7 % w/w DW obtained by dos Santos Nascimento et al. [69] using water as solvent and 5.52 % w/w DW with a mixture of MeOH:H_2O (50:50 v/v) obtained by Sohgaura et al. [87]. Here, the high difference might be related to both the solvent and the extraction method, highlighting a higher efficiency of blending in water as compared to the Soxhlet extraction with the MeOH:H_2O solution. Considering the other species, the extract obtained from *Kalanchoe pinnata* are the highest, followed by *Kalanchoe tomentosa* [83]

and *Kalanchoe gastonis-bonnieri* [75]. When the total phenolic content was considered as efficiency parameter, Bogucka-Kocka et al. found a higher content in *Kalanchoe pinnata* (79.11 μg/g DW) over *Kalanchoe daigremontiana* (39.22 μg/g DW), *milloti* (48.04 μg/g DW) and *nykae* (9.61 μg/g DW) using simple maceration in ethanol [71]. At the same time, when the maximum TPC they obtained was with *Kalanchoe daigremontiana* extracted by accelerated solvent extraction with EtOH (124.68 μg/g DW), more than three times higher than the maceration in EtOH of the same species. Finally, it is worth to notice that Asiedu-Gyekye et al. found a better efficiency both in terms of both TPC and TFC of *K. integra* and over *K. pinnata* [81]. Furthermore, they obtained better performance of methanol over water using simple blending. From all these considerations, it emerges that among the solvents, water seems to be an effective solution compared to methanol and ethanol to extract high-value components from the *Kalanchoe* leaves. At the same time, pressing should also be taken into account, as it is a solvent-free extraction method.

Up to now most of the works have been focused on the identification of the different rather than on the quantification of the compounds. In order to make the extraction profitable at larger scales, it is needed to move the focus on the quantification of the compounds identified and the optimization of the extraction process. For example, dos Santos Nascimento et al. optimized the aqueous extraction of Quercetin 3-O-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranoside (QAR), known to be a strong anti-inflammatory, antiedematogenic and antinoceptive compound, from *Kalanchoe pinnata* leaves [69]. In the optimal conditions, they obtained a QAR content of 20.8 ± 2.3 mg/g.

3.3 Downstream processing

Due to the original use of the plant in folk medicine, the application of the extracts from *Kalanchoe* leaves is in general oriented towards medical applications. The efficiency of the extracts on a large variety of pathologies has been successfully tested in different works, which are resumed in Table 11.

Table 11 List of different application of the extracts from different Kalanchoe leaves

Application	Reference
Evaluate the local anti-inflammatory efficacy of *K. pinnata* and *K. brasiliensis* in a formulation for topical administration.	[68]
Determine reparing effects of aqueous extract of *K. pinnata* on lupus nephritis mice.	[78]
Assess potential *in vitro* CaOx crystal dissolution and crystal growth inhibition properties of *K. pinnata*	[87]
Establish the action mechanism involved in the gastroprotective activity of *K. brasiliensis* and *K. pinnata* leaf juices against gastric lesions induced by ethanol and by indomethacin in rats.	[70]
Test the α-glucosidase inhibitory activity of two flavonoid glycosides from *K. pinnata* leaves.	[80]
Verify the cytotoxicity of *Kalanchoe* extracts against various tumor cell lines and evaluate the antioxidant activity of extract prepared from *K. daigremontiana, K. pinnata, K. milloti* and *K. nyikae.*	[71]
Cytotoxic evaluation against P-388 murine leukemia cells of the compounds isolated from *K. prolifera* and *K. tomentosa*.	[82, 83]
Evaluate the ability of the chemical constituents of *K. brasiliensis* and *K. pinnata* species to inhibiti local effects induced by *Bothrops jararaca* snake venom.	[72]
Evaluation of herbal tablets formulation of hydroalcoholic extracts of *K. pinnata* and *Rotula aquatica* and their combination for antilithiatic *in vitro* method.	[88]
Explore preliminary antioxidant, antimicrobial, cytotoxic and thrombolytic activities of extracts from *K. pinnata.*	[84]
Study the effect of *K. pinnata* leaf press juice and its chemical fractions on the response of human myometrial strips.	[77]

Commercial products based on extracts from *Kalanchoe* leaves are already present, as described in the review by Hamburger et al. [89]. In particular, different Galenical formulations exist for *K. pinnata* extracts, such as “Bryophyllum 50% powder” and “Bryophyllum chewable tables 350 mg 50%”, both containing leaf pressed juice adsorbed to lactose or “Bryophyllum Dilutio 33%”, which is an ethanolic leaf extract for oral application. The same review reports that the *K. pinnata* preparations are used to treat premature labor and other medical conditions like sleep disorders and recurrent inflammation in the metabolic system [89]. At the same time the kalanchoe agro-gardening bio-waste do not have at present an industrial application. Since the beneficial properties

of the extracts from *Kalanchoe* leaves are now evident thanks to the numerous works present in the literature, it is desirable the development of an industrial process or the optimization of the already existing ones. Moreover, a further analysis on possible applications for the exhaust leaves residue, such as its possible use in the biogas production, is also necessary, in order to create a Biocascade loop for this biomass.

3.4 Biocascade plant for Kalanchoe leaves processing

Fig. 2 shows the proposed biocascade plant for the kalanchoe leaves. The leaves are first pressed in a screw press obtaining a liquid phase (Juice) and a solid phase (Press Residue). The first one contains the components of interest and is then purified using a series of membranes, while the latter is directly sent to anaerobic digestion for biogas production. For the purification, an initial microfiltration stage is used to separate major particles from the liquid phase and the residue is sent to the anaerobic digestion unit. The extract of the microfiltration is further sent to a reverse osmosis unit where the water level of the extract is reduced and the components of interest are concentrated in the liquid phase. This stream can then be processed for production of nutraceuticals in various formulations.

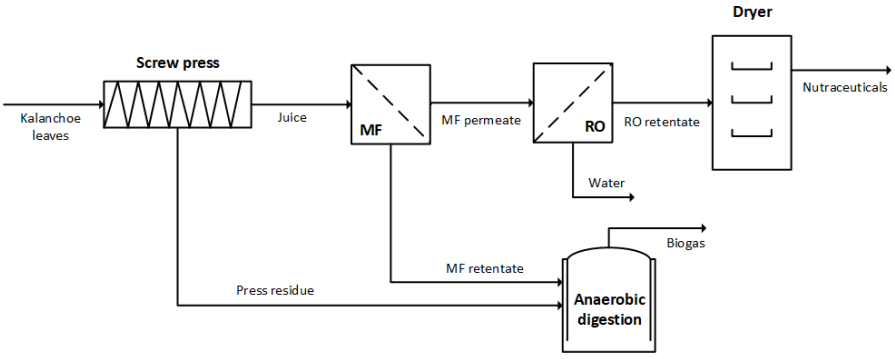

Fig. 2 Biocascade approach for *kalanchoe* leaves

4. Red clover

Red clover (*Trifolium pratense* L.) is a forage legume that grows on temperate regions all around the world. Red clover became of utmost relevance in Europe in the 17th and 18th centuries, when the increase in population and consequent demand for agricultural products, resulted in fast nutrient depletion from the soil [90]. A well-known remedy to this issue was crop rotation, in which cultivation of cereals and legumes would take place on recurring succession on the same land. Legumes and specially red clover were ideal candidates for crop rotation, since they improve soil fertility by converting atmospheric nitrogen into nitrates by symbiotic N_2-fixation [91, 92]. Crop rotation allowed the abolishment of fallow, and increased soil productivity. At the same time, red clover proved to be an excellent cattle fodder, which increased the livestock population and consequent production of milk and dairy products. The increase in productivity reduced to some extent the need for forest clearing for agricultural land-use, and helped sustain the growing population with less resources [90]. Red clover therefore played a substantial role in changing the agricultural landscape distribution in Europe. In fact, this influence has been said to be larger than that of any other forage crop, and even the potato [93]. An old Flemish proverb cited "Without clover no man in Flanders would presume to call himself a farmer" [94].

By the beginning of the 20th century, the development of the Haber-Bosch method allowed the catalytic synthesis of ammonia directly from air. The use of industrially produced fertilizers increased dramatically after WWII, and nitrogen-fixating legumes including red clover started to disappear from the fields and remain mostly in organic agriculture [90]. It can be said that technological innovation produced both the rise and fall of red clover. However, following the recent environmental challenges and the change of paradigm towards a bio-based economy, red clover is gaining interest once again. One of the most promising applications of red clover is the production of plant-based protein.

Present day livestock production in Europe is highly dependent on the importation of soya bean meal protein from South America [95]. This has several drawbacks including the environmental

impact of long-distance transportation, insecurity of supply, and price volatility. The exploration of alternative protein sources is therefore of utmost relevance, and has been subject of different scientific reviews [96, 97]. Leaf protein concentrates from legumes can be produced by mechanical pressing [98-102], resulting in a juice rich in soluble proteins, carbohydrates, organic acids and minerals. The proteins can be recovered from the juice by precipitation to produce leaf protein concentrate [98, 102]. Red clover plants (including all above ground organs) contain a typical crude protein content between 150 - 200 g/kg DM [101, 103-105]. However, variations are expected due to the influence of the cultivar [105], plant maturity at harvest [106] and post-harvest storage methods [107].

The production process of red clover leaf-protein concentrates results in different bio-wastes with high potential for valorization, namely the press cake and the residual juice. In the present review, the components in red clover and their potential to produce new products are described. In the light of this potential, a bio-cascade methodology is proposed as an integral approach that exploits the by-products from red clover leaf protein concentrate production as a source of specialty chemicals like phytoestrogens, and ultimately production of biogas and soil conditioners.

According to the European Seed Certification Agencies Association [108], the production of clover seeds in Europe covered 81,490 hectares in 2018, out of which 46 % corresponded to red clover. Hermansen et al. [102] reported a biomass yield for red clover of 2.3 tons/hectare in dry matter basis. Using these data, the European production of red clover in 2018 can be estimated to account for at least 16,298 tons dry biomass. In the same study, Hermansen et al. [102] predicted that in Denmark alone, green biomass has the potential cover 25 % of the need for feed protein and therefore reduce the dependence from importation. In that scenario, the valorization of the associated by-product streams by means of Biocascading would have a positive impact in the revenue per weight biomass processed and make red clover leaf-protein concentrates an even more attractive alternative to imported soybean meal.

4.1. Recoverable components

Red clover is naturally rich in isoflavones, a sub-class of secondary plant metabolites that belong to the family of flavonoids. The main isoflavones in red clover, namely formononetin, biochanin A, genistein, glycitein and daidzein, are also referred to as phytoestrogens due to their estrogenic or antiestrogenic activities similar to those of estradiol hormones of mammals.

In fact, formononetin was blamed for the so-called "clover disease", in which sheep feeding on subterranean clover (*Trifolium subterraneum*) became infertile in Australia in the 1930s [109]. However, phytoestrogens must be found in large amounts in the animal diet before this effect is observed [110]. A more recent study did not find differences in fecundity of sheep feeding on red clover before, during and after breeding season [111].

Due to their estrogenic effect, isoflavones have been proposed as an alternative to hormone replacement therapy in postmenopausal women [112, 113]. The reported effects range from decreased bone loss [114] to reduced incidence of hot flushes [115-117]. Likewise, studies performed in men have linked the intake of dietary isoflavones with decreased cholesterol levels [118] and prevention or slower progression of prostate cancer [119, 120]. Besides their medicinal application, red clover extracts could also find application in the food and beverage industry. For instance, Talcott et al. (2005) reported that isoflavonoids in red clover enhanced color and stability of anthocyanins in muscadine wine via co-pigmentation [121].

Most of the isoflavones in legumes are generally found in the form of glucosides, and only 2 – 5 % are found as aglycones, the biologically active form [122]. However, the glucosides undergo hydrolysis in the gastrointestinal tract [122] or by simple crushing of the leaves and consequent hydrolysis catalyzed by endogenous enzymes [98]. Red clover isoflavones have been reported mainly in the leaves, but also on the flowers, sprouts, petioles, stems and even the roots [123-127]. High variations in isoflavone content are also found within cultivars, growing conditions and maturity stage at harvest. In general, higher isoflavone content is observed when the plants are harvested in early stages of maturity [125, 128] and before storage either as silage or hay [129].

Red clover is typically harvested as the entire plant, and no further fractionation of its components is performed prior to pressing. Therefore, only the isoflavone content of the combined above-ground part of the plants will be considered in this review.

4.2 Extraction methods and performance

An overview of the research works published according to the solvent, extraction methodology, extraction parameters and isoflavone content is reported in Table 12. Generally, the polarity of the solvent is the most crucial factor in the extraction efficiency. For extraction and quantification of isoflavones, the preferred solvents are 70-80% alcohol in water mixtures. In some cases, acidification of the solvent is performed to hydrolyze the glucosides into the aglycones and ease quantification. Long maceration times at room temperature [130] can be substituted by shorter extractions under reflux [128, 131], sonication [132], or enzymatic treatment [103]. Klejdus et al. (2005) reported extraction of isoflavones by supercritical fluid extraction using CO_2 and a water/methanol polarity modifier. Whereas supercritical CO_2 is an ideal solvent for the extraction of non-polar products due to its relatively mild critical temperature (31.1 °C) and pressure (7.39 MPa), very high flowrates of modifier are needed to extract isoflavones [133]. This does not only lower immensely the concentration of the resulting extract, which requires further downstream concentration, but it is also overall detrimental to the economy and sustainability of the process.
As shown in Table 12, the isoflavone content varies remarkably among the research works reported. No study was found that compared different extraction methods performed on the same material.

Table 12 Overview of the published works according to extraction method (SFE: supercritical fluid extraction), solvent used, operating conditions (T: temperature, P: pressure) and performance (TPC: Total phenolic content, TFC: Total flavonoid content, fresh: evaluated with reference to the fresh weight, dry: evaluated with reference to the dry weight, GAE: gallic acid equivalent, querc: quercetin)

Extraction Process	Solvent Composition	T [°C]	P [MPa]	Solid/Solvent	Time	Formononetin (g/kg_{dry})	Genistein (g/kg_{dry})	Glycitein (g/kg_{dry})	Biochanin A (g/kg_{dry})	Daidzein (mg/kg_{dry})	Total isoflavones (g/kg_{dry})	Refs.
Enzyme-assisted extraction twice Conventional Conventional	H_2O EtOH 70% EtOH-H_2O	-	-	5g/140 mL+100 mL +200 mL +100 mL	4 nights	2.1-5.0	0.14-10.68	N.r.	0.7-3.3	0.13-0.33	3.07-9.31	[134]
Ultrasound + enzyme-assisted extraction	55% MeOH-H_2O	80	-	0.5 g/25 mL	10 min	2.05±0.2	0.306±0.04	-	1.77 ± 0.3	0.127 ± 0.02	4.25±0.58	[103]
Reflux	80% MeOH-H_2O acidified with HCl	90	-	10 mg/900 uL	1 h	6.2-8.24	0.11-1.07	-	2.82-4.11	0.23 – 1.7	9.76 – 12.8	[128]
Reflux	70% EtOH-H_2O	-	-	5 g/100 mL	1 h	-	-	-	-	-	Only qualitative	[131]
Maceration	70% MeOH-H_2O	Room T	-	0.25 g/10 mL	16 h	1.52-11.24	-	5.85-36.72	-	-	17.14-55.63	[130]
Conventional	80% EtOH-H_2O acidified with 0.3M HCl	37	-	1 g/20 mL	30 min	9.02±0.36	-	-	5.44±0.68	-	14.47±1.02	[129]
SFE	CO_2 with 5% MeOH-H_2O v/v modifier	38	30	0.1 g /850 mL/min	20 min	0.725	0.625	Trace	-	Trace	-	[133]
Ultrasound-assisted extraction under reflux	75% MeOH, acidified with HCl	75	-	3g /50 mL	30 min	0.877	0.239	-	1.06	0.133	-	[132]

4.3. Downstream processing

Based on the reviewed information, a biocascade approach is proposed to fully utilize the bio-wastes from the production of leaf-protein concentrates from red clover. As shown in Fig. 3, the leaf protein concentrate production process consists of a pressing step to produce the juice, followed by precipitation of the soluble protein. Two main by-product streams result from this process: the insoluble cake from the pressing step, and the residual juice after protein precipitation.

The residual juice is perhaps the most interesting of the two streams, since it contains most of the high value phytoestrogens, that are mostly found in their water-soluble form. In the present review, a process based in membrane filtration is suggested for their recovery. Membrane-based operations offer the advantage of using mild operation conditions, no need of organic solvents or phase change, and low energy consumption. Xu et al. (2005) proposed a method to recover isoflavones from red clover flowers consisting of alkaline extraction and solid phase adsorption; and recovered more than 50% of the initial isoflavones in the flowers [135]. One year later, the same group proposed a membrane-based process for recovery of isoflavones from red clover flowers consisting of extraction, ultrafiltration, reverse osmosis, evaporation, decantation and freeze-drying [136]. In their two articles Xu et al. emphasize the poor water solubility of the isoflavones, hence suggesting that the glucosides are probably hydrolyzed into non-soluble aglycones. The changes in form and therefore solubility are factors of paramount importance and should be considered and controlled when designing a recovery process.

As shown in Fig. 3, a microfiltration step is recommended to separate the extract from particles and bacteria that might be present in the residual juice. The permeate rich in phytoestrogens could then be concentrated by reverse osmosis prior to final drying. The final product, a dry red clover extract could then be formulated and marketed as nutraceuticals for hormone replacement therapy. Dietary supplements based on red clover extracts are readily available in the market with different isoflavone composition and quantity [112, 115, 137]. The supplements typically contain 40 - 80 mg

isoflavones per capsule, which agrees with the recommended daily doses of 40-50 mg/day [113] or 40-82 mg/day [116].

The cake or insoluble fraction form the pressing step contains mainly cellulose, hemicellulose, lignin and structural cell-wall proteins. Firstly, residual phytoestrogens (if any) could be further recovered by an extraction step and processed in the same way as the residual juice. Furthermore, the insoluble proteins could be recovered by techniques such as ammonia fibre expansion (AFEX) [138-140] or enzymatic treatment [141, 142]. On the other hand, this fraction could be directly turned into pellets to feed ruminants [104] or used as feedstock for production of biofuels. Santamaria-Fernandez et al. (2018) evaluated the biogas potential of the press cake of red clover after protein extraction and found methane yields in the range of 218-375 ml-CH_4 g-VS^{-1}. They also observed that co-digestion of the press cake with the residual juice resulted in increased methane yields [143]. However, the high value phytoestrogens should be recovered from the residual juice prior to co-digestion in order to maximize value per kg biomass and improve the overall economy of the process. The microfiltration retentate, for instance, could be used for co-digestion of the press cake and production of methane. The resulting digestate after biogas production could be further used as soil amendment in the form of biochar as it has been reported for wheat straw, rice husks or herbaceous plant cuttings, among others [144].

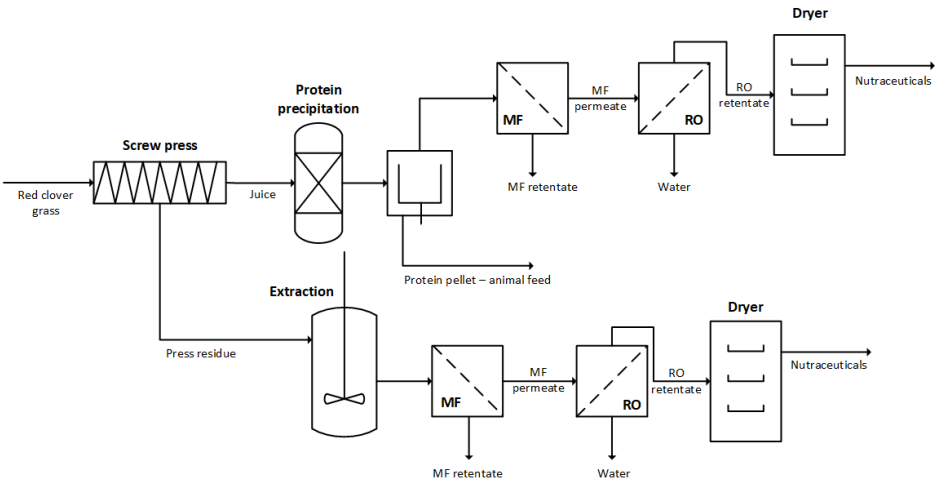

Fig. 3 Biocascade approach for red clover (*Trifolium pratense L.*)

5. Conclusions

Bio-compounds derived by natural resources is fast-expanding market due to the healthy style of life embraced by the moder society. Their recovery from bio-waste represents an interesting opportunity to increase companies´ revenue reducing simultaneously the ammont of wastes to handle.

However this potential win-win situation where a profit is realized by waste exploitation, presents challenges.

Three target bio-wastes named sour cherry pomace, kalanchoe leaves and red clover, have been considered as representative of three different productions. Sour cherry pomace is representative of food industry, kalanchoe leaves are representative of gardening and red clover of feed animal industry. For all of them a characterization-isolation-utilization approach has been described. In the characterization step the target compounds have been identified and their isolation discussed in terms of extraction methods. The isolation or final purification has been also reviewed according to unit operations suitable to preserve the components´properties.

The main challenge in the recovery of natural compounds remains the transposition of the results obtained at laboratory level to a larger scale. In particular extraction processes need to be optimized and integrated within the purification step envisaged. For the three bio-wastes considered, using a biocascade approach, possible process flowsheets have been proposed aiming to catalyze new research efforts in analyzing the recovery process as a whole. It is possible to notice that these flowsheets have a common skeleton of unit operations, with adaptations to each individual case. In particular, membrane separation was given a prominent role in dealing with sensitive components due to mild operation conditions, low energy requirements, high productivity and easy scale-up. This overlapping between the flowsheets opens the possibility in treating multiple bio-wastes in the same plant taking advantage of the seasonal availability of the feedstock.

References

1. Beeton, I.M., *Mrs Beeton's Cookery Book: New and Enlarged Edition*. 1899, London, UK: Ward Lock & Co.
2. Schneider, F. *The History Of Food Wastage*. in *Waste - The Social Context - People, Policies, Persuasion and Payoffs*. 2011. Edmonton, Canada.
3. Batori, V., L. Lukitawesa, and R. Nair, *Food waste: Potential source for bioenergy and bio-products*. 2017, Mycorena AB: Sweden.
4. Girotto, F., L. Alibardi, and R. Cossu, *Food waste generation and industrial uses: A review.* Waste Management, 2015. **45**: p. 32-41.
5. RedCorn, R., S. Fatemi, and A.S. Engelberth, *Comparing End-Use Potential for Industrial Food-Waste Sources.* Engineering, 2018. **4**(3): p. 371-380.
6. Kumar, V. and P. Longhurst, *Recycling of food waste into chemical building blocks.* Current Opinion in Green and Sustainable Chemistry, 2018. **13**: p. 118-122.
7. Bharat Helkar, P., A.K. Sahoo, and N.J. Patil, *Review: Food Industry By-Products used as a Functional Food Ingredients.* International Journal of Waste Resources, 2016. **6**(3): p. 1000248.
8. Paritosh, K., et al., *Food Waste to Energy: An Overview of Sustainable Approaches for Food Waste Management and Nutrient Recycling.* BioMed Research International, 2017. **2017**: p. 2370927.
9. Baiano, A., *Recovery of Biomolecules from Food Wastes - A Review.* Molecules, 2014. **19**(9): p. 14821-14842.
10. European Commission, *Directive 2008/98/EC on waste (Waste Framework Directive).* 2016.
11. Md Salim, N.S., A. Singh, and V. Raghavan, *Potential Utilization of Fruit and Vegetable Wastes for Food through Drying or Extraction Techniques.* Novel Techniques in Nutrition & Food Science, 2017. **1**(2).
12. Galanakis, C.M., *Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications.* Trends in Food Science & Technology, 2012. **26**(2): p. 68-87.
13. Talekar, S., et al., *From waste to wealth: High recovery of nutraceuticals from pomegranate seed waste using a green extraction process.* Industrial Crops and Products, 2018. **112**: p. 790-802.
14. Socaci, S.A., et al., *Food Wastes as Valuable Sources of Bioactive Molecules*, in *Superfood and Functional Food - The Development of Superfoods and Their Roles as Medicine*. 2017, IntechOpen.

15. Ravindran, R. and A.K. Jaiswal, *Exploitation of Food Industry Waste for High-Value Products.* Trends in Biotechnolgy, 2016. **34**(1): p. 58-69.
16. Galanakis, C.M., *Emerging technologies for the production of nutraceuticals from agricultural by-products: A viewpoint of opportunities and challenges.* Food and Bioproducts Processing, 2013. **91**(4): p. 575-579.
17. Principato, L., et al., *Adopting the circular economy approach on food loss and waste: The case of Italian pasta production.* Resources, Conservation and Recycling, 2019. **144**: p. 82-89.
18. Zucchella, A. and P. Previtali, *Circular business models for sustainable development: A "waste is food" restorative ecosystem.* Business Strategy and the Environment, 2018. **28**(2): p. 274-285.
19. Ellen MacArthur Foundation, *Toward the Circular Economy: Economic and Business Rationale for Accelerated Transition*. 2015, Ellen MacArthur Foundation: Isle of Wight, United Kingdom.
20. Brar, S.K., S.J. Sarma, and K. Pakshirajan, *Platform chemical biorefinery: future green chemistry*. 2016: Elsevier.
21. Venselaar, J., *Sustainable Growth and Chemical Engineering.* Chemical Engineering & Technology, 2003. **26**(8): p. 868-874.
22. Venselaar, J., W.F.W.M. van Heugten, and J.J.M. Mulderink, *Integrated Plant Conversion and BIOCASCADE optimising use of biomass as renewable resource in sustainable development*, in *CHISA2000*. 2000: Prague.
23. Kusch-Brandt, S., et al., *Valorization of Residues From Beverage Production*, in *Processing and Sustainability of Beverages*. 2019. p. 451-494.
24. *FAOSTAT, Food and Agriculture Organization of the United Nations*. 2020; Available from: http://www.fao.org/home/en/.
25. Nemes, A., et al., *Determination of Flavonoid and Proanthocyanidin Profile of Hungarian Sour Cherry.* Molecules, 2018. **23**(12): p. 3278.
26. Muchagato Maurício, E., et al., *Evaluation of Industrial Sour Cherry Liquor Wastes as an Ecofriendly Source of Added Value Chemical Compounds and Energy.* Waste and Biomass Valorization, 2018.
27. Švarc-Gajić, J., et al., *Bioactive compounds of sweet and sour cherry stems obtained by subcritical water extraction.* Journal of Chemical Technology & Biotechnology, 2018. **93**(6): p. 1627-1635.
28. Tumbas Šaponjac, V., et al., *Encapsulation of sour cherry pomace extract by freeze drying: characterization and storage stability.* Acta Chimica Slovenica, 2017. **64**(2): p. 283-289.

29. Woźniak, Ł., K. Marszałek, and S. Skąpska, *Extraction of phenolic compounds from sour cherry pomace with supercritical carbon dioxide: Impact of process parameters on the composition and antioxidant properties of extracts.* Separation Science and Technology, 2016. **51**(9): p. 1472-1479.
30. Tumbas Šaponjac, V., et al., *Sour cherry pomace extract encapsulated in whey and soy proteins: Incorporation in cookies.* Food Chem, 2016. **207**: p. 27-33.
31. Yilmaz, F.M., M. Karaaslan, and H. Vardin, *Optimization of extraction parameters on the isolation of phenolic compounds from sour cherry (Prunus cerasus L.) pomace.* Journal of Food Science and Technology, 2015. **52**(5): p. 2851-2859.
32. Adil, İ.H., M.E. Yener, and A. Bayındırlı, *Extraction of Total Phenolics of Sour Cherry Pomace by High Pressure Solvent and Subcritical Fluid and Determination of the Antioxidant Activities of the Extracts.* Separation Science and Technology, 2008. **43**(5): p. 1091-1110.
33. Rødtjer, A., L.H. Skibsted, and M.L. Andersen, *Antioxidative and prooxidative effects of extracts made from cherry liqueur pomace.* Food Chemistry, 2006. **99**(1): p. 6-14.
34. Petrović, J., et al., *Encapsulated sour cherry pomace extract: Effect on the colour and rheology of cookie dough.* Food Science and Technology International, 2019. **25**(2): p. 130-140.
35. Kopjar, M., et al., *Phenolics Content and Antioxidant Activity of Sour Cherry Extracts with Sugar Addition.* Acta Alimentaria, 2017. **46**(4): p. 501-507.
36. Issaad, F.Z., et al., *Flavonoids in Selected Mediterranean Fruits: Extraction, Electrochemical Detection and Total Antioxidant Capacity Evaluation.* Electroanalysis, 2017. **29**(2): p. 358-366.
37. Ahmad, I., S. Shamsi, and R. Zaman, *A review on sour cherry (Prunus cerasus): A high value Unani medicinal fruit.* International Journal of Green Pharmacy, 2017. **11**(1): p. 1-6.
38. Homoki, J.R., et al., *Anthocyanin composition, antioxidant efficiency, and alpha-amylase inhibitor activity of different Hungarian sour cherry varieties (Prunus cerasus L.).* Food Chemistry, 2016. **194**: p. 222-229.
39. Kopjar, M., M. Orsolic, and V. Pilizota, *Anthocyanins, Phenols, and Antioxidant Activity of Sour Cherry Puree Extracts and their Stability During Storage.* International Journal of Food Properties, 2014. **17**(6): p. 1393-1405.
40. Piccolella, S., et al., *Antioxidant Properties of Sour Cherries (Prunus cerasus L.): Role of Colorless Phytochemicals from the Methanolic Extract of Ripe Fruits.* Journal of Agricultural and Food Chemistry, 2008. **55**: p. 1928-1935.
41. Chaovanalikit, A. and R.E. Wrolstad, *Total Anthocyanins and Total Phenolics of Fresh and Processed Cherries and Their Antioxidant Properties.* Journal of Food Science: Food Chemistry and Toxicology, 2004. **69**(1): p. 67-72.

42. Toydemir, G., et al., *Industrial processing effects on phenolic compounds in sour cherry (Prunus cerasus L.) fruit.* Food Research International, 2013. **53**(1): p. 218-225.
43. Roda-Serrat, M.C., et al., *Enzyme-Assisted Extraction and Ultrafiltratio of Value-Added Compounds from Sour Cherry Wine Pomace.* Chemical Engineering Transactions, 2019. **74**: p. 811-816.
44. Gligor, O., et al., *Enzyme-assisted extractions of polyphenols – A comprehensive review.* Trends in Food Science & Technology, 2019. **88**: p. 302-315.
45. Türkyılmaz, M., F. Hamzaoğlu, and M. Özkan, *Effects of sucrose and copigment sources on the major anthocyanins isolated from sour cherries.* Food Chemistry, 2019. **281**: p. 242-250.
46. Oancea, A.-M., et al., *Functional evaluation of microencapsulated anthocyanins from sour cherries skins extract in whey proteins isolate.* LWT - Food Science and Technology, 2018. **95**: p. 129-134.
47. Carvalho, V.V.L., et al., *Separation of anthocyanins extracted from red cabbage by adsorption onto chitosan films.* International Journal of Biological Macromolecules, 2019. **131**: p. 905-911.
48. Korlesky, N.M., et al., *Extraction and Characterization of Montmorency Sour Cherry (Prunus cerasus L.) Pit Oil.* Journal of the American Oil Chemists' Society, 2016. **93**(7): p. 995-1005.
49. Górnaś, P., et al., *Composition of bioactive compounds in kernel oils recovered from sour cherry (Prunus cerasus L.) by-products: Impact of the cultivar on potential applications.* Industrial Crops and Products, 2016. **82**: p. 44-50.
50. Özcan, M.M., A. Ünver, and D. Arslan, *A research on evaluation of some fruit kernels and/or seeds as a raw material of vegetable oil industry.* Quality Assurance and Safety of Crops & Foods, 2015. **7**(2): p. 187-191.
51. Yılmaz, C. and V. Gökmen, *Compositional characteristics of sour cherry kernel and its oil as influenced by different extraction and roasting conditions.* Industrial Crops and Products, 2013. **49**: p. 130-135.
52. Popa, V.-M., et al., *Characterization of sour cherries (Prunus cerasus) kernel oil cultivars from Banat.* Journal of Agroalimentary Processes and Technologies, 2011. **17**(4): p. 398-401.
53. Bak, I., et al., *Isolation and Analysis of Bioactive Constituents of Sour Cherry (Prunus cerasus) Seed Kernel: An Emerging Functional Food.* Journal of Medicinal Food, 2010. **13**(4): p. 905-910.
54. Matthäus, B. and M.M. Özcan, *Fatty Acids and Tocopherol Contents of Some Prunus Spp. Kernel Oils.* Journal of Food Lipids, 2009. **16**: p. 187-199.
55. Di Pasquale, M.G., *The Essentials of Essential Fatty Acids.* Journal of Dietary Supplements, 2009. **6**(2): p. 143-161.

56. EFSA Panel on Dietetic Products, N.a.A.N., *Scientific Opinion on the substantiation of a health claim related to 3 g/day plant sterols/stanols and lowering blood LDL-cholesterol and reduced risk of (coronary) heart disease pursuant to Article 19 of Regulation (EC) No 1924/2006.* EFSA Journal, 2012. **10**(5): p. 2693-2705.
57. Salimi, A., et al., *Toxicity Evaluation of Microemulsion (Nano Size) of Sour Cherry Kernel Extract for the Oral Bioavailability.* Jundishapur Journal of Natural Pharmaceutical Products, 2014. **9**(1): p. 16-23.
58. Mahmoud, F., et al., *Sour cherry (Prunus cerasus) seed extract increases heme oxygenase-1 expression and decreases proinflammatory signaling in peripheral blood human leukocytes from rheumatoid arthritis patients.* International Immunopharmacology, 2014. **20**(1): p. 188-96.
59. Bak, I., et al., *Cardioprotective mechanisms of Prunus cerasus (sour cherry) seed extract against ischemia-reperfusion-induced damage in isolated rat hearts.* American Journal of Physiology-Heart and Circulatory Physiology, 2006. **291**(3): p. H1329-H1336.
60. Pap, S., et al., *Evaluation of the adsorption potential of eco-friendly activated carbon prepared from cherry kernels for the removal of Pb^{2+}, Cd^{2+} and Ni^{2+} from aqueous wastes.* Journal of Environmental Management, 2016. **184**: p. 297-306.
61. Gheju, M., I. Balcu, and P. Jurchescu, *Removal of hexavalent chromium from aqueous solutions by use of chemically modified sour cherry stones.* Desalination and Water Treatment, 2015. **57**(23): p. 10776-10789.
62. Angin, D., *Utilization of activated carbon produced from fruit juice industry solid waste for the adsorption of Yellow 18 from aqueous solutions.* Bioresource Technology, 2014. **168**: p. 259-266.
63. Angin, D., *Production and characterization of activated carbon from sour cherry stones by zinc chloride.* Fuel, 2014. **115**: p. 804-811.
64. Švarc-Gajić, J., et al., *Simultaneous dispersive liquid-liquid microextraction derivatisation and gas chromatography mass spectrometry analysis of subcritical water extracts of sweet and sour cherry stems.* Analytical and Bioanalytical Chemistry, 2018. **410**(7): p. 1943-1953.
65. Milad, R., S. El-Ahmadi, and A. Singab, *Genus Kalanchoe (Crassulaceae): A Review of Its Ethnomedicinal, Botanical, Chemical and Pharmacological Properties.* European Journal of Medicinal Plant, 2014. **4**(1): p. 86-104.
66. George, L.O., H.R. Radha, and B.V. Somasekariah, *In vitro anti-diabetic activity and GC-MS analysis of bioactive compounds present in the methanol extract of Kalanchoe pinnata.* Indian Journal of Chemistry, 2018. **57B**: p. 1213-1221.

67. Madriz-Ordeñana, K., et al., *First report of Kalanchoe leaf and stem spot caused by Corynespora cassiicola in Denmark.* Plant Disease, 2017. **101**(3): p. 505-505.
68. de Araújo, E.R.D., et al., *Local anti-inflammatory activity: Topical formulation containing Kalanchoe brasiliensis and Kalanchoe pinnata leaf aqueous extract.* Biomedicine & Pharmacotherapy, 2019. **113**: p. 108721.
69. dos Santos Nascimento, L.B., et al., *Optimization of Aqueous Extraction from Kalanchoe pinnata Leaves to Obtain the Highest Content of an Anti-inflammatory Flavonoid using a Response Surface Model.* Phytochemical Analysis, 2018. **29**(3): p. 308-315.
70. de Araújo, E.R.D., et al., *Gastroprotective and Antioxidant Activity of Kalanchoe brasiliensis and Kalanchoe pinnata Leaf Juices against Indomethacin and Ethanol-Induced Gastric Lesions in Rats.* International Journal of Molecular Sciences, 2018. **19**(5): p. 1265.
71. Bogucka-Kocka, A., et al., *Phenolic acid content, antioxidant and cytotoxic activities of four Kalanchoë species.* Saudi Journal of Biological Sciences, 2018. **25**(4): p. 622-630.
72. Fernandes, J.M., et al., *Inhibitory Effects of Hydroethanolic Leaf Extracts of Kalanchoe brasiliensis and Kalanchoe pinnata (Crassulaceae) against Local Effects Induced by Bothrops jararaca Snake Venom.* PLoS ONE, 2016. **11**(12): p. e0168658.
73. de Oliveira Costa, A.C., et al., *Quantification of Chemical Marker of Kalanchoe brasiliensis (Crassulaceae) Leaves by HPLC–DAD.* Journal of Liquid Chromatography & Related Technologies, 2015. **38**(7): p. 795-800.
74. El Abdellaoui, S., et al., *Bioactive molecules in Kalanchoe pinnata leaves: extraction, purification, and identification.* Analytical and Bioanalytical Chemistry, 2010. **398**(3): p. 1329-1338.
75. Costa, S.S., M.F.P. Corrêa, and L.M. Casanova, *A New Triglycosyl Flavonoid Isolated from Leaf Juice of Kalanchoe gastonis-bonnieri (Crassulaceae).* Natural Product Communications, 2015. **10**(3): p. 433-436.
76. Nascimento, L.B.D.S., et al., *Ultraviolet-B radiation effects on phenolic profile and flavonoid content of Kalanchoe pinnata.* Journal of Photochemistry and Photobiology, 2015. **148**: p. 73-81.
77. Wächter, R., et al., *Leaf press juice from Bryophyllum pinnatum (Lamarck) Oken induces myometrial relaxation.* Phytomedicine, 2011. **19**(1): p. 74-82.
78. Indriyanti, N., A. Nuryanti Garmana, and F. Setiawan, *Repairing Effects of Aqueous Extract of Kalanchoe pinnata (Lmk) Pers. on Lupus Nephritis Mice.* Pharmacognosy Journal, 2018. **10**(3): p. 548-552.

79. Fürer, K., et al., *Two New Flavonol Glycosides and a Metabolite Profile of Bryophyllum pinnatum, a Phytotherapeutic Used in Obstetrics and Gynaecology.* Planta Medica, 2013. **79**(16): p. 1565-1571.
80. Sofa, F., et al., *Isolation and Identification of Quercetin Derivatives and their α-Glucosidase Inhibitory Activities from Bryophyllum pinnatum.* Research Journal of Chemistry and Environment, 2018. **22**(II): p. 114-119.
81. Asiedu-Gyekye, I.J., et al., *Comparative study of two kalanchoe species: Total flavonoid and phenolic contents and antioxidant properties.* African Journal of Pure and Applied Chemistry, 2012. **6**(5): p. 65-73.
82. Aisyah, L.S., et al., *Flavonoid Compounds from the Leaves of Kalanchoe prolifera and Their Cytotoxic Activity against P-388 Murine Leukimia Cells.* Natural Product Sciences, 2017. **23**(2): p. 139-145.
83. Aisyah, L.S., et al., *Flavonoids from the Fresh Leaves of Kalanchoe tomentosa (Crassulaceae).* Open Chemistry Journal, 2015. **2**: p. 36-39.
84. Sharker, S.M., et al., *Chemical and biological studies of Kalanchoe pinnata (Lam.) growing in Bangladesh.* Asian Pacific Journal of Tropical Biomedicine, 2012. **2**(3): p. S1317-S1322.
85. Rosli, N.H.M., et al., *Determination of Quercetin in Local Kalanchoe Pinnata Extract Using High Performance Liquid Chromatography*, in *UMT 11th International Annual Symposium of Sustainability Science and Management*. 2012: Terengganu, Malaysia.
86. Muzitano, M.F., et al., *Influence of cultivation conditions, season of collection and extraction method on the content of antileishmanial flavonoids from Kalanchoe pinnata.* Journal of Ethnopharmacology, 2011. **133**(1): p. 132-137.
87. Sohgaura, A.K., P. Bigoniya, and B. Shrivastava, *In Vitro Antilithiatic Potential of Kalanchoe pinnata, Emblica officinalis, Bambusa nutans, and Cynodon dactylon.* Journal of Pharmacy & BioAllied Sciences, 2018. **10**(2): p. 83-89.
88. Gilhotra, U.K., G. Mohan, and A.J.M. Christina, *Antilithiatic activity of poly-herbal formulation tablets by in-vitro method.* Journal of Applied Pharmaceutical Science, 2013. **3**(5): p. 43-48.
89. Hamburger, M., et al., *Bryophyllum pinnatum - Reverse Engineering of an Anthroposophic Herbal Medicine.* Natural Product Communications, 2017. **12**(8): p. 1359-1364.
90. Kjærgaard, T., *A Plant that Changed the World: The rise and fall of clover 1000-2000.* Landscape Research, 2003. **28**(1): p. 41-49.
91. Rasmussen, J., et al., *N2-fixation and residual N effect of four legume species and four companion grass species.* European Journal of Agronomy, 2012. **36**(1): p. 66-74.

92. McKenna, P., et al., *The use of red clover (Trifolium pratense) in soil fertility-building: A Review.* Field Crops Research, 2018. **221**: p. 38-49.
93. Fergus, E. and E. Hollowell, *Red clover,* in *Advances in Agronomy*. 1960, Elsevier. p. 365-436.
94. Weir, W.W., *Soil productivity as affected by crop rotation*. 1926: US Department of Agriculture.
95. Van Krimpen, M., et al., *Cultivation, processing and nutritional aspects for pigs and poultry of European protein sources as alternatives for imported soybean products*. 2013, Wageningen UR Livestock Research.
96. Boland, M.J., et al., *The future supply of animal-derived protein for human consumption.* Trends in Food Science & Technology, 2013. **29**(1): p. 62-73.
97. Wilkins, R. and R. Jones, *Alternative home-grown protein sources for ruminants in the United Kingdom.* Animal Feed Science and Technology, 2000. **85**(1-2): p. 23-32.
98. Glencross, R., G. Festenstein, and H. King, *Separation and determination of isoflavones in the protein concentrate from red clover leaves.* Journal of the Science of Food and Agriculture, 1972. **23**(3): p. 371-376.
99. Pirie, N.W., *The extended use of fractionation processes.* Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 1977. **281**(980): p. 139-151.
100. Mandl, M.G., *Status of green biorefining in Europe.* Biofuels, Bioproducts and Biorefining: Innovation for a sustainable economy, 2010. **4**(3): p. 268-274.
101. Damborg, V.K., et al., *Protein value and degradation characteristics of pulp fibre fractions from screw pressed grass, clover, and lucerne.* Animal Feed Science and Technology, 2018. **244**: p. 93-103.
102. Hermansen, J.E., et al., *Green Biomass-Protein production through bio-refining*. 2017: DCA-Nationalt Center for Fødevarer og Jordbrug.
103. Daems, F., et al., *Changes in the isoflavone concentration in red clover (Trifolium pratense L.) during ensiling and storage in laboratory-scale silos.* Animal Feed Science and Technology, 2016. **217**: p. 36-44.
104. Solati, Z., et al., *Crude protein yield and theoretical extractable true protein of potential biorefinery feedstocks.* Industrial Crops and Products, 2018. **115**: p. 214-226.
105. Hoekstra, N., et al., *Red clover varieties of Mattenklee type have higher production, protein yield and persistence than Ackerklee types in grass–clover mixtures.* Grass and forage science, 2018. **73**(2): p. 297-308.

106. Åman, P., *Chemical composition and in vitro degradability of major chemical constituents in botanical fractions of red clover harvested at different stages of maturity.* Journal of the Science of Food and Agriculture, 1985. **36**(9): p. 775-780.

107. Grabber, J., *Protein fractions in forage legumes containing protein-binding polyphenols: Freeze-drying vs. conservation as hay or silage.* Animal feed science and technology, 2009. **151**(3-4): p. 324-329.

108. *ESCAA European Seed Certification Agencies Association. Seed production in EU 2018*. 2020; Available from: http://www.escaa.org/index/action/page/id/7/title/seed-production-in-eu---2018.

109. Marshall, T., *Clover disease: What do we know and what can we do.* Journal of the Department of Agriculture, Western Australia, Series 4, 1973. **14**(3): p. 198-206.

110. Reed, K., *Fertility of herbivores consuming phytoestrogen-containing Medicago and Trifolium species.* Agriculture, 2016. **6**(3): p. 35.

111. Mustonen, E., et al., *Fertility and growth of nulliparous ewes after feeding red clover silage with high phyto-oestrogen concentrations.* animal, 2014. **8**(10): p. 1699-1705.

112. Dornstauder, E., et al., *Estrogenic activity of two standardized red clover extracts (Menoflavon®) intended for large scale use in hormone replacement therapy.* The Journal of steroid biochemistry and molecular biology, 2001. **78**(1): p. 67-75.

113. Beck, V., U. Rohr, and A. Jungbauer, *Phytoestrogens derived from red clover: an alternative to estrogen replacement therapy?* The Journal of steroid biochemistry and molecular biology, 2005. **94**(5): p. 499-518.

114. Atkinson, C., et al., *The effects of phytoestrogen isoflavones on bone density in women: a double-blind, randomized, placebo-controlled trial.* The American Journal of clinical nutrition, 2004. **79**(2): p. 326-333.

115. Myers, S.P. and V. Vigar, *Effects of a standardised extract of Trifolium pratense (Promensil) at a dosage of 80mg in the treatment of menopausal hot flushes: A systematic review and meta-analysis.* Phytomedicine, 2017. **24**: p. 141-147.

116. Coon, J.T., M.H. Pittler, and E. Ernst, *Trifolium pratense isoflavones in the treatment of menopausal hot flushes: a systematic review and meta-analysis.* Phytomedicine, 2007. **14**(2-3): p. 153-159.

117. Gartoulla, P. and M.M. Han, *Red clover extract for alleviating hot flushes in postmenopausal women: a meta-analysis.* Maturitas, 2014. **79**(1): p. 58-64.

118. Nestel, P., et al., *A biochanin-enriched isoflavone from red clover lowers LDL cholesterol in men.* European journal of clinical nutrition, 2004. **58**(3): p. 403.

119. Jarred, R.A., et al., *Induction of apoptosis in low to moderate-grade human prostate carcinoma by red clover-derived dietary isoflavones.* Cancer Epidemiology and Prevention Biomarkers, 2002. **11**(12): p. 1689-1696.

120. Engelhardt, P.F. and C.R. Riedl, *Effects of one-year treatment with isoflavone extract from red clover on prostate, liver function, sexual function, and quality of life in men with elevated PSA levels and negative prostate biopsy findings.* Urology, 2008. **71**(2): p. 185-90; discussion 190.

121. Talcott, S.T., J.E. Peele, and C.H. Brenes, *Red clover isoflavonoids as anthocyanin color enhancing agents in muscadine wine and juice.* Food research international, 2005. **38**(10): p. 1205-1212.

122. D'Alessandro, T.L., et al., *Metabolism of phytoestrogen conjugates.* Methods in enzymology, 2005. **400**: p. 316-342.

123. Drenin, A.A., E.K. Botirov, and Y.P. Turov, *A new isoflavone glycoside from Trifolium pratense L.* Russian Journal of Bioorganic Chemistry, 2011. **37**(7): p. 862-865.

124. Tsao, R., et al., *Isoflavone profiles of red clovers and their distribution in different parts harvested at different growing stages.* Journal of agricultural and food chemistry, 2006. **54**(16): p. 5797-5805.

125. Saviranta, N.M., et al., *Red clover (Trifolium pratense L.) isoflavones: determination of concentrations by plant stage, flower colour, plant part and cultivar.* Journal of the Science of Food and Agriculture, 2008. **88**(1): p. 125-132.

126. Wu, Q., M. Wang, and J.E. Simon, *Determination of isoflavones in red clover and related species by high-performance liquid chromatography combined with ultraviolet and mass spectrometric detection.* Journal of chromatography A, 2003. **1016**(2): p. 195-209.

127. Budryn, G., et al., *Evaluation of estrogenic activity of red clover (Trifolium pratense L.) sprouts cultivated under different conditions by content of isoflavones, calorimetric study and molecular modelling.* Food Chem, 2018. **245**: p. 324-336.

128. Gikas, E., et al., *Determination of isoflavones in the aerial part of red clover by HPLC–diode array detection.* Journal of Liquid Chromatography & Related Technologies, 2008. **31**(8): p. 1181-1194.

129. Sivesind, E. and P. Seguin, *Effects of the environment, cultivar, maturity, and preservation method on red clover isoflavone concentration.* Journal of Agricultural and Food Chemistry, 2005. **53**(16): p. 6397-6402.

130. Krähmer, A., et al., *Characterization and quantification of secondary metabolite profiles in leaves of red and white clover species by NIR and ATR-IR spectroscopy.* Vibrational Spectroscopy, 2013. **68**: p. 96-103.
131. He, X.-g., L.-z. Lin, and L.-z. Lian, *Analysis of flavonoids from red clover by liquid chromatography—electrospray mass spectrometry.* Journal of chromatography A, 1996. **755**(1): p. 127-132.
132. Zgórka, G., *Ultrasound-assisted solid-phase extraction coupled with photodiode-array and fluorescence detection for chemotaxonomy of isoflavone phytoestrogens in Trifolium L.(Clover) species.* Journal of separation science, 2009. **32**(7): p. 965-972.
133. Klejdus, B., et al., *Supercritical fluid extraction of isoflavones from biological samples with ultra-fast high-performance liquid chromatography/mass spectrometry.* Journal of separation science, 2005. **28**(12): p. 1334-1346.
134. Booth, N.L., et al., *Seasonal Variation of Red Clover (Trifolium pratense L., Fabaceae) Isoflavones and Estrogenic Activity.* Journal of Agricultural and Food Chemistry, 2006. **54**(4): p. 1277-1282.
135. Xu, L., et al., *Production of an isoflavone concentrate from red clover flowers.* Journal of food science, 2005. **70**(8): p. s558-s562.
136. Xu, L., et al., *Recovery of isoflavones from red clover flowers by a membrane-based process.* Innovative Food Science & Emerging Technologies, 2006. **7**(3): p. 251-256.
137. Andres, S., et al., *Determination of the isoflavone composition and estrogenic activity of commercial dietary supplements based on soy or red clover.* Food & function, 2015. **6**(6): p. 2017-2025.
138. Luis, B., et al., *Integrated production of ethanol fuel and protein from coastal bermudagrass.* Applied biochemistry and biotechnology, 1994. **45**(1): p. 483-497.
139. Bals, B., et al., *Extraction of proteins from switchgrass using aqueous ammonia within an integrated biorefinery.* Applied Biochemistry and Biotechnology, 2007. **143**(2): p. 187-198.
140. Dale, B.E., et al., *Protein feeds coproduction in biomass conversion to fuels and chemicals.* Biofuels, bioproducts and biorefining, 2009. **3**(2): p. 219-230.
141. Dotsenko, G. and L. Lange, *Enzyme enhanced protein recovery from green biomass pulp.* Waste and biomass valorization, 2017. **8**(4): p. 1257-1264.
142. Sari, Y.W., M.E. Bruins, and J.P. Sanders, *Enzyme assisted protein extraction from rapeseed, soybean, and microalgae meals.* Industrial Crops and Products, 2013. **43**: p. 78-83.

143. Santamaría-Fernández, M., et al., *Biogas potential of green biomass after protein extraction in an organic biorefinery concept for feed, fuel and fertilizer production.* Renewable Energy, 2018. **129**: p. 769-775.
144. El-Naggar, A., et al., *Biochar application to low fertility soils: A review of current status, and future prospects.* Geoderma, 2019. **337**: p. 536-554.