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Protein fractionation of broccoli (*Brassica oleracea*, var. Italica) and kale (*Brassica oleracea*, var. Sabellica) residual leaves — A pre-feasibility assessment and evaluation of fraction phenol and fibre content

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ABSTRACT

This pre-feasibility study evaluates the use of residual leafy green biomass from broccoli (*Brassica oleracea*, var. Italica) and kale (*Brassica oleracea*, var. Sabellica) as feedstock for protein fractionation and potential application of the fractions in food and feed products. The protein concentration, protein recovery potential and the content of phenols and dietary fibre in these biomass sources and fractions were investigated. Field produce and side-stream analysis showed that among broccoli and kale side-streams the potentially suitable leaves for protein fractionation constitute up to 16 and 1.9 t/ha (DM content), respectively. Fractionation demonstrated that between 34–42 and 25–34 kg total protein could be extracted per t DM of broccoli and kale residue leaves, respectively. The amount of protein was generally high in green protein fraction (GPF) and the white protein concentrate (WPC) of both crops, although significantly higher in broccoli compared to kale. The recovery of bound and free phenolic compounds was up to 18% in the GPF of both crops, while only 0.4% ended up in the WPC. The economic assessment showed that the feedstock and processing costs of producing GPF and WPC, as well as of the combined protein fraction (CPF) 1.9–6.0 and 1.3–3.9 times higher than expected revenues for broccoli and kale, respectively, indicating that the production of protein fractions is not economically feasible with the current production scheme. However, potentially higher revenues may be obtained if value-added products such as fractionated phenols and dietary fibre components are also included and investigated in future production schemes. The pathway investigated, that included a direct drying and milling of leaf biomass showed a low processing cost and thereby the most favourable economic alternative, with approx. 7–30% profit for kale, while for broccoli revenues covered only 44–47% of the costs due to the extra harvest cost of the broccoli leaves.

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1. Introduction

Fruits and vegetables are an essential part of the human diet, with a high content of health promoting compounds and a significant correlation between their intake and human health has been proven (Liu, 2003). The consumption of cruciferous vegetables has been associated with health benefits, and are suggested to have both anticancer and antioxidant properties (Liu et al., 2018; Melchini and Traka, 2010). Broccoli (*Brassica oleracea*, var. Italica) and kale (*Brassica oleracea*, var. Sabellica) are two commonly consumed vegetables, offering a high nutritive and dietetic value with their suitable content of proteins, bioactive compounds (e.g. polyphenols and glucosinolates), vitamins, minerals and dietary fibre (Campas-Baypoli et al., 2009; Lisiewska et al., 2008). However, during harvesting, sorting and processing of these two crops, a significant portion of the plant is not utilized, which is either discarded in the field or in the processing facility. Thus, for broccoli, the leaves, stalks and stems (together ca. 70% of the plant) are left on the fields after the harvest of the heads/florets (Liu et al., 2018; Zhang et al., 2017). Similarly, during harvesting and factory sorting of kale leaves, up to 50% of the kale plant is discarded in the form of green residues (leaves, stalks and stems), which is ploughed back into the field as green fertilizer (Berndtsson et al., 2019). Such a waste of valuable resources is both a loss of nutritious green biomass, and of investments in the form of limited resources such as water, fertilizer, farmland and energy, which contributes to greenhouse gas emissions (Röös et al., 2020).

Recent developments in bio-refining technologies to valorize agro-industrial side-streams into added-value products create opportunities for a climate-smart and sustainable use of the above described underutilized biomass. The fractionation of plant proteins into valuable, bioactive compound-rich food products from green leaves is a possible pathway to improved use of the leafy green crop residues (Berndtsson, 2019; Berndtsson et al., 2020). Interest in plant proteins from fractionation of green biomass, especially leaves, for food and feed uses is currently growing by: (i) a demand for plant-protein based food products from the increasing number of flexitarians, vegetarians and vegans, (ii) ethical and environmental issues regarding meat production (Pojić et al., 2018; Rosenfeld and Burrow, 2017), (iii) an interest to reduce food waste in field production and the whole production chain, (iv) a wish to contribute added-value to agricultural side-streams (Berndtsson et al., 2020, 2019) and (v) an increased desire to produce proteins for feed locally, reducing the dependency on imported feed meals (e.g. soy protein import to Europe) (de Visser et al., 2014). This interest is reflected in several ongoing projects targeting green biorefining including at Aarhus University in Foulum, Denmak (dca.au.dk/en/current-news/news/show/artikel/indvielse-af-bioraffineringsanlaeg-paa-au-foulum/), at Töreboda, Sweden under the EU GreenValleys project (vgregion.se/f/naturbruk/utveckling-och-innovation/pagaende-projekt/green-valleys—testpilot-for-gron-bioraffinering) the project Biorefinery Glas in Ireland (biorefinery-glas.eu/) and new commercial scale ventures in Denmark (dlf.com/about-dlf/news-and-press-releases/article/danish-cooperatives-join-forces-on-green-protein?Action=1&PID=1905) all apparently focussed on protein for animal feed. Other projects such as the GreenProteinProject headed by Wageningen University in Netherlands (greenproteinproject.eu) and the PlantProteinFactory at the Swedish University of Agricultural Sciences in Alnarp, Sweden (vinnova.se/en/p/plantproteinfactory-step-2) use a hybrid food/feed approach. Projects aimed at green biomass from several crops, such as alfalfa (Colas et al., 2013) and sugar beet leaves (Tenorio et al., 2016), have been evaluated as source for protein concentrate/isolate production for food and feed applications. Similar to other green biomasses, the underutilized leaves obtained as residue from broccoli and kale production could be a potential source for plant protein production using a biorefinery/fractionation approach.

In addition to proteins, the residual leaves from broccoli and kale contain bioactive compounds and fibre that can be of value for fractionation into food and feed ingredients. Biochemical analyses of broccoli side-streams have shown that the composition of bioactive compounds (e.g. polyphenols and glucosinolates), vitamins, dietary fibre and minerals in leaves resembles that found in the florets (Berndtsson et al., 2020; Zhang et al., 2017). Owing to their attractive nutritional profile, broccoli leaves have been studied as a food ingredient in pasta (Angiolillo et al., 2019), bread (Ranawana et al., 2016), green tea (Campas-Baypoli et al., 2009; Dominguez-Perles et al., 2011) and as functional food ingredient for delivery of specific compounds (Shi et al., 2020), thereby providing added value to food. In kale leaves, a high content of glucosinolates, polyphenols, vitamin C and minerals has been demonstrated (Biegańska-Marecik et al., 2017; Lisiewska et al., 2008). However, studies on the composition and content of bioactive compounds found in kale leaves rejected from the factory sorting process are still lacking (Berndtsson et al., 2019). Since most rejected kale leaves in the factory sorting process are discarded only due to their poor aesthetic appeal to consumers and retailer packaging demands, it is fair to assume that they possess a similar nutritional profile compared to marketed leaves. Therefore, alternative protein and bioactive compound-rich feed and food products from residue leaves of broccoli and kale would not only contribute with consumer-desired products but also increase value for such side-streams. An increased understanding on protein recovery and chemical compositions of different fractions produced from broccoli and kale residual leaves is needed for their commercial application. In addition, economic feasibility studies on the production of proteins for food and feed using broccoli and kale residual leaves in a biorefinery/fractionation concept are still lacking.

In this study, the use of broccoli and kale leaf residue for the extraction of proteins, fibre and phenolic compounds for potential use in food and feed products was evaluated. To our knowledge, this is the first study comparing phenolic and dietary fibre contents in different fractions after fractionation of broccoli and kale leaf residues. To understand such an opportunity, a complete analysis of total proteins, phenolics and dietary fibre was performed to estimate their content in residual leaves and in different fractions produced during a protein extraction process. Based on the amount of different compounds in broccoli and kale leaves, a prefeasibility assessment was carried out on an up-scaled fractionation process of multiple value-added products, evaluating the economic viability of protein extraction and its use in food and feed.

2. Materials and methods

2.1. Determination of amount of field residues

For broccoli, the amount of field residues was determined on August 29, 2018, at a commercial farm in north-western Skåne, Sweden, according to Strid et al. (2014). For this purpose, three squares (1.5 m × 1.5 m) were randomly placed in the field and 10 broccoli plants in each square were cut 2 cm above the ground, weighed, and then divided into different fractions (heads, leaves and stalks), which were individually weighed. The mean weight per 2.25 m^2 square for the different fractions and for the whole plants was calculated.

The amount of residual leaves from kale was determined in October 2020, at a commercial farm, Viklunda farm, in north-western Skåne, Sweden. On commercial harvesting and sorting of kale, plants were cut 40 cm above the ground and brought to a sorting facility, with the remaining stems left unharvested in the fields. Thereafter, kale plants were divided into three fractions; (i) leaves that could be sold, (ii) leaves rejected for sale on the fresh market, and (iii) residual stem remaining after all leaves were picked from the top stem in the manual sorting operation. For determination of the residual leaves, kale plants were randomly picked from an ongoing sorting process, weighed and divided into the above described fractions.

2.2. Plant material

For lab analysis of protein content, bound and free phenolic compounds and dietary fibre, leaves from broccoli (*Brassica oleracea*, Italica group) and kale (*Brassica oleracea*, Sabellica group) were collected from six commercial production fields, in north-western Skåne, Sweden (56°24′38.5″N 12°39′34.5″E). The broccoli and kale plants were collected during the autumn of 2017 and 2018, within 24 h after the last harvest of the main produce (2 and 23 October 2017, and 30 October 2018 for broccoli, 23 October and 6 December 2017, and 12 November and 11 December 2018 for kale) to minimise deterioration of the leaves. Plants of broccoli and kale were cut approximately 2 cm above ground (excluding most woody part of the stems). Leaves already laying on the ground were not collected. Plants collected in 2017 and 2018 were only used for lab analysis.

The plant samples were washed to remove dirt and thereafter the leaves were collected and the other parts were discarded. Leaves were stored at −80 °C until further analysis. Dry matter content was measured by weighting the frozen samples before and after lyophilisation. Prior to analyses of protein content, dietary fibre and bioactive compounds such as bound and free phenolics, the samples were lyophilised.

2.3. Fractionation of the leaf biomass

The fractionation procedure to obtain a green protein fraction (GPF) and a white protein concentrate (WPC) from leaf biomass is depicted in Fig. 1 as pathway B. Similarly, Fig. 1 shows the fractionation procedure to obtain a combined protein fraction (CPF) as pathway C. Both fractionation procedures have been used previously for intermediate crops (Muneer et al., 2021). In the present study, analysis and characterization of proteins, phenols and fibre, was carried out on different fractions obtained along the fractionation pathway to produce GPF and WPC (Fig. 1). The full protein fractionation procedure is fully described in Nynäs et al. (2021). In short, a green juice (GJ) was separated from the leaf pulp (P) through screw pressing of green residue leaves. From GJ, the GPF was thermally precipitated at 55 °C and collected through centrifugation. The WPC was thereafter obtained from the supernatant (white juice — WJ) through acid precipitation (pH 4.5) and collected through centrifugation leaving a supernatant (brown juice — BJ).

2.3.1. Determination of dry matter and protein content

Dry matter and nitrogen/protein content were evaluated for the P, GJ, WJ, BJ, GPF and WPC. For dry matter content evaluation, ~30 ml of each of the juices and ~30 g of each of the protein fractions were weighed before and after lyophilisation. The nitrogen content was analysed on dried samples, in triplicate, using the Dumas method on a Flash 2000 NC Analyser (Thermo Scientific, USA). The protein content was estimated by applying a nitrogen conversion factor of 5.6 (Mariotti et al., 2008).

2.3.2. Determination of total free and bound phenolics content

The amount of total free and bound phenolics was evaluated in triplicate for each of the P, GJ, WJ, BJ, GPF and WPC fractions of broccoli and kale leaves, following the extraction procedure of Dinelli et al. (2009). All samples were lyophilised and milled prior to analysis.

Thus, for free phenolic acids extraction, 1 ml 80% ethanol was added to 50 mg (DM) of sample, vortexed for 10 s and thereafter, ultrasonically treated (Bandelin sonorex digitec, Germany) at 35 kHz for 10 min at room temperature (RT), followed by centrifugation (2500 RCF, 5 min). The resulting supernatant was transferred to a new tube, and the pellet re-extracted using the same procedure. The supernatants were pooled and thereafter evaporated using a SpeedVac SVC 100 (Savant, USA) for 60 min. The samples were cooled in a freezer (−20 °C), reconstituted in cold solution (0.5 ml of 50% ethanol and 2% acetic acid (v/v)) and stored in the freezer for further analysis.

Extraction of bound phenolics was subsequently carried out using alkali and acidic procedures on the remaining pellets after extraction of free phenolic acids. The pellet was dispersed in 1.2 ml water and vortexed, followed by addition of 0.5 ml of 10 M NaOH. The samples were then stored at room temperature overnight (16 h). Thereafter, the samples were centrifuged (16.2k RCF, 20 min), and the supernatants transferred to new tubes before further extraction three times with 0.6 ml ethyl acetate followed by centrifugation (16.2k RCF, 20 min). The ethyl acetate layer (top) was removed by pipette, and the three supernatants were pooled and thereafter evaporated by use of N_2, cooled, reconstituted and frozen as described above until analysis.

The pellets remaining after alkali hydrolysis were acidified by the addition of 0.2 ml 37% HCl and heated in a heating block at 85 °C in an oven for 30 min. Thereafter, the samples were cooled to RT, gently shaken using a vortex and the pH adjusted to below 2 using 37% HCl. The tubes were centrifuged (16.2k RCF, 20 min) and the supernatants were transferred to new tubes. The supernatants were further extracted and stored as described for the alkali extracted samples.

The phenolic content of the samples produced as described above was determined according to Singleton and Rossi (1965), with some modifications (Dewanto et al., 2002; Gao et al., 2000). A standard solution of gallic acid (2 mg/ml in methanol) was used for making a six-point standard curve (10, 20, 50, 100, and 200 μg/ml diluted in 5% ethanol). The prepared extracts were diluted with Millipore water to get readouts within the standard range. A total of 12 μl of extract or standard solution was mixed with 50 μl of Millipore water directly in a 96-well plate, and 12 μl of Folin–Ciocalteau reagent (Sigma-Aldrich, Sweden) was added to the wells. After 6 min of incubation 125 μl of 7% (w/v) Na_2CO_3 was added. The samples were incubated for 75 min and the absorbance measured at 765 nm with a spectrophotometer (ThermoFisher Multiskan GO, USA). An empty well was used as a blank. The concentration of phenolic compounds in the samples was expressed as mg gallic acid equivalents based on the standard curve.

2.3.3. Determination of fibre content

Total content of dietary fibre was analysed in lyophilised and milled samples of the P, GJ, WJ, BJ, GPF and WPC by the ISO/IEC 17025:2005 SWEDAC 1977 accredited laboratory Eurofins Food & Feed Testing Sweden (Lidköping, Sweden) using the standard method (AOAC 991.43).

2.4. Economic assessment

A cost-benefit analysis was conducted on the use of broccoli and kale leaves for the valorisation of leaf proteins for food and feed applications. Calculations were carried out as a step-by-step assessment that included all necessary machinery operations in the field, transport, storage and processing in a theoretical protein extraction plant based on the nec-

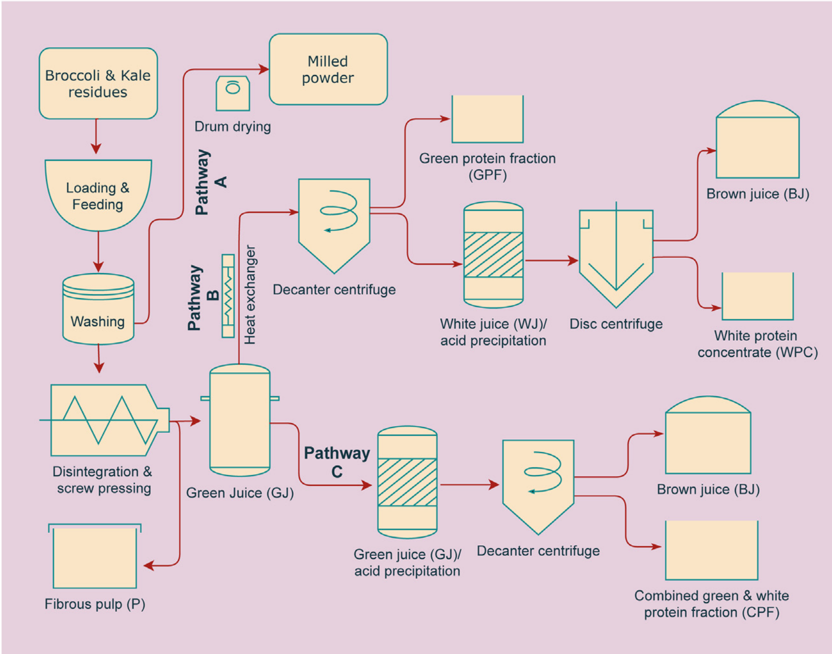

Fig. 1 – Overview of proposed use of broccoli and kale residual leaves as dried and milled biomass (pathway A), and material flow in protein extraction pathways (B and C), with different fractions and side products.

essary operations described below. Results of this type of pre-feasibility study usually have an error margin of up to ±30% (Bals and Dale, 2011). To present also the variation of data in the results, a low and a high range analysis for cost and revenue structure for each fractionation pathway was employed.

2.4.1. *Feedstock supply*

The amount of available broccoli and kale leaf biomass was estimated based on typical wet yields of marketable product (broccoli florets and kale leaves), corresponding total above-ground biomass wet yields and typical proportions between marketable product and leaves suitable for protein extraction. Data used for the further economic assessment is presented in the results section. A conversion factor of 1 SEK = 0.0938 € was applied.

For the cost assessment in the case of broccoli, data from both conventional and organic cultivation systems was considered. The harvest of broccoli leaves was assumed to be added as an additional manual harvest operation. Labour and machinery costs were considered for harvest and transport operations (Table 1). Transport of the leaves to the protein processing plant was accounted for assuming a distance of 150 km. To avoid degradation and assure compliance with regulations regarding the microbial safety of food and feed products, broccoli leaf biomass was assumed to be transported without cooling to the processing plant within 4 h after harvest.

For kale, costs based on the already occurring sorting practice in the sorting facility at the farm was estimated. Instead of only sorting kale leaves into marketable and non-marketable leaves, the non-marketable fraction would be further sorted into leaves suitable for protein extraction and leaves to be discarded. This distinction was assumed to be done based on a visual judgement and would result in slightly damaged and discoloured leaves to be used for protein extraction, while heavily damaged leaves and leaves with microbiological defects would be discarded, which could be used in a biogas plant. The useful feedstock was considered to have no additional costs for harvest, only for transport with the same assumptions as for broccoli leaves.

2.4.2. *Protein extraction pathways*

Three production pathways were evaluated in this study: (A) milled biomass, (B) production of green protein fraction (GPF) and white protein concentrate (WPC) and (C) total recoverable combined protein fraction (CPF, both green and white proteins) (Fig. 1). All three pathways assume a processing capacity of 100 t/h. In a previous study, economic assessment has been carried out on application of pathways B and C, respectively, on intermediate crops (Muneer et al., 2021). In the present study, the same setup was followed, however additional data on fibre and phenolic contents in different protein fractions is presented for the crops investigate here. However, since it is unknown if the presence of phenolic compounds in different protein fractions have a positive or negative health effect, their economic value has not been considered. Fibre was considered to be part of the final product and fibre content was used to compare to other products on the market.

For the economic assessment of pathway A, broccoli and kale leaves were assumed to be dried in a drum dryer to a moisture content of approx. 6%, and then milled to a fine powder with an assumed long shelf-life. Initial moisture content of broccoli was assumed to be 88 and 74% for the low and high case, respectively, and 86 and 77% for kale.

For the economic assessment of pathways B and C (Fig. 1), the production of the different fractions follows the same procedure as previously have been described for intermediate crops (Muneer et al., 2021). Thus, in the protein extraction plant, the leaf biomass is directly fed to a washing basin to remove contaminants, e.g. soil particles. From the washing step, the biomass is fed into a screw-press designed to disrupt the cell wall structure and to separate the material into a P and GJ fraction. The P is ensiled for later use, for example to biogas production or used as cattle feed. In pathway B, the GJ is heated to 55 °C to coagulate and precipitate the GPF. In a decanter centrifuge the GPF is separated from the WJ, which is transferred

Table 1 – Working time requirements and related costs for harvest of broccoli leaves based on Ascard et al. (2008).

Parameter	Unit	Harvest: labour		Harvest: machinery		Transport: labour & machinery[a]	
		Low	High	Low	High	Low	High
Work	[h/ha]	67	75	13	15		
Cost	[€/ha]	1257	1407	146	169	169	253

[a] Estimated at approx. 2.8 €-ct/kg, which corresponds to a transport of 150 km in a full truck (Ascard et al., 2008).

to a tank for further extraction of WPC. The GPF collected in this step is dried to a green powder using a drum dryer. The pH of the WJ is adjusted to approximately pH 4.5 to precipitate the white protein fraction, which is separated using a disk centrifuge. This WPC is later dried to obtain a white protein powder. The clarified BJ produced in this process is stored for later use e.g. in biogas production. In pathway C, to obtain a CPF, the pH of the GJ is adjusted to approximately pH 4.5 to precipitate both green and white proteins. The precipitated CPF is then separated using a decanter centrifuge and the BJ fraction obtained in this process is stored for use in biogas production.

Economic data on an extraction process with mechanical screw-pressing for fraction separation were used as presented by Bals and Dale (2011) (Table 2). However, the processes differ somewhat, e.g. the Bals and Dale process includes additional milling for further cell disruption of the switchgrass feedstock used in the study and a secondary pressing step, both of which are energy and capital intensive (Bals and Dale, 2011). Milling was considered not necessary as broccoli and kale leaves are less fibrous compared to switchgrass. A cost reduction of 31 and 39% for capital and operational cost was suggested by Bals and Dale (2011). Simulating the CPF pathway (C), a simpler process with direct protein precipitation and no milling was assumed. To not overestimate the cost of the avoided milling step, a 20% cost reduction was assumed here. Protein fractions were dried before sale as products to an average moisture content of 6%.

2.4.3. *Final products*

The fine powder produced through pathway A, is assumed to be suitable for a product that could be used in food industry either as a bulk food additive or as a niche health product. As economic revenue differs extremely between these two markets, milled biomass from broccoli and kale leaves is assessed for both applications.

For the production pathway B, WPC powder is intended as a product for human consumption, e.g. as food ingredient in the food industry. The DM protein content (and yield) depends strongly on precipitation conditions and typically ranges between approx. 0–30% (Bals et al., 2012). In this study, a protein content in the WPC of 29% and 16% for broccoli and kale-derived white protein, respectively, was assumed, following the results of the lab analyses. This protein concentration was assumed to be increased to 85% in the final product assuming additional purification steps (Edwards et al., 1975; Tenorio et al., 2016). The product is an off-white powder dried to a moisture content of 4–8% resulting in a long shelf-life. A protein profile suitable for human consumption was assumed. Monetary valuation considered only the nutritional value, with no functional value attached to the proteins.

Both green protein fractions (from production pathways B and C) were assumed to be refined into a green powder intended for use as feed or feed ingredient. Based on lab analyses, the protein content in the protein precipitates was 24–26% for products from both broccoli and kale. The final product is a green powder dried to a moisture content of 4–8% assumed to result in a long shelf-life. Although a protein profile suitable for use as animal feed for both monogastric animals and ruminants was assumed, the economic assessment was carried out for the use as horse feed, specifically as high-protein horse feed additive. However, similar products available on the market have a considerably lower protein content, 11–17% (Appendix Table A1). The kale product had a fibre content of 16%, whereas the broccoli product had a lower fibre content, 11%, which compares to a fibre content in commercial products that ranges 7–27%.

Fibre pulp from production pathways B and C is ensiled at a moisture content of 30% and intended for use as cattle feed. Protein content is approx. 4.3 and 3.0% wet basis for broccoli and kale, respectively, and a protein profile suitable for use as animal feed for ruminants (Dolores Megías et al., 2014; Yi et al., 2015) was assumed.

Brown juice from production pathways B and C is a residue product with potential use as biogas substrate. However, due to the low dry matter content (approx. 6–7%), transport costs are high. Treatment to increase DM content needs to be balanced against product value. Depending on the transport distance, this by-product can be a cost or produce revenues. Therefore, revenues from this by-product have not been included in the economic assessment. The estimations of revenue from the different fractions were carried out based on market reviews for the corresponding applications (Table 3).

3. Results and discussion

3.1. *Field produce and side-streams*

Broccoli harvest following Nordic routines means that only florets of 10–15 cm in diameter and with a weight of approx. 300 g are harvested, although several harvests per year occur in the same field, which allows for continued growth and harvest. The present study showed that field production of broccoli in Southern Sweden resulted in a high variability in the size of the broccoli heads (140–300 g) and in the total biomass of broccoli heads (13–21%; including those being too small to be marketed) within the same field of production. A total of 43–87% of the biomass was leaves and stems suitable to be used as side-streams for fractionation into different products. This corresponds with previous studies on Swedish broccoli production systems, reporting above ground broccoli biomass yield in the field of 49–160 t wet weight per hectare, of which only 10–33 t per hectare are marketable, leaving 32–138 t of harvest residues (Fink et al., 1999). Additional side-streams are produced during processing, corresponding to 45–50% of the initial broccoli head weights (Campas-Baypoli et al., 2009). In the present study, broccoli leaves constituted 43–78% of the wet weight of the broccoli plants and 64–84% of the crop residues after removal of the broccoli heads. Another

Table 2 – Cost given as range per t of initial feedstock for protein extraction and drying for final product formulation.

Fraction	Crop	Operational cost [€/t]	Investment cost[a] [€/t]	Technology used	References
Milling (pathway A)	Broccoli and kale	6.6–8.1	2.2–2.7	Disc mill	Bals and Dale (2011)
Extraction					
White and green protein (pathway B)	Broccoli and kale	18.7–23.5	8.0–9.6	Mech. separation	Bals and Dale (2011)
Total recoverable green protein (pathway C)	Broccoli and kale	15.0–18.8	6.4–7.7	Mech. separation	Bals and Dale (2011)
Drying					
Milled biomass	Broccoli	12.1– 31.9	5.5–12.8	Mechanical dewatering & thermal drying	Own estimation[b]
	Kale	12.5–32.5	5.6–13.0		
White protein	Broccoli	0.6–3.8	0.3–1.5	Spray drying	Own estimation[b]
	Kale	0.7–4.7	0.3–1.9		
Green protein fraction	Broccoli	1.9–6.8	0.9–2.7	Drum drying	Own estimation[b]
	Kale	2.1–7.3	0.9–2.9		
Total recoverable combined protein fraction	Broccoli	4.6–16.2	2.1–6.5	Drum drying	Own estimation[b]
	Kale	4.1–14.5	1.9–5.8		

[a] For the drying processes estimated as 40 and 45% of high and low operational costs, respectively.
[b] Estimated based on the energy consumption of 3–7 MJ/kg evaporated water (Baker and McKenzie, 2005) and energy prices of 1.0–1.8 €-ct/MJ (SCB, 2019).

Table 3 – Product revenues per kilogram protein as assumed for the economic assessment.

Product	Application	Chosen value [€/kg] (market range)
Green protein, GPF	Horse feed	8.5 (6.6–10.4)
White protein, WPC	Food for human consumption	11.2 (8.6–13.8)[a]
Total green protein, CPF	Horse feed	8.5 (6.6–10.4)
Fibre pulp; P	Feed for ruminants	0.21 (0.14–0.28)[b]
Milled broccoli leaves	Health product (protein value only)	1.7 (1.3–2.1)[c]
Milled kale leaves	Health product (protein value only)	2.1 (1.6–2.6)[c]

GPF = green protein fraction; WPC = white protein concentrate; CPF = combined protein fraction; P = pulp.
[a] Range as analysed on Alibaba.com (8 June 2019) for plant-based protein; when a default price of 1 US$ kg^{-1} product was given as the lower price range, this was corrected by assuming the lower price limit being at 50% or the upper price limit of the same product.
[b] Assumed to have the same value as that of untreated ley crop biomass used as ruminant feed.
[c] Based on a protein content of 11 and 14% in the final product from broccoli and kale, respectively, and the protein value of white protein.

study has reported leaf shares of 74–85% of the wet weight of greenhouse-grown broccoli (Domínguez-Perles et al., 2010). Dry matter (DM) content of leaf biomass varied between 12.5–25.7% in the present study and an average DM content of 15% was assumed for the economic assessment. The economic feasibility study here is focusing on using the leaves as a suitable side-stream as broccoli stems were determined less suitable, being hard and fibrous and thereby difficult to process in a plant protein factory. Based on above mentioned yield related parameters for Southern Sweden, a total yield of 3.8–16.0 t DM per hectare of broccoli leaves was selected as a basis for the pre-feasibility calculations. If not used as a side-stream, broccoli residues are normally ploughed into the soil as green fertiliser. Broccoli florets are normally harvested by hand and leaves as a side-stream can also be harvested by hand, simultaneously with the last floret harvest. Another option would be to harvest the top leaves with the top stem, mechanically, after the manual harvest of the last florets. Here, our pre-feasibility study was based on a simultaneous hand harvesting of leaf residues with the final harvest of the florets.

The kale harvest includes manual cutting and collection of the top, which is transported to the facility for sorting and packaging of the marketable leaves. The rejected leaves correspond to ca. 16% of the whole kale plant, which means that a mean weight of ca. 1.6 kg/kale plant and on average 30,000 plants/ha per, will result in ca. 7.7 t/ha of rejected residue leaves for protein fractionation. Based on the experience of kale producers (personal communication), approx. 50% of the weight of the kale plant is marketable leaves while ca. 10–20% are residual leaves and ca. 30–40% are stem parts. Thus, in the economic assessment carried out here, these assumptions were used. These results correspond well with results from Fink et al. (1999) on the Swedish production system for kale with a total aboveground biomass yield of 21–65 t wet weight per hectare, of which 10–26 t per hectare are marketable, leaving 10–49 t of harvest residues per hectare. Dry matter content of leaf biomass varied between 14.0–22.8% in the present study and an average DM content of 15% was assumed for the economic assessment. Based on the above mentioned parameters for Southern Sweden, a total yield of 0.32–1.95 t DM per hectare of kale leaves was selected as a basis for the pre-feasibility calculations. Within the current harvesting system, discarded kale leaves, which can be used for extraction of added-value compounds, can be collected simultaneously as marketable kale leaves are collected, and thereby no extra harvest operation is required.

3.2. *Composition of fractions*

3.2.1. *Dry matter, protein content and nitrogen recovery*

Dry matter (DM) content varied for both crops and in the different fractions (Table 4). Generally, higher DM content was observed in kale than in broccoli, and higher DM content in kale stems than in kale leaves. Furthermore, for both broccoli and kale the highest DM content was obtained in the P (277 and 313 g kg^{-1}), and rather high values were found in the GPF (195 and 183 g kg^{-1}), while generally low values were found in the GJ, WJ, BJ and WPC (65−84 g kg^{-1}), respectively.

Interestingly, a high protein content was found in all the fractions obtained, although with the highest content in the GPF and WPC in both crops (Table 4). Corresponding to the dry matter content, the protein content in the various fractions varied similarly for the two crops evaluated. However, the protein content was consistently lower in all fractions for kale compared to broccoli, which also corresponds to previous reports on total amino acid contents in the crops with significantly lower values for kale than for broccoli (Campas-Baypoli et al., 2009; Lisiewska et al., 2008). Inconsistent with the previous findings, leaves of kale showed higher protein content than those of broccoli in the present study. However, the values for leaves are based on a single measurement. Then, a larger amount of leaves of each crop was processed into the different fractions from which three separate samples were taken for analyses. Thus, the discrepancies in the protein content between the raw material and the fractions might be the result of a single sample being analysed from the raw material. Broccoli is known as a high-protein vegetable (Kmiecik et al., 2010), which is not the case for kale, but both crops have an excellent amino acid profile (Campas-Baypoli et al., 2009; Lisiewska et al., 2008). The dominating protein in all green biomass is RuBisCO, that catalyses the uptake of CO_2 in photosynthesis, which is considered to be the most abundant protein in the world (Andersson and Backlund, 2008). RuBisCO should have the same amino acid profile independent of crop background (Udenigwe et al., 2017), and previous studies have indicated alanine, glycine, glutamate and leucine to be the major amino acids (Udenigwe et al. (2017). However, different green biomasses have been shown to contain varying amino acid profiles, due to the fact that other proteins are present in the green biomass. In broccoli and kale parts, the dominant amino acids are aspartic acid, glutamic acid and proline (Campas-Baypoli et al., 2009; Lisiewska et al., 2008). Studies reporting amino acid composition in various fractions are scarce, although high levels of essential amino acids have been reported for the WPC (Hojilla-Evangelista et al., 2017; Kaszás et al., 2020; Merodio and Sabater, 1988; Wang and Kinsella, 1975). Recent results (unpublished) from our lab on hemp and red clover biomass, have indicated an increased accumulation in the relative content of essential amino acids in the P, GPF and WPC (ca. 55% essential amino acids in each), in comparison to the dry biomass (48–49% essential amino acids), while the WJ and BJ were low in relative content of essential amino acids (15–35%).

Nitrogen recovery from the original leafy green biomass to the different fractions was similar for the two crops evaluated. Thus, more than 50% of the N in the green biomass ended up in the P, around 30% ended up in the GPF, 15% in the BJ and only around 2% in the WPC (Table 5). The fact that broccoli and kale behaved similarly when it comes to protein content and N recovery in various fractions after fractionation, does not necessarily mean that this also is the case for other green biomasses. A recent study has in fact shown the opposite, i.e. that the fractionation process must be optimized in relation to different green biomass to obtain reasonable protein content in the WPC (Nynäs et al., 2021). Furthermore, what fractionation processes are being used and type of WPC product compared is also of relevance when evaluating protein content in various fractions as discussed by Nynäs et al. (2021).

From the present study, it is clear that the GPF and WPC both have a generally high protein content (Table 4) and a valuable amino acid composition, which makes them suitable as food and feed sources. In addition, the P and the GJ hold a considerable content of proteins and a good amino acid profile. Therefore, P and GJ should also be considered and further analysed as sources for food and feed products in a protein factory concept. However, the proteins in the P are known to be captured in cell wall components, and as insoluble proteins retained in fibrous scaffold (Damborg et al., 2020). In this study, more than 50% of the N in the green biomass ended up in the P and the protein content in the P was actually 20–50% higher per kg DW as compared to unprocessed plant biomass, which makes the P an attractive feed material for ruminants. For the BJ, previous studies have indicated it contains mainly non-protein components, small peptides and free amino acids, separated during the extraction process (Damborg et al., 2020; Santamaría-Fernández et al., 2017). However, results from Nynäs et al. (2021) indicated the presence of proteins in the BJ, verified by SDS-PAGE. Here, BJ was reported to contain proteins, although measurements were carried out on nitrogen content and then converted to protein by the use of a conversion factor. Thus, the protein content value presented includes non-protein nitrogen and the actual protein content of the BJ requires further investigation.

Based on the results of the analyses presented in Table 4, assumptions were made on the amount of protein to become available in the final products (Table 5). This follows a low/high approach that represents the variation in the lab analyses. For the combined green protein fraction, some of the protein that could be precipitated in a heat treatment as in pathway B would be precipitated in the direct acid treatment of pathway C. The additional amount of protein compared to the GPF was estimated to be 15 and 20% for the low and high case, respectively.

3.2.2. *Phenolics*

Strikingly, phenolic compounds are clearly present in all the fractions and with equal levels for both the crops. The measured content of the free and bound phenolics of the broccoli and kale biomass corresponded well with previous studies (Berndtsson et al., 2020; Goupy et al., 1990; Liu et al., 2018; Olsen et al., 2009).

The highest contents are found in the juices (GJ, WJ and BJ) and in the WPC (Table 4) for both crops and for both bound and free phenolic compounds. Highest recovery of the phenolic compounds was found in the juices (GJ, WJ, and BJ), although also a relatively high recovery was found in the P (Table 4). Recovery was similar for bound and free phenolics and in both crops, with 33–43% of the phenolics ending up in the P (somewhat higher values for kale than broccoli), 50–66% in the juices, with higher values in the GJ than in the WJ and BJ (larger differences for broccoli than for kale), 4–18% in the GPF (larger values for broccoli than kale), and 0.3–0.4% in the WPC (Table 4).

Previous studies evaluating the health benefits of phenolics have shown that a human diet rich in phenolics contributes to

Table 4 – Average content of analysed compounds; dry matter, protein (average of triplicates, except for stems and leaves, measured as N content and thereafter transferred to protein content using a conversion factor of 5.6), total phenolic content, free phenolic content, and fibre (n = 1), and nitrogen, phenolics and fibre recovery from the original biomass (leaves), in the different fractions in the process. Numbers give the mean value and the standard deviation (in parentheses), where analysis was based on triplicates. N = not analysed.

Crop	Component	Unit	Leaves	P	GJ	GPF	WJ	BJ	WPC
Broccoli	Dry matter content	[g/kg]	125	277 (±43)	84 (±2)	195 (±5)	65 (±1)	65 (±0)	81 (±14)
	Protein content	[g/kg DM]	120	142 (±11)	164 (±0)	272 (±10,2)	110 (±0,7)	110 (±0)	304 (±132)
	Bound phenolics content	[mg GAE/g DM]	8.2 (±0.4)	4 (±0.1)	10.2 (±0.6)	6.0 (±0.9)	11.2 (±1.3)	11.6 (±0.6)	13.6 (±0.3)
	Free phenolics content	[Fe^{2+} μmol/g DM]	108.4 (±7.2)	40.1 (±1.1)	114.3 (±4.7)	57 (±6.6)	128.7 (±4.8)	135.3 (±2.7)	153.1 (±4.9)
	Dietary fibre content	[g/kg DM]	352	431	63	116	47	30	N
	Nitrogen recovery from biomass	[%]	–	56 (±20)	44 (±2)	29 (±1)	16 (±2)	15 (±2)	0.31 (±0.17)
	Bound phenolics recovery from biomass	[%]	–	36 (±0.7)	64 (±1.3)	18 (±1.9)	45 (±1.6)	45 (±0.02)	0.4 (±0.007)
	Free phenolics recovery	[%]	–	34 (±0.6)	66 (±90.9)	16 (±1.5)	50 (±0.5)	50 (±0.01)	0.4 (±0.01)
	Fibre recovery from biomass	[%]	–	91	9.2	5.9	3.2	3.2	0
Kale	Dry matter content	[g/kg]	143	313 (±23)	76 (±1)	183 (±6)	68 (±2)	68 (±1)	88 (±8)
	Protein content	[g/kg DM]	150	99 (±4.5)	120 (±3.1)	258 (±1.9)	89 (±0.3)	88 (±0)	167 (±4.6)
	Bound phenolics content	[mg GAE/g DM]	7.7 (±0.2)	4.3 (±0.1)	10.6 (±0.4)	4.7 (±0.2)	11 (±0.1)	10.7 (±0.1)	15.7 (±0.7)
	Free phenolics content	[Fe^{2+} μmol/g DM]	87.8 (±1.7)	47.4 (±0.5)	115.2 (±2.7)	42.3 (±2.2)	141.7 (±1.1)	141.9 (±3.2)	166.3 (±8.8)
	Dietary fibre content	[g/kg DM]	407	602	49	171	N	N	N
	Nitrogen recovery from biomass	[%]	–	61 (±2)	39 (±2)	18 (±1)	21 (±1)	21 (±1)	0.20 (±0.11)
	Bound phenolics recovery from biomass	[%]	–	43 (±0.9)	57 (±0.9)	6.2 (±0.2)	51 (±0.06)	51 (±0.003)	0.4 (±0.02)
	Free phenolics recovery from biomass	[%]	–	43 (±0.3)	57 (±0.6)	4.4 (±0.2)	53 (±0.03)	52 (±0.01)	0.3 (±0.02)
	Fibre recovery from biomass	[%]	–	96	4.3	4.3	N	N	N

GAE = gallic acid equivalent; P = pulp, GJ = green juice, GPF = green protein fraction, WJ = white juice, BJ = brown juice, WPC = white protein concentrate, CPF = combined protein fraction.

Table 5 – Recoverable protein in the different fractions relative to the initial amount of protein in the leaf biomass as used in the economic assessment.

Parameter	Unit	Broccoli		Kale	
		Low	High	Low	High
White protein fraction (WPC)	[%]	0.11	0.41	0.09	0.31
Green protein fraction (GPF)	[%]	28.0	29.3	16.5	19.0
Combined green protein (CPF)	[%]	32.2	35.2	19.0	22.8
Brown juice (BJ)	[%]	13.7	17.0	20.1	21.8
Fibre pulp (P)	[%]	54.5	57.9	59.0	63.1

improved cardiovascular health (Wang et al., 2011), decreased risk of developing some forms of cancer (Kyle et al., 2010) and a decreased mortality due to cancer (Ivey et al., 2015) or by cardiovascular diseases (Manach et al., 2005; Williamson, 2017). Furthermore, phenolic compounds have been suggested to have a positive impact on the gut microbiota in humans (Selma et al., 2009), and flavonoids, such as quercetin and kaempferol, have shown some possible positive impact on ruminant health by reducing inflammation (Olagaray and Bradford, 2019). Also, positive impact on human health has been reported from the intake of phenolic compounds of vegetable origin when compared to synthetic antioxidants added to food (Peschel et al., 2006). Due to all the positive benefits from consumption of plant based phenolics, the content of phenolics reported here in the different fractions are highly relevant if some fractions are to be used for food purposes as e.g. as nutritional additives. Another opportunity is to carry the fractionation process further and extract the phenolics from the rich fractions for further use as plant phenolic concentrates.

The present study did not evaluate the composition of the specific phenolic compounds in the different fractions. Thus, for further studies, this will be an important topic in order to understand where and in what amount beneficial phenolic compounds are present in the different fractions. The current results indicate that there might be a difference in the composition between the P and the juices and protein fractions. Phenolics found in the P might be such types that are more thoroughly bound to dietary fibre. Earlier studies have indicated human health benefits from combined phenolic-dietary fibre complexes (Saura-Calixto, 2011). Phenolics soluble in the GJ seem to mainly continue through the process in the juice fractions and phenolics found in the protein fractions (GP and WPC) might be bound to the proteins. Earlier studies have shown that there are high levels of kaempferol and quercetin in kale leaves (Olsen et al., 2009; Schmidt et al., 2010), two compounds that might have different health benefits (Martinez et al., 2017). The fact that the phenolics are found together with dietary fibre (Saura-Calixto, 2011) or protein (Foegeding et al., 2017) could have an impact on both bioavailability and on extractability, as the co-occurrence of these groups of compounds are often needed. Such issues require further study.

3.2.3. *Fibre*

The broccoli leaves in this study contained 35 g dietary fibre/100 g DW, which is in line with earlier studies (Berndtsson et al., 2020). Kale leaves contained higher levels of dietary fibre compared to the broccoli leaves, with 41 g/100 g DW, and this content was similar to what has been found in previous studies (Thavarajah et al., 2019).

The highest fibre content (>90%) was clearly seen in the P fraction for both crops and second highest level in the GPF (Table 4). Dietary fibre as a supplement in food and feed is of interest because of the suggested health benefits, improving human gastrointestinal and cardiovascular health (Kim and Je, 2016), e.g. lowering blood cholesterol levels (Surampudi et al., 2016). Furthermore, fibre improves the gastrointestinal health and the immune system in animals (Jha et al., 2019). However, for animals the dietary fibre might also be considered as an anti-nutritional factor, as it increases satiety (Jha et al., 2019) which could reduce total caloric intake. Dietary fibre also positively influences the bioavailability of phenolic compounds by entrapping them, leading to more phenolic compounds reaching the gut microbiota (Edwards et al., 2017).

To further estimate the value or possible health benefits of fibre from the broccoli and kale fractions, the proportions of soluble and insoluble dietary fibre, as well as the composition of dietary fibre needs to be evaluated. Also, a larger data set is required, since the current data set is minimal and serves to demonstrate the presence of interesting opportunities in these kinds of biomasses.

3.2.4. *Anti-nutritional components*

In this study, a chemical analysis to identify potential anti-nutritional components was not performed, although literature indicates that the presence of such components needs to be evaluated before any fractions can be used for food and feed purposes. The total content and distribution of anti-nutritional compounds may vary according to genera and species of plants used for protein extraction, although major anti-nutritional factors commonly found in green leafy vegetables are nitrates, oxalates, phytates, tannins and saponins (Gupta and Wagle, 1988; Natesh et al., 2017; Satheesh and Workneh Fanta, 2020). Presence of such anti-nutritional compounds may have a direct or indirect impact on the health of an ingesting human or animal (Natesh et al., 2017). In general, the amount of anti-nutritional compounds e.g. nitrates, oxalates, phytates and tannins, are relatively low in kale and broccoli as compared to other leafy vegetables such as spinach (Natesh et al., 2017). However, during fractionation anti-nutritional compounds can possibly be accumulated in specific fractions, resulting in some of the fractions being less useful or even harmful for food and feed purposes. Our preliminary results indicate accumulation of nitrates and nitrites in all of the juice fractions. Therefore, it would be highly relevant to further evaluate the accumulation of these compounds in the different fractions and to improve the separation processes in future work.

3.3. *Economic evaluation*

Economic assessment evaluating the use of broccoli and kale leaves as milled biomass (pathway A) and extraction of white and green protein following pathways B and C showed large differences in both costs and revenues for the investigated range of low and high yields in field production and pro-

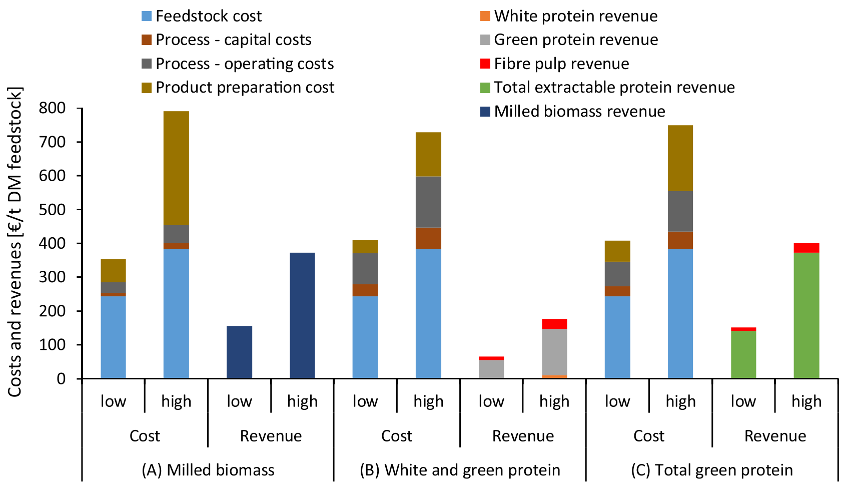

Fig. 2 – Cost and revenues [€/t DM of feedstock] of broccoli leaf-derived products in the three production pathways given as low–high range. 'Process' refers to extraction of proteins and production formulation refers to drying or ensiling for the different product fractions. Revenue for milled biomass refer to use as a bulk food additive, revenues from application in health products is presented in the text.

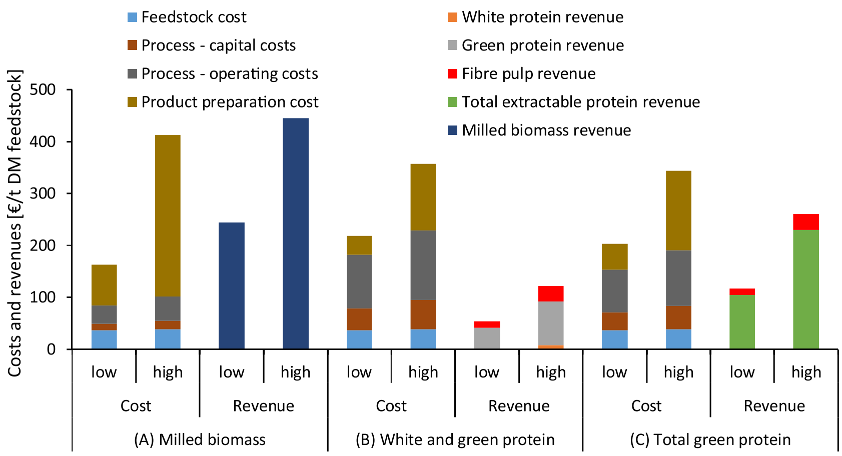

Fig. 3 – Cost and revenues [€/t DM of feedstock] of kale leaf-derived products in the three production pathways given as low–high range. 'Process' refers to extraction of proteins and 'production formulation' refers to drying or ensiling for the different product fractions. Revenue for milled biomass refers to use as a bulk food additive, revenues from application in health products are presented in the text.

tein extraction combined with variability in the process data (Figs. 2 and 3).

3.3.1. Costs

3.3.1.1. Broccoli. Feedstock costs ranged between 240−380 €/t DM and represented the largest cost for production of protein products from broccoli leaves (Fig. 2). Feedstock costs were the same for all three production pathways with 48–69% of the total cost. Process capital costs, process operating costs and product preparation corresponded to 2–9, 7–22 and 9–43% of the total costs, respectively (Fig. 2). Capital and operating process costs in the less intense processing of the milled biomass pathway (A) were approx. 2–3 and 7–9% of the total cost, respectively. Due to a large amount of material requiring drying, product preparation in the milled biomass pathway corresponded to a higher share of total cost of 1 9–43% compared to the 9–26% in the production of white and green protein fractions. Processing of white and green protein that included an additional step for white protein precipitation, was 25% more expensive per t of feedstock compared to production of the green CPF. Product preparation of white and green protein had a 32–39% lower cost due to the lower amount of product to be dried per t of feedstock.

3.3.1.2. Kale. Feedstock cost of kale leaves were approx. 40 €/t DM (Fig. 3), which was considerably lower than the feedstock costs for broccoli leaves. Feedstock costs were the same for all three production pathways and represented 9–22% of the total cost, which was much lower compared to the broccoli leaf feedstock. The much smaller absolute cost is a consequence of that the leaves were available from the sorting facility without further harvest costs. Process capital costs, process operating costs and product preparation corresponded to 4–19, 12–47 and 17–75% of the total costs. Similar to the broccoli case, the less intense processing in the milled biomass pathway (A) resulted in a considerably lower range of relative capital and operating process costs of 4–7 and 12–22%, respectively. Again, due to a large amount of material requiring drying, product preparation in the milled biomass pathway showed a much higher relative cost of 49–75%. Compared to the CPF production pathway for broccoli leaves, product preparation costs per t of feedstock for production of white and green protein fractions were 16–27% lower. Similar to the broccoli case, this can be explained by the lower extraction efficiency for white protein extraction and corresponding lower drying requirements.

3.3.2. *Revenues*

Revenues from milled biomass marketed as a health food product (pathway A) ranged from approx. 160–370 and 240–440 €/t DM of feedstock for broccoli (Fig. 2) and kale (Fig. 3) leaves, respectively. For the assessment, value was attributed only to the protein content and not to any health effect of the fibre or phenolic content of the biomass. However, if health effects based on the phenolic content can be substantiated, as has been shown with similar products, e.g. wheatgrass (Rana et al., 2011) or pulse shoots (Ghumman et al., 2017), the value and therefore the pricing of the product could be increased. Even without this health claim, milled biomass products from broccoli and kale leaves show an approx. 70–180 and 90–210 times higher protein price, respectively, compared to the protein value assumed here and based on our market analysis.

Revenues from the production of white and green protein (pathway B), ranged from approx. 50 to 180 €/t DM of feedstock for both broccoli (Fig. 2) and kale (Fig. 3) leaves. Here, the proportion of revenue originating from the WPC was extremely low, 2–6%, for both broccoli and kale. This was based on lab experiments that aimed at extracting protein with a high functional value (e.g. foaming properties). Here, the revenues from the GPF represented 69–84% of the total revenues. The P contribution to revenues ranged between 5–25%.

Revenues from the production of total recoverable CPF (pathway C) ranged from approx. 120 to 400 €/t DM of feedstock for both broccoli (Fig. 2) and kale (Fig. 3) leaves. Here, the proportion of revenue originating from the CPF varied little and was 88–94% of the total revenues, for both broccoli and kale leaves. Revenues from use as horse feed varied mainly due to a large price variability of the Swedish market (Appendix Table A1). The P contributed the remaining approx. 11–12% of revenue. Early technological assessments and economic estimates of leaf protein concentrates as presented in the 1970s–80 s, e.g. using alfalfa for chicken feed production (Enochian, 1980; Vosloh, 1976), predicted good profitability. A more recent study on plant protein concentrates from alfalfa employing a process comparable to the CPF process of the present study has found similar discrepancies between feedstock cost and corresponding revenues, at higher yields of total recoverable combined protein but lower protein value (Sinclair and MacManus, 2009). Similar to the CPF production from broccoli presented here, Hermansen et al. (2017) found feedstock costs for purpose-grown grass-clover leys corresponding to 76–83% of the resulting revenues when the green protein concentrates were valorised as pig feed and fibrous pulp as feed for ruminants.

3.4. *Economic feasibility*

3.4.1. *Broccoli*

For the milled biomass and total green protein production pathways, revenues in the high case were similar to the cost in the low case, but much lower than the costs in the high case, indicating that a more detailed assessment is required for evaluation if there is a potential to develop these pathways commercially. The focus of a more detailed assessment should be on reducing the feedstock costs and improving the product quality enabling a better value assessment and market placement. The extraction of WPC is not an economically feasible option under the investigated conditions. This is mainly due to the extremely small fractions of protein that was recovered.

None of the investigated production pathways were economically viable without an adjustment of the current practices of harvesting broccoli florets as the additional harvest operations for recovering broccoli leaves were costly. The potential to reduce feedstock supply costs for additionally harvested broccoli leaves is regarded as low, since this interferes with current practise of quality-driven harvest operations picking only florets suitable for the fresh market. Alternative harvest methodologies similar to the kale harvest could entail the harvest of the larger part of the broccoli plant with a facility-based sorting procedure. Another alternative is a mechanised leaf harvest after the last floret harvest. This could be viable since the broccoli plants continue to grow after harvest of the florets. However, cuts from floret removal may become subject to infections and mould, which could cause problems with food safety in the downstream process. In order to determine if this can be a viable option, detailed field studies are required to investigate if the feedstock quality could be adequate with mechanical harvest and how this would affect the value of the resulting products.

3.4.2. *Kale*

Economic feasibility of the milled biomass using kale leaves as feedstock is much more likely to be achieved compared to broccoli, since most leaves used are harvested in the same step as harvesting kale leaves for conventional marketing as a fresh vegetable. The leaves that are made available for protein extraction are derived from the quality-based sorting step in the leaf processing facility and imply no further harvesting costs, with the exception of transport costs.

For a milled biomass product (pathway A), costs and revenues are comparable when the milled biomass is marketed for only the nutritional value of the protein, indicating that a more detailed assessment is required to evaluate if there is a potential to develop this pathway commercially. Still, the simple process of drying and milling the leaves to prepare a health product seems to be an interesting option mostly for kale leaves, since the current production setup does not require costly field operations for additional harvest. A simple process adjustment can provide the feedstock with only transportation costs straining the economic balance. If health benefits from fibre and phenolic compounds can be substantiated, the economic feasibility of such a milled product could improve considerably.

White and green protein extraction (pathway B), is not an economically feasible option under the investigated conditions. Similar to broccoli, this is mainly due to the small fractions of protein that was recovered. The literature on the topic suggests the application of an ultrafiltration (UF) step or similar as one way of increasing the white protein recovery (Koschuh et al., 2004). From a cost perspective, a major part of UF cost is related to membrane replacement (Yu et al., 2020), but Bals and Dale (2011) suggested a low-cost and effective way to restore fouled membranes, which could decrease UF cost. However, the present study showed that more than 50% of the protein was still retained in the pulp after the juicing step, indicating additional fractionation early in the process (e.g. additional juicing steps or enzymatic treatments) are needed to reach feasibility for the protein fractionation. Also, mining other components, such as bioactive components and fibre would contribute positively to process economic feasibility.

Table A1 – Overview of commercial horse feed products.

Product	Digestibility [g]	Fibre content [%]	Protein content [%]	Protein price [€/kg]	Source
Krafft Groov Original, 20 kg	81.8	18	11	8.9	https://www.granngarden.se/hastfoder-krafft-groov-original-20-kg/p/1235439
Krafft Groov Protein, 20 kg	85.2	16	13.5	8.0	https://www.granngarden.se/hastfoder-krafft-groov-protein-20-kg/p/1235440
Krafft Groov Extra Protein, 20 kg	82.4	16	17	6.6	https://borjes.se/stall-skotsel/hastfoder-stro/foder/krafft-groov-extra-protein-20kg/270
Best Horse Basic Pellets	90.0	?	11	7.9	https://www.foderonline.se/hastfoder/best-horse-basic-pellets.html
Best Horse Müsli Classic	90.0	?	11.4	8.0	https://www.foderonline.se/hastfoder/best-horse-musli-classic.html
Best Horse Müsli Classic, havrefritt	90.0	?	11.8	7.9	https://www.foderonline.se/hastfoder/best-horse-musli-classic-havrefritt.html
RS Mustang Protein Müsli	85.0	9	14	9.3	https://www.hooks.se/hast/hastfoder/20-kg-protein-musli-rs-mustang
RS Mustang Trottning	97.0	10	11.5	9.3	https://www.hooks.se/hast/hastfoder/hastfoder-2/pellets-20-kg-trotting-rs-mustang
RS Mustang Diet Pellets	112.0	20	14.7	7.6	https://www.hooks.se/hast/hastfoder/hastfoder-2/20-kg-diet-pellets-rs-mustang
RS Mustang Fibre Original Müsli	93.0	12	11.2	10.4	https://www.hooks.se/hast/hastfoder/hastfoder-2/musli-fiber-orginal-rs-mustang
RS Mustang Lusernpellets	0.0	27	15	7.0	https://www.hooks.se/hast/hastfoder/hastfoder-2/lusern-rs-mustang
RS Mustang Breed Pellets	95.0	14	12	7.8	https://www.hooks.se/hast/hastfoder/hastfoder-2/pellets-breed-rs-mustang
RS Mustang Active Pellets	90.0	8	10.5	9.8	https://www.hooks.se/hast/hastfoder/hastfoder-2/pellets-active-rs-mustang
RS Mustang Slobber Mash	90.0	7.2	11.2	10.0	https://www.hooks.se/hast/hastfoder/hastfoder-2/slobber-mash
RS Mustang Alround Müsli	85.0	9	10.7	10.0	https://www.hooks.se/hast/hastfoder/hastfoder-2/musli-allround-rs-mustang
Minimum	**0.0**	**7.2**	**10.5**	**6.6**	
Maximum	**112.0**	**27.0**	**17.0**	**10.4**	
Average	**84.4**	**13.9**	**12.4**	**8.6**	

For the combined protein fraction (pathway C), marketing as a horse feed has a good potential to achieve economic feasibility but requires further investigation. The horse feed market in Sweden is relatively large with a high number of horses kept for recreational and tournament purposes. As this requires that the feed product is safe for animals as a large component of their diet, further research is needed to investigate if the product possesses an acceptable content of anti-nutritional components. However, other specific nutritional or animal health-related components are interesting to investigate in order to motivate the higher product price required to reach economic sustainability.

For all three production pathways, the focus of a more detailed assessment should be on product quality enabling a better value assessment and market placement. This should also include an assessment of the stability of dried products.

4. Conclusions

Both broccoli and kale cultivation result in substantial amounts of residuals, in terms of stems and leaves, with the potential to be used as a raw material for producing protein-rich or other health promoting products for humans and animals, in particular in countries with large production volumes. The leaves of the two crops behave similarly when fractionated, with dry matter, protein, phenolics and fibre content and recovery similarly divided into the different fractions. Thus, for both crops, a high protein and a significant phenolic content is obtained in all fractions, although the protein content is higher in all fractions of broccoli than in the corresponding fractions of kale. The highest protein content is obtained in the GPF and WPC for both crops making these fractions interesting for food and feed production purposes. However, the protein recovery is clearly highest in the P fraction of both crops, with around 50% of the proteins ending up in this fraction thereby calling for an improved protein fractionation from the P. All juice fractions contain high amounts of phenolics indicating these fractions to be of importance for phenolics fractionation after a more thorough evaluation of their composition and solubility. A significant content of dietary fibres is only present in the P fraction of both crops.

Protein fractionation from broccoli and kale residuals results in large differences in costs and revenues depending on the planned products. For both crops, the most economically feasible use of the crop residues, such as the leaves, is a direct milling of the leaves to produce a flour to be used as a food additive with health claim. Higher feasibility is obtained for kale than for broccoli, due to a lower feedstock production cost of kale than broccoli. For broccoli, the production cost of the biomass to feed the protein fractionation facility is a large part of the cost, due to the fact that an extra harvest of the broccoli leaves is needed. A change in this procedure so that the leaves can be harvested together with the florets and thereafter sorted (similar to the current situation for kale), or a cheaper harvest procedure used, should reduce the cost for protein fractionation of broccoli. For kale, the cost for drying of the products produced is a significant part of the costs.

The revenues for the full fractionation of the broccoli and kale residual leafy biomass are extremely low, mainly due to the fact that the protein recovery in the WPC is very low, thereby resulting in substantially higher revenues for a limited protein fractionation with a CPF as the final product. The full fractionation resulting in a GPF and a WPC is only economically feasible if feedstock costs are significantly decreased (i.e. the leaf harvest procedure changed) and/or nitrogen recovery to the WPC significantly increased (i.e. by higher nitrogen recovery from the P fraction). Also, additional fractionation to develop an increased number of added-value products e.g. phenolics and dietary fibres, would contribute to economic feasibility for the full fractionation of broccoli and kale leafy residues.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A

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